Acta Scientiarum
http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v38i3.28111

Optimization of protein extraction process from jackfruit seed flour by reverse micelle system

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ABSTRACT. The extraction of protein from flour of jackfruit seeds by reverse micelles was evaluated. Reverse micelle system was composed of sodium dodecyl sulfate (SDS) as surfactant, butanol as solvent, and water. The effects of stirring time, temperature, molar ratio H_2O SDS^{-1}, concentration of butanol (mass percentage) and flour mass were tested in batch systems. Based on the adjusted linear regression model, only butanol concentration provided optimum extraction conditions (41.16%). Based on the analysis of surface response, the best extraction yield could be obtained at 25°C, stirring time of 120 min, mass of flour of 100 mg, and a ratio H_2O SDS^{-1} of 50. Experimental results showed that a 79.00% extraction yield could be obtained.

Keywords: sodium dodecyl sulfate (SDS), butanol, DCCR, *Artocarpus Heterophyllus* Lam.

Otimização do processo de extração da proteína da farinha de semente de jaca utilizando sistema de micela reversa

RESUMO. Nesse trabalho foi avaliado o processo de extração de proteína da farinha de semente de jaca utilizando micelas reversas. O sistema de micelas reversas utilizado foi composto por dodecil sulfato de sódio (SDS) como surfactante, butanol como solvente, e água. Foram testados os efeitos do tempo de mistura, temperatura, razão molar H_2O SDS^{-1}, concentração de butanol (porcentagem em massa) e massa de farinha. Baseado no modelo de regressão linear ajustado, apenas o fator concentração de butanol apresentou uma condição ótima de extração que foi de 41,16%. Baseado na análise de superfície de resposta, em 25°C, no tempo de mistura de 120 min, na massa de farinha de 100 mg e na razão H_2O SDS^{-1} de 50, é possível obter o melhor rendimento de extração. Os resultados experimentais mostraram que foi possível alcançar rendimentos de extração de 79,00%.

Palavras-chave: dodecil sulfato de sódio (SDS), butanol, DCCR, *Artocarpus Heterophyllus* Lam.

Introduction

In the wake of utilizing wastes from food industries as an alternative protein source, several research works have been conducted to fulfill daily nutritional needs for the general public with efficiency and accessibility. Protein food of animal origin has been replaced by that of plant origin as new food sources with technological and nutritional properties (Nunes, Batista, Raymundo, Alves, & Sousa, 2003).

Jackfruit seeds, the fruit´s byproduct (averaging 15 - 25% of the fruit), are used as food, cooked or roasted or baked on coal. They are not only nutritive, but also tasty (Silva, Ribeiro, & Silva, 2007). The flour from seeds is a fruit residue featuring alternative protein, carbohydrate and fiber source.

Reverse micelles, a possible alternative as a protein liquid–liquid extraction method from jackfruit seed, are surfactant molecule aggregates of nanometric size, with their polar groups concentrated in the interior, while their hydrophobic parts extend into and are surrounded by the organic solvent, thermodynamically stable and optically transparent system (Silber, Biasutti, Abuin, & Lissi, 1999; Nandini & Rastogi, 2009). The technique offers several advantages such as low interfacial tension, ease of scale-up and continuous mode of operation. The biotechnological importance of these structures is due to their capacity of increasing solubility of hydrophilic molecules, such as proteins, in their polar sites (Sun, Zhu, & Zhou, 2008).

Several research groups are involved in studies on protein extraction by reverse micelles. Sun, Zhu, and Zhou (2008) studied the use of reverse micelles containing AOT (bis-2-ethyl-hexil sodium sulfosuccinate) as surfactants and isooctane as an organic solvent, for the protein extraction from non-fat wheat germ, using the surface response method for the optimization of extraction. As a rule, authors verified the following advantages in protein extraction by reverse micelles: surfactants and organic solvents may be repetitively used after recovering the same, with reduced extraction costs, and polar sites of reverse micelles maintain activities which are characteristics of dissolved proteins. Nascimento et al. (2008) optimized extraction of lectin from the bark of *Crataeva tapia* from a crude extract using anionic surfactant sodium bis (2-ethylhexyl) sulfosuccinate (AOT) in isooctane reversed micelles. The back-extraction to a final aqueous phase was made by adding butanol. The overall yield obtained for the process was 80% for lectin activity and 56% for protein recovery.

