THERMAL STUDY AND PHYSICO-CHEMICAL CHARACTERIZATION OF SOME FUNCTIONAL PROPERTIES OF GUAVA SEEDS PROTEIN ISOLATE (*PSIDIUM GUAJAVA*)

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The guava seed protein isolate (PI) was obtained from the protein precipitation belonging to the class of the gluteline (Ip 4.5). The conditions for the preparation of the PI were determined by both the solubility curve and simultaneous thermogravimetry-differential thermal analysis (TG-DTA): pH 11.5, absence of NaCl and whiteners and T=(25±3)°C. Under these conditions a yield of 77.0±0.4%, protein content of 94.2±0.3, ashes 0.50±0.05% and thermal stability, T=200°C, were obtained. The TG-DTA curves and the PI emulsification capacity study showed the presence of hydrophobic microdomains at pH 11.5 and 3.0 suggesting a random coil protein conformation and, to pH 10.0, an open protein conformation. The capacity of emulsification (CE), in the absence of NaCl, was verified for: 1 – pH 3.0 and 8.5, using the IP extracted at pH 10.0 and 11.5, CE≥343±5 g of emulsified oil/g of protein; 2 – pH 6.60 just for the PI obtained at pH 11.5, CE≥140±8 g of emulsified oil/g of protein.

Keywords: *glutelins, guava seed, protein isolate, TG-DTA, TG/DTG*

Introduction

The production and industrialization of guava in America needs a great amount of raw material. Only in Brazil, around 202 thousands tons of guava are processed per year. A 6% part of it (12 thousand tons a year) corresponds to the seeds that are discharged as waste in the environment [1]. Nowadays this waste disposal creates an environmental problem to the industrial fruit processing companies.

In spite of the environmental problems caused by the disposal ‘in nature’ generating handling costs, this product represents important fiber and protein sources, and has been the object of studies aiming at its protein characterization and evaluation of its functional properties. Recently Nicanor *et al*. [2] and El-Aal [3] have studied the extraction process of guava seed protein isolate as well as the functional and nutritional quality of this food product.

Thermal analysis has been an important technique used for quality control in food processing [4–8]. TG-DTG and DSC have been applied to study of protein isolates [8–10]. μ-DSC and DSC techniques have been used to study heat-induced structural changes in proteins [11], in meal (wheat flour suspensions and doughs) [12], and to study protein foaming properties and protein thermal denaturation [13, 14].

Magoshi *et al.* [8] have studied the thermal properties (glass transition, crystallisation, thermal stability, etc.) of films prepared from the protein isolate (prolamine) of rice seeds, wheat and soya, employing TG and DSC and also auxiliary techniques (XRD and IR).

Ross [5] has studied the thermal properties of concentrated food systems (low water content and frozen food) trying to find out the relation between the food properties and the variations in the quality parameter.

Recently, Amorim *et al.* [4] pointed out the annual dimension of the rejection of rice husks (2.8 millions of ton/per year) discarded in Brazil. The authors used TG/DTG and DSC techniques to study the gelatineous state of the starch, water contents, ash in rice and also the kinetic study of the water elimination.

The present work tries to investigate the thermal behavior (TG-DTA) and the characteristics of some functional properties of the protein isolate obtained from guava seed flour (Psidium guajava) in relation to food preparation and waste treatment.

Experimental

Materials and methods

Guava seeds were kindly supplied by Predilecta Foods (São Lourenço do Turvo, SP, Brasil). They were

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washed with water, the residual attached pulp was removed (1) and then dried at ambient temperature (2). The dried seeds were previously triturated in mill knives, using a 1 mm hole piece, for 10 min (3), defatted by successive extraction with ethylic ether reflux (4), and air desolventized (5). The dried material obtained at step (5) was ground in a liquefier, at least, for one minute, to remove only the skins (6). The skins were separated using a 0.250 mm sieve so that the guava seed flour could be obtained (7). This procedure allowed to separate the skin from the cotyledon and endosperm of the seed, as well as to quantify the total protein content in absence of skins. This is the main difference in comparison with the procedures presented in the literature [2, 3, 13].

Centesimal composition

The determination of moisture, ash, fibers, crude fat and protein in guava seed flour was carried out according to procedures described by AOAC, 1995 [15].

Influence of the pH and salt concentration in the solubilization of the protein

The solubility of the protein isolate was determined following the method used by Kinsella *et al*. (1976) [16].

