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Guava seed storage protein: Fractionation and characterization

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Abstract

In the present work, guava seed storage proteins have been fractionated and characterized. Glutelins (86–90 g/100 g) and globulins (≈10 g/100 g) are the main components of the protein extract. Albumins and prolamins are minor components (≈2 g/100 g). Guava seed glutelin extracts, like rice and amaranth glutelins, are legumine-like proteins that, due to their solubility properties, have to be extracted using extreme pH (borate buffer, pH 10, Gt-Bo; NaOH pH 12 Gt-Na), denaturing (borate buffer plus sodium dodecyl sulfate, Gt-BoSDS) or reducing conditions (borate buffer plus 2-mercaptoethanol, Gt-BoME; borate buffer plus sodium dodecyl sulfate and 2-mercaptoethanol, Gt-BoSDSME). The highest yield was obtained with SDS extraction, suggesting that proteins in the seeds form aggregates stabilized mainly by non-covalent interactions. Glutelins are mainly composed of 65 and 67 kD subunits, with a lower proportion of 55 kD subunits. These subunits are formed by disulfide bond-linked polypeptides with molecular masses 40–45 kD, 22–27 kD and 23–25 kD, respectively. The guava seeds protein isolate (GSI) exhibited a polypeptide profile very similar to that of the glutelin fraction.

The guava seed could be an alternative source of protein for human and animal consume, additional to this to solve at least in part the pollution problem that fruit processing industry has for discarding this material.

Keywords: Guava seed; Protein fractions; Glutelins

1. Introduction

One of the most common problems in food processing industry is the disposal of the sub products generated. This ''waste material'' produces ecological problems related to the proliferation of insects and rodents, and an economical burden because of transportation to repositories. Therefore, strategies for the profitable use of these materials are needed. Several studies on the use of waste products generated by the food industry (Bernardino-Nicanor, Ortíz, Martínez, & Dávila-Ortíz, 2001; Liadakis, Constantine, Vassiliki, & Christos, 1995; Ravindran & Sivakanesan, 1996), have shown that these products are an alternative source of oil and protein for human and animal feeding. Examples of such products are tomato seeds (Liadakis et al., 1995) and sesame seeds (Adawy, 1997). Guava fruit, usually consumed in Mexico, belongs to a dicotyledoneous family. The pulp (88 g/100 g of fruit weight) is used for juice production, but seeds (12 g/100 g of fruit weight) are discarded. The in vitro digestibility of storage proteins of guava seeds is higher than that of the soybean isolate (94.8 g/100 g vs. 89.9 g/100 g). Except for lysine content, the essential amino acid profile is above that recommended in the FAO/WHO (1985) pattern for adults (Bernardino-Nicanor et al., 2001).

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Seed storage proteins were initially classified according to their solubility properties into albumins (water soluble), globulins (saline soluble), prolamins (alcohol soluble), and glutelins (residue) (Osborne, 1924). Based on more recent and extensive molecular and biochemical analysis of storage proteins and their genes, these proteins have been classified into two major groups, globulins and prolamins (Shewry & Casey, 1999, Chaps. 2, 6, 13, 22, 23, and 24). According to this classification, the guava seed proteins could be considered globulins, like most of dicotyledonous storage proteins.

The objective of the present work was to obtain protein fractions of the guava seed and determine partially their chemical and molecular characteristics. Another goal was to use these data to locate the guava seed storage proteins within the proposed classifications.

2. Materials and methods

2.1. Materials

Guava pomace was obtained from a guava processing plant (Boing industry located in Queretaro, México). It was sundried (20–30 °C, 3 days). The skins were removed using a 1 mm sieve. The seeds were pulverized in a stone mill and passed through a 0.5 mm sieve, producing guava seeds meal (GSM). Defatted GSM (GSMd) was obtained by treatment with anhydrous ether in a Soxhlet apparatus (AOAC, 1995).