Bu et al. (2012) used three kinds of reverse micelle systems, namely, anionic surfactant AOT, sodium dodecyl sulfate (SDS) and cationic surfactant cetyl trimethyl ammonium bromide (CTAB) to extract soy protein. The effects of soy flour concentration, Wo ($[H_2O]/[AOT]$), temperature, time, pH, ionic strength and ultrasonic power on forward extraction efficiency of soy protein were investigated. The three methods were effective in the extraction of soy protein.

Hasmann, Cortez, Pessoa Júnior, and Roberto (2003) studied the β-xylosidase recovery by reversed micelles using CTAB cationic surfactant and butanol. The optimization of β-xylosidase recovery was made by response surface methodology. Gaikaiwari, Wagh, & Kulkarni (2012) presented an optimized methodology for reverse micellar extraction and purification of *Aspergillus allahabadi* intracellular tannase. Under optimized conditions, a 81.2% recovery of tannase was obtained.

Since jackfruit seeds are not employed in food industries and since there is a lack of studies on protein extraction from jackfruit seeds by reverse micelles, current research develops an alternative method for protein extraction from jackfruit seed flour using reverse micelles, with sodium dodecyl sulphate (SDS) as surfactant and butanol as organic solvent. SDS is an anionic surfactant and an FDA-approved food additive (FDA 21 CFR 172.822) (Predmore & Li, 2011). With the addition of the short chain alcohol butanol, a stable micellar system may be established at room temperature and the alcohol is used in protein recovery (Krei & Hustedt, 1992; Hemavathi, Hebbar, & Raghavarao, 2007).

A fractional factorial was employed to evaluate effects of butanol concentration (mass percentage), stirring time, temperature, H_2O SDS^{-1} ratio and flour mass in batch systems to optimize protein extraction process. Further, Central Composite Rotatable Design (CCRD) for testing significant factors at 5% level was employed on the fractional factorial. Further, the Methodology of Surface Response (MSR) was applied to optimize protein extraction conditions of jackfruit seed flour by the reverse micelle system.

Material and methods

Material

Seeds were obtained from jackfruits commercialized in the neighborhood of Itapetinga BA Brazil. Sodium dodecyl sulfate (SDS) and 1-butanol were obtained from Vetec Química Fina (Rio de Janeiro, Rio de Janeiro State, Brazil). All reagents were of analytical degree.

Preparation of jackfruit seed flour

Seeds were cleaned in fresh water, crushed with water (1:3) and filtered in a cotton filter (1 mm gap) to remove starch. The procedure was repeated six times. The cake was dried at 50°C (trail dryer) for 24 hours. Then, it was crushed in a hammer mill, screened in a 20-mesh sieve to obtain the desired granulometry. The flour without starch (FWS) was stored in plastic recipients at room temperature.

Chemical analysis

The proximate composition (moisture content, total fat, protein and ash) of FWS was determined according to official methods (Association of Official Analytical Chemists [AOAC], 1990), with three replications, each in triplicate.

Preparation of reverse micelle system and protein extraction

Reverse micelle systems were made from SDS, butanol and water, prepared according to Gallego, Bravo-Diaz, and Romero (2004). Each reagent was separately weighed. SDS was mixed to butanol and water was added in the specific amount for each system. SDS concentration have always been above the critical micelle concentration (CMC) (4.2 mmol L^{-1}) obtained from Dubey (2008).

Variables in the extraction process were H_2O SDS^{-1} molar ratio, butanol percentage, temperature, time and flour mass for the fractional factorial; and H_2O SDS^{-1} molar ratio, concentration of butanol (mass percentage) and flour mass for CCRD.

All forward extraction experiments were conducted at 40 mL Falcon tubes. The protein was extracted directly from FWS. The tubes were agitated at 40 rpm in orbital equipment during previously set intervals. A BOD incubator (Tecnal TE-184, Brazil) was used to control temperature. The non-dissolved residue was separated from the micellar system by centrifugation (5804, Eppendorf, Germany) at 2000 × g, during 15 min.

Extraction efficiency

Protein was quantified by Semi-micro Kjeldahl method (conversion factor of 6.25) in the micellar systems collected to estimate extraction efficiency (EE), calculated by Equation 1.

$$EE\,(\%) = \frac{VS \times PM}{MF \times PF} \times 100 \quad (1)$$

where:
VS is the micellar system volume (mL);
PM is the protein concentration in micellar system (mg mL^{-1});
MF is the FWS mass (mg);
PF is the protein concentration (mg mg^{-1}).

Experimental design

A 2^{5-1} fractional factorial design with 4 replications at the central point was implemented to evaluate the influence of H_2O SDS^{-1} molar ratio, concentration of butanol (mass percentage) ([Butanol]), temperature, time and flour mass in the percentage of protein extracted from flour (dependent variable) by reverse micelles. Table 1 shows rates of variables and their levels.

