

Determination of Inhibitory Activity of the Bioactive peptides, derived from PINEAPPLE (*Ananas comosus* L.) fruit and juice, towards Angiotensin-converting enzyme

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Presented by:

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INTRODUCTION

❖ PINEAPPLE and BROMELAIN

❖ BIOACTIVE PEPTIDES

❖ ANGIOTENSION CONVERTING ENZYME



INTRODUCTION

“Pineapple”

- ✓ One of the most important fruits in the world
- ✓ Also processed as canned juices, chunks, and slices which produce wastes brought by the unconsumed parts such as the peel and stem.
- ✓ 80% moisture and 20% sugars





INTRODUCTION

“Pineapple”

- ✓ The nutritional facts of pineapple based on the Malaysian Food Nutrition Composition (1982)
- ✓ Major protein group (cysteine proteases) is the *bromelain*

COMPONENTS	100 gram/edible part
<i>Energy</i>	45 cal
<i>Moisture</i>	87.8 g
<i>Protein</i>	<i>0.5 g</i>
<i>Fat</i>	0.1 g
<i>Carbohydrate</i>	10.6 g
<i>Fiber</i>	0.6 g
<i>Ash</i>	0.4 g
<i>Beta-carotene</i>	270 ug
<i>Vitamin B1</i>	0.7 mg
<i>Niacin</i>	0.1 mg
<i>Vitamic C</i>	15.2 mg



INTRODUCTION

“Bromelain”

- ❖ **BROMELAIN** is a crude aqueous extract from the stems and immature fruits of pineapples, constituting an unusually complex mixture of different thiol endopeptidases.
- ✓ Stem bromelain is the major protease present in extracts of pineapple stem
- ✓ Fruit bromelain is the major enzyme fraction present in the juice of the pineapple fruit (Kelly, 1996).



INTRODUCTION

“Bromelain”

❖ BROMELAIN

- ✓ Safe, therapeutic drugs for oral systemic treatment of inflammatory, blood-coagulation-related and malignant diseases (Maurer, 2001).

- ✓ 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

“Bioactive Peptides”

- ✓ inactive within the sequence of the native protein, and could be released through enzymatic hydrolysis *in vivo* and *in vitro* (Correa *et al.* 2011)
- ✓ Possess antihypertensive, anti-inflammatory, and anticancer properties
- ✓ Other types of these peptides were isolated from egg, cereals, and soybean (Wang & Gonzales, 2005)

INTRODUCTION



“Angiotensin-converting enzyme”

- ✓ One of the major components in the renin-angiotensin system
- ✓ Facilitates conversion of angiotensin I to angiotensin II (vasoconstrictor)
- ✓ *Inhibition of ACE* is considered to be an important therapeutic approach for controlling hypertension.
- ✓ ACE inhibitors (ACEI) are originally synthesized in pit viper venom.



