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Original

Thermal properties of defatted meal, concentrate, and protein isolate of baru nuts (*Dipteryx alata* Vog.)

Propriedades térmicas de farinha desengordurada, concentrado e isolado proteico de baru (Dipteryx alata Vog.)

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Abstract

Baru (*Dipteryx alata* Vog.), a species of legume found in the Brazilian savannas, was investigated in this study for the composition of its flesh and seed. Thermal analyses, Thermogravimetry (TG), and Differential Scanning Calorimetry (DSC) were used to investigate the proteins in defatted meal, concentrate, and protein isolate. The protein concentrate was extracted at pH 10, followed by a precipitation at the isoelectric point to obtain the isolate that was spray dried. The thermogravimetric curves were obtained under a nitrogen atmosphere with a 100 mL/minute flow. The initial, final and peak temperatures and mass loss were analyzed. Within the performed temperature ranges studied, the defatted meal and concentrate presented four steps of mass loss, while the isolate showed only two steps. The protein content of defatted meal from Baru nuts was higher than that of the isolate. On the other hand, there was a reduction in enthalpy, which suggests that the process applied to obtain the baru concentrate and isolate led to protein denaturation.

Keywords: *protein denaturation; thermal analysis; endothermal peaks; legumes.*

Resumo

Componentes de polpa e de semente de baru (*Dipteryx alata* Vog.), leguminosa do cerrado brasileiro, foram objetos de estudo neste trabalho. Análises térmicas, Termogravimetria (TG) e Calorimetria Exploratória Diferencial (DSC) foram utilizadas na investigação de proteínas em farinha desengordurada, concentrado e isolado proteico. A extração do concentrado proteico foi em pH 10, seguida de precipitação no ponto isoelétrico para obter o isolado, o qual foi seco por atomização. As curvas termogravimétricas foram obtidas em atmosfera de nitrogênio em vazão de 100 mL/minuto. As temperaturas iniciais, finais e de pico foram analisadas, assim como a perda de massa. Na faixa de temperatura avaliada, a farinha desengordurada e o concentrado apresentaram quatro etapas de perda de massa, enquanto que o isolado apenas duas etapas. O conteúdo de proteína da farinha desengordurada da semente de Baru foi mais alto do que do isolado. Por outro lado, ocorreu redução da entalpia, sugerindo que o processo aplicado na obtenção do concentrado e isolado de baru levou à desnaturação das proteínas.

Palavras-chave: *desnaturação proteica; análises térmicas; picos endotérmicos; leguminosas.*

1 Introduction

Brazil has the largest biological diversity on the planet with about 30% of the known species of plants and animals distributed in various ecosystems (AVIDOS; FERREIRA, 2000). Brazilian savanna is the second largest biome in South America with a large number of endemic plant including more than 40% of species of woody plants.

Baru (*Dipteryx alata* Vog.) is a Brazilian savanna fruit that belongs to the Family *Leguminosae* and is abundant in the States of Mato Grosso, Mato Grosso do Sul, Goiás and Minas Gerais; it is considered as a drupe (fleshy fruit with a single seed or nut). A portion of 100 g of baru nuts provides 574.8 kcal and contains 33.2 g of protein, 24.65 g of carbohydrate, and 38.3 g of lipids (JUNQUEIRA; FAVARO, 2004). This elevated protein content suggests possible applications as an isolated ingredient, like soybean, the most commonly consumed legume in the world, with a protein content of about 39 g.100 g^{-1} (VIEIRA et al., 1999). Like soy, the proteins in baru lack sulphurated amino acids (TOGASHI; SGARBIERI, 1995).

Defatted meal, concentrates, and protein isolates from legumes constitute a potential source of proteins that are widely studied for their applications in the food industry as functional ingredients.

