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Functional Properties of Guava Seed Glutelins

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Five guava seed glutelin extracts were obtained with different buffer solutions: $Na_2B_4O_7$ alone (Glut.Bo) or containing SDS (Glut.BoSDS), 2-mercaptoethanol (Glut.Bo2-ME), or a combination of both (Glut.BoSDS2-ME) and NaOH (Glut.Na). All borate buffer solutions were at pH 10. The higher yield of glutelins corresponded to the Glut.BoSDS extract (81.9% dry basis) and the lower to Glut.Bo (6.8%). The functional properties of the five guava seed glutelin extracts were determined. Glut.BoSDS, Glut.BoSDS2-ME, and Glut.Na showed high values for several properties, including surface hydrophobicity (7.7, 10.8, and 0.6, respectively), solubility at pH 10 (91.1, 77.9, and 96.7, respectively), water-holding capacity at pH 3.6 (1.7, 2.5, and 2.8, respectively), emulsifying activity index (pH 10; 503.5, 238.2, and 838.0, respectively), and foaming properties (pH 10; $V_0 = 0.14$, 0.25, and 0.19, respectively; $V_{max} = 6.1$, 5.59, and 4.51, respectively; $t_{1/2} = 266$, 255.3, and 94 s, respectively). These results suggest that the denaturing reagent (SDS or NaOH) during extraction conferred on the proteins a structure that facilitated the development of their functional properties.

KEYWORDS: **Guava seed glutelins; functional properties; glutelins**

INTRODUCTION

Vegetable proteins have found widespread utilization in the food industry for many years, where they serve as functional ingredients in food formulations.

Guava seed proteins have been reported to be a suitable ingredient in food for human and animal consumption. Some functional properties of the guava seed protein isolate have also been reported, suggesting its possible application in food formulations and for food processing (*1*).

Functional properties of proteins have been studied in great detail for a vast variety of seeds such as cowpea (*2*), soy (*3*−*5*), corn (*6*), and wheat (*7*).

The most important functional property of proteins is the solubility, such that loss of solubility has been used as an indication for loss of functionality. Generally, superior functional attributes for most applications in food processing are directly related to the solubility of proteins (*8*, *9*).

Emulsifying properties are also important in many food applications of proteins, and several methods have been proposed to evaluate these properties. The most popular is the emulsifying capacity (EC) measurement. However, the emulsifying activity index (EAI) proposed by Pearce and Kinsella (*10*) has been also used by several authors (*2*, *7*, *11*, *12*), and it is considered to be a more relevant and quick measure of the emulsifying capacity of the proteins as it estimates roughly the droplet size and the ability of the protein to aid in the dispersion of the oil phase (*11*).

The vegetable proteins vary in their ability to form and stabilize foams, reflecting differences in composition, conformation, molecular flexibility, and physicochemical properties (*13*). Protein foams are important in many processes in the beverage and food industries, and this has stimulated the study of their formation and stability. Foams are also used to improve the texture, consistency, and appearance of food and are commonly found in baked, confectionery, and other goods (*14*).

Taking into account that glutelins represent ∼86% of the total protein content of the guava seed (*15*), the main objective of the present work was to contribute to the knowledge of the functional properties of the guava seed glutelins.

MATERIALS AND METHODS

Materials. Guava pomace was obtained from a guava-processing industry (Boing industry located in Queretaro, Mexico). It was sun-dried (20−30 °C, 3 days). The major part of the skins was removed using a 1 mm sieve. The seeds were pulverized in a stone mill and passed through a 0.5 mm sieve. Guava seed meal was prepared by defatting ground seeds with anhydrous ether in a Soxhlet apparatus (*16*). The percentage of protein content of the defatted guava seed meal was determined according to the micro-Kjeldahl method ($f = 6.25$) (*16*).