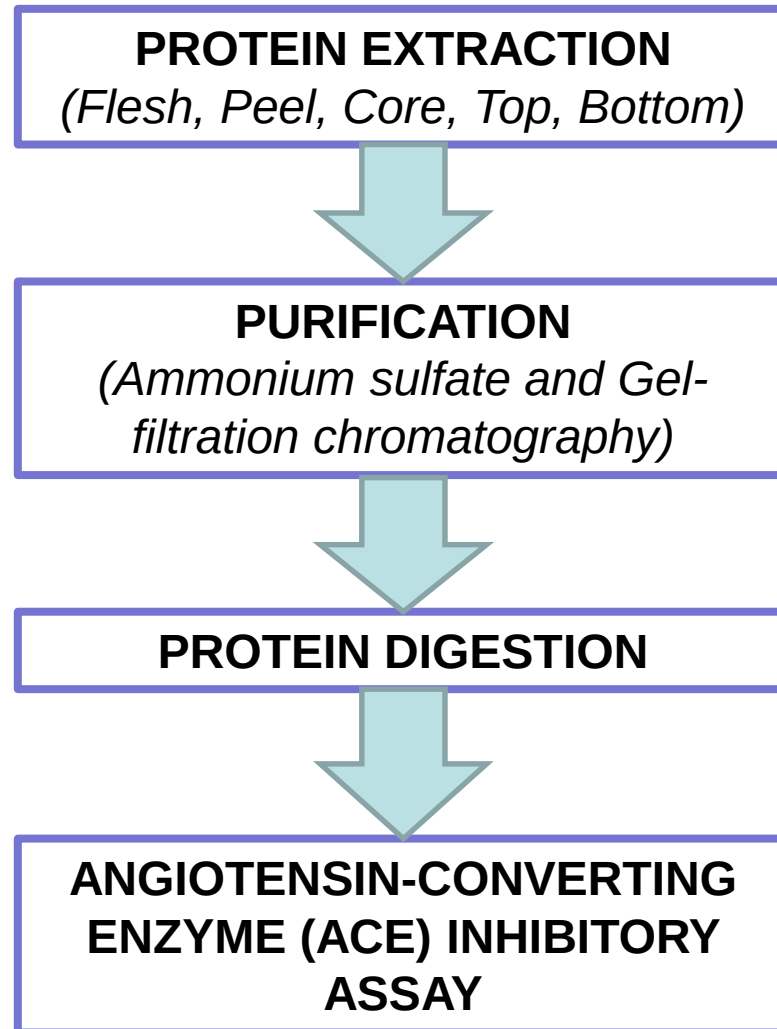
OBJECTIVES

The objectives of the study are:

- ✓ to generate protein/enzyme/peptide/free amino acids profiles of different portions and ripeness levels of the pineapple fruit;
- ✓ to compare the protein concentrations of two major brands of pineapple juice and its variants, and;
- ✓ to determine the inhibitory activity of the hydrolyzed purified bromelain towards ACE which leads to the decrease of blood pressure.



METHODOLOGY



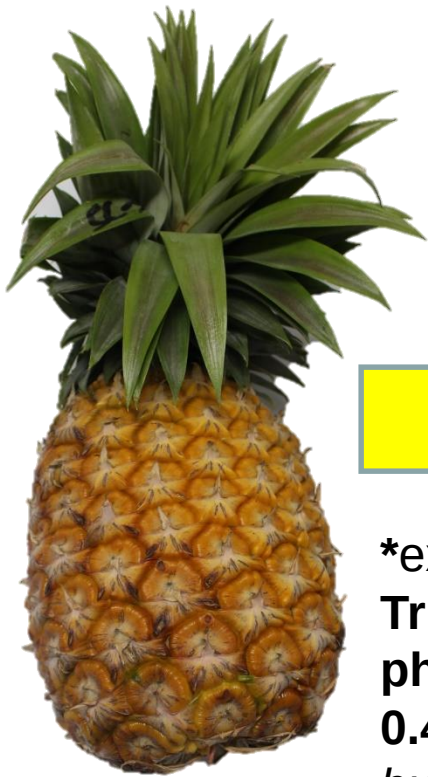


RESULTS

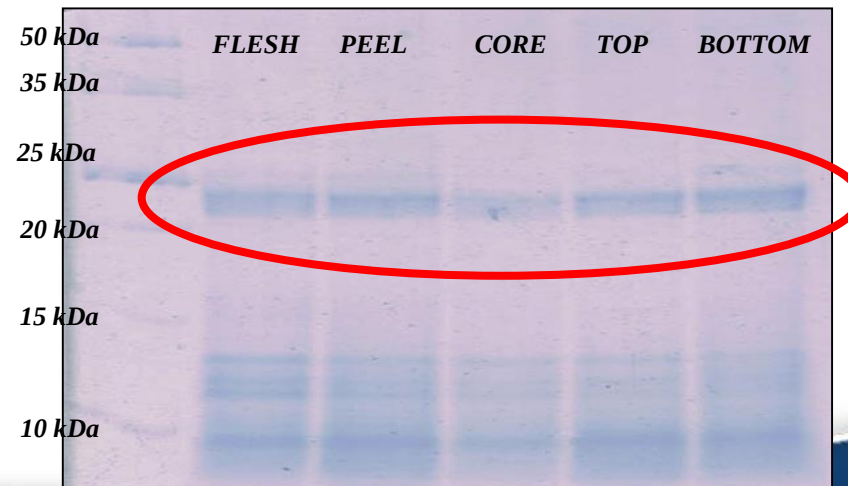
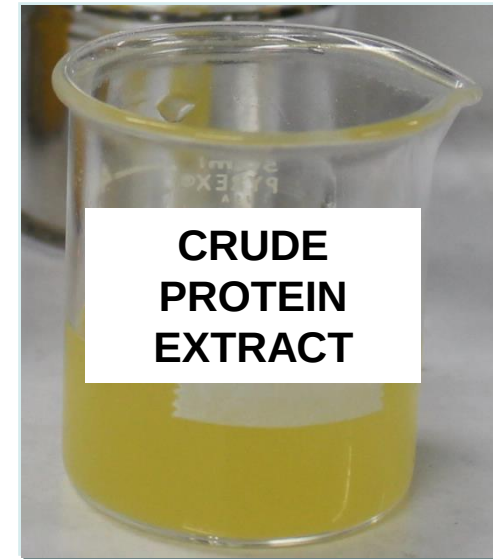