Thermal analysis, Thermogravimetry (TG), and the Differential Scanning Calorimetry (DSC) are efficient in the study of proteins' behavior in a linear temperature scale providing useful results (MAGOSHI et al., 2002) for the study of the conformation transitions of biological macromolecules. This methodology allows the assessment of the transition phenomena of biomolecules in terms of temperature, enthalpy, and heat capacity during the process (GIANCOLA, 2008). In the food industry, it plays an important role since most processing chains involve heating steps that could cause changes in the functional and nutritional characteristics of the final product. One of the most important events which can take place with proteins is denaturation. Protein unfolding is an endothermic event which can be performed by DSC (GIANCOLA, 2008).

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Additionally, events such as mass loss related to proteins, lipids, carbohydrates, water, and volatile fractions can be thoroughly studied with TG.

In the present study, the effect of thermal properties, TG, and DSC on defatted meal, concentrate, and protein isolate of baru nuts were analyzed in order to understand the behavior of their proteins at various temperatures.

2 Material and methods

2.1 Raw material

The fruits were collected in the city of Campo Grande, Mato Grosso do Sul State, Brazil. The nuts were oven-dried with air-circulation at 70 °C for 24 hours to facilitate peeling, which was done by hand. The cleaned nuts were ground in a blender, sifted, and defatted in a Soxhlet extractor with hexane.

2.2 Concentrate and protein isolate

The protein concentrate was obtained by extracting the proteins in the meal at pH 10 with maximum solubility in the proportion of m/v 1:20, followed by centrifugation at 1520 g 20/minute. The supernatant was dried by spray drying (Büchi, Model B 290) at a sample entrance temperature of 165 °C, aspiration 100%, and injection pump pressure of 25%.

The protein isolate was obtained from the defatted meal dispersed in distilled water in the ratio of 1:20 (mass/volume) and the pH maintained constant at 10 by the addition of NaOH 0.1N. The suspension was centrifuged at 3000 rpm/30 minute; the supernatant was collected and the protein precipitated at pH 4.8, defined as the isoelectric value for baru proteins. The protein was then centrifuged at 1520 g 40/minute, and the precipitate was lyophilized resulting in the isolate.

2.3 Protein content

The protein contents of the defatted meal, concentrate and isolate of baru nuts were determined by the Kjeldahl method (ASSOCIATION…, 1997).

2.4 Thermal analysis

The TG and DSC thermogravimetric curves were obtained using the adapted protocols of Carrasco (1996), Conceição (2007), Faria (2002) and Oliveira (2006). In a thermobalance SDTQ600 (TA Instruments), an average mass of 8.85 mg of samples were placed into aluminum pans and heated from 25 to 350 °C at a heating rate of 20 °C/minute. The atmosphere used was nitrogen, with a 100 mL/minute flow. The calibration was carried out with indium and zinc. An opened pan was used as reference. The thermogravimetric curves obtained were processed with the software Tasys. Enthalpy values were expressed as $J.g^{-1}$ sample and the peak temperature (T_p) was considered as the denaturation temperature (T_d) (TANG; MA, 2009).

3 Results and discussion

3.1 Protein content

The defatted meal of baru nuts presented 49.01 $g.100\ g^{-1}$ of protein. The solubilization procedure led to a concentrate with 55.03 $g.100\ g^{-1}$ of protein, and after the precipitation, at the isoelectric point, the protein content rose to 84.11 $g.100\ g^{-1}$ in the isolate. The amount of protein increased 72% from the defatted meal to the isolate.

3.2 Thermal analysis: TG and DSC

The thermal behavior, in the evaluated range of temperature, was similar among the samples assayed (Figure 1). A major endothermic peak was observed for all of them. The average initial temperature of this first peak was 29.3 °C and the final temperature was 161.7 °C. A mass loss was observed simultaneously to this endothermic event (Table 1). The mass loss was about 5.2 $g.100\ g^{-1}$, which probably corresponds to moisture and other volatiles since the unfolding of proteins does not involve mass loss.

It cannot be stated that all energy absorbed in this first event is due to protein denaturation since other endothermic reactions occurred within that temperature range, such as water and volatile loss and starch gelatinization. At higher temperatures a

Table 1. Mass loss and heat flow of defatted meal, concentrate, and protein isolate of baru nuts.

