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Assessment of the hemoaglutinant and digestive enzyme inhibitory activity of extracts obtained from different parts of atemoia

Alexandre Coelho Serquiz[1], Raphael Paschoal Serquiz[2], Thayane Kerbele Oliveira das Neves Peixoto[3], Elizeu Antunes dos Santos[4], Hugo Alexandre Oliveira Rocha[5]

ABSTRACT - The benefits of various foods, such as fruits and vegetables, have been the focus of several studies aimed at improving welfare, health, and reducing the incidence of diseases. Therefore, the present paper objectified to investigate the presence of molecules of biotechnological and pharmacological interest in peel, seed, and pulp of *Annona x atemoya Mabb*. Aqueous extracts of the fruit parts were obtained with different buffers and assessed as to their protein and phenolic compounds content. The three parts of the fruit presented different proportions of these compounds when subject to different extraction conditions, with the highest concentrations of proteins being found in the seed and phenolic compounds in the peel of the studied fruit. Bioactive proteins (protease inhibitors and lectins) were detected through inhibitory tests for trypsin and chymotrypsin and hemagglutinating activity tests with human erythrocytes. A variation of 400 to 9600 inhibition units for the trypsin in the analyzed extracts, whereas for chymotrypsin a variation of 200 to 2500 in the inhibition units for the three fruit parts, considering the different extraction conditions were identified. The extracts obtained from the seeds and the peel presented titers higher than 0.9 hemagglutination units, suggesting that the by-products from the processing of *A. x atemoya* are potential sources of bioactive molecules.

Index terms: *Annona x atemoya*; residues; protease inhibitors; lectins.

Avaliação da atividade hemoaglutinante e inibidora de enzimas digestórias de extratos obtidos de diferentes partes da atemoia

RESUMO – Os benefícios de diversos alimentos, como frutas e hortaliças, têm sido foco de vários estudos que visam a melhorar o bem-estar, a saúde e a reduzir a incidência de doenças. Dessa forma, no presente estudo, averiguou-se a presença de moléculas de interesse biotecnológico e farmacológico em casca, semente e polpa de *Annona x atemoya Mabb.* Extratos aquosos das três partes do fruto foram obtidos com diferentes tampões e avaliados quanto a seu conteúdo de proteínas e compostos fenólicos. As três partes do fruto apresentaram proporções diferentes destes compostos quando submetidos a distintas condições de extração, sendo que as maiores concentrações de proteínas foram encontradas na semente, e as de fenólicos, na casca, do fruto estudado. Proteínas bioativas (inibidores de proteases e lectinas) foram detectadas por ensaios inibitórios para tripsina e quimotripsina, e ensaios de atividade hemaglutinante com eritrócitos humanos. Identificou-se variação de 400 a 9.600 unidades de inibição para a tripsina nos extratos analisados; já para a quimotripsina foi vista variação nas unidades de inibição de 200 a 2.500 unidades nas três partes do fruto e nas diferentes condições de extração. Os extratos obtidos das sementes e da casca apresentaram títulos superiores a 0,9 unidades de hemaglutinação, sugerindo que os resíduos do processamento de produtos derivados de *A. x atemoya* são potenciais fontes de moléculas bioativas.

Termos para indexação: *Annona x atemoya*; resíduos; inibidores de proteases; lectinas.

Corresponding author:
E-mail: alexandreserquiz@gmail.com

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[1]PhD candidate, Graduate Program in Health Sciences, Federal University of Rio Grande do Norte, Natal-RN, Brazil. E-mail: alexandreserquiz@gmail.com
[2]Master's Degree candidate, Graduate Program in Biochemistry, Federal University of Rio Grande do Norte, Natal-RN, Brazil. E-mail: raphaserquiz@hotmail.com
[3]BSc Nutrition and Food Sciences, Federal University of Rio Grande do Norte, Natal-RN, Brazil. E-mail: thayanekerbele@gmail.com
[4]PhD,Department of Biochemistry, Federal University of Rio Grande do Norte, Natal-RN, Brazil. E-mail: elizeu@ufrnet.br
[5]PhD, Department of Biochemistry, Federal University of Rio Grande do Norte, Natal-RN, Brazil. E-mail: hugo@cb.ufrn.br

Introduction

Urbanization, industrialization and globalization had great influence on the lifestyle, diet and, consequently, the nutritional status of Brazilians (TARDIDO, FALCÃO, 2006; BRASIL, 2012). This influence led to a decrease in poverty and social exclusion, bringing in a decrease in figures related to hunger and malnutrition among the poor population. On the other hand, a vertiginous increase in weight excess is observed in all the layers of the population, pointing towards a new scenario of problems related to food and nutrition, such as chronic non-communicable diseases (BRASIL, 2012). In view of this, the role of Nutrition today is to maximize physiological functions and ensure increased health and well-being with reduced risk of disease (SMET; VOSSEN, 2016).