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Glutelin Extraction. The glutelin fraction was extracted from the residue obtained after albumins, globulins, and prolamin extraction according to Osborne's method. For glutelin extraction five different extracting agents were tested: (a) $Na_2B_4O_7$ (0.1 M) (yielding the Glut.Bo fraction), (b) $Na_2B_4O_7$ (0.1 M) + SDS (1% w/v) (Glut.BoSDS), (c) $Na_2B_4O_7$ (0.1 M) + 2-ME (0.6% v/v) (2-mercaptoethanol) (Glut.Bo2-ME), (d) $Na_2B_4O_7$ (0.1 M) + SDS (1% w/v) + 2-ME (0.6% v/v) (Glut.BoSDS2-ME), all at pH 10, and (e) NaOH (0.1 M) (Glut.Na). Samples were suspended in the buffer solutions by magnetic stirring during 1 h and centrifuged at 10000*g* for 30 min. Supernatants were dialyzed against acetic acid (1% v/v) (to avoid the possible formation of hydrogen bonds and molecular aggregation) for 5 days according to the method used by Barba de la Rosa et al. (*17*) for amaranth glutelin extraction. The dialysis buffer was changed daily, and the final dialysate was freeze-dried. A micro-Kjeldahl method (*16*) was used to determine protein content ($f = 6.25$). The yield of protein fractions and residues obtained after each extraction was expressed as percentage on dry basis.

The different protein fractions obtained (albumins, globulins, prolamins, and glutelins) were characterized by electrophoresis under nonreducing and reducing conditions (*15*).

Surface hydrophobicity (H_0) was determined with the hydrophobicity flourescence probe 1-anilino-8-naphthalenesulfonate (ANS) according to the method of Kato and Nakai (*18*). Serial dilutions in 0.1 M borate buffer were prepared at pH 10 to a final concentration of 0.012−0.34 mg/mL; in all cases the final concentration corresponds to protein in the soluble fraction. Sixty microliters of ANS (8 mM) was added to 1.5 mL of each dilution, and the fluorescence intensity (FI) was measured at 363 nm (excitation) and 484 nm (emission) using Perkin-Elmer 2000 equipment (Perkin-Elmer, Norwalk, CT). The initial slope of the FI versus protein concentration (mg/mL) plot (calculated by lineal regression analysis) was used as an index of surface hydrophobicity (H_0).

Water-Imbibing Capacity (WIC). The WIC of guava seed glutelins was determined using a modification of Baumann apparatus (*19*). Fifty milligrams of spray-dried sample was used, and the WIC was expressed as milliliters of water imbibed per gram of samples.

Water-Holding Capacity (WHC). Samples were dispersed (1% of protein w/v) in distilled water by magnetic stirring and occasional vortex agitation for 1 h at room temperature (~20 °C). The final pH reached for samples was 3.6. Then they were centrifuged at 10000*g* for 30 min at 15 °C. WHC was calculated as

$$\mathrm{WHC} = (m_{\mathrm{hip}} - m_{\mathrm{tp}} + m_{\mathrm{sp}})/m_{\mathrm{tp}}\delta$$

where m_{hip} is the mass of the hydrated insoluble protein fraction obtained, m_{tp} is the mass of total protein in sample, considering that the sample is protein totality, then m_{tp} is equal to m_s (mass of the sample), m_{sp} is the mass of soluble protein content in sample determined in the supernatant according to the biuret method (*20*), and δ is the water density at room temperature. WHC was expressed as milliliters of H_2O retained by the insoluble protein fraction per gram of total protein (*21*).

Solubility was determined using the method of Maruyama et al. (*22*). The guava seed glutelin extracts were dissolved in buffers at different pH values (0.1 M borate for pH 8−10; 0.1 M citrate for pH 3−7). The protein solutions (0.8 mg/mL) were kept at 4 °C for 18 h. After centrifugation, protein concentrations in the supernatant were determined using the method of Bradford (*23*). Solubility was expressed as a percentage of the total protein content in the sample.

Emulsifying Activity Index (EAI). The spectroturbidimetric method of Pearce and Kinsella (*10*) was used with slight modifications to determine the EAI. Emulsions were prepared by homogenizing 1.5 mL of 0.05% (w/v) protein dispersion with 0.5 mL of refined sunflower oil, at pH 5 and 10. The dispersion was sonicated to 100 W for 30 s at 25 °C. Aliquots (30 μL) of emulsion were drawn from the test tube bottom immediately and diluted in 20 mL of 0.1% (w/v) SDS solution. Absorbance of the diluted emulsions was measured at 500 nm against a 0.1% (w/v) SDS blank. The EAI was expressed as interfacial area per unit weight of protein ($m^2\ g^{-1}$).

Foaming Properties. Assays were performed as described previously (*3*). N_2 was sparged at a flow rate of 1.70 mL s^{-1} through 6 mL of 1.0 mg mL^{-1} of sample in buffer solutions (0.1 M acetate buffer, pH 3 and 5; and borate buffer, pH 10). Bubbling was continued until after a maximum elapsed bubbling time of 90 s. The initial rate of liquid incorporation to the foam (V_0, mL min^{-1}) and the maximum volume of liquid incorporated to the foam (V_{max}, mL) were determined. The time for half-drainage of the liquid that had been incorporated to the foam at the end of the bubbling period ($t_{1/2}$, s) was also measured. Determinations were performed in triplicate.