Table 1. (2^{5-1}) Fractional factorial design: variables and levels.

Variable	Level		
	-1	0	1
H_2O/SDS	32.2	36.5	42.7
[Butanol] (% m m^{-1})	45.0	62.5	80.0
Temperature (°C)	15.0	25.0	35.0
Stirring time (min)	30.0	180.0	330.0
Flour mass (mg)	200.0	300.0	400.0

Results obtained from experiments were submitted to ANOVA variance analysis and effects were considered significant at $p < 0.10$.

From ANOVA results for fractional factorial experiment, the first central rotational composed design was used to optimize or verify optimization tendency of protein extraction from flour.

Significant effects were studied at two levels of four repetitions at the central point, used for evaluating experimental error and model fitness. For each independent variable, higher value (codified value: +1) and lower value (codified value: -1), for axial points higher value (codified value: +1.68) and lower value (codified value: -1.68), defined points were selected according to results obtained from fractional factorial planning.

With a second order polynomial model (Equation 2), experimental data and regression coefficients were adjusted and regression coefficients were obtained by multiple linear regression.

$$K = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (2)$$

From CCRD results, a new CCRD was installed with new variable values to optimize extraction process. Variables of this new design were the same variables from the past.

However, values tended to the optimal region. In both CCRD, results were statistically analyzed by variance analysis and regression, taking into consideration lack of adjustment, parameters´ significance and regression coefficient. By surface response methodology, the best conditions for protein extraction were determined for intervals of the experimental conditions employed.

All statistical analyses were conducted with *Statystical Analysis System®* 9.0, RSREG procedure (SAS Institute Inc., Cary NC USA).

Results and discussion

Gross composition of flour

Moisture content of jackfruit seed flour was 9.40% (± 1.34). In their studies on methods to obtain jackfruit seed flour, Mukprasirt and Sajjaanantakul (2004) reported a moisture content of 13.16%. Vanna, Kanitha, Prapa, and Nongnuj (2002) analyzed jackfruit seed flour of the hard and soft varieties and obtained moisture contents 12.67 and 9.67%, respectively. Difference in moisture content is due to different methods to obtain flour used by each author.

Protein content of jackfruit seed flour was 12.00% (± 0.02), and lipid content was equal to 8.89%. Protein content was higher than the percentage registered by Mukprasirt and Sajjaanantakul (2004), who obtained 10.37%, and that recorded by Vanna et al. (2002) who evaluated protein content in jackfruit flour seed of the hard and soft varieties, with 5.05 and 5.14%, respectively. Variations were due to technological differences in the preparation of samples.

Flour ash content was equal to 1.53% (± 0.13), or rather, it was lower than rate by Mukprasirt and Sajjaanantakul (2004) equal to 3.21 and by Vanna et al. (2002) who obtained ash content equal to 3.92.

Effects on H2O SDS-1 molar ratio, concentration of butanol (mass percentage), temperature, time and flour mass in protein extraction from jackfruit seed flour

Fractional factorial evaluation

Variance analysis (ANOVA) and parameter estimate analysis (Table 2) were conducted for the 2^{5-1} fractional factorial to evaluate which factors had a significant effect on the protein extraction process of meal jackfruit using reverse micelle of SDS, water and butanol.

Table 2. Estimate of parameters for extraction yield using 2^{5-1} factorial.

Variable	FD	Estimates	t value	Pr > t
[Butanol] (% m m^{-1})	1	-10.5504	-3.52	0.0037
Temperature (°C)	1	1.9397	0.65	0.5283
Time (min)	1	2.2666	0.76	0.4624
H_2O SDS^{-1}	1	5.4802	1.83	0.0901
Flour mass (mg)	1	-6.0362	-2.02	0.0649

Probability rates in Table 2 showed that only concentration of butanol (mass percentage) ([Butanol]), H_2O SDS^{-1} molar ratio and flour mass (M.F.) had significant effects on protein extraction at $p < 0.10$ by t test.

Results demonstrated that when concentration of butanol and flour mass varied between 45.0 and 80.0 % m m^{-1} and between 200.00 and 400.00 mg, respectively, the extraction efficiency decreased. According to Sun et al. (2008), effect of flour mass was perhaps the limitation of the quantity and size of reverse micelles so that excess protein may not be solubilized in reverse micelles and forward extraction efficiency decreased. It has also been demonstrated that H_2O SDS^{-1} molar ratio had a positive effect on protein extraction, i.e., an increase in the rate of the variable causes an increase in extraction efficiency. This fact may be due to higher water amounts in the reverse micelles and consequently increase in protein solubility.