RESULTS

Isolation and Purification of Bromelain



***extracted with 0.05 M Tris-HCl, 34mM sodium phosphate buffer and 0.4 M NaCl (optimized buffer for maximum yield of desired protein)**





RESULTS

Crude protein concentration of MD2 variety
(Bradford assay)

PARTS	Concentration, mg/mL		
	MD2, Green skin	MD2, 50% yellowing of skin	MD, 100% yellowing of skin
Pulp	0.819 ± 0.030 ^a	0.751 ± 0.011 ^a	0.516 ± 0.014 ^a
Peel	0.765 ± 0.129 ^a	0.866 ± 0.047 ^a	0.374 ± 0.011 ^a
Core	0.537 ± 0.039 ^{a,b}	0.461 ± 0.017 ^{a,c}	0.384 ± 0.020 ^{a,b,c}
Top	0.962 ± 0.034 ^a	0.800 ± 0.131 ^{a,b}	0.379 ± 0.056 ^b
Bottom	0.997 ± 0.122 ^a	0.739 ± 0.002 ^{a,b}	0.419 ± 0.052 ^a

*Values are means of 2 trials and expressed as mean ± SD

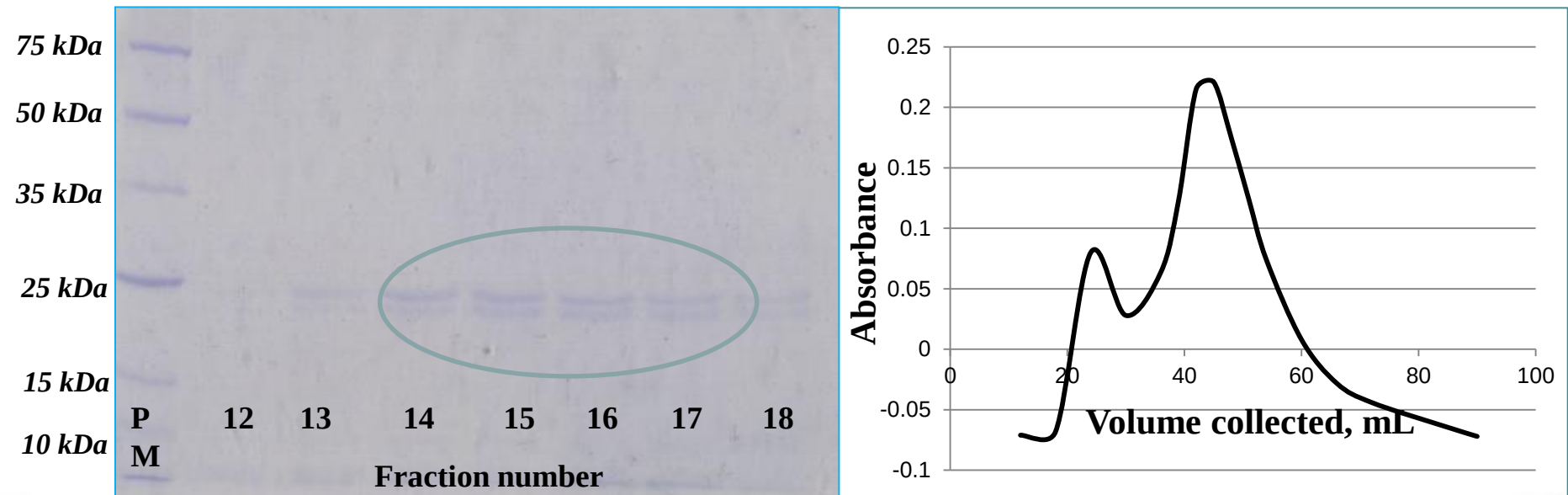
¹Mean of 2 determinations, means in the columns followed by the same letters are not significantly different at 5% level of significance using Ordinary one-way ANOVA followed by Tukey's test.



RESULTS

Purification and Digestion

- The ammonium sulfate precipitates were purified using gel-filtration chromatography with Sephacryl S-100 as resin.
- Bromelain elutes between 39-51 mL of the fractionation.