Statistical Analysis. All analyses were carried out in triplicate. The data so obtained were statistically evaluated by variance analysis (ANOVA). The comparison of means was done by the least significant difference (LSD) test at a significance level (α) of 0.05. Both tests were carried out using the statistical analysis package SYSTAT (*24*).

Table 1. Yield of Guava Seed Glutelins Obtained with Five Buffer Solutions[a]

buffer solution	g of protein/ 100 g of protein	g of protein/ 100 g of residue
0.1 M $Na_2B_4O_7$, pH 10 (Glut.Bo)	6.8 ± 0.4	79.1 ± 0.5
0.1 M $Na_2B_4O_7$ + 0.6% (v/v) 2-ME, pH 10 (Glut.Bo2-ME)	25.7 ± 0.5	60.1 ± 0.4
0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS, pH 10 (Glut.BoSDS)	81.9 ± 0.2	4.1 ± 0.2
0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME, pH 10 (Glut.BoSDS2-ME)	72.7± 0.9	13.1 ± 0.3
0.1 M NaOH (Glut.Na)	59.1 ± 0.5	26.9 ± 0.8

[a] Protein contents in seed and extracts were determined according to Kjeldahl (N × 6.25). Values are expressed as the mean and standard deviation of three replicates, dry weight basis.

RESULTS AND DISCUSSION

Glutelin Extraction. Table 1 shows the results of guava seed glutelin extraction with different buffers. The higher yield of glutelins was obtained with $Na_2B_4O_7$ + SDS (81.9%) (Glut-.BoSDS), followed by $Na_2B_4O_7$ + SDS + 2-ME (Glut.BoSDS2-ME) (72.7%). Whereas the yield of guava seed glutelin obtained with 0.1 M NaOH (Glut.Na) was only 59.1% (dry basis), that obtained with 0.1 M $Na_2B_4O_7$ buffer solution (Glut.Bo) was even lower (6.8%, dry basis) (**Table 1**). A similar behavior was reported by Abugoch et al. (*25*) when glutelins of amaranth seeds were obtained with the same buffers.

The differences observed among protein yields were attributed to different levels of glutelin extractions because according to previous results these samples were free of other guava seed protein fractions (albumins, globulins, and prolamins) (*15*).

The difference between the extraction yields of Glut.Na and Glut.Bo could be attributed to the different actions of NaOH and borate buffer on the molecular structure of proteins. At variance with borate, NaOH can provoke the denaturation and dissociation of protein molecules, favoring their extraction (*25*).

The addition of 2-ME to the borate buffer did not improve the extraction yield, probably because disulfide bonds are not involved in glutelin structure and/or glutelin−glutelin aggregates. Similar results were obtained by Barba de la Rosa et al. (*17*) for amaranth seed glutelins.

The low yield of Glut.Bo (6.8%), compared to that obtained by Barba de la Rosa et al. (*17*) for amaranth seed glutelins (22.5%), suggested that the guava seed glutelins have a more compact structure, requiring a higher degree of denaturation for their solubilization and, consequently, for their extraction.

Consequently, the results obtained suggest that glutelins in seeds should be present as polymers of high molecular mass stabilized by non-covalent bonds, hydrophobic interactions, and hydrogen bonds.