Obtaining the protein isolate (PI)

The protein isolate, freezed-dried, was obtained according to the methodology described by Liadakis *et al*. [17].

Thermal analysis – how TG–DTA and TG/DTG curves were obtained

TG-DTA curves were obtained with an instrument model SDT 2960, from TA Instruments, New Castle, DE, USA in dynamic air atmosphere (100 mL min^{-1}) using a platinum crucible without a lid; sample mass was 4 mg; the temperature range was from 30 to 900°C and heating rate (β) of 20°C min^{-1}.

Emulsifying capacity

The emulsifying capacity was determined by the Wang and Kinsella method [16] and the results were expressed in g of emulsified oil/g of protein.

Results and discussion

Table 1 presents the results of the percentual composition analysis of the constituents of the guava seed. The table shows that the water and ash yields, obtained by thermogravimetric methods, demonstrate better repetitivity. The determination of the moisture yield is important, as it is related to the control of the deterioration process during the storage of the flour or PI. On the other hand, the ash content is indicative of the mineral content and, thus, it shows the PI and flour impurities.

Table 1 Centesimal composition of the Brazilian guava seed flour using proposed (1) and literature (2)* procedures

Method/analyses	(1)/ mass/mass%	(2)/ mass/mass%
	Conventional**	
moisture	9.6±0.5	6.2±0.4
mineral residue (ash)	1.9±0.3	0.7±0.2
an ethereal extract (fat)	0.6±0.1	0.5±0.2
protein	22.7±0.8	8.4±0.1
fibers	50.7±1.5	60.9±0.9
	Thermogravimetry	
moisture	9.74±0.05	
mineral residue (ash)	2.06±0.04	

*[2]; **[15]

Another important aspect shown in Table 1 refers to the high content of the total protein percentage (22.7±0.8) when compared to the value presented in the literature regarding guava seeds, Psidium guava, cultivated in Mexico 7.60±0.20% [2], in Egypt 10.4% (species not determined) [3] and in India, *Psidium guava* L., 9.0% [18]. This high content of the total protein in Brazilian guava seed was ascribed to the difference in the first step of the sample preparation, in comparison with the methods proposed in the specialised literature [2, 3–14]. Table 1, in addition, shows the centesimal composition results obtained in the present work employing the original samples prepared according to the experimental procedures described by Nicanor *et al*. [2].

Figure 1 illustrates the solubility variation of the protein as a function of the pH and of the saline concentration. It can be observed from the figure that: 1 – the protein solubility increases considerably as the

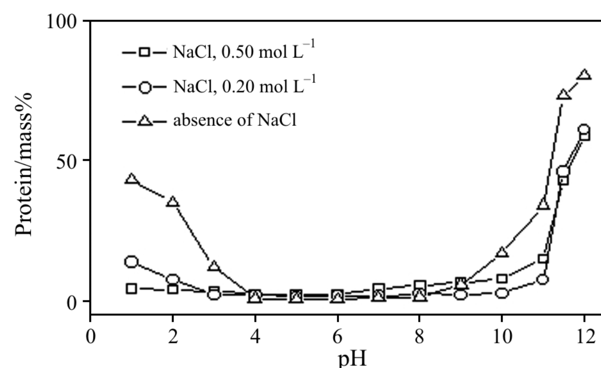

Fig. 1 Protein solubility curves from guava seed flour achieved for 1≤pH≤12 and sodium chloride concentration varying from 0 to 0.5 mol L^{-1}, T=25±3°C

Table 2 Experimental results from PI extractions (yield and protein content) and data from TG-DTA; m_i=4 mg; β=20°C min^{-1}; gas flow: 100 mL min^{-1}; Pt crucible

pH	Treatment	Yield*	Content protein*	TG-DTA					
				m_i/mg	Δm_1	Δm_2	Δm_3	Δm_4	m_r
3.0	–	4.7±0.5	95±3	3.92	4.53	38.27	53.68	1.15	2.39
10.0	–	53.1±0.8	93±2	3.70	9.47	37.27	46.06	–	7.30
	0.25% Na_2SO_3	33.2±0.4	98.0±0.4	4.03	9.90	41.50	48.39	–	0.21
11.0	–	69.0±0.3	93.0±0.7	4.36	6.63	37.35	50.83	–	5.19
11.5	–	77.0±0.4	94.2±0.3	4.14±0.03*	5.10±0.02*	41.17±0.03*	53.23±0.02*	–	0.50±0.05*
	0.25% Na_2SO_3	71.3±0.2	91.1±0.8	4.56	9.08	39.21	47.80	–	3.91
	1.0% Na_2SO_3	71.2±0.3	94.8±0.7	–	–	–	–	–	–
	1.0% AA	70.2±0.4	96.3±0.5	4.17	7.15	39.57	50.74	–	2.54

