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Bio-utilization of Pineapple (*Ananas comosus*) waste for Bromelain extraction

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Abstract
This study was conducted on Bio utilization of pineapple waste for bromelain extraction. The skin has bromelain, which is powerful enzyme anti-inflammatory in the nature. It can be used to reduce swelling post surgery or an injury. It is also believed to help with digestion. It is said that the peels can fight intestinal parasites and help with constipation. Pineapple is a potential source of bioactive compounds like vitamin C, Carotenoid, Phenolic compound, Flavonoids. These compounds have antioxidant activity as well as other biological activities.

Keywords: Indian mustard, path coefficient analysis

Introduction
The bromelain is an enzyme which can be obtained from the extraction of pineapple waste which has an ability to separate proteins and amino acids by breaking of peptide bonds. Bromelain is often used in food industry as meat softening since it can facilitate digestion of proteins. The purpose of this study is to extract the bromelain enzyme from the pineapple waste. These findings also could increase the utilization of pineapple waste.
The results of this study revealed that for dried pineapple peel powder, the moisture content is (6.39%), Ash content (5.8%), Protein content (6.93%) Fat content (3.56%), Crude fiber content (2.88%), Colour values L*(48.98), a*(11.61) & b*(29.90), Water holding capacity (85%), pH (3.11%), Cooking yield of chick peas for normal cooking (116%), cooking yield of chick peas with bromelain (136%).
Thus, from this study it can be concluded that value addition of PPP can be used for production of various nutrition & delicious products and also for the bromelain extraction. Bio- utilization of this PPP & bromelain has got many advantages in the food industries as a functional and nutraceutical component.

Material and Methods
Samples of Pineapple fruit were obtained from the farms and supermarket located near Bodhan and Varni areas. As soon as the samples were collected, they were stored in refrigerator to retain the freshness of the samples till the drying process began.

Proximate analysis of DPPP
Determination of ash content
The total ash content of given food sample was determined by using (AOAC, 2016) method.

Working principle
The principle of Ashing is to burn off the organic matter and to determine the inorganic matter remained. Heating is carried out in two stages: firstly, to remove the water present and to char the sample thoroughly and finally ashing at 550 °C in a muffle furnace.

Procedure
1. Set the temperature of the muffle furnace to 600 ºC and heat crucibles for 1 hour and transfer into a desiccator, cool them to room temperature and weigh (W_1).
2. Weigh about 5 g to 10 g of sample (defatted) into the crucible of known weight (W_2).
3. Heat the crucible cautiously at low flame until the material begins to char, and continue till charring is complete.

4. Then transfer the crucible to muffle furnace which is already heated to 550 ºC-600 ºC continue Ashing until a light grey or white ash is obtained. It may require 4 – 6 hours.
5. Transfer the crucibles in to the desiccators and cool them to room temperature and weigh (W_3). Weigh immediately to prevent moisture absorption.
6. Continue Ashing, if a portion of ash is not completely Ashed.
7. Repeat the experiment for duplicate.

Analysis of Moisture Content

The moisture content was determined using a technique advised by the Association of Official Analytical Chemists (AOAC, 2016)

Procedure

In oven drying method, the sample is heated under specified conditions, and the loss of weight is used to calculate the moisture content of the sample.

- Weigh the empty Petri dish (W_1). Take 5 g of the sample and place in weighed empty Petri dish.
- Note the weight (Petri dish + sample) (W_2). Pre-heat the oven to 100C. Now place the sample in the oven at 105 ºC for 4 hours.
- Take the sample from the hot air oven and place it in desiccators for some time.
- Weigh the sample (dried sample + Petri dish) (W_3).

Estimation of Crude Fiber

Principle

The crude fiber was calculated using the AOAC method (2016).