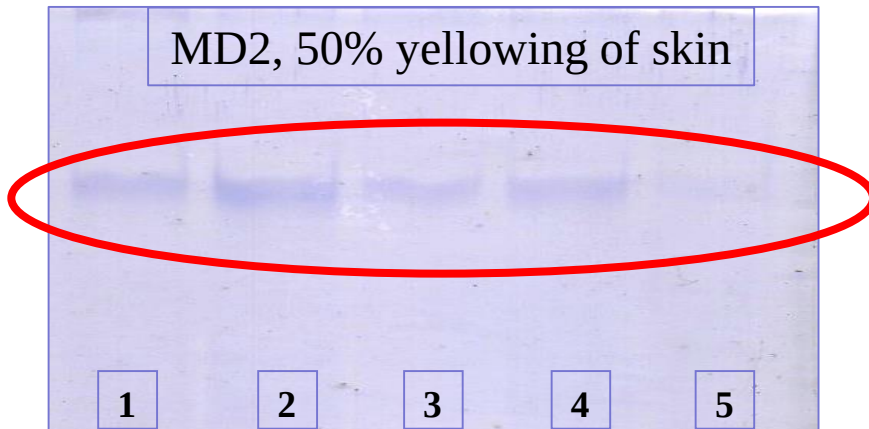
RESULTS

Purification and Digestion

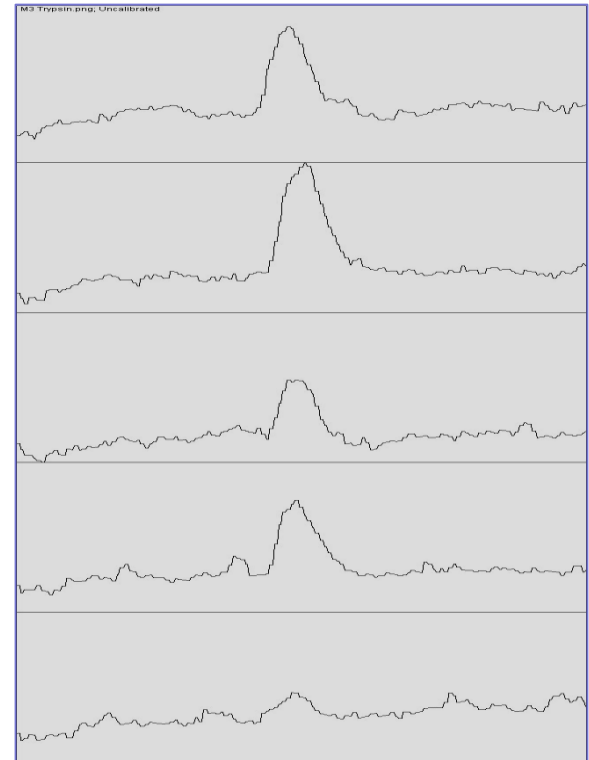
- ✓ The purified bromelain was hydrolyzed for 24 hours using digestive enzymes.

Bromelain digest

MD2, 50% yellowing of skin



Legend: Lanes: 1 – 5min; 2 – 20min;
3 – 30min; 4 – 60min; 5 – 24hr)





RESULTS

Bradford Assay

<i>VARIETY</i>	<i>Protein Concentration, mg/mL</i>
MD2, Green skin	0.071 ± 0.001^a
MD2, 50% yellowing of skin	0.218 ± 0.175^b
MD2, 100% yellowing of skin	0.056 ± 0.009^a



RESULTS

ACE Inhibition Assay

- ✓ Spectrophotometric assay using the method of Cushman and Cheung (1971) with some modifications
- ✓ Hippuryl-Histidyl-Leucine (HHL) was used as the substrate