*n=3; Δm/%; m_i=initial mass/mg; m_r= residual mass/%

concentration of NaCl decreases and also when the pH varies, what can be seen in the acidic (1.0≤pH≤3.0) and in alkaline (8.0≤pH≤12.0) regions; 2 – the protein precipitation occurs at pH 4.5, this value being in accordance with the results obtained in literature for precipitates of guava seeds, Ip 5.5 [2] and Ip 4.5 [3] and for precipitates from other proteins such as safflower Ip 5.5 [19] and tomato seeds Ip 3.9 [17].

Proteins are classified according to their solubility [20]. Therefore, the characteristics described in the previous paragraph suggest, for the protein in focus (in this study), that it belongs to the gluteline group: soluble proteins in acidic and alkaline medium.

For the preparation of the protein isolate, the method of Liadakis *et al*. [17], was employed with some modifications: pH conditions, temperature and addition of bleaching agents in the process; pH at intervals from 10≤pH<12 and pH 3, so that they could be compared. The absence of salt addition led again to a growth of yield (77.0±0.4%) and of protein content (94.2±0.3%), both very high for the isolate prepared at pH 11.5 (Table 2). The values of the table are in accordance with those observed in literature: growth yield (78.25±0.07%) and protein content (96.78±0.07%) for the isolate obtained at pH 12 [2]; growth yield 9.73% and protein content 91.20%, for the isolate obtained at pH 10 [3].

Figure 2 presents TG-DTA and DTG curves of the protein isolate prepared using pH 11.5, absence of NaCl and of bleaching agents. The results of TG curves obtained under different experimental conditions used to obtain the protein isolate (pH, addition of bleaching agents – Na_2SO_3 0.25 and 1 mass/mass% and also to obtain the ascorbic acid 1 mass/mass% along with the yield percentual of the extraction process of the protein isolate and of the protein content (total protein) can be seen on Table 2.

TG-DTA curve in Fig. 2 shows three steps in the thermal decomposition process of the isolate, having the three of them fast kinetics. The first step occurs in the temperature range of 30≤T≤180°C, Δm=5.10±0.02%, and it can be ascribed to an endothermic process, in accordance with DTA curve. This first step was attributed to the water removal, which was present in the freeze-dried isolate.

The second and third steps are consecutive and they occur at temperature intervals of 205≤T≤405°C, Δm=41.17±0.03%, and 450≤T≤680°C, Δm=53.23±0.02%, respectively. The initial temperature of the second step suggests high temperature of the thermal stability at PI (T=200°C).

The second and third steps correspond to the thermal decomposition of the protein isolate. DTA curve shows that the second step occurs through simultaneous thermal processes, starting with an endothermic peak (T_{peak}=220°C), possibly associated to the water removal or to the elimination of the water that belongs to the isolate and the consequent denature of the protein [9], followed by a sequence of consecutive peaks exothermic as well as endothermic, leading to the carbonization of the isolate. The third step occurs with an intense heat release (T_{peak}=595°C), as expected to protein species. This third step was ascribed to further carbonization.

The resulting residue was of 0.50±0.05%, formed by oxides of alkaline metals and earth alkaline.

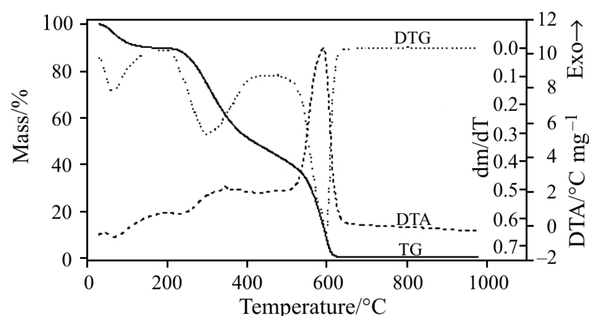

Fig. 2 TG-DTA and DTG curves of the PI obtained prepared using pH 11.5, non-addition of NaCl and of bleaching. m_i=4 mg; β=20°C min^{-1}; gas stream (air): 100 mL min^{-1}; crucible: Pt

Table 2 shows that the treatment employed for the bleaching of the protein isolate at pH 10.0 using Na_2SO_3, 0.25 mass/mass%, presents, on one hand, the lowest content of ash (0.21%), high water content (9.90%) as well as high content of protein (98.0±0.4%); however, on the other hand, it shows low yield for the protein isolate (33.2±0.4%). Low yields make the industrial processing of the residues totally impracticable.

