Universidad Continental

FACULTAD DE INGENIERÍA

Escuela Académico Profesional de Ingeniería Ambiental

Tesis

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Para optar el Título Profesional de
Ingeniera Ambiental

Huancayo, 2022

IOP Conf. Series: Earth and Environmental Science 1072 (2022) 012010 doi:10.1088/1755-1315/1072/1/012010

Bromelain and Papain from Organic Residues of *Ananas Comosus* and *Carica Papaya*

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Abstract. In Peru there are no minimization proposals for the large amount of organic waste discarded daily by economic activities such as in the case of the El Tambo food market. In the present work we use *Ananas comosus* and *Carica papaya* peels and seeds to obtain the enzymes bromelain and papain, using the protocol [1] with the following steps: obtaining the juice, enzymatic extraction with ethanol and NaCl, protein concentration and finally the enzymatic activity test is performed by coagulation. From 1 kilogram of residues, 0.3851g with ethanol and 2.123g with NaCl of lyophilized bromelain and 0.3172g with ethanol and 1.0032g with NaCl of lyophilized papain were obtained; it was observed that the treatments with NaCl allowed obtaining a greater amount of product. The tests showed that it is possible to obtain the enzymes from the peels of *Ananas comosus* and *Carica papaya*, which poses a possible alternative for the reuse of these residues as nutritional complements for food.

1. Introduction

In recent years, environmental problems have worsened due to population growth, causing concern in almost all countries of the world. The report issued by the World Bank "A global vision of solid waste management until 2050", explains that waste worldwide will grow by 70% compared to what we generate now, this will occur as a result of rapid population and urban growth. Countries with a high economic development generate approximately 34% of the world's waste, in places like Asia and the Pacific together they produce 23% of the world's waste [2].

There are few alternatives to minimize the large amount of organic waste generated by the different economic activities; one of those with a large production of organic waste are the markets where fruits and vegetables are sold [3]. Throughout Peru, markets lack management systems for the waste they produce and become sources of contamination, ignoring their productive potential. This is even more so in the jungle cities that, due to the nature of their production, generate organic waste from these vegetables.

This research proposes the reuse of the remains of *Ananas Comosus* and *Carica papaya*, as peels and seeds, but not the pulp [4]. Therefore, we hypothesized that bromelain and papain enzymes can be obtained from peels, pips and other fruit residues. We want to obtain the mentioned enzymes from 1 kilogram of each fruit, after performing the coagulation test to see the activity of these proteins and in the future we hope to be able to include them with subsequent tests in animal feed and why not human beings as biological additives.

2. Methods and materials

The amount of waste generated per day in the department of Junín and its nine provinces is 764.4 tons per day, where the provinces that generate the most waste are Huancayo and Chanchamayo with 307 and 137 tons, respectively [5].

Currently in the province of Huancayo, district of El Tambo, there is a model market called "El Tambo", where daily wholesale and retail marketing of inputs such as fruits and vegetables, discarding a considerable amount of organic waste per day, specifically are disposed of by juice stalls and greengrocers [6].

For the acquisition of the primary inputs of the research, the juice stalls of the market were randomly selected, from where 1 kg of residues of each fruit was collected.

The processing of the collected inputs to obtain the freeze-dried enzymes in the case of both fruits, is carried out according to the protocol of [1].

2.1. Obtaining the juice of Ananas comosus and Carica papaya

The pulp is not separated from the peel, since only peels and seeds are present [7]; and for filtration a medium sieve is used instead of the filtering sieve.

2.2. Bromelain and papain enzyme extraction

Two types of treatments are used for the protein juices with 9% NaCl and 96% ethanol in quantities of 5 ml each, as shown in Table 1. A Hatch centrifuge at 4000 rpm was used for the separation of solids and liquids.

Table 1. Amount of treatment for each sample.

Type of sample	Ethanol	NaCl 9%.
Ananas comosus	5 ml	5 ml
Carica papaya	5 ml	5 ml

2.3. Protein concentration

It is placed in the oven for 48 hours at 40°C [1], is allowed to cool for 10 minutes at room temperature, in order to obtain better values of moisture, dry matter and freeze-drying product. An initial weight M1 and a final weight M2 are then obtained. With these data, the moisture percentage is determined, and then the dry matter value is found, using the formulas of [1].

$$\%humidity = \frac{M_1 - M_2}{M}.100\% \quad (1)$$

M_1 : Weight of capsule plus wet sample
M_2 : Weight of capsule plus dry sample
M: Sample weight

$$\%dry\ matter = 100 - \%humidity \quad (2)$$

2.4 Enzyme activity test: coagulation

This test determines the amount of enzymes present in a protein concentrate, this is done by the milk coagulation method (Balls and Hoover) [8] which indicates that the greater the amount of clots, the higher the enzymatic activity. For the research, it is carried out as follows:

First in the analytical balance 8.61 grams of powdered milk are weighed and diluted in 60 milliliters of lukewarm water and 5 ml of acetic acid. In 3 beakers 20 ml of the diluted milk is separated; which will have the name of test 1, test 2 and test 3.