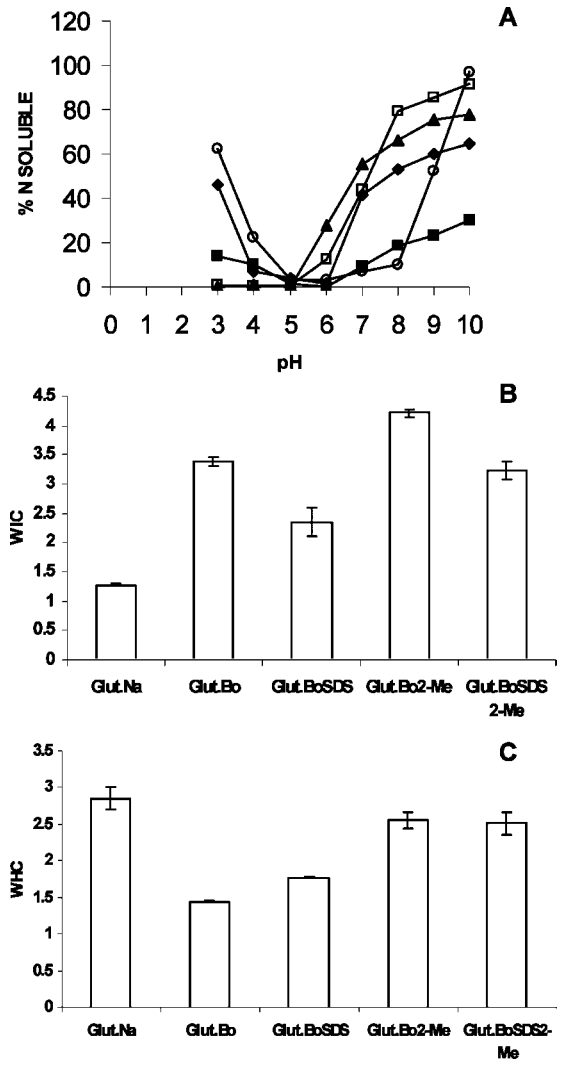

Figure 1. (**A**) Solubility of the guava seed glutelin samples: ◆, Glut.Bo; □, Glut.BoSDS; ■, Glut.Bo2-ME; ▲, Glut.BoSDS2-ME; ○, Glut.Na. (**B**) WIC of the guava seed glutelin samples. (**C**) WHC of the guava seed glutelin samples.

Solubility. Solubility profiles of guava seed glutelins are shown in **Figure 1**. Glutelins presented a characteristic "U" profile (*1*, *5*, *26*), with the lowest solubility in the pH range of 5−6, which includes the p*I*, and the highest values at alkaline pH.

The glutelin fractions with the highest solubilities at alkaline pH were Glut.Na, Glut.BoSDS, and Glut.BoSDS2-ME, which suggests that the presence of a denaturing reagent (SDS or NaOH) during extraction can provoke a conformational change of the protein molecule, which facilitates its solubilization within the pH range here tested. The lowest solubility corresponded to Glut.Bo2ME. It is probably that during the extraction of this sample 2-ME provoked the reduction of disulfide bonds of proteins, and then a SS/SH rearrangement could have occurred during the freeze-drying of this fraction, probably reducing its solubility.

Glut.BoSDS and Glut.BoSDS2-ME showed low solubility at acid pH.

Surface Hydrophobicity (H_0). Table 2 shows the surface hydrophobicity of the five guava seed glutelin extracts; Glut-.BoSDS2-ME showed the highest H_0, followed by Glut.BoSDS. This behavior suggests that SDS provoked—during the extraction process—at least a partial irreversible exposure of hydrophobic groups hidden in the folded structure of the protein and that this effect was increased by 2-ME.

Table 2. Surface Hydrophobicity of the Guava Seed Glutelin Samples at pH 10

glutelin extract	H_0[a]
Glut.Bo	0.88 ± 0.04
Glut.Bo2-ME	ND[b]
Glut.BoSDS	7.7 ± 0.3
Glut.BoSDS2-ME	10.8 ± 0.2
Glut.Na	0.61 ± 0.02

[a] Values are expressed as the mean and standard deviation of three replicates. [b] Not determinable.

The high H_0 presented by Glut.BoSDS2-ME and Glut.BoSDS seems to be contradictory because proteins of high H_0 would be expected to show low solubility (**Figure 1A**). Because of their natural tendency to aggregate by hydrophobic interactions, these behaviors could suggest that the solubility is not exclusively determined by the degree of exposure of hydrophobic zones, as similar results was reported for the soy protein isolate (*27*). On the other hand, the low H_0 of Glut.Na was expected for its high solubility.

Glutelin extracts obtained with borate buffer (with the exception of Glut.Bo2-ME) and NaOH showed a low H_0. It was not possible determine the H_0 of Glut.Bo2-ME, because of the sponge structure and low solubility (30.1%, **Figure 1A**) of the sample.

Water-Imbibing Capacity. Glut.Bo2-ME presented the best WIC (**Figure 1B**), which can explained if the WIC of proteins in general is examined. According to the literature, the WIC is determined mainly by the content and the level of hydration of the insoluble fraction of a protein isolate. On the other hand, the isolates with better solubility exhibit lower WIC, because they contain a low proportion of protein insoluble fraction (*28*).