The utilization of the pH higher than pH 10.0, with or without bleaching agents, results in an increase of the yield in the process to obtain the protein isolate when compared to pH≤10.0 values. On the other hand, considerable increase in the protein content could not be observed. Therefore, taken into account the parameters, yield, protein content and ash content, the best conditions to obtain a practical protein isolate for industrial processing would be pH at 11.5 and bleaching agents should not be used (Table 2).

Table 3 presents the evaluation results of the emulsification, employing the protein isolates obtained at pH 10.0 and 11.5, with no bleaching agents, selected from the results of Fig. 2 and Table 2.

The results of the table show that the emulsions prepared from the protein isolates freeze-dried and suspended in water in the absence of NaCl can be considered as follows.

Table 3 Emulsifying capacity * from PI obtained from PI extracted in pH 10.0 and 11.5, without bleaching agents; T=25±3°C

Solution	pH	g (oil)/g (protein)	
		isolated – pH 10.0	isolated – pH 11.5
H_2O	3.0	387±6	343±5
	6.6	37±2	140±8
	8.5	363±6	388±6
NaCl	3.0	57±3	67±7
	6.6	32±3	39±4
	8.5	66±7	53±5

*methodology [16]

- at pH 3.0 and 8.5, high capacity of emulsification can be found 343≤CE≤388 g of emulsified oil/g of protein, regardless of the original conditions of the isolate preparation (pH 10.0 or 11.5);
- at pH 6.6 (close to neutrality), the formation of the emulsion occurs only for the protein isolate obtained at pH 11.5, CE=140±8 g of emulsified oil/g of protein. The high emulsifying capacity of the IP observed at pH 3.0 and 8.5 is associated to the formation of hydrophobic-microdomains induced by the adjustment of the medium pH, which favors the process. On the other hand, the emulsion formation close to neutrality, verified just for the isolate prepared at pH 11.5, occurs due to the pre-existence of hydrophobic-microdomains.

The suspension of the protein isolates prepared in the presence of NaCl, at pH 3.0, 6.6 and 8.5 present low emulsifying capacity and an emulsifying profile similar to that observed in the absence of NaCl. This low capacity may be due to the saline effect that induces the decrease of hydrophobic-microdomains, favors the aggregation and the precipitation of the aggregate, lowering, this way, the capacity of emulsification.

Conclusions

The protein isolate could be obtained from the flour sieved guava seed at a high content (22.7±0.8%) of total protein, using the proposed method to separate the skin of the seed from the cotyledon and endosperm. The precipitate protein was regarded as belonging to the gluteline class, presenting Ip 4.5. The conditions for which the isolate preparation could be defined from the solubility curve and from thermogravimetry (TG/DTA) as being: pH 11.5, absence of both NaCl and of bleaching agents and temperature of 25±3°C. Such conditions allowed to obtain a PI with extraction yield of 77.0±0.3%, high protein content 94.2±0.3%, besides low content of moisture 5.10±0.02% as well as of ash percentage 0.50±0.05%, and high temperature of thermal stability (200°C). Water content and thermal stability parameters permit the control of the deterioration process during the storage period and also the handling of the isolate at other temperatures than the usual ones.

The effects of the formation and presence of hydrophobic-microdomains on the emulsification of the PI freeze-dried could be suggested from thermal analysis results and found out by the results of the emulsifying experiments obtained at pH 6.60, in the absence of NaCl. Thus, for the protein isolate prepared at pH 11.5, it was possible to suggest the presence of hydrophobic-microdomains which induce the protein to have random coil conformation. This random coil conformation leads, therefore, to an increasing emulsifying capacity of the protein isolate from 37±2 g of emulsified oil/g of protein, when in pH 10.0, to 140±8 g of emulsified oil/g of protein at pH 11.5, when in absence of NaCl.

The emulsifying capacity studies accomplished in NaCl (2.0%) show that the salt effect acts hindering the hydrophobic microdomains formation lowering the capacity of emulsification.

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