Given these facts, a large number of novel products which supposedly provide health improvement ha e been presented by the food industry, due to the concern on food and nutrition (ANJO, 2004). The growing speed at which this occurs gives rise to new terms in this field as, for instance, functional foods. There is still no consensus regarding the concept of "functional food" by the National Agency for Sanitary Surveillance (ANVISA), however, its records state that "a claim of functional property is that concerning the metabolic or physiological role played by the nutrient or non-nutrient in the growth, development, maintenance and other normal functions of the human organism" (ANVISA, 1999). The concept of functional foods is broad and still advocates the assumption that the diet can reduce the risk of involvement by morbidities (BORGES, 2000).

According to Roberfroid (2002), the functional food can be: 1. Natural food. 2. Food to which a component has been added. 3. Food from which a component has been removed. 4. Food in which the nature of one or more components has been modified. 5. Food in which the bioavailability of one or more components has been modified.

Therefore, functional property is present in several foods, such as fruits and vegetables. Fruits are the subject of studies worldwide, aiming to elucidate the properties and effects of substances in these foods, including certain phenolic compounds, lectins and trypsin inhibitors (VASCONCELOS; OLIVEIRA, 2004). Phenolic compounds have an antioxidant function which attracts a special attention to fruits regarding the treatment and prevention of various diseases, including degenerative diseases (GARDNER et al., 2000, LEONG & SHUI, 2002; KUSKOSKI et al., 2006). Lectins are proteins capable of binding to glycogen and glycoconjugates (HATAKEYAMA et al., 1995), and by doing so those can modulate the function of these molecules. As an example, by associating with the glycoproteins in the surface of certain tumor cells, lectins can lead to a change in cellular metabolism, by modulating various intracellular signal transduction pathways (PARK et al., 2000) and, as shown by several in vitro and in vivo studies, lectins can inhibit various stages of the carcinogenesis process (HOLLMAN, KATAN, 1997; RABELO et al., 2012; MAESTRI, 2014).

Nevertheless, despite the efforts, the "functional" properties of many fruits are still unknown (RIBEIRO et al., 2015). One of them is atemoya (*Annona x atemoya Mabb*.). This is a hybrid fruit resulting from the cross between cherimolia (*Annoma cherimola Mill*.) and sugar apple (also known as earl fruit or ata) (*Annoma squamosa L*.) (STENZEL et al., 2003). It is intentionally produced because it is easy to plant and highly adaptable to the tropical climate, further to being described as tastier, containing fewer seeds and having a longer post-harvest life when compared to cherimolia and sugar apple (MARCELLINI et al., 2003; RABLLO et al., 2014). The main destination of atemoya fruits is in natura consumption. Although considered very tasty fruits, their production and commercialization are still limited and there is little availability of fresh fruits in the Brazilian and world markets. Moreover, there are few studies about their benefits to human health (PEREIRA et al., 2011).

If little is known about the atemoya fruit, to date, there are no data referring to atemoya wastes: peel and seed. They account for about 40% of the fruit's weight and are discarded by consumers.

Discarded waste from food could have a beneficial purpose for man and the environment. Some fruits, during processing, have their seeds discarded. However, they could be used, minimizing this waste. One way this could be done would be to obtain higher added value products of the seeds, like, for instance, vegetable oils (KOBORI; JORGE, 2005). In addition, some researchers have shown the presence of phytochemicals in fruit peel and seed (AL-DAIHAN; BHAT, 2012), which brings functional value to these types of food residues.

The properties of atemoya and its residues are still unknown, thus the importance to explore and investigate their biotechnological and pharmacological potentials. Thereby, for this work, extracts of atemoya peel, pulp and seed were obtained and the protein and phenolic compound contents of the extracts were evaluated, as well as their hemagglutinating agents and protease inhibitors activities, in order to initiate filling the gap regarding the knowledge about the potential of atemoya and its residues.

Materials and Methods

The atemoya (*Annona cherimola Mill. X A. squamosa L.)* fruit samples were obtained in supermarkets in the city of Natal-RN in February 2014 and their parts (seed, pulp and peel) were manually separated.

