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Biological activity of proteins from pulps of tropical fruits

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Abstract

Proteins present in fruit pulps were extracted and concentrated to 90% saturation point with ammonium sulfate. The total protein content (mg/g) from each fruit pulp was: Assai (0.20), Suriname Cherry (0.10), Mangaba (0.62), Yellow Mombim (0.15), Acerola (0.17), Cupuassu (0.54), Melon (0.24) and Passion fruit (0.80). Inhibitory activity against digestive enzymes, bovine trypsin, human salivary α-amylase and porcine pancreatic α-amylase, were assessed. Results show that inhibitory activity against pancreatic α-amylase was greater in yellow mombim and cupuassu pulps with 0.0345 and 0.0335 mg inhibitor/g pulp, respectively. Melon and assai pulps had the greatest inhibitory activity towards salivary α-amylase with 0.042 and 0.142 mg inhibitor/g pulp, respectively. Antitryptic activity was detected in Assai, mangaba, yellow mombim, suriname cherry, acerola and cupuassu. The levels of inhibitory activity were low for most fruits and those with highest activities were assaí (0.054 mg/g), mangaba (0.0395 mg/g) and cajá (0.0328 mg/g).

Keywords: Tropical fruit pulps; Trypsin inhibitors; α-Amylase inhibitors

1. Introduction

Fruits are important sources of nutrients in human diet. They have high contents of carbohydrates (glucose, fructose, other soluble sugars and pectin), vitamins, minerals and organic acids although they are usually very low in proteins and fats (Ornellas, 1988). Plant proteins may be classified as albumins or globulins, according to their solubility. Albumins are metabolically active proteins, such as enzymes, lectins and enzyme inhibitors (Higgins, 1984); the globulin fraction is made up of reserve or storage proteins, of allergenic proteins and of some lectins (Shewry, 1995).

Compositional, nutritional and functional properties are essential parameters for defining food quality. Composition is described as the quantities or proportions of the various components. Nutritional properties are characterized by both abundance of essential nutrients and by their bioavailability, as also by the lack of toxic or antinutritional substances (Sgarbieri, 1996). Functional properties of some substances present in plant storage organs, such as seeds and tubers, may inflict a negative impact on the nutritional quality of proteins (Chrispeels & Raikhel, 1991; Liener, 1994). Some of these substances are proteins themselves, that act as defence weapons during storage (e.g. cell wall proteins, β-glucanases, lectins and proteinase inhibitors). The latter molecules play double roles in metabolism, preventing undesirable protein hydrolysis and protecting tissue, fluids and cell proteins from foreign hydrolytic enzymes (Mills, 1988; Richardson, 1991; Ryan, 1990).

There are few published articles describing proteins from pericarps of tropical fruits. With this in mind, we used biochemical methods to detect and assess the biological activity and particularly the enzyme inhibitory activitys of proteins present in pulp of tropical fruits.

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2. Material and methods

2.1. Biological material

The following tropical fruits were acquired at local markets and samples were taken from their pulps and frozen: Yellow mombim (*Spondias lutea L.*), yellow Passion fruit (*Passiflora sp*), Melon (*Cucumis melo L*), Suriname cherry (*Eugenia uniflora L.*), Mangaba (*Hancomia speciosa*), Assai (*Euterpe oleracea*), Acerola (*Malpighia glabra*), and Cupuassu (*Theobroma grandeflorum*).

2.2. Enzymes

Human salivary α-amylase was generously supplied by the Laboratory of Protein Chemistry and Function of the Federal University of Rio Grande do Norte, Natal-Brazil. Bovine trypsin was acquired commercially from Sigma Chemical Co, St. Louis, USA. Porcine pancreatic α-amylase was acquired commercially from Boehringer Mannheim, Germany.