Against this background, the low WIC of Glut.Na could be explained by the higher solubility at acid pH and, in consequence, the lower insoluble fraction.

According to their solubility in the range of acid pH values, the WIC of Glut.BoSDS and Glut.BoSDS2-ME should be the highest. However, they were intermediate between those of Glut.Bo2-ME and Glut.Na. The presence of SDS during the extraction process could induce the formation of micelles, thus reducing the WIC of these glutelin samples.

The WIC values of guava seed glutelin samples are in a range reported for soy protein isolates (*28*).

Water-Holding Capacity. This property is also inversely related with the protein solubility. A lower solubility corresponds with a high WHC (*21*).

Glut.Na samples showed the highest WHC, followed by Glut.BoSDS2-ME and Glut.BoSDS2-ME (**Figure 1C**).

The low solubilities of Glut.Bo2-ME, Glut.BoSDS2-ME, and Glut.BoSDS, at acidic pH, are related with their high WHC (2.6, 2.5, and 1.8, respectively), just as the high solubility of Glut.Bo is related with its low WHC (1.4).

On the other hand, the high solubility of Glut.Na (>40%) does not correlate with its high WHC (2.8), suggesting that, for this particular case, a significant reduction of solubility is not necessary to produce a high WHC. In this way the insoluble proteins retain a higher amount of water.

The guava seed glutelin samples presented lower WHC (1.4−2.8 mL of H_2O/g of sample) than soy protein isolates obtained

Table 3. EAI of the Guava Seed Glutelin Samples

glutelin extract	EAI[a] (m^2/g) pH 5	pH 10
Glut.Bo	108 ± 3	253 ± 2
Glut.Bo2-ME	86 ± 3	115 ± 3
Glut.BoSDS	639 ± 1	504 ± 2
Glut.BoSDS2-ME	730 ± 1	238 ± 1
Glut.Na	374 ± 1	838.± 1

[a] Values are expressed as the mean and standard deviation of three replicates.

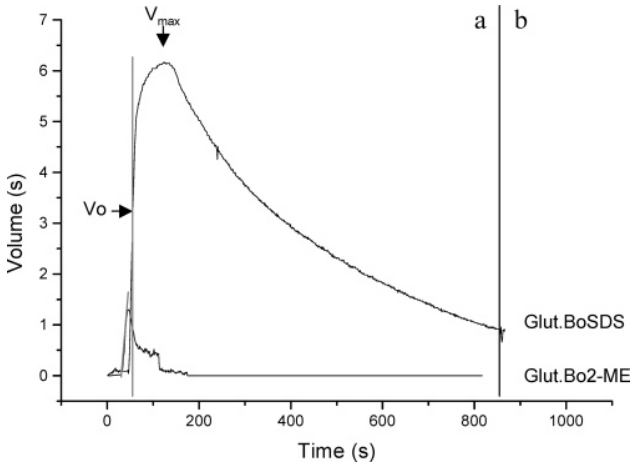

Figure 2. Volume of liquid incorporated into the foam as a function of time. Assays were performed with guava seed glutelin samples Glut-.BoSDS and GlutBo2-ME at pH 10: zone a, foam formation; zone b, foam liquid drainage.

under thermal treatment at pH 9 (20.5−30.7 mL of H_2O/g of isolate) (*21*).

Emulsifying Activity Index. Glut.BoSDS and Glut.BoSDS2-ME exhibited the highest EAI (**Table 3**) at acid pH. It is probably that some of the SDS used during the extraction procedure was not removed during dialysis, and then proteins may be present as micelles, favoring the interaction with the interface.

Glut.Na showed an increase of the EAI at alkaline pH (>8), which relates directly with its high solubility. This latter property facilitates the diffusion of the protein to the water−oil interface. The high values of solubility, as well as the possible presence of SDS, explain the EAI corresponding to Glut.BoSDS and Glut.BoSDS2-ME at pH 10.

The guava seed glutelin samples showed lower absorbance values than reported for soy proteins, sodium caseinate, and egg albumin at pH 6 and 7 (*11*), for gluten hydrolysates (*29*), and for wheat glutelins at the same conditions (*7*).

Foaming Properties. Figure 2 shows, as examples, the formation and destabilization profiles of a foam of Glut.BoSDS and Glut.Bo 2-ME samples, which showed the best and worst foaming properties, respectively.

Changes in protein structural properties such as surface hydrophobicity, molecular mass, and net charge as results of the use of different buffer solutions during the extraction procedure could originate specific surface characteristics and functional properties.

