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Proteolytic Activity in Stems of 'Vitória', 'Smooth Cayenne' and Pérola' Pineapple Plants

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Abstract

Pineapple is one of the most important tropical fruits, not only due to its quality as fresh and processed product, but also as a source of subproducts such as bromelain, a proteolytic enzyme mixture with large applications in pharmaceutical and food industries. Looking for solutions for disease problems, Brazilian research institutions have developed new pineapple cultivars such as the 'Vitória', released for farmers' use in the State of Espirito Santo in 2006. 'Vitória' plants are resistant to fusariosis, the main pineapple disease in Brazil, and show agronomic characteristics similar or even superior when compared to the traditional cvs. 'Pérola' and 'Smooth Cayenne'. In this work the total protein contents and the proteolytic and specific activities of proteolytic enzymes in pineapple stems of cvs. 'Vitória', 'Pérola' and 'Smooth Cayenne' were determined. Results indicate that the new cv. 'Vitória' presented protein and enzymatic values in stem tissues similar to those found in the 'Smooth Cayenne' cultivar.

INTRODUCTION

Pineapple (*Ananas comosus* var. *comosus)* is a monocot plant of the Bromeliaceae family, and presents a fruit symbolic for tropical and subtropical regions, of great acceptance throughout the world as both fresh and processed fruit.

The fruit is rich in a group of proteolytic enzymes, known by the general name of bromelain, which is also present in other parts of the pineapple plant. Bromelain has wide applications in the food industry, but it also has clinical use for many different purposes, such as antitumoral agents, immune modulation, cleaning of wounds, increase of antibiotic effect, mucolitic action, aid in digestion, applications in cardiovascular and circulatory illnesses, surgical procedures and treatment of wounds of skeletal musculature. Moreover, bromelain has anti-inflammatory effect (Cooreman et al., 1976).

Enzymes catalyze reactions in biological systems and have a extraordinary catalytic efficiency superior to that of chemical catalyzers. They also speed up specific chemical reactions and remain functional in watery solutions and conditions of small variation of temperature and pH (Voet and Voet, 1995).

The proteolytic enzymes or peptidases make up about 50% of industrially used enzymes (García-Carreño and Del Toro, 1997). The bromelains are hydrolases that cleave peptide bounds for water addition and belong to the subclass of cysteine (or tiol) peptidases, as they present a residue of cysteine.

The wide use of bromelain in pharmaceutical industries, cosmetics and food increases the importance of studies to evaluate proteolytic activities in different cultivars of pineapple. Moreover, knowledge of proteolytic activity in new genotypes of pineapple resistant to *Fusarium* disease (Ventura et al., 2006), may indicate opportunities to minimize losses and aggregate values in the pineapple agribusiness, with generation of more jobs, higher income and better profits for the productive sector.

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The objective of this work was to determine total protein contents and proteolytic and specific activities of proteolytic enzymes in stems from 'Vitória', 'Smooth Cayenne' and 'Pérola' pineapple plants.

MATERIALS AND METHODS

Plant samples of three pineapple cultivars ('Vitória', 'Smooth Cayenne' and 'Pérola') were obtained from Incaper Sooretama Experimental Farm, Sooretama-ES, Brazil. The samples were processed and analyzed in the analytical chemistry laboratory of Univix, in Vitória city from July to November 2006.

The total protein content was determined by the biuret method cited by Freiman and Sabaa (1999). The standard curve was determined by a relation of absorbance and amount of bovine albumin solution prepared together with the biuret solution in assay pipes (tubes). The blank (control) was prepared by adding to an assay pipe the biuret solution with distilled and deionized water.

The prepared solutions were homogenized and transferred to buckets and read in a UV/visible Pharmacia Biotech® - Ultrospec 2000, at the wavelength of 540 nm. The experiment was done in triplicate and an average of the absorbance readings was obtained. Results were submitted to statistical analysis and by linear regressions the coefficients of determination (R^2) and the equations were determined.

Proteolytic activity was determined by the method of Depeau (1976) with some modifications, where two series of assay cuvettes were prepared, one used for analysis of the extract of the cultivars and the other one for the blank. The experiment was performed in triplicate.

The enzymatic extract was prepared by separating the plant material from the extract previously acquired in a microcentrifugal machine CEINTEC®, with maximum rotation at 14,000 rpm and 4°C for 10 minutes.

The test pipe consisted of 5 ml of casein solution, 0.2 ml L-cysteine solution and 0.8 ml of enzymatic extract. These cuvettes were placed into a water bath QUIMIS® at 35°C for 30 minutes and thereafter 5 ml of proteic precipitant solution was added. After 30 minutes at room temperature, this solution was filtered and the absorbance read in a spectrophotometer UV/visible Pharmacia Biotech® - Ultrospec 2000, adjusting the wave length to 280 nm. The blank consisted of 5 ml of casein solution, 0.2 ml of L-cysteine solution, 0.8 ml of distilled water and 5 ml of solution of the proteic precipitant. These cuvettes were placed into a water bath QUIMIS® at 35°C for 30 minutes and the solutions were left at room temperature for additional 30 minutes. The solutions were filtered and absorbance read at 280 nm.