Model adjustment (CCRD results)

Employing information from fractional factorial evaluation, CCRD was used twice with factors which obtained significant effects on the protein extraction process of jackfruit seed flour to optimize values of significant variables concentration of butanol (mass percentage) (X_1), H_2O SDS^{-1} molar ration (X_2) and M.F. (X_3), in the extraction process. Factors with no effect, such as temperature and time, were fixed in CCRD at 25°C and 120 min respectively. Extraction results from the first CCRD (Table 3) showed that by fixing other factors, in a general mode, yield extraction decreased and butanol percentage increased when the micelle system was employed.

Table 3. Data for the 1st CCRD with factors which affected fractional factorial evaluation.

Sample	X_1	X_2	X_3	x_1	x_2	x_3	% Extraction
1	45.00	30.20	200	-1	-1	-1	63.92
2	80.00	30.20	200	1	-1	-1	37.61
3	45.00	46.70	200	-1	1	-1	81.65
4	80.00	46.70	200	1	1	-1	42.77
5	45.00	30.20	400	-1	-1	1	36.36
6	80.00	30.20	400	1	-1	1	38.64
7	45.00	46.70	400	-1	1	1	46.43
8	80.00	46.70	400	1	1	1	41.48
9	33.10	38.45	300	-1.68	0	0	46.78
10	91.90	38.45	300	1.68	0	0	14.07
11	62.50	24.59	300	0	-1.68	0	47.23
12	62.50	52.31	300	0	1.68	0	47.28
13	62.50	38.45	132	0	0	-1.68	20.30
14	62.50	38.45	468	0	0	1.68	25.17
15	62.50	38.45	300	0	0	0	23.17
16	62.50	38.45	300	0	0	0	24.08
17	62.50	38.45	300	0	0	0	24.06
18	62.50	38.45	300	0	0	0	25.39
19	62.50	38.45	300	0	0	0	24.78
20	62.50	38.45	300	0	0	0	23.80

When other factors were fixed and H_2O SDS^{-1} ratio increased, yield extraction tended to increase. While keeping butanol percentage and H_2O SDS^{-1} ratio and increasing flour mass, yield extraction tended to decrease.

Based on this information, a new CCRD (Table 4) was built, decreasing concentration of butanol (mass percentage), increasing H_2O SDS^{-1} ratio and decreasing MF. These rates were chosen to acquire higher extraction yields.

Table 4. Data from 2nd CCRD adjusting parameters to rates which obtained acceptable extraction yield from the 1st CCRD.

Sample	X_1	X_2	X_3	x_1	x_2	x_3	% Extraction
1	30.00	35.00	150	-1	-1	-1	32.05
2	60.00	35.00	150	1	-1	-1	35.75
3	30.00	45.00	150	-1	1	-1	47.91
4	60.00	45.00	150	1	1	-1	49.39
5	30.00	35.00	300	-1	-1	1	19.64
6	60.00	35.00	300	1	-1	1	29.18
7	30.00	45.00	300	-1	1	1	49.83
8	60.00	45.00	300	1	1	1	49.52
9	19.80	40.00	225	-1.68	0	0	29.19
10	70.20	40.00	225	1.68	0	0	3.76
11	45.00	31.60	225	0	-1.68	0	64.97
12	45.00	48.40	225	0	1.68	0	79.43
13	45.00	40.00	99	0	0	-1.68	54.72
14	45.00	40.00	351	0	0	1.68	21.41
15	45.00	40.00	225	0	0	0	71.63
16	45.00	40.00	225	0	0	0	32.04
17	45.00	40.00	225	0	0	0	33.07
18	45.00	40.00	225	0	0	0	32.46
19	45.00	40.00	225	0	0	0	32.01
20	45.00	40.00	225	0	0	0	50.26

Results experimentally obtained from the 2nd CCRD were submitted to ANOVA and Multiple Linear Regression Analysis to verify factors´ influence on extraction yield.

The Equation 3 for extraction efficiency (Y) as a function of non-corded variables X_1 (concentration of butanol (mass percentage)), X_2 (H_2O SDS^{-1} ratio) and X_3 (flour mass) was:

$$Y=54.9200-7.2124X_1+11.6281X_2-10.2147X_3- -14.0861X_1^2 \quad (3)$$

Extraction efficiency decreased with an increase in butanol. Although the exact mechanism of the co-solvent is still not quite clear, there are reports which indicate that co-solvent molecules might have inserted between the molecules of the surfactant, thereby decreasing the interaction between surfactant head groups (Hemavathi et al., 2007). Hemavathi, Hebbar, and Raghavarao (2007) studied the effect of butanol concentration on forward extraction of bromelain of pineapple using CTAB or AOT and butanol systems and reported that a decrease in extraction efficiency occurred above 15% ($v\ v^{-1}$) of butanol concentration.