2.2. Quantification of macrocomponents

The protein content of meals, isolate and different fractions was determined by the Kjeldahl method (AOAC, 1995). The protein/nitrogen coefficient used was 6.25 (Method 955.04). The proximate analysis was completed with crude fiber (Method 962.09), crude fat (Method 920.39), moisture (Method 934.01) and ash (Method 923.03) (AOAC, 1995).

2.3. Protein isolate (GSI)

The method of Liadakis et al. (1995) (as modified by Bernardino-Nicanor et al. (2001)), was used. The meal:water ratio was 1:20. The pH of the suspension (11.5) was kept constant during the extraction procedure by the addition of 0.1 mol/l NaOH. The temperature (40 °C) was regulated with a water bath. After 30 min, the slurry was centrifuged at 2600*g* for 30 min at 4 °C. The supernatant was collected and the pH was adjusted to its isoelectric point (pH 5.0) using 0.1 mol/l HCl. The protein precipitate was separated by centrifugation at 2600*g* for 30 min at 4 °C and freeze-dried.

2.4. Guava protein fractions

2.4.1. Obtained by Osborne's method (Osborne, 1924)

Albumins: Defatted guava meal, GSMd, was first extracted with distilled water (0.1 g/ml) by two stirring steps of 1 h at 4 °C and then centrifuged at 10 000*g* for 30 min at 4 °C. Supernatant was freeze-dried and called Alb_O.

Globulins: The residue from albumins extraction was extracted under magnetic stirring for 1 h with NaCl (10 g/100 g) at 4 °C and centrifuged at 10 000*g* for 30 min at 4 °C. Supernatant was dialysed against distilled water for 5 days, changing water dialysis every day and freeze-dried. It was called Glb_O.

Prolamins: The residue resulting from globulin extraction was extracted under magnetic stirring for 1 h at 4 °C with 70 ml/ 100 ml aqueous 2-propanol and centrifuged at 10 000*g* for 30 min at 4 °C. Supernatant was dialysed against acetic acid (1 ml/ 100 ml) for 5 days, with daily changes of solution, and freeze-dried (Pro_O).

Glutelins: After prolamins were obtained by the method of Osborne (Osborne, 1924), the glutelin fraction was extracted with one of the following extracting agents: (a) $Na_2B_4O_7$ (Gt-Bo), (b) $Na_2B_4O_7$ + SDS (sodium dodecyl sulfate) (Gt-BoSDS), (c) $Na_2B_4O_7$ + 2-ME (2-mercaptoethanol) (Gt-BoME), (d) $Na_2B_4O_7$ + SDS + 2-ME (Gt-BoSDSME), all at pH 10, and (e) NaOH (Gt-Na), pH 12. Samples were suspended in the buffer solutions by magnetic stirring during 1 h and centrifuged at 10 000*g* for 30 min at 4 °C. Supernatants were dialyzed against acetic acid (1 ml/100 ml) for 5 days, with daily changes of solution, and freeze-dried. The $Na_2B_4O_7$ and NaOH concentrations were 0.1 mol/l, SDS concentration was 1 g/100 ml and that of 2-ME was 0.6 ml/100 ml.

2.4.2. Obtained by Barba de la Rosa's method (Barba De La Rosa, Paredes-López, & Guéguen, 1992)

Albumins+Globulins: A suspension of flour in 0.1 mol/l $Na_2B_4O_7$ pH 7.0 (0.1 g/ml) was stirred for 1 h at room temperature and centrifuged at 10 000*g* for 30 min at 4 °C. Then, a second extraction was done with the same reagent. Supernatants were collected and dialysed at 4 °C against deionized water for 5 days; with daily changes of water. The content of dialysis tubes was centrifuged at 10 000*g* for 30 min at 4 °C. Supernatant was albumin fraction (Alb_{BR}) and pellet was globulin fraction (Glb_{BR}). Both were freeze-dried.

Prolamins: The residue of albumins and globulins extraction was mixed with 70 ml/100 ml aqueous 2-propanol for 1 h at 4 °C and centrifuged at 10 000*g* for 30 min at 4 °C. Supernatant was dialysed against acetic acid (1 ml/100 ml) for 5 days, with daily changes of solution, and freeze-dried.