Extraction - to obtain the extracts of the different parts of atemoya, fruits were selected and those containing signs of infestation, poor state of conservation or advanced maturation were discarded. The fruits chosen were opened and divided into three parts: pulp, peel and seed. The first two were frozen (-20ºC) and only defrost at the time of obtaining the extracts. The seeds were dried in a ventilated oven at 60ºC, peeled for tegument removal, and the cotyledon was then ground in an industrial mill until the seed meal, which was then cooled, was formed. All samples were through extraction proceses in three buffers, separately - 0.05 M sodium acetate, pH 4.3; 0.05 M tris-HCl, pH 7.5 and 0.05 M tris added to 0.05 M glycine, pH 11.5 – at 1:10 (w/v) ratio, for seed extraction, and 1:2 (w/v) for peel and pulp, and maintained for four hours under constant stirring. Subsequently, the samples were centrifuged at 4ºC for 30 minutes at 8000 x g. The precipitate was discarded and the supernatant obtained was named crude extract.

Protein Dosage - all protein determinations were performed by the method of Bradford (1976) with modifications. In a 96-well flat bottom plate, 10 µL of the extracts was placed in different titrations. Subsequently, 200 µl of the Bradford reagent was added and the plate was rested for approximately ten minutes before being read on a microplate reader at 595 nm. Bovine albumin was used to determine the standard curve and wells containing only 10 µL of the extraction buffers were used as white.

Total phenolic compounds assessment - the total phenolic content was measured in duplicate, and the tests were repeated three times for each extract as described by Melo-Silveira et al. (2014). An analytical curve containing 500, 400, 300, 200, 100, 50, 10 and 0 mg/ml of gallic acid was used as an equivalence reference. 200 µl of extracts was then separately added to the test tubes, then 1400 µl of ultrapure water, and 100 µl of Folin reagent. The tubes were then shaken in a tube shaker and rested for ten minutes at room temperature. Sequentially, 50 µl of 20% sodium bicarbonate was added to the tubes, which were again stirred and placed in a water bath at 40°C for 20 minutes. Whites containing only the respective extraction buffers from each crude extract were made to exclude residual absorbance. The absorbance readings were performed at 765 nm in a spectrophotometer. The results were expressed as gallic acid equivalents (EAG)/g of sample dry weight.

Trypsin inhibitory activity - the trypsin inhibitory activity test, for the three fruit parts, was performed in triplicate, being repeated three times and determined as described by Migliolo et al. (2010). Thus, 10 µL of the enzyme was added into the tubes and then 120 µL of 2.5 mM hydrochloric acid and 100 µL of the extracts, separately. Subsequently, 0.05 mM Tris-HCl buffer, pH 7.5 was added to obtain the final volume of 790 µl. An extract free tube was determined as the standard for enzymatic activity. After this procedure, the tubes were kept in a water bath at 37oC for 10 minutes, and 500 µL of 1.25 mM BAPNA substrate was added to the water bath. The reaction was then halted with the addition of 120µl of 30% acetic acid. The whites were made into sample tubes, where the substrate was only added after the reaction stopped. The result was measured by spectrophotometer reading at 410 nm. The results were expressed in specific inhibitory activity obtained in three sequential assays with the same samples. The specific activity was calculated as the ratio between the sample's inhibition units and the amount of proteins or phenolic compounds present in the test samples. An inhibitory unit was defined as the amount of inhibitor decreasing enzyme activity by 0.01 of the absorbance at 410 nm.

Inhibitory activity for chymotrypsin - the inhibitory activity on chymotrypsin was measured according to the methodology proposed by Oliveira et al. (2007) with adaptations. Thus, 20 µL of bovine chymotrypsin (0.1 mg / mL in 50 mM Tris-HCL, pH 7.5, containing 20 mM CaCl2) was added to 100 µL of the inhibitor and incubated for 15 minutes at 37°C. After this, the reaction was started by adding 200 mL of 1% azocasein. After 30 minutes, the reaction was halted by adding 300 mL of 20% TCA solution. The reaction mixture was centrifuged at 12,000 x g for 10 minutes and the supernatant basified with 2M NaOH at 1:1 ratio. The absorbance was measured in a spectrophotometer at 440 nm. White tests were performed and the tests were done in triplicate. The results were expressed in specific activity, calculated in the same manner as for the trypsin testes.

Hemagglutinating activity - the hemagglutinating activity was verified following the methodology adapted by Medeiros et al. (2010). Three 1 mL aliquots of human types A, B and O erythrocytes were washed with 12 mL of 0.9% saline solution and centrifuged at 3500 rpm for five minutes until a serum free erythrocyte mass was obtained. Two aliquots of each blood type were subjected to enzymatic treatment - one with papain and one with trypsin - to increase exposure of the red blood cell binding sites and the other was maintained as native blood. The treated aliquots were rewashed for complete removal of the enzymes. The hematocrit was performed and 4% erythrocytes solutions were prepared in saline.