In each dilution beaker, the protein concentrates, previously separated into 5, 10 and 15 ml, are added. [9]. Finally, place the 6 covered samples in a container with warm water and wait 45 to 55 minutes for coagulation.

3. Results

The results are obtained by determining the percentage of moisture, for which the weights of wet and dry samples are needed, as detailed in Table 2. The best results obtained were 3.6133 grams with *Carica papaya* in wet sample and with 9% NaCl and 2.123 grams in dry sample and also with 9% NaCl.

Table 2. Weights in grams of bromelain and papain of the protein concentrates.

	Capsule weight	Wet sample weight	Weight of capsule plus wet sample	Dry sample weight	Weight of capsule plus dry sample
***Anana comosus* + Etanol**	81.9089	0.5556	82.4645	0.3172	82.2261
***Anana comosus* + NaCl**	81.9089	0.506	82.4149	0.3851	82.294
***Carica papaya* + Etanol**	81.9089	1.1787	83.0876	1.0032	82.9121
***Carica papaya* + NaCl**	81.9089	3.6133	85.5222	2.123	84.0319

Equation (1) is applied to find the percentage of moisture and from this equation (2) is applied to find the percentage of dry matter, as shown in Table 3, considering that from the sample of *Carica papaya* plus Ethanol the maximum percentage of 85.11% is obtained and the minimum of the sample of *Ananas comosus* with Ethanol is 57.09%.

Table 3. Percentage of dry matter obtained based on the percentage of humidity.

	Humidity (%)	Dry matter (%)
***Ananas comosus* + Ethanol.**	42.909%	57.09%
***Ananas comosus* + NaCl**	23.893%	76.11%
***Carica papaya* + Ethanol**	14.889%	85.11%
***Carica papaya* + NaCl**	41.245%	58.76%

The lyophilized product is obtained with 40 ml of each of the protein concentrate samples, as shown in Table 4, the *Carica papaya* samples with ethanol produce 1.0032 grams and with NaCl 2.123 grams. For the samples of *Ananas comosus* the maximum value obtained is 0.3172 grams with NaCl.

Table 4. Quantity in grams of freeze-dried product obtained in samples

	Sample milliliters	Grams obtained
***Ananas comosus* + Ethanol**	40	0.3172
***Ananas comosus* + NaCl**	40	0.3851
***Carica papaya* + Ethanol**	40	1.0032
***Carica papaya* + NaCl**	40	2.123

As a result of the coagulation process presented in Table 5, *Ananas comosus* with Ethanol and *Carica papaya* with NaCl have presented clots of different amounts and sizes, contrary to the case of the 5 ml sample of *Ananas comosus* with NaCl that does not present clots, and the 5 ml and 10 ml of *Carica papaya* with Ethanol if there is presence of these clots.

Table 5. Descriptive results of the coagulation test

	Ananas comosus + Ethanol	Clot	Clot size	Remarks
Evaporated milk	5 ml	YES	Small	In small quantity
	10ml	YES	Medium and large	Dense liquid
	15 ml	YES	Small	Diluted
	Ananas comosus + NaCl	Clot	Clot size	Remarks
Evaporated milk	5 ml	NO		
	10ml	YES	Medium and large	Dense liquid
	15 ml	YES	Small	Diluted
	Carica papaya + Ethanol	Clot	Clot size	Remarks
Evaporated milk	5 ml	NO		
	10ml	NO		
	15 ml	YES	Small	Diluted
	Carica papaya + NaCl	Clot	Clot size	Remarks
Evaporated milk	5 ml	YES	Great	Dense liquid
	10ml	YES	Medium and large	Dense liquid
	15 ml	YES	Small	Diluted

4. Discussion of result

For [1] who experimented with the fruit pulp of Ananas comosus with NaCl 10% and Ethanol, obtained promising results for the use of bromelain in the food and pharmaceutical industry; in the present research the peels of *Ananas comosus* and *Carica papaya* are used, obtaining in the same way recommendable results for the application in these industries. In addition, in the same research it was found that the highest percentage, with respect to dry matter, was the bromelain concentrate with NaCl with 94.4% and Ethanol had 91.9%; being in our research a similar result for the protein concentrates, and different with NaCl with 76.11% and Ethanol with 57.09% of dry matter. It is worth mentioning that the results for papain concentrates are inverse, with Ethanol with 85.11% and NaCl with 58.76% of dry matter.

For the *Ananas comosus* pulp sample used in this study, a sample of [1], the sample with ethanol, losing more water, is more concentrated and therefore significant, even though it has less dry matter, and the same is true for this research; however, in the case of the *Carica papaya* sample, the concentrate with NaCl is the one that loses more water and therefore less dry matter..

5. Conclusions

From 1 kg of *Ananas comosus* residues and 9% NaCl, 0.3851 grams of bromelain is obtained, likewise from 1 kg of Carica papaya and 9% NaCl, the highest amount of lyophilized papain is obtained, which is 2.123 grams, therefore the treatment with 9% NaCl produces the highest amount of enzymes. Ethanol treatment was not as effective.

The research shows that enzymes can also be obtained from peels and other fruit remains. The enzyme activity test shows that we obtain the highest amount of bromelain with ethanol and the highest amount of papain with NaCl.

6. References

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