The highest foaming capacity was obtained for Glut.BoSDS2-ME and the lowest for Glut.Bo2-ME at all pH values assayed. The pH of the dispersing medium markedly affected foam capacity by its direct effect on the net charge and conformation

Table 4. Foaming Properties of the Guava Seed Glutelin Samples[a]

glutelin extract	V_0 (mL/s)	V_{max} (mL)	$t_{1/2}$ (s)
pH 3			
Glut.Bo2-ME	0.3 ± 0.1	3.2 ± 0.6	4.0 ± 0.5
Glut.BoSDS	0.36 ± 0.02	3.7 ± 0.2	2.5 ± 0.5
Glut.BoSDS2-ME	0.46 ± 0.03	2.9 ± 0.2	4.0 ± 0.0
Glut.Na	0.36 ± 0.03	4.4 ± 0.1	6.3 ± 0.5
pH 5			
Glut.Bo2-ME	0.09 ± 0.01	1.2 ± 0.3	3.5 ± 0.7
Glut.BoSDS	0.31 ± 0.03	4.29 ± 0.01	4.5 ± 0.7
Glut.BoSDS2-ME	0.33 ± 0.04	4.2 ± 0.2	42 ± 4.2
Glut.Na	0.12 ± 0.01	1.5 ± 0.3	3.7 ± 0.5
pH 10			
Glut.Bo2-ME	0.10 ± 0.03	1.3 ± 0.3	2.0 ± 0.0
Glut.BoSDS	0.14 ± 0.03	6.10 ± 0.09	266 ± 1
Glut.BoSDS2-ME	0.25 ± 0.00	5.59 ± 0.07	255.3 ± 37.1
Glut.Na	0.19 ± 0.00	4.51 ± 0.09	94 ± 3

[a] Assays were performed with sample solutions at 1.0 mg/mL in 0.1 M borate buffer (to pH 10) and 0.1 M acetate buffer (pH 3 and 5). Parameters V_0, V_{max} (foam formation), and $t_{1/2}$ (foam stabilization) as described under Materials and Methods.

of proteins. Glut.BoSDS2-ME and Glut.BoSDS exhibited the highest V_0, at pH 10 (**Table 4**); this suggests that the presence of a denaturating reagent (SDS) during the extraction produced a conformational change and generated a more flexible protein structure, allowing the protein to be adsorbed at the interface and enhancing the formation and stabilization of the foam (*5*, *30*). It is not possible to disregard the presence of some SDS in the samples after dialysis, which can improve the foaming capacity of these glutelin extracts.

These guava seed samples showed the better foam stability at pH 10, perhaps because these samples presented better solubility at this pH. Foaming proteins should be soluble in the aqueous phase; they should concentrate at the interface, unfold to form cohesive layers around air burbles, and possess enough viscosity and mechanical strength to prevent rupture and collapse (*3*). On the other hand, if it is considered that the foaming capacity and foam stability are related with the surface hydrophobicity (H_0) of the proteins and that Glut.BoSDS2-ME and Glut.BoSDS exhibited the highest H_0, then these samples should present high foaming capacity and foam stability (*5*, *30*).

At pH 3 all glutelin fractions assayed exhibited good foaming capacity but a lower stability than foams obtained at pH 10. Proteins present in the soluble fraction could be dissociated as a consequence of the extreme acid pH; the reduction in the molecular mass could favor the migration of the molecules at the interface but not the stability of the foam.

The guava seed glutelins presented lower foaming capacity than the soluble fraction of soy protein isolate (*31*) and soybean whey and isolate proteins (*32*).

The guava seed glutelin samples presented better foam stability than the reported for the soy glycinin; the latter, however, had higher foaming capacity (*4*). The guava seed glutelin samples showed V_0 and V_{max} values within the range reported for soy hydrolysates. However, the time for half-drainage ($t_{1/2}$) of the guava seed glutelin samples was high (*5*, *33*).

The results discussed indicate that the best functional properties were exhibited for the glutelins extracted with Bo.SDS and BOSDS 2-ME and the worst for those extracted with Bo.ME. Due to residual amounts of SDS that could remain in the samples after dialysis, these extracts cannot be used as functional ingredients. Otherwise, glutelins extracted with NaOH showed intermediate functional properties (high WHC, good solubility

at acid and alkaline pH values, and good foaming and emulsifying properties); this fraction could be a suitable ingredient in food formulations.

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