2.3. Total protein extraction from fruit pulps and fractionation by ammonium sulfate

Samples were suspended in 0.1 M NaCl/ 0.05 M phosphate buffer, pH 7.5 (1:1 pulp to buffer ratio) and agitated for 2 h at room temperature. After centrifugation at 10,000×*g* for 30 min at 10 °C, proteins in the supernatant were fractionated by ammonium sulfate precipitation up to 90% saturation point. After another centrifugation at 10,000×*g* for 30 min and 10 °C the precipitate obtained from 0 to 90% saturation (F0/90 fraction) was dialyzed against water for 24 h at 9 °C, and then frozen and used in assays.

2.4. Protein assessment

Protein content of each sample was measured by the Bradford method (1976), using bovine serum albumin as standard.

2.5. Pancreatic α-amylase inhibitory activity

The porcine pancreatic α-amylase inhibitory activity was determined. Pulp samples from each fruit (60 μl) were pre-incubated with 10 μl of enzyme solution (2 mg/ml) in 130 μl of 3 mM NaCl /0.1 M acetate buffer, pH 5.5, in a water bath at 37 °C for 10 min, prior to the addition of 2000 μl substrate solution (1% soluble starch). After 60 min at 30 °C, the reaction was terminated by immersion in an ice-water bath. Aliquots (25 μl) from each sample were added to a 2.5 ml lugol solution (1 mM iodine plus 24 mM KI_2) and the hydrolysis products were assessed when A_{565nm} was measured. One unit of inhibitory activity was defined as the amount of inhibitor that decreases absorbance by 0.01 at 565 nm after correction of enzyme blanks in the assay conditions. All the assays were made in triplicate.

2.6. Salivary α-amylase inhibitory activity

The human salivary α-amylase inhibitory activity was also determined in fruit pulps. Pulp samples from each fruit (60 μl) were pre-incubated with 30 μl of enzyme solution (2 mg/ml) in 110 μl of 3 mM NaCl/0.1M acetate buffer pH 5.5 in a water bath at 37 °C for 10 min, prior to the addition of 2000 μl substrate solution (1% soluble starch). After 60 min at 30 °C, the reaction was terminated by immersion in an ice-water bath. Aliquots (25 μl) from each sample were added to a 2.5 ml lugol solution (1 mM iodine plus 24 mM KI_2) and the hydrolysis products were assessed when A_{565nm} was measured. One unit of inhibitory activity was defined as the amount of inhibitor that decreases absorbance by 0.01 at 565 nm after correction of enzyme blanks in the assay conditions. All the assays were made in triplicate.

2.7. Trypsin inhibitory activity

The tryptic inhibitory activity was also assessed in these tropical fruit pulps. Pulp samples from each fruit (60 μl) were pre-incubated with 20 μl of trypsin enzyme solution (0.3 mg/ml in 0.0025 M HCl) in 225 μl of 0.0025 M HCl plus 190 μl of 0.1 M phosphate buffer pH 7.8 in a water bath at 37 °C for 10 min, prior to the addition of 500 μl 1% azocasein substrate solution. After 30 min in a water bath at 37 °C, reaction was stopped by addition 500 μl of 20% TCA solution. The mixture was centrifuged at 12,000×*g* for 10 min and allowed to stand for a further 10 min at room temperature; afterwards, 500 μl of the supernatant was alkalinized with 500 μl of 2 N NaOH. The hydrolysis products were assessed when A_{440nm} was measured. One unit of inhibitory activity was defined as the amount of inhibitor that decreases absorbance by 0.01 at 440 nm after correction of enzyme blanks in the assay conditions. All the assays were made in triplicate.

3. Results and discussion

Plant storage organs, such as seeds and tubers, are rich in storage proteins and defence-related proteins, such as enzyme inhibitors, allergenic proteins and lectins (Xavier-Filho, 1993). Proteinase inhibitors play different roles in these organs. They inhibit hydrolysis of proteins that accumulate during organ development, by endogenous enzymes, and are also involved in defence mechanisms through inhibition of a predator's digestive enzymes (Xavier-Filho, 1993). These proteinase inhibitors are easily extracted from plant tissues by means of