Sample	Mass loss (TG)				Heat flow (DSC)			
	Event	Ti	Tf	% loss	T_{onset}	T_{endset}	T_p	$J.g^{-1}$ sample
Defatted meal	1	29.95	172.07	4.26	30.01	166.01	64.47	1585.00
	2	172.07	254.29	11.33				
	3	254.29	361.73	37.17				
	4	361.73	493.89	20.65				
Protein concentrate	1	31.33	126.67	4.86	31.12	147.90	66.36	1527.00
	2	126.67	188.71	5.21				
	3	188.71	258.83	14.19				
	4	258.83	493.89	48.19				
Protein isolate	1	26.52	183.30	6.80	28.68	148.97	67.97	1215.00
	2	183.30	337.25	31.40				

*Ti: initial temperature, T_f: final temperature, T_p: peak temperature.

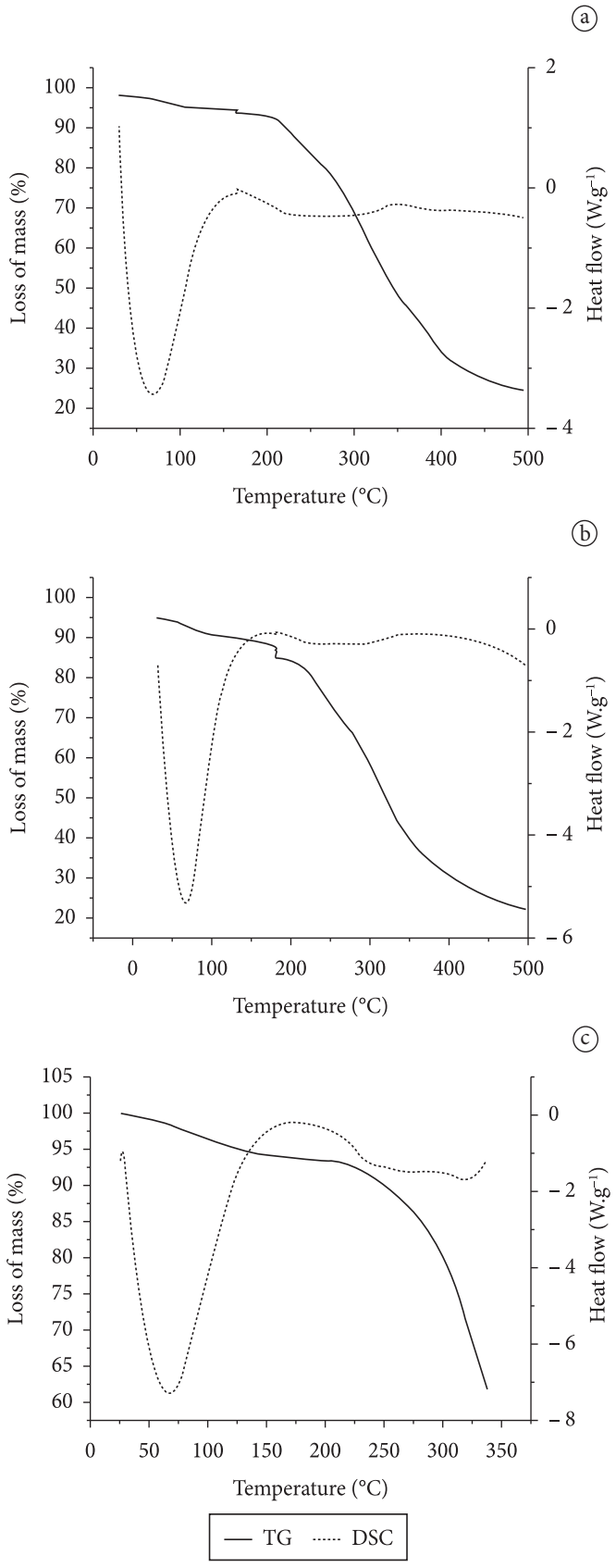

Figure 1. Loss of mass and heat flow of the samples of defatted meal, concentrate and protein isolate where: a) defatted meal; b) protein concentrate; and c) protein isolate of baru.

marked mass reduction was observed simultaneously with the endothermic peaks (Table 1).