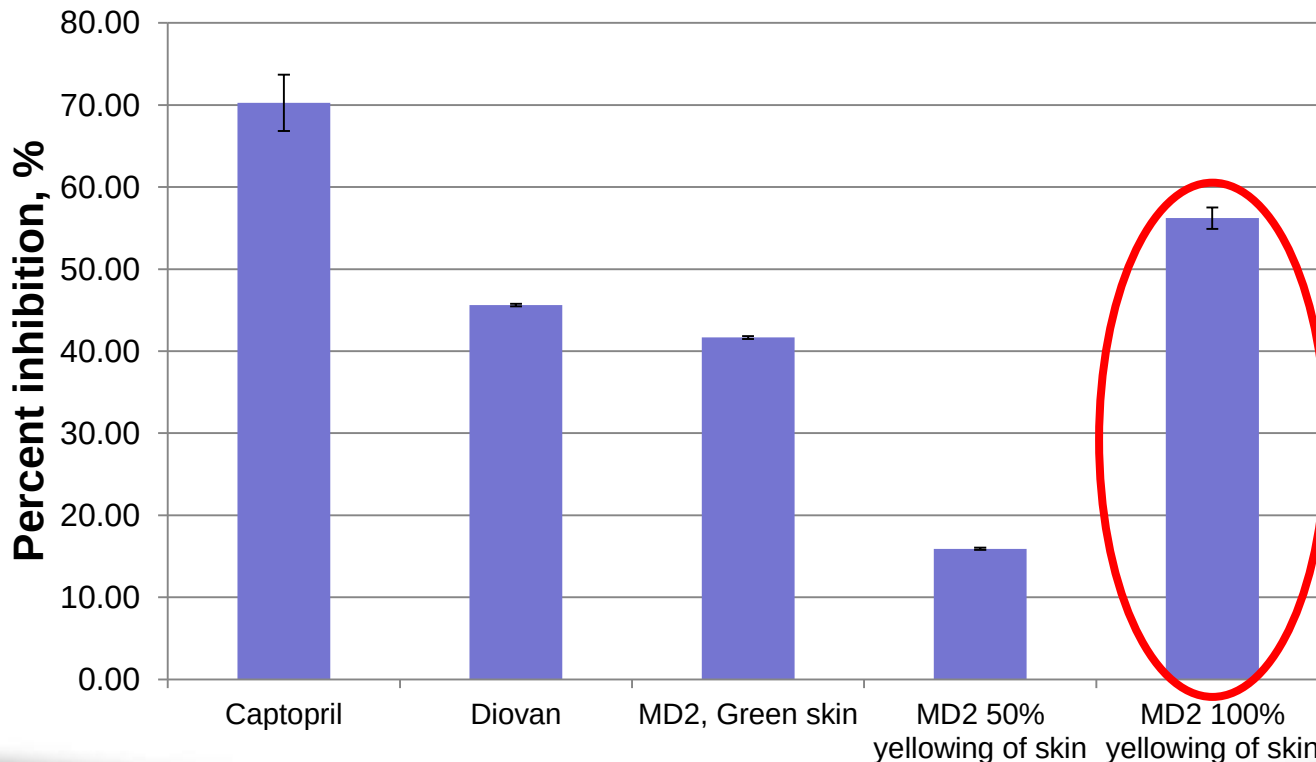
Crude ACE extract





RESULTS

- Percent ACE inhibition activity (*Flesh* part) of **bromelain** digests (mixture of enzymes) in MD2 pineapple variety





RESULTS

Protein Concentration of Canned Pineapple Products

Product Name	Concentration, mg/mL ¹	
	Brand A	Brand B
Pineapple Juice	0.095 ± 0.005 ^a	0.459 ± 0.007 ^e
Pineapple Orange	0.086 ± 0.005 ^a	0.178 ± 0.012 ^{b,c}
Four Seasons	0.069 ± 0.014 ^a	0.258 ± 0.013 ^d
Fortified Pineapple Juice 1	0.161 ± 0.016 ^b	
Fortified Pineapple Juice 2	0.211 ± 0.031 ^c	

*Values are means of 2 trials and expressed as mean ± SD

¹Mean of 2 determinations, means in the columns and rows followed by the same letters are not significantly different at 5% level of significance using Ordinary one-way ANOVA followed by Tukey's test.



RESULTS

ACE Inhibition Activity of Canned Pineapple Products

Product Name	Inhibition activity, % ¹	
Captopril	70.27 ± 3.43^g	
Diovan	45.63 ± 0.15^{b,d}	
	Brand A	Brand B
Pineapple Juice	53.55 ± 0.18 ^{a,b}	38.40 ± 1.17 ^{c,d}
Pineapple Orange	36.75 ± 0.23 ^{c,d}	25.81 ± 0.29 ^f
Four Seasons	15.65 ± 0.41 ^e	34.19 ± 1.40 ^{c,f}
Fortified Pineapple Juice 1	57.72 ± 1.64 ^a	
Fortified Pineapple Juice 2	15.31 ± 4.49 ^e	

¹Mean of 2 determinations, means in the columns and rows followed by the same letters are not significantly different at 5% level of significance using Ordinary one-way ANOVA followed by Tukey's test.



POSSIBLE PATTERNS FOR ACE INHIBITORS

SOURCE	AMINO ACID SEQUENCE	REFERENCES
<i>Seaweed</i>	1. Ala-Ile-Tyr-Lys 2. Tyr-Lys-Tyr-Tyr 3. Ls-Phe-Tyr-Gly 4. Tyr-Asn-Lys-Leu	Suetsuna et al., 200
<i>Bean</i>	1. Asp-Leu-Pro 2. Asp-Gly	Wu et al., 2002
<i>Rice</i>	Thr-Gln-Val-Phe	Li et al., 2007



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**THANK YOU FOR
LISTENING!**