Hemagglutination tests were performed by serial dilution in "V' bottom 96-well plates. In each well 25µl of saline solution, 25µl of the 12 titrations serially diluted samples were added. Thus 25µL of a 4% erythrocyte suspension was added and the reaction was incubated at room temperature for 1 hour and visually analyzed after that period. Each sample was tested with type A, B and O blood – trypsin treated, papain treated and native - separately.

The specific hemagglutinating activity (HU/µg) was determined by the ratio between the hemagglutinating units and the amount of protein or phenolic compounds contained in the evaluated sample. The hemagglutinating unit was obtained as the maximum titer at which

agglutination of the red blood cells was noted.

Statistical analysis – the data were tabulated and analyzed using descriptive statistics, through central trend and dispersion measures (mean and standard deviation) with the Microsoft Excel® program, version 2015.

Results and discussion

The results presented in Table 1 refer to the quantification data of soluble proteins and phenolic compounds present in the atemoya extracts.

Regarding the content of phenolic compounds, it was observed that the three extracts obtained from the peel are the ones with the greater content of this component, followed by the pulp extracts. When comparing the three buffers studied, the largest quantities of phenolic compounds, regardless of source, were always extracted using the tris-glycine buffer. It is believed that the peel extracts stand out because they are the main reservoirs of phenolic compounds in most of the studied fruits (Heim et al., 2002). These molecules are considered as one of the main responsible for the antioxidant action of fruits and are indicated as preventive agents of chronic-degenerative diseases and other biological processes related to oxidative stress (ALMEIDA et al., 2011). Finding them in atemoya, especially in the peel, is a display of the potential lost by the population when using the fruit, since it is common not to use that part of atemoya as food.

Regarding proteins (Table 1), when comparing the amount of protein obtained from each part of the fruit using the same buffer, it is verified that, regardless of the buffer, more protein is always obtained from the seed than from other parts This occurred mainly when the tris-glycine buffer was used, a condition at which ten times more proteins were obtained from the seeds than from the pulp and the peel. Also with respect to such buffer it has been found that the amounts of proteins extracted from the pulp and the peel are similar. In the other conditions, the amount of protein extracted from the peel was higher than those extracted from the pulp.

These data corroborate with those obtained by Cruz (2011), since they show that the seed, followed by the peel, would be the main protein reservoirs of atemoya. This would reflect, therefore, the higher amount of proteins found in the extracts obtained from these parts in comparison to the extracts obtained from the pulp. With the tris-glycine buffer, regardless of the fruit part used, more proteins were always extracted than with the other buffers. An explanation for these facts is probably the characteristic of the proteins extracted and of their amino acids. The tris-glycine buffer was the only one to maintain the basic pH, around 11. This value enables the ionization of basic amino acids, which increases the solubility of their proteins (HOVING et al., 2002). Therefore, it is believed that more proteins were extracted with the tris-glycine buffer because atemoya would be rich in proteins comprising basic amino acids in their constitution, which favors their solubilization at basic pH.

Figure 1 shows the specific inhibition activity of the trypsin enzyme in IU/µg of protein (Figure 1A) and IU/µg of phenolic compounds (Figure 1B).

With respect to the seed extracts it can be observed that with those rich in proteins, i.e. extracts obtained with tris-glycine and tris-HCl (Table 1), no detectable rates of trypsin enzyme inhibition were observed when the protein component was analyzed. This index was only observed when the phenolic component was analyzed. For the acetate extract, the best trypsin inhibition rates were obtained both with respect to the protein and phenolic components (Figures 1A and B). It is also worth noting that this extract had a high specific activity for phenolic compounds, which is an unusual fact, given the fact that this extract, among the three, has the lowest content of phenolic compounds, indicating a high specificity of the phenolic compounds extracted.

Table 1 – Soluble proteins and phenolic compounds in aqueous extracts of three parts of the atemoya fruit, for three extraction buffers. ND - levels lower than the Bradford reaction sensitivity.

Fruit Part	Extraction Buffer	**Soluble Proteins** mg·mL^{-1}	**Phenolic Compounds** EAG/g
Seed	Tris-Glycine	19,072 (± 0,022)	0,117 (± 0,001)
	Tris-HCl	14,506 (± 0,039)	0,042 (± 0,004)
	Sodium Acetate	0,501 (± 0,007)	0,047 (± 0,001)
Pulp	Tris-Glycine	1,898 (± 0,021)	0,391 (± 0,057)
	Tris-HCl	0,093 (± 0,01)	0,124 (± 0,068)
	Sodium Acetate	ND	0,117 (± 0,009)
Peel	Tris-Glycine	1,865 (± 0,044)	1,375 (± 0,005)
	Tris-HCl	0,608 (± 0,017)	0,328 (± 0,009)
	Sodium Acetate	0,435 (± 0,008)	0,717 (± 0,018)

ND- levels lower than the Bradford reaction sensitivity.