As H_2O SDS^{-1} ratio increased, the extraction efficiency also increased. The above result is consistent with report by Bu et al. (2012) using surfactant AOT for soy protein extraction by reverse micelles systems. The authors verified the forward extraction efficiency increased with an increase of the molar ratio of water to surfactant ($[H_2O]/[AOT]$). Ratio increase caused an increase in the reverse micelle size. Meanwhile, protein solubilization strongly depended on the reverse micelle size. The size of micelle relative to the size of a protein was critical to the ability of the micelle to solubilize protein (Sechler, Delsole, & Deak, 2010). The addition of protein to reverse micelles did not appreciably solubilize the protein until the diameter of the reverse micelle was similar to that of the protein (Matzke, Creagh, Haynes, Prausnitz, & Blanch, 1992). With an increase in $[H_2O]/[AOT]$, larger reverse micelles were formed which were able to include plural protein molecules.

Similar results on flour mass were also reported by Bu et al. (2012). They verified that when soy flour concentration increased, the forward extraction efficiency decreased gradually in the reverse micellar systems studied. It was due to soy protein which could enter the micelle in a limited way. These results were also consistent with conclusion by Sun et al. (2008).

The statistical significance of Equation 3 was verified by F test and the results of ANOVA Variance Analysis are in Table 5.

Table 5. ANOVA for surface response model (CV = 36.48% and $R^2 = 0.6267$).

FV	GL	SQ	QM	F Cal.	p < 0.005
Model	4	6879.838	1719.959	6.30	0.0035
Lack of fit	10	2875.677	287.567	1.18	0.4552
Error	15	4098.390	273.226		
Total	19	10078.000			

Due to the fact that F rate of the model has low probability rate ($p = 0.0035$), the model is highly significant.

The test for lack of fit, associated with central point errors, was not significant in this case ($p < 0.05$). Regression analysis (Table 6) for experimental data of the 2nd CCRD showed that, according to t test for parameters significance, molar ratio and flour mass factors had a significant linear effect on protein extraction yield, while concentration of butanol (mass percentage) was significant on quadratic effect. Estimates for independent variables and the correspondent p rates suggested that, although X_1 (concentration of butanol (mass percentage) had no significant effect on Y (R.E. of flour protein), its quadratic term had a significant effect on Y response. According to Gallego et al. (2004), concentration of butanol (mass percentage) is a fundamental factor for reverse micelles formation and was able to influence micelle concentration. A positive coefficient for X_2 revealed a linear effect for increasing Y response, while negative coefficients for X_1 and X_3 showed a linear effect for decreasing Y response; X_{11} coefficient showed a quadratic effect on decreasing Y response. X_1, X_2, X_3 and X_{11} were the significant model factors in current research.

Table 6. Regression coefficients´ significance for response (Y).

Model term	Coefficient	Standard deviation	t value	p value
X_0	54.9200	4.7284	11.61	< 0.0001
X_1	-7.2124	4.4748	-1.61	0.1278
X_2	11.6281	4.4748	2.60	0.0202
X_3	-10.2147	4.4748	-2.28	0.0375
X_{11}	-124.0861	4.3226	-3.26	0.0053

ANOVA results for the complete model is shown in the Pareto Graph (Figure 1), in which absolute amplitude and estimate standard effect (estimative effect divided by standard deviation) values of each factor were plotted in an decreasing order when compared to a 95% reliability of the minimum significant factor ($p = 0.05$), represented by the vertical line.

The Pareto Graph on protein extraction of jackfruit flour seed (Figure 1) showed that the quadratic relation of concentration of butanol (mass percentage - [Butanol]) had a greater effect on yield. Further, H_2O SDS^{-1} molar ratio and flour mass (MF), without their interaction, also had an important effect on extraction yield since these factors are related to the amount of reverse micelle and to the amount of dissolved protein in reverse micelles.