Procedure for Crude Fiber

a. Weigh 2 g of sample
b. Addition of 200 ml of H_2SO_4
c. Boiling for 30 minutes
d. Filter the sample with hot water
e. Transfer the residue into 200 ml of NaOH & boiling fo 30 minutes
f. Filter the sample with hot water
g. Weigh the sample in crucible and keep it in hot air oven at 105 ºC for 3 hours
h. Weigh the crucible with residue and place in the muffle furnace at 550 ºC for 3 hours Cool in desiccator and weigh it.

Estimation of Crude Fat

The amount of fat was determined by using Soxhlet apparatus method given by (AOAC, 2016).

Procedure

1. We assemble the apparatus
2. We take the weight of round bottom flask
3. We fill the round bottom flask with solvent petroleum ether (50 ml)
4. We keep the thimble containing 5g of dried pineapple peel powder sample into extraction tube
5. After that we attached the extraction tube with flask containing flask
6. Attached a condenser with the extraction tube and run the water
7. We fixed the soxhlet apparatus on hot plate and heat the flask containing solvent
8. The solvent starts to evaporate and falls in the extraction tube after condensing
9. Continued this process till the fat is extracted.
10. We assemble the apparatus
11. We take the weight of round bottom flask
12. We fill the round bottom flask with solvent petroleum ether (50 ml)
13. We keep the thimble containing 5g of dried pineapple peel powder sample into extraction tube
14. After that we attached the extraction tube with flask containing flask
15. Attached a condenser with the extraction tube and run the water
16. We fixed the soxhlet apparatus on hot plate and heat the flask containing solvent
17. The solvent starts to evaporate and falls in the extraction tube after condensing
18. Continued this process till the fat is extracted.

Determination of protein content

Protein content was calculated as the sum of individual amino acid residues. The procedure for estimation of protein content is done by Kjeldhal method is as follows:

Procedure

Sample preparation

- Solid foods are to pass a 20-mesh screen.
- Sample for analysis should be homogeneous 2 ml of liquid samples or 0.2-0.3 g solid samples are sufficient for digestion.

Digestion

Distillation and Neutralization

Titration

Determination of Protease Activity

The protease activity of the bromelain extract was determined according to the method in Murachi (1976) using tyrosine as a standard. The bromelain activity was determined using casein (1.5%, w/v) as a substrate in the presence of cysteine and EDTA at 37 ºC and pH 7.0 for 10 min. After exactly 10 min, the reaction was stopped by adding 3.0 mL of 5% (w/v) TCA. Precipitated protein was removed by centrifugation at 10 000 rpm for 10 min. The absorbance of the clear supernatant was measured at 275 nm. One unit of protease activity is defined as the amount of enzyme releasing product equivalent to 1 mmol of tyrosine min/mL under the assay conditions.

Determination of water holding capacity (WHC)

Flow chart for WHC

↓

Take 20 g of sample

↓

Add required amount of water

↓

Mix it well

↓

Making a round

↓

Record the volume of water

Effect of Bromelain on cooking yield (CY) of foods

The treated samples (10 g) were steamed for 1 min and then cooled at room temperature. The cooked sample was surface-Dried with a filter paper and reweighed using an analytical balance. The cooking yield was calculated by the difference in raw and cooked weights as following:

$$\text{Cooking Yield (\%)} = \frac{\text{Weight of cooked chunks}}{\text{Weight of raw chunks}} \times 100$$

Determination of pH for DPPP

The pH value in meat product is very important as it influences other physio-chemical properties like WHC, juiciness, tenderness etc.

Flow Chart for pH

↓

Calibration of pH meter by using buffer solution

↓

Take 10 g of dried pineapple peel powder

↓

Addition of 100 ml distilled water

↓

Mixture to stand for 2-3 minutes

↓

Determination of pH of sample

Estimation of Bulk Density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed.

Procedure for Bulk Density

1. We take a measuring cylinder of 100ml capacity and fill it with dried pineapple waste for which density is to measured.
2. The measuring cylinder should be filled to its highest mark.
3. Adjust the level of dried pineapple waste sample by repeated tapping take the weight of these sample in a digital/analytical balance.
4. We repeated the readings five times
5. Final weight of that dried pineapple waste sample in 100ml measuring cylinder

Estimation of True Density

True density is the measure of the solid particles in a powder or granule. Pycnometry uses helium gas to fill the voids in the powder bed to measure the volume taken up by the powder. True density is the density of a material at 0% porosity.