Considering the peel, it was possible to inhibit the trypsin activity with all of its extracts. Amongst them, it was with the peel extract obtained with acetate that the best specificity of trypsin inhibition was verified, considering the specific activity of the protein component.

For the pulp extract obtained with the tris-glycine buffer, very low rates of trypsin inhibition were observed for both the protein and phenolic components. The pulp extract obtained with the Tris-HCl buffer presented high specific activity of the protein components, the highest obtained among all the extracts evaluated herein (Figure 1).

Trypsin inhibitors of protein origin have already been found in various vegetable sources, such as peanuts (ARAUJO, 2014), in pulps and seeds of tropical fruits, such as white and red varieties of guava, passion fruit, watermelon (BEZERRA, 2014), and tamarind (RIBEIRO et al., 2015). With regard to atemoya, only one other study evaluating extracts of this fruit as trypsin inhibitors was identified. In this case, pulp and peel extracts have proven to possess trypsin inhibitory activity. However, the individual influence of both the protein and phenolic components in this inhibition was not determined (CRUZ, 2011).

In these foregoing studies, the use of different extraction conditions for inhibitors of existing sources was not evaluated. However, it can be stated that use of the tris-glycine buffer, at least in the case of atemoya, decreases the extraction of trypsin inhibitors.

Trypsin inhibitors are known as antinutritional substances because they have inhibitory action of digestive enzymes. This inhibition results in decreased protein digestion, compromising the absorption of amino acids, and leading to loss of peptides through feces. This generates negative nitrogen balance, a fact that leads to losses to individuals (VAZ PATTO et al., 2015). Trypsin inhibitors are described in several plant sources, including beans, the most consumed pulses in Brazil (Vaz Patto et al., 2015; Yamamoto, IKENAKA, 1967; GU et al., 2014). Phenolic compounds are also reported as being trypsin inhibitors in three grape varieties (CARVALHO et al., 2014).

The low trypsin concentration in foods may contribute to the bioavailability of the protein nutrients found in these foods, favoring their digestion and absorption processes, thus contributing to the added nutritional value of the food (Silva et al., 2000). However, from the studies carried out with inhibitors, there is still little practical knowledge on how to reduce the amount of these antinutritional factors. Therefore, it is of paramount importance to find extraction media that allow the acquisition of smaller inhibitor amounts, which is the case observed when the tris-glycine buffer was used. Based on the data obtained, it is suggested that studies to be carried out with fruits evaluate different conditions for the extract obtention, in order to identify their real nutraceutical potential.

The seed extracts obtained with acetate and the pulp extracts obtained with tris-HCl, in their turn, contain a greater specific inhibitory activity of trypsin (Figure 1) and can be used in research aimed at the purification and characterization of trypsin inhibitors. These molecules are identified as possessing properties such as anticancer, anti-inflammatory and anticoagulant (KENNEDY, 1998; OLIVA et al., 2000). They may help to control glycemia (SERQUIZ et al., 2016) and suppress the regulation of pancreatic secretion with greater release of the cholecystokinin hormone (CCK) through the intestinal mucosa, a hormone known as satietyogenic (LIENER, 1994; SERQUIZ, 2012). However, not all of them have the same activities or the same efficiency as bioactive agents. This drives the search for the discovery of new trypsin inhibitors which may have different or more efficient physiological actions, even presenting less side effects. The extraction of these compounds in different buffers is fast, reproducible and inexpensive, and can also be used to select fruits presenting higher indexes of inhibitors in the peel and seed in order to select cultivars that are more resistant to pests.

Chymotrypsin inhibitors, as for the trypsin inhibitors, were extracted in different amounts for each extract (Figure 2).

Regarding the protein inhibitors (Figure 2A), it was verified that the seed is the source with the least amount of these inhibitors, as well as for trypsin, whereas in the peel and in the pulp the highest values were observed. In this case, with the acetate buffer and the Tris-HCl buffer, respectively.

For the phenolic compounds, it was with the seed extract that the highest value of specific inhibition of the enzyme was obtained (Figure 1B and 2B), however, the phenolic composition was not elucidated. It is assumed that these extracts would have the presence of alkaloids and tannins (SANTOS, 2007) and, therefore, the predominance of the specific activity in the seed extract may be a compensatory effect for the specific activity of these molecules. Regardless, it is worth noting that inhibitory activity was found for all extracts.