The denaturation temperature, here assumed as the peak temperature, of a protein in a solution is usually below 100 °C and increases with higher water contents (KITABATAKE; TAHARA; DOI, 1989). The samples of baru were obtained with low amount of water. Considering the mass loss of the first event as the moisture content, this was only up to 6.8 g.100 g^{-1} (Table 1). Further studies are suggested to investigate the thermal behavior of baru proteins with higher moisture content to evaluate conditions of industrial processing, such as extrusion, to produce textured vegetable proteins.

The defatted meal and concentrate presented two steps of mass loss, while the isolate showed one single step from 175 to 380 °C. The process to isolate the proteins could remove compounds which were decomposed at that temperature range.

Studies on crude maize meal have shown that in the TG curve, the loss of moisture took place between 60 and 110 °C representing a mass loss of 10% (MOTHÉ et al., 2005) similar to the results observed in this study.

In an inert atmosphere of nitrogen, the DSC curves showed a slight increase in the T_d according to the higher amounts of protein in the samples of baru (Table 1). On the other hand, the enthalpy decreased with higher protein contents. Generally, the T_d is not affected by the protein content (KITABATAKE; TAHARA; DOI, 1989). The T_d for soybean proteins occurs at about 100 °C (KITABATAKE; TAHARA; DOI, 1989), which is higher than those predetermined for the baru proteins.

Higher levels of protein suggest major gains in energy to break the disulphide bonds. However, according to the results obtained in this study, it was seen that an increase in the concentration of proteins was not accompanied by an increase in enthalpy. The defatted meal containing 49.01% of protein showed the greatest enthalpy during the first endothermic event. In the concentrate, on the other hand, in which the protein content increased to 55.03%, there was a slight drop in enthalpy. The protein isolate containing 84.11% of protein showed the lowest enthalpy. It can be inferred from this inverse relationship between the increase in protein concentration and enthalpy reduction that the process applied to obtain the concentrate and isolate led to the denaturation of the proteins.

Probably the acid pH in which the isolate was maintained led to the conformational change in the macromolecules causing a low enthalpy. The high temperatures applied in the spray drying process to produce the isolate of baru, could also have contributed to protein unfolding. Protein isolate from kidney (*Phaseolus vulgaris* L.) bean showed a decrease in stability with an increase of heat time (TANG; MA, 2009). According to the authors, the decrease in enthalpy suggests thermal denaturation since partially unfolded protein would require less heat energy.

The bean (*Phaseolus vulgaris* L.) protein isolates heated for 15 and 30 minutes and the controls showed T_d at 98.1, 97.6, and 98.8 °C, respectively (TANG; MA, 2009). This phenomenon contradicts the general view, and the authors suggested that the thermal stability of the globulin fraction is improved since

the thermal treatment may form protein groups resulting in a more compact structure with an elevated thermal stability. The major fraction of baru nuts is globulin, followed by albumin (GUIMARÃES, 2009), and further research could clarify whether there is some relationship between Td and stability of the protein fractions in this nut.

Chickpeas (*Cicer arietinum*) protein isolates obtained at low temperature, or enriched with the albumin fraction, showed denaturation peaks at 97 to 103 °C, respectively (PAPALAMPROU et al., 2009). These temperatures may be related to denaturation peaks of the legume fractions, vicilins, and albumins with the latter being the major fraction in this legume. Endothermic peaks in prolamina films occur at 228, 250, and 270 °C for rice, wheat, and soybeans, respectively (MAGOSHI et al., 2002) indicating heat resistant proteins.

4 Conclusion

Baru proteins showed an increase in T_d, and thus the protein content of defatted meal was higher than that of the isolate. The reduction of enthalpy with higher amounts of protein suggests that the process applied to obtain the baru concentrate and isolate led to protein denaturation.

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