Procedure

1. Take 10 grams of dried pineapple waste sample.
2. For this take a 50ml capacity measuring cylinder and fill it with toulene to a predetermined level.
3. Drop 10 grams of dried pineapple waste sample in the cylinder and note the change in volume accurately.
4. This gives the volume of 10grams of that sample.
5. Now weight these 10 grams of that sample in an analytical balance/digital balance.
6. Record the readings.

Extraction of crude Bromelain

Four extractants were used in this experiment: distilled water (DI), distilled water with 15 mM cysteine and 2 mM EDTA (DI-CE), 100 mM sodium phosphate buffer pH 7.0 (PB) and 100 mM sodium phosphate buffer pH 7.0 with 15 mM cysteine and 2 mM EDTA (PBCE).

The pineapple peel was blended in cold extractants at a 1:1 ratio (w/v) for 3 min and then filtered through a cheese cloth. The filtrate was centrifuged at 10000 rpm for 20 min at 4 ºC The obtained supernatant was referred to as 'crude bromelain extract: BE' and was used for the experiments.

Results and Discussion

The present study "Bio utilization of pineapple peel waste for bromelain extraction" was carried out in the department of Food Process Technology, Food Process Engineering & Food Safety and Quality Assurance laboratories in the College of food science and technology, Rudrur. The estimation of proximate analysis, protease activity and colour analysis is discussed.

Proximate analysis

Moisture, Ash, Fiber & Fat Content of DPPP

The results for the moisture, ash, fiber & fat percentage for the DPPP were depicted in the

Table 1: Show the sample of DPPP

Sample	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)
DPPP	6.39	5.8	2.88	3.56

Protein and Protease Activity Content

Protein content was estimated using kjeldhal method based on the analysis of amino acid residues and calculated using formulas. The protein content for DPPP were depicted in table.

Table 2: Sample Protein

Sample	Protein
DPPP	6.93%

The results of Protein and protease activity for bromelain were depicted in table

Table 3: Protein and Protease activity

Sample	Protein (%)	Protease activity (%)
Bromelain	48.1%	2.27%

Water holding capacity, cooking yield & pH content

The results of water holding capacity, cooking yield for chick pea by adding Bromelain and normal cooking & pH were depicted in table

Table 4: Normal cooking & pH were depicted

Sample	WHC (%)	Cooking Quality (%)		pH (%)
DPPP	85	116	136	3.11

Colour Analysis

The Hunter lab Colorimeter was used to measure colour of edible spoons. The final values are given in L*, a* and b*. The L*a*b* values produced by the analysis were used to measure chroma, hue and ΔE. ΔE is the difference between

the displayed color and the original color standard of the input content. The results of colour analysis for DPPP is depicted in the Table.

Table 5: The results of colour analysis for DPPP is depicted

Sample	L*	a*	b*
DPPP	48.98	11.61	29.90

Conclusion

The present study on "Bio utilization of pineapple (Ananas comosus) waste for bromelain extraction".

Bromelain is often used in the food industries as meat softening since it can facilitate digestion of proteins.

From the above study it could be concluded that the enzyme present in the crude extract looses it activity if it is stored for longer duration. Storage upto 1 or 2 days is possible under cold storage condition (4 ºC).

It is recommended that extract is to be immediately taken for further processing to preserve its activity.

This study also showed that bromelain content was high in crown part of the fruit and then peel and core.

These findings could also increases the utilization of pineapple waste. It can be recommended in the house hold level and it can be a commercial in business as a ready-to-use product. Thus, from this study it can be concluded that value addition of PPP can be used for production of various nutrition & delicious products and also for the bromelain extraction.

Bio- utilization of this PPP & bromelain has got many advantages in the food industries as a functional and nutraceutical component.

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