To date, chymotrypsin inhibitors have, admittedly, a range of possibilities of diverse functions and properties for living organisms and previously unexplored biotechnological applications, of which biomedicine and environmental applications can be highlighted. Several plants were selected for generations in order to choose the cultivars with the greatest number of chymotrypsin inhibitors, which would therefore be more resistant to insect-plague attack (PADUL et al., 2012). In the biomedical field, it is possible to highlight the potential of these inhibitors in the control of diseases - of which AIDS and cancer are highlighted - due to coagulation as well as to the immune system related effects (GUPTA et al., 2013).

The tris-glycine extracts of the peel, seed and pulp of the atemoya fruit, despite presenting a great amount

of protein in relation to the other components evaluated (Table 1), showed low specific inhibition activity, both for trypsin and chymotrypsin, when compared to other fruits described in the literature (BEZERRA, 2014). This indicates that these extracts have a high nutritional value, since they present a higher amount of protein and lower amount of inhibitors, favoring their digestion and absorption process. Future studies of digestibility for these extracts could confirm this hypothesis.

The presence of proteins in the extracts indicated the existence of lectins in them. Lectins are carbohydrate-recognizing proteins with high biotechnological potential (SHIMOKAWA et al., 2016) and a rapid way to identify their presence is to use the blood cell agglutination test, since several lectins are known as agglutinins (POP et al. al., 2015b).

Table 2 shows the specific hemagglutinating activity of the extracts obtained from the seed, peel and pulp of atemoya. Using tris-glycine buffer, the haemagglutinating activity molecule was not extracted.

Another noteworthy fact was the absence of hemagglutinating activity in the three extracts obtained from the atemoya seeds (Table 2). This data does not agree with others in the literature, which show that seeds are good sources of lectins (NG TZI BUN et al, 2015), and can lead to two notices. There are actually no lectins in the atemoia seed, after all three extraction conditions were used, and in the three conditions no agglutinating activity was identified. Another thing Is that the possibility of agglutinating lectins or other agglutinating agents in the atemoia seed can not be totally excluded, since there is a possibility that the extraction conditions were not favorable to extract those, or disadvantage their activities, fact which has already been described by other authors when studying other fruits (POP et al., 2015a). It is hoped

Table 2 – Specific Hemagglutinating Activity (UH/µg) in extracts obtained from the seed, peel and pulp of atemoya for different extraction buffers; calculated based on the amount of proteins and phenolic compounds. Each blood type (A, B and O) was evaluated natively (N), papain treated (P) and trypsin treated (T), separately.

Protein		**Blood Type**								
		A			**B**			**O**		
Part	**Extract**	**N**	**P**	**T**	**N**	**P**	**T**	**N**	**P**	**T**
Semente	Sodium Acetate	-	-	-	-	-	-	-	-	-
	Tris-HCl	-	-	-	-	-	-	-	-	-
	Tris-Glycine	-	-	-	-	-	-	-	-	-
Polpa	Sodium Acetare	-	-	-	-	-	-	-	-	-
	Tris-HCl	-	19,9	1,2	-	39,8	5,08	-	9,9	2,5
	Tris-Glycine	-	-	-	-	-	-	-	-	-
Casca	Sodium Acetate	71,8	287,1	287,1	143,5	287,1	287,1	143,5	287,1	143,5
	Tris-HCl	140,0	140,0	140,0	140,0	140,0	140,0	140,0	140,0	140,0
	Tris-Glycine	-	-	-	-	-	-	-	-	-

Phenolic Compounds		**A**			**B**			**O**		
Part	**Extract**	**N**	**P**	**T**	**N**	**P**	**T**	**N**	**P**	**T**
Seed	Sodium Acetate	-	-	-	-	-	-	-	-	-
	Tris-HCl	-	-	-	-	-	-	-	-	-
	Tris-Glycine	-	-	-	-	-	-	-	-	-
Pulp	Sodium Acetate	3,2	103,7	25,9	6,5	207,3	25,9	3,2	207,3	13,0
	Tris-HCl	-	14,8	0,9	-	29,6	3,7	-	7,4	1,9
	Tris-Glycine	-	-	-	-	-	-	-	-	-
Peel	Sodium Acetate	14,3	57,1	57,1	28,6	57,1	57,1	28,6	57,1	28,6
	Tris-HCl	124,9	124,9	124,9	124,9	124,9	124,9	124,9	124,9	124,9
	Tris-Glycine	-	-	-	-	-	-	-	-	-

to use other conditions in the future to clarify whether or not there are agglutinants in atemoya seeds.

In the pulp and the peel (Table 2), the binding activity was evidenced in different intensities. It stands out that, when obtained with the sodium acetate or tris-HCl buffers, they were able to agglutinate all three types of blood (treated or native), which highlights the possibility of having one more binding molecule and/or of aglutinin binding non-specificity. This data also points to the existence of lectins in the extracts. Lectins are molecules with great potential, as for example, being apoptosis inducing agents in tumor cells (PARK et al., 2000; RABELO et al., 2012).