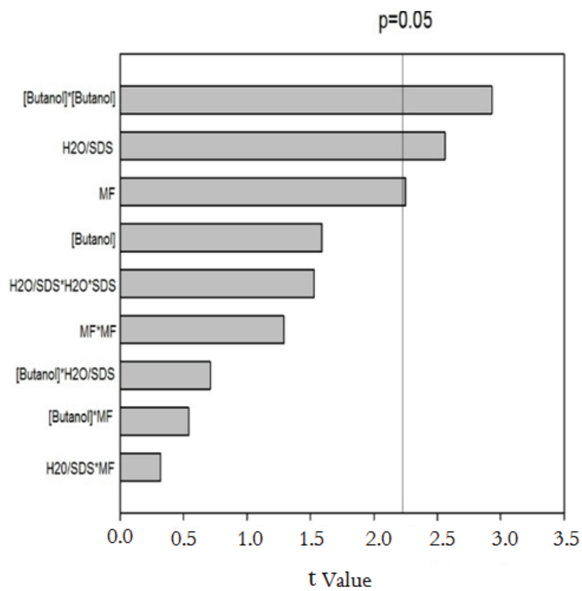

Figure 1. Pareto Graph for standard effects of variables concentration of butanol (mass percentage), (X_1), H_2O SDS^{-1} ratio (X_2), flour mass (X_3) on protein extraction rate of jackfruit flour seed.

Effect of independent variables on response variable

Using the Method of Surface Response (MSR), the relationship between factors and response may be better understood, exposing each effect behavior on the extraction yield.

In the MSR graph (Figure 2), butanol concentration rates (mass percentage) were fixed, with variations in H_2O SDS^{-1} ratio and flour mass only. It has been observed that an increase of water amount in reverse micelle system causes an increase of protein extraction yield (Y) of jackfruit seed flour.

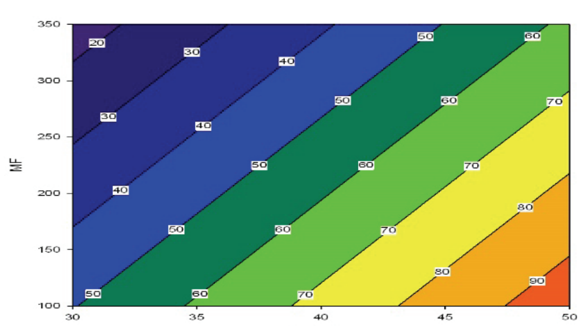

Figure 2. Surface Response Graph for H_2O SDS^{-1} ratio (X_2) and MF (X_3) effects on protein extraction yield.

The graph shows that for the optimal extraction with fixed butanol concentration (mass percentage) the H_2O SDS^{-1} ratio rates are around 50, and MF, under studied conditions, may be around 100 mg, for approximate optimal protein extraction rate. Protein extraction increases by reverse micelles with H_2O SDS^{-1} ratio increase for rates close to 50, due to the necessity of an equilibrium between water and surfactant amount for reverse micelle formation in this type of system. According to Gallego et al. (2004), H_2O SDS^{-1} ratio must be around 42.70. According to these authors, the rate was calculated by Jobe, Dunford, Pickard, and Holwarth (1989) in phase diagram studies of compound reverse micelle systems by SDS, butanol and water.

To evaluate the concentration of butanol (mass percentage) (X_1) and MF (X_3) effects on extraction yield, a Surface Response Graph (Figure 3) was drawn, fixing H_2O SDS^{-1} ratio. Graph analysis reveals that extraction yield is close to optimal point when butanol concentration (mass percentage) is around 40.0% for the systems analyzed. Moreover, in a wide range of butanol concentration (mass percentage) rates, between 20.0 and 60.0%, extraction yield varies from 70.0 to 95.0%, with an optimal extraction point around 40.0% of butanol. In the case of MF, RE optimal point is near 100.0 mg of flour. It is important to point out that according to the previous graph (Figure 2), extraction yield decreased as mass flour increased under current conditions.

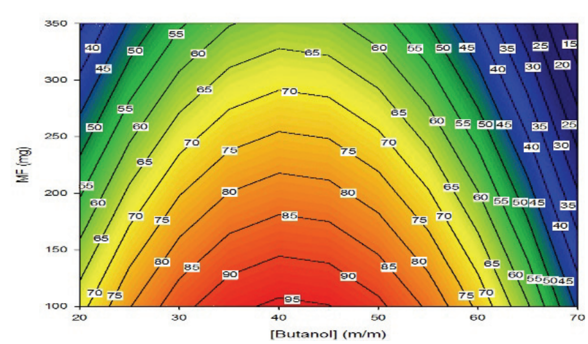

Figure 3. Surface Response Graph for ([Butanol]) (m m^{-1}) (X_1) and MF (X_3) effects on protein extraction yield.

