IN VITRO STUDY OF BROMELAIN ACTIVITY IN ARTIFICIAL STOMACH JUICE AND BLOOD

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## Introduction

### Bromelain

- Found in pineapple (*Ananas comosus*), *Bromeliaceae* family.
- Mixture of cysteine proteases and non proteases.
- Cysteine proteases: stem bromelain, fruit bromelain, ananain, comosain (Rowan et al., 1990).
- Catalytic activity- sulfhydryl group (SH) at the active site.
Introduction

Bromelain

- Safe, therapeutic drugs for oral systemic treatment of inflammatory, blood-coagulation-related and malignant diseases (Maurer, 2001).
  - Rodents: LD50 of bromelain is >10g/kg (Kelly, 1996).
  - Human: 12g/day of bromelain without major side effects (Castell et al., 1997).

- Pharmacological actions:
  - Antiedematous, antithrombotic, fibrinolytic (Moss et al., 1963; Pirotta and Giuli-Morghen 1978).
  - Anti-inflammation, promotion of absorption of antibiotic drugs, inhibition of tumor cells growth (Desser et al., 1994; Tinozzi and Venegoni, 1978; Grabowska et al., 1997).
Introduction

Absorption of bromelain

- Pineapple fruit provides health beneficial effects due to presence of bromelain.

- Is bromelain absorbed following oral application?
  - Rabbits: increase plasmin serum level and prolong prothrombin and antithrombin times (Smyth et al., 1962).
  - 40% of labelled bromelain is absorbed from intestine in high molecular form (Siefert et al., 1979).
  - Bromelain is detected and retains its proteolytically activity in plasma (by immunoassay and proteolysis of model substrates) (Castell et al., 1997).
Introduction

Absorption of bromelain
Introduction

Absorption of bromelain

- In blood, bromelain is complexed with antiproteinases (α-2-macroglobulin and α-1-antichymotrypsin) (Castell et al., 1997).

- Clinical study:
  - Oral administration of 400mg-800mg tablets, 4 times daily up to 4 days.
  - Specific activity of bromelain in plasma is proportionally correlated with the respective dosage (Mai et al., 1996).

- Enzymes are absorbed from gastrointestinal tract in a functionally intact form.
- Relatively high doses is well tolerated.
  (Maurer, 2001)
Introduction

- Choose bromelain in pineapple juice rather that purified bromelain.
  - Natural form, ease of processing, easy consume
- Consume sufficient pineapple juice → considerable amount of bromelain absorbed by body → beneficial to health.
- However, bromelain are hydrolyzed by gastric acid and proteinases after oral administration.

In this study:
- How do conditions of body fluid (stomach juice and blood) influence the bromelain concentration and activity after oral consumption of pineapple juice containing bromelain?
Objective

- To investigate the bromelain activity in pineapple juice after being exposed to artificial stomach juice and blood.
Methodology

Measurement of clotting time and enzymatic activity

- Milk clot unit (MCU) assay (Balls et al., 1937)
  - Enzymatic activity is related to time required to clot substrate in milk.
  - Standard curve:
    - clotting time (s) versus standard bromelain concentration (mg/ml)
  - Sample activity
    - Milk Clotting Unit (U/ml) = Enzyme concentration (mg/ml)/ clotting time (s)

Pineapple juice processing

- Collect cores of Gandul pineapple
- Juicing, centrifuge
- Measure clotting time of clarified juice
Analyze bromelain concentration in artificial stomach juice

Artificial stomach juice preparation
- Contain pepsin (proteases), acidic solution (HCl, water).
- Measure clotting time (negative control).

Pineapple juice with different bromelain concentration in artificial stomach juice
- Dilute pineapple juice into different concentration.
- Diluted juice + stomach juice, 37°C, 4 hours.
- Measure clotting time.

Pineapple juice in artificial stomach juice for 8 hours
- Pineapple juice + stomach juice, 37°C, 8 hours.
- Measure clotting time every hour interval.
- Determine bromelain concentration for every hour sample.
Analyze bromelain concentration in artificial blood

Artificial blood preparation
- Fetal bovine serum (FBS)
- Measure clotting time (negative control).

Pineapple juice in artificial blood for 8 hours
- Pineapple juice + serum, 37°C, 8 hours
- Measure clotting time every hour interval.
- Determine bromelain concentration for every hour sample.
Clotting time decreased with increasing bromelain concentration.

High amount of bromelain could coagulate casein in milk at faster rate as compared to small concentration.
Bromelain concentration decreased gradually from beginning (9.61mg/ml) to fourth hour (3.66mg/ml). Concentration remained constant after 4 hours reaction.

- 61.91% of bromelain being hydrolyzed in first 4 hours.
## Result and Discussion

| Bromelain concentration in pineapple juice within 8 hours reaction with artificial stomach juice |

- Reduction in activity (from beginning to $4^{th}$ hour)
  - due to inactivation and degradation of bromelain by gastric enzymes (pepsin) and low pH (acidic phase solution).

- Remain constant in activity (from $4^{th}$ to $8^{th}$ hour)
  - Bromelain of 3.66mg/ml could survive the reaction against gastric inactivation and became stable in artificial stomach juice condition.
  - May due to pepsin-bromelain reaction in acidic phase solution reached saturation point.

- Bromelain of 3.66mg/ml could be passed to blood for further reaction.
**Result and Discussion**

**Bromelain concentration in pineapple juice within 8 hours reaction with artificial blood**

- Bromelain concentration decreased gradually from beginning (3.66mg/ml) to fourth hour (2.44mg/ml). Concentration remained constant after 4 hours reaction.
- 13% loss in bromelain concentration in first 4 hours.
Result and Discussion

Bromelain concentration in pineapple juice within 8 hours reaction with artificial blood

- Reduction in activity (from beginning to 4th hour)
  - due to degradation of bromelain by serum globulins or other components in blood.

- Bromelain of 2.44mg/ml is predicted to be absorbed by body for further reaction.
Result and Discussion

Bromelain concentration and activity before and after 4 hours exposure to artificial stomach juice and blood.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bromelain concentration (mg/ml)</th>
<th>Bromelain (mg) in 250ml pineapple juice</th>
<th>% reduction in bromelain concentration</th>
<th>Residual bromelain activity (MCU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting</td>
<td>9.61</td>
<td>2402.50</td>
<td>0.00</td>
<td>600.63</td>
</tr>
<tr>
<td>After 4 hours exposure to artificial stomach juice</td>
<td>3.66</td>
<td>915.00</td>
<td>61.91</td>
<td>228.75</td>
</tr>
<tr>
<td>After 4 hours exposure to artificial blood</td>
<td>2.44</td>
<td>610.00</td>
<td>74.61</td>
<td>152.5</td>
</tr>
</tbody>
</table>
Conclusion

- Bromelain concentration and activity in artificial stomach juice and blood was determined. Obtain a better understanding of bromelain activity in human body.

- Bromelain concentration of 3.66mg/ml became stable after 4 hours reaction in artificial stomach juice.

- Bromelain concentration of 2.44mg/ml remained after 4 hours reaction in artificial blood. This concentration of bromelain is predicted to be absorbed by body for further reaction.

- If 250ml pineapple juice is consumed, the estimated bromelain activity absorbed by body remained 152.5MCU, about 74.61% activity loss as compared to that of original pineapple juice.
References

Thank you