The other component of the extracts are the phenolic compounds. There are authors who show that some phenolic compounds may have agglutinating action (CAROCHO, 2013; CALUETE, et al., 2015). Therefore, the possibility that the phenolic compounds of the atemoya extracts can act as agglutinins can not be ruled out. It is hoped to subject these extracts to purification steps in the future in order to characterize the phenolic compounds and the lectins present in the atemoya.

The molecules discussed in this paper, probably present in the pulp, peel and seed extracts of atemoya, have great interest both for biomedical research and for environmental science studies. Acquiring knowledge about them has the effect of increasing the added value of atemoya and its residues (peel and seed), thus strengthening the production chain around the production and use of this fruit.

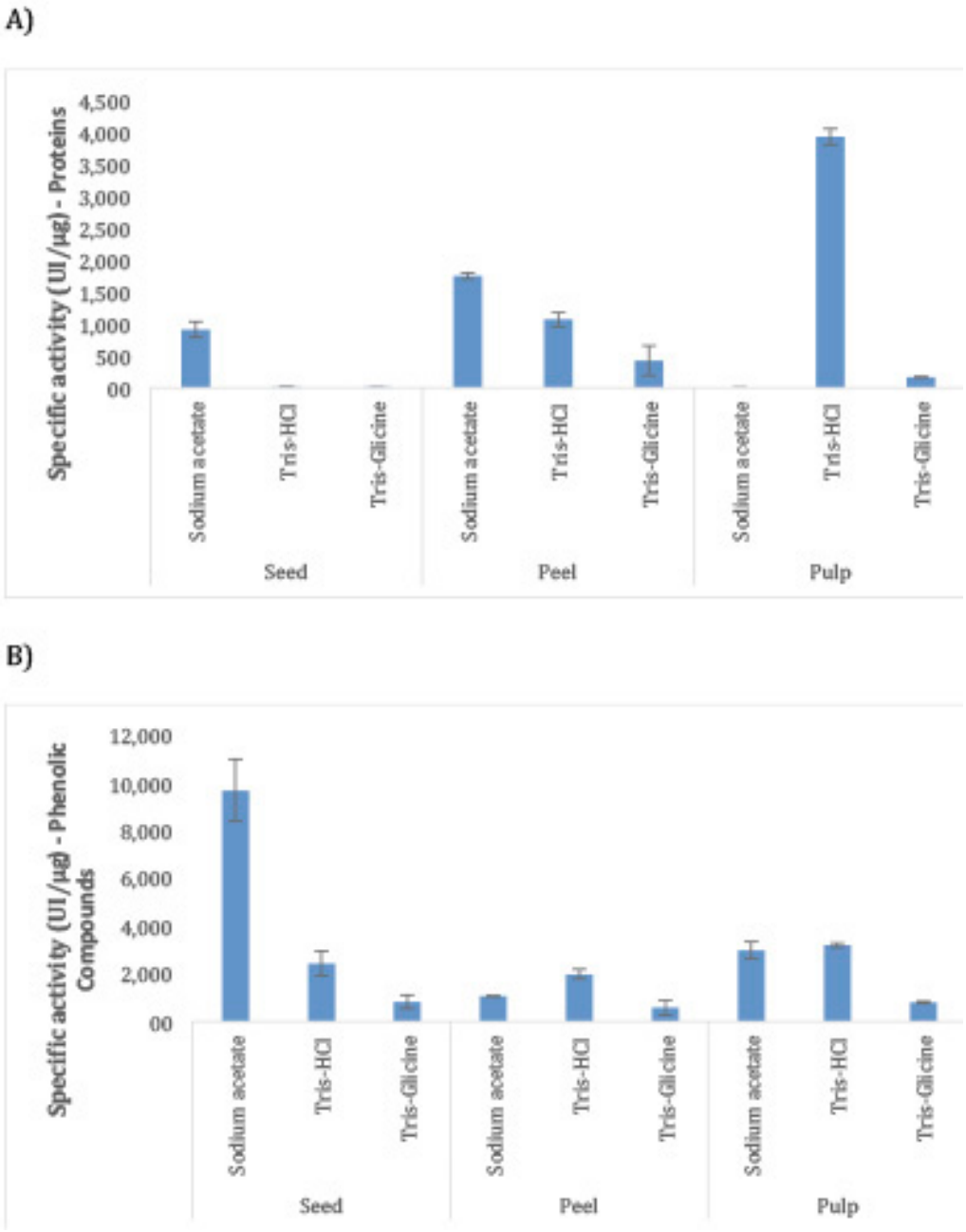

Figure 1. Specific inhibitory activity of the seed, peel and pulp extracts of the atemoya fruit for three extraction buffers, tested against trypsin, calculated based on the amount of proteins (A) and phenolic compounds (B). IU – inhibition unit