Butanol acts on the reverse micelle system as an organic solvent. Therefore, if concentration of butanol (mass percentage) reaches above optimal rates (40.0%), the system amount of water and surfactants tends to decreased; consequently, the amount of reverse micelle able to extract protein from jackfruit seed flour also tends to decrease, with a reduction in yield extraction.

To evaluate butanol concentration (mass percentage) and H_2O SDS^{-1} ratio influence, the flour mass rate was fixed on the surface of the response graph (Figure 3). The optimal yield extraction for butanol concentration (mass percentage) was close to 40.0%, as previously discussed; and the optimal point for H_2O SDS^{-1}

ratio was close to 50, confirming results shown in Figure 4.

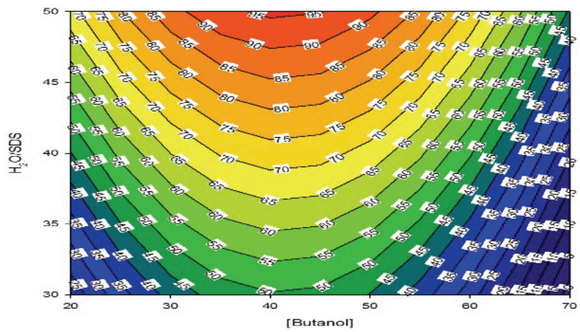

Figure 4. Surface Response Graph for butanol concentration (mass percentage) ([Butanol]), (X_1) and H_2O SDS^{-1} ratio (X_2) on protein extraction yield for jackfruit seed flour.

Data from Surface Response Graph reveals information that clarifies the optimal region for MF and H_2O SDS^{-1} ratio factors. Since only concentration of butanol (mass percentage) had a quadratic effect, it is the only factor for which an optimal rate was obtained by the derivative of the model with non-coded rates (Equation 4):

$$Y = -11259738+5.15358X_1+2.32562X_2- -0.13620X_3-0.06260X^2_1 \quad (4)$$

Equation 5 is obtained by derivation of Equation 4 in relation to X_1,

$$\frac{dY}{dX_1} = 5.1536 - 0.1252X_1 \quad (5)$$

Through dY dX_1^{-1} = 0, the optimal point is obtained, or rather, butanol % in which the extraction yield is maximum and X_1 = 41.16%.

Therefore, the best conditions for protein extraction from jackfruit seed flour by the Method of Surface Response comprise 41.16% of butanol, 100 mg of flour mass and H_2O SDS^{-1} ratio = 50.

For these variable rates, efficiency extraction was 96.13%. The rate is similar to that reported by Yu, Chu, and Ji (2003) and higher than that registered by Zhao, Liu, Chen, and Liu (2012) and Zhao, Chen, Chen, Wang, and Wang (2015) to forward extraction yeast-lipase, protein from sesame seeds and peanut protein by AOT-reverse-micelles, respectively.

Model validation

For the validation of the model, jackfruit seed flour protein was extracted under ideal conditions and determined. The experimental value was compared to the value predicted to determine the model´s validation. Optimal extraction points obtained from the model were: 41.16% butanol, H_2O SDS^{-1} ratio = 50 and flour mass = 100 mg. Efficiency prediction for extraction equaled 96.13%, while the experimental rate under the same conditions was 91.50%. Results indicated that experimental rate (91.50%) was close to the predicted one (96.13%).

Conclusion

Experimental results confirmed the viability of protein extraction from jackfruit seed flour using reverse micelle systems constituted by butanol, SDS and water. This extraction method is considered viable from the point of view of extraction yield, at 79.0%. The rate which maximizes extraction was only reported for butanol concentration (mass percentage), equal to 41.16%. Further studies must be conducted to see whether extracted proteins may have their properties modified by system reagents and if extraction process costs are viable.

References

Association of Official Analytical Chemists. (1990). *Official methods of analysis* (15th ed.). Arlington, TX: AOAC.

Bu, G., Liu, H., Chen, F., Liu, K., Yang, Y., & Gao, Y. (2012). Effects of different factors on the forward extraction of soy protein in reverse micelle systems. *African Journal of Biotechnology, 11*(28), 7247-7257.

Dubey, N. (2008). A conductometric study of Interaction between sodium dodecyl sulfate and 1-propanol, 1-butanol, 1-pentanol and 1-hexanol at different temperatures. *Journal Surface Science Technology, 24* (3-4), 139-148.

Gaikaiwari, R. P., Wagh, S. A., & Kulkarni, B. D. (2012). Extraction and purification of tannase by reverse micelle system. *Separation and Purification Technology, 89*, 288-296.