A solution of tyrosine at 50 µg/ml was prepared and the absorbance read at 280 nm. Absorbances for the test pipes, blank and tyrosine solution were determined and enzyme units per ml (U/ml) calculated for each pineapple cultivar studied as follows (1):

$$\text{U/ml of enzyme} = \frac{(At - Ab) \times \mu\text{mol tyrosine} \times V2}{A\ \text{tyrosine} \times V1} \quad (1)$$

Where:
At: absorbance of the solution tested for each cultivar
Ab: absorbance of the blank
A tyrosine: absorbance of the tyrosine
µmol tyrosine: concentration of the solution of tyrosine in the bucket
V1: volume placed in the cuvette of the enzymatic extract
V2: total volume in the cuvette

The experiments were organized in a completely randomized design, and results submitted to statistical analyses using the analysis of variance, the homogeneity test of Bartellets and the test of Duncan at the level of 5% of probability.

The proteolytic activity of commercial bromelain was determined with 10 mg of bromelain diluted in 100 ml of L-cysteine solution. The same procedure was performed for determination of the proteolytic activity in the pineapple cultivars. The specific

activity of proteolytic enzymes in the three pineapple cultivars was determined based on the relationship between proteolytic activity and total protein values as **(2)**:

$$\text{Specific activity} = \frac{\text{Proteolytic activity (U/mg)}}{\text{Total Protein (mg/ml)}} \quad (2)$$

RESULTS AND DISCUSSION

The standard curve obtained by analyses of different bovine serum albumin solutions of different concentrations indicated a good adjustment of precision. The regression equation established the functional relationship between the values of albumin and the sample absorbance.

The model proposed was adequate to study the total protein as confirmed by the coefficient of determination ($R^2 = 0.99$) (data not shown).

A correlation was obtained between the amount of protein determined in the stem samples of the pineapple genotypes studied, expressed in g/kg of tissue, and the albumin concentrations in mg/ml analyzed.

Total protein contents did not significantly differ among cultivars ($P<0.05$) (Table 1). There was a variation from 2.7278 g/kg in cultivar 'Pérola' to 2.9404 g/kg in cultivar 'Smooth Cayenne'.

Pineapple stems are considered plant residues that are left in the field after fruit harvest and planting material removal or destroyed by cutting and incorporation into the soil. The results obtained in this work confirm that pineapple stems are a potential protein source for animal food of high nutritional value, and for extraction of proteolytic enzymes such as bromelain, with applications in food and drug industries.

The analysis of variance made it possible to decompose the total variation between all components of known and independent causes, with those of random nature having a high level of significance ($F \leq 0.01$) among the different samples.

Enzyme values were significantly different among the cultivars studied (Table 2). There was a variation from 19,716.6 U/ml in cultivar 'Smooth Cayenne' to 26,743.5 U/ml for 'Pérola'. The latter was superior to both 'Smooth Cayenne' and 'Vitória', without statistical difference between the latter two. The test of Bartellets confirmed the homogeneity of the variance, without needing data transformation.

As expected, the commercial bromelain Sigma® (average of 5,158.0 U/ml), presented lower values than those obtained for the plant material studied. When comparing the results for enzymes and for total protein contents, it can be observed that the values are inversely proportional, with 'Pérola' presenting the highest enzyme value but also the lowest protein content. In both cases the cv. 'Vitória' presented intermediate values close to those of the cv. 'Smooth Cayenne'.

The results obtained for the specific activity of proteolytic enzymes were similar to those of enzyme values, with significantly higher values for the cv. 'Pérola' and no statistical difference between 'Vitória' and 'Smooth Cayenne' (Table 3). Values ranged from 6,705.36 to 9,705.03 U/mg.

Based upon the data obtained in this study, it cannot be affirmed that the amount of bromelain in pineapple stems is proportional to its total protein content.

Values of proteolytic activity and specific activity of the cultivar 'Pérola' were superior to those of the other cultivars. This result indicates that a large amount of the proteolytic enzymes consists of bromelain.

CONCLUSIONS

The proteolytic and specific activities of proteolytic enzymes in stems of 'Pérola' pineapple plants were superior to those obtained for 'Smooth Cayenne' and 'Vitória'.

The new cultivar 'Vitória', a hybrid derived from 'Smooth Cayenne', presented protein content, proteolytic and specific activities of proteolytic enzymes similar to those of 'Smooth Cayenne'. These results indicate that 'Vitória' stems may be an economically viable source for bromelain extraction.

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Tables

Table 1. Total protein values (g/kg) in stems of pineapple genotypes.

Cultivar	Total Protein (g/kg)[1]	
Smooth Cayenne	2.9404	a
Vitória	2.9323	a
Pérola	2.7278	a

[1]Averages of three replications. Means followed by the same letter are not significantly different by the Tukey test ($P \leq 0.05$).

Table 2. Enzyme values (U/mL) obtained in stems of pineapple genotypes.

Cultivar	Enzyme (U/ml)[1]	
Smooth Cayenne	19,716.6	a
Vitória	20,246.6	a
Pérola	26,473.5	b

[1] Averages of three replications. Means followed by the same letter are not significantly different by the Duncan test ($P \leq 0.05$).

Table 3. Values of specific activity of proteolytic enzymes (U/mg) in pineapple genotypes.

Cultivar	Enzyme (U/mg)[1]	
Smooth Cayenne	6,705.36	a
Vitória	6,904.66	a
Pérola	9,705.03	b

[1] Averages of three replications. Means followed by the same letter are not significantly different by the Duncan test ($P \leq 0.05$).