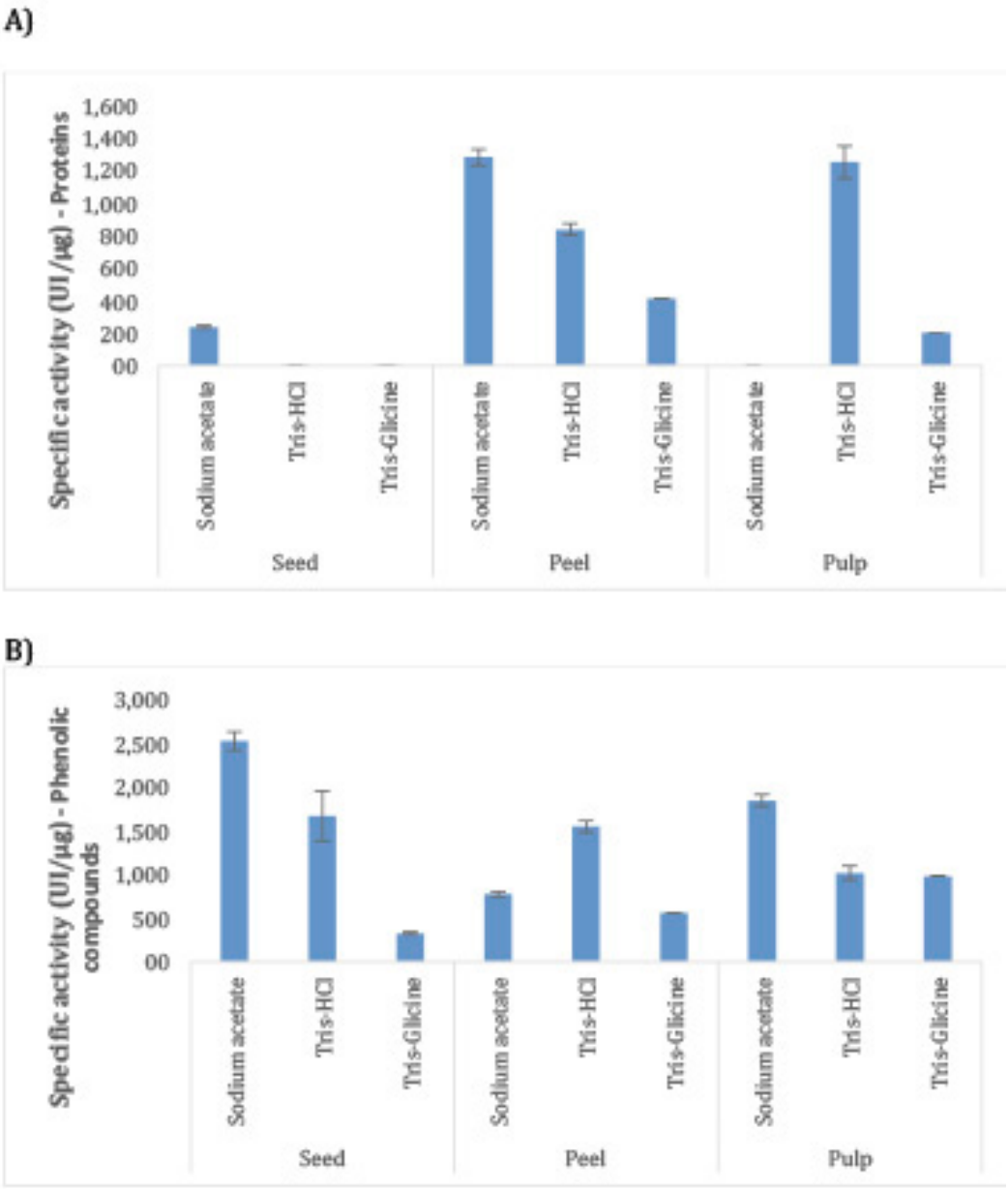

Figure 2 – Specific inhibitory activity of the seed, peel and pulp of the atemoia fruit extracts for different extraction buffers, tested against chymotrypsin, calculated on the basis of the amount of proteins (A) and phenolic compounds (B). IU - inhibition unit.

Conclusion

The atemoya, including its currently neglected parts, has several biological molecules, from which trypsin and chymotrypsin inhibitors, and haemagglutinating molecules can be highlighted.

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