Gallego, M. J. P., Bravo-Diaz, C., & Romero, G. E. (2004). Fluorimetric determination of structural parameters of Bu OH/SDS/H_2O reverses micelles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 249*(1-3), 25-28.

Hasmann, F. A., Cortez, D. V., Pessoa Júnior, A., & Roberto, I. C. (2003). Optimization of β-xylosidase recovery by reversed micelles using response surface methodology. *Electronic Journal of Biotechnology, 6*(2), 153-157.

Hemavathi, A. B., Hebbar, H. U., & Raghavarao, K. S. M. S. (2007). Reverse micellar extraction of bromelain from *Ananas comosus* L. Merryl. *Journal of Chemical Technology and Biotechnology, 82*(11), 985-992.

Jobe, D. J., Dunford, H. B., Pickard, M., & Holwarth, J. F. (1989). *Reactions in compartmental liquids*. Helldelberg, DE: Springer-Verlag.

Krei, G. A., & Hustedt, H. (1992). Extraction of enzymes by reverse micelles. *Chemical Engineering Science, 47*(1), 99-111.

Matzke, S. F., Creagh, A. L., Haynes, C. A., Prausnitz, J. M., & Blanch, H. W. (1992). Mechanisms of protein solubilization in reverse micelles. *Biotechnology and Bioengineering, 40*(1), 91-102.

Mukprasirt, A., & Sajjaanantakul, K. (2004). Physico-chemical properties of flour and starch from jackfruit seeds (*Artocarpus heterophyllus* Lam.) compared with modified starches. *International Journal of Food Science and Technology, 39*(3), 271-276.

Nandini, K. E., & Rastogi, N. K. (2009). Reverse micellar extraction for downstream processing of lipase: Effect of various parameters on extraction. *Process Biochemistry, 44*(10), 1172-1178.

Nascimento, C. O., Costa, R. M. P. B., Araújo, R. M. S., Chaves, M. E. C., Coelho, L. C. B. B., Paiva, P. M. G., ... Carneiro-da-Cunha, M. G. (2008). Optimized extraction of a lectin from *Crataeva tapia* bark using AOT in isooctane reversed micelles. *Process Biochemistry, 43*(7), 779-782.

Nunes, M. C., Batista, P., Raymundo, A., Alves, M. M., & Sousa, I. (2003). Vegetable proteins and milk puddings. *Colloid Surface B, 31*(1-4), 21-29.

Predmore, A., & Li, J. (2011). Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Applied and Environmental Microbiology,* 77(14), 4829-4938.

Sechler, T. D., Delsole, E. M., & Deak, J. C. (2010). Measuring properties of interfacial and bulk water regions in a reverse micelle with IR spectroscopy: a volumetric analysis of the in homogeneously broadened OH band. *Journal of Colloid Interface Science, 346*(2), 391-397.

Silber, J. J., Biasutti, A., Abuin, E., & Lissi, E. (1999). Interactions of small molecules with reverse micelles. *Advances in Colloid and Interface Science, 82*(1-3), 189-252.

Silva, J. H. V., Ribeiro, M. L. G., & Silva, E. L. (2007). Efeitos da inclusão do farelo de sementes de jaqueira Artocarpus heterophyllus Lam.) na ração sobre a produção, pigmentação da gema e umidade fecal em codornas. *Ciência e Agrotecnologia, 31*(2), 523-530.

Sun, X. H., Zhu, K. X., & Zhou, H. M. (2008). Protein extraction from defatted wheat germ by reverse micelles: Optimization of the forward extraction. *Journal of Cereal Science, 48*(3), 829-835.

Vanna, T., Kanitha, T., Prapa, S., & Nongnuj, J. (2002). Some physicochemical properties of jackfruit (*Artocarpus heterophyllus* Lam) seed flour and starch. *Science Asia,* 28, 37-41.

Yu, Y.-C., Chu, Y., & Ji, J.-Y. (2003). Study of the factors affecting the forward and back extraction of yeast-lipase and its activity by reverse micelles. *Journal of Colloid and Interface Science, 267*(1), 60-64.

Zhao, J., Liu, D., Chen, F., & Liu, G. (2012). Optimization of forward extraction of protein from sesame seeds by reverse micelles. *Journal of the Chemical Society of Pakistan, 34*(4), 849-855.

Zhao, X., Chen, J., Chen, X., Wang, X., & Wang, Y. (2015). Separation of peanut protein by reverse micelles: optimization of the forward extraction. *Journal of Food Processing and Preservation, 39*(6), 1457-1465.

Received on June 9, 2015.
Accepted on February 15, 2016.