buffer systems and concentrated through precipitation with salts under saturating conditions, such as ammonium sulfate (AS). In this work, proteins present in fruit pulps were extracted and concentrated to 90% saturation point with AS. The total protein content of each fruit pulp was measured by the Bradford method (1976) and is shown in Table 1. These values are below those found for packing products. Inhibitory activities against mammalian digestive enzymes (bovine trypsin, human salivary α-amylase and porcine pancreatic α-amylase) were also assessed (Table 1). Results show different protein contents from different fruit pulps. Passion fruit had the greatest content protein and no inhibitory activity. Inhibitory activity of pancreatic α-amylase (Table 2) was greater in yellow mombim and cupuassu pulps with 0.0345 and 0.0335 mg of inhibitor/g of pulp, respectively. Meanwhile, melon and assai pulps had the greatest inhibitory activity towards salivary α-amylase with 0.042 and 0.142 mg of inhibitor/g pulp, respectively (Table 3). A possible involvement of these inhibitors with plant resistance to pests has aroused interest in many research groups who try to take advantage of it through plant breeding programmes (Pueyo et al., 1995; Reis et al., 1997). In human diet, the importance of α-amylase inhibitors is not very clear (Sgarbieri, 1996), although scientists affirm that medical use of these inhibitors causes a reduction in starch absorption in the intestinal lumen that could, theoretically, influence digestion of carbohydrates in diabetic or obese patients (Layer, Carlson, & DiMagno, 1985).

Table 1
Protein contents of pulps of tropical fruits and detection of biological activity as enzyme inhibition (I): bovine trypsin (BT), α- porcine pancreatic amylase (α-PPA) and α- human salivary amylase (α-HSA)

Fruit pulp	mg Protein/g pulp	I-BT	I-α-PPA	I-α-HSA
Assai	0.20±0.05	+	–	+
Suriname Cherry	0.10±0.01	+	–	–
Mangaba	0.62±0.06	+	–	–
Yellow Mombim	0.15±0.01	+	+	–
Acerola	0.17±0.02	+	–	–
Cupuassu	0.54±0.03	+	+	–
Melon	0.24±0.01	–	–	+
Passion fruit	0.80±0.09	–	–	–

Table 2
Assessment of porcine pancreatic amylase inhibitors in pulps of tropical fruits

Fruit pulp	mg Inhibitor/g pulp
Yellow Mombim	0.0345±0.0004
Cupuassu	0.0335±0.0002

Table 3
Assessment of human salivary amylase inhibitors in pulps of tropical fruits

Fruit pulp	mg Inhibitor/g pulp
Melon	0.042±0.0003
Assai	0.142±0.04

Table 4
Assessment of bovine trypsin inhibitors in pulps of tropical fruits

Fruit pulp	mg Inhibitor / g pulp
Assai	0.054±0.005
Suriname Cherry	0.013±0.001
Mangaba	0.0395±0.0002
Yellow Mombim	0.0328±0.0001
Acerola	0.0234±0.0002
Cupuassu	0.0047±0.00

Antitryptic activity was detected in six of the fruit samples (Assai, mangaba, yellow mombim, suriname cherry, acerola and cupuassu). The levels of inhibitory activity were relatively low for most fruits (Table 4) and those with highest activities were assaí (0.054 mg/g), mangaba (0.0395 mg/g) and cajá (0.0328 mg/g).

Interest in protease inhibitors is not only due to their adverse and complex effects on digestion, but also due to their high concentrations in animal diets that could affect digestive processes, influencing animal development and growth (Richardson, 1991; Ryan, 1990). For humans, enzyme inhibitors are not a real problem since the level of inhibitory activity is mostly reduced through cooking. When denatured, these inhibitors present themselves as digestible proteins with essential amino acids, important to the diet, such as lysine, cysteine and methionine (Ryan, 1989). The very small amounts of enzyme inhibitors present in endocarp of ripe fruits may be explained by studies with apples. Apples, in the first stages of development, had very small amounts of protease inhibitors which increased as they grew and decreased again as they matured (Ryan, Liang, & McManus, 1998). It seems that, during seed formation, inhibitors have physiological activity and play a role in defence and, after seeds are formed, their low concentrations allow seed dispersal by animals.

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