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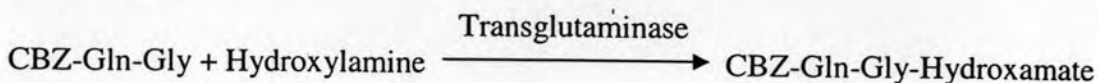


APPENDICES

APPENDIX A

ENZYMATIC ASSAY OF TRANSGLUTAMINASE

PRINCIPLE:



Abbreviations used: CBZ = N-Carbobenzoxy

CONDITIONS: T = 37°C, pH = 6.0, A525 nm, Light path = 1 cm

REAGENTS: (Sigma)

- A 1000 mM Tris Buffer, pH 6.0 at 37°C
- B CBZ-Glutaminylglycine (CBZ-Gln-Gly) (Sigma Prod. No. C-6154)
- C 200mM Hydroxylamine with 20 mM Glutathione Reduced Form Solution (HA/Glut) were prepared fresh using Hydroxylamine Hydrochloride (Sigma Prod. No. H-9876, and Glutathione, Reduced Form, Sigma Prod. No. G-4251)
- D 1000 mM Calcium Chloride Solution (CaCl₂)
- E 10 mM L-Glutamic Acid γ -Monohydroxamate Solution (std)
- F 12% (v/v) Trichloroacetic Acid Solution
- G 5% (w/v) Ferric Chloride Solution
- H 100 m M Hydrochloric Acid
- I Transglutaminase Enzyme Solution
(Immediately before use, prepare a solution containing 2 units/mL of Transglutaminase in cold deionized water.)

PROCEDURE:

The reaction cocktail was prepared by combining the following reagents into a suitable container:

Reagent B (CBZ-Gln-Gly)	120 mg
Reagent A (Buffer)	2.00 mL
Reagent C (HA/Glut)	5.00 mL

Then reagent D (CaCl_2 , 0.05 mL) was added and the solution was mixed by inversion, and adjusted to pH 6.0 at 37°C with 100 mM NaOH. Then deionized water was added to make a final volume of 10.0 mL. The assay was performed by pipetting the following reagents in to suitable containers:

	Test			Std.
	<u>Test</u>	<u>Blank</u>	<u>Std.</u>	<u>Blank</u>
Reaction Cocktail	0.20	-	-	-
Equilibrate to 37°C . Then add:				
Reagent I (Enzyme Solution)	0.03	-	-	-
Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:				
Deionized Water	-	-	-	0.10
Reaction Cocktail	-	0.20	-	-
Reagent E (Std)	-	-	0.10	-
Reagent F (TCA)	0.50	0.50	0.05	0.50
Reagent I (Enzyme Solution)	-	0.03	-	-
Mix by inversion. Then add:				
Reagent G (FeCl_3)	0.50	0.50	0.50	0.50

The resulting solutions were mixed by inversion, centrifuged for 5 minutes, and the solutions were transferred to suitable cuvettes. Then $A_{525\text{nm}}$ was recorded for the Standard, Test and Blank samples. The activity of TGase was calculated as shown below,

$$E_{\text{mM}}^1 = (A_{525\text{nm}} \text{ Std.} - A_{525\text{nm}} \text{ Std. Blank}) \quad (1.1)$$

$$\text{Units/mg enzyme} = \frac{(A_{525\text{nm}} \text{ Test} - A_{525\text{nm}} \text{ Test Blank}) \quad (1.23)}{(E_{\text{mM}}) (\text{mg enzyme/RM}) (10)}$$

1.1 = Volume of Standard (in milliliters)

1.23 = Volume of Color Mix

RM = Reaction Mix (Volume = 0.23 mL)

10 = Time of reaction in minutes

Unit Definition:

One unit is defined as the amount of enzyme which will catalyze the formation of 1.0 μ mole of hydroxamate per minute from N α -CBZ-Glutaminyglycine and hydroxylamine at pH 6.0 at 37°C. (L-Glutamic acid γ -monohydroxamate is the standard.)

In a 0.23 mL reaction mix, the final concentrations are 174 mM Tris, 31 mM CBZ-glutaminyglycine, 87 mM hydroxylamine, 8.7 mM glutathione, reduced form, 4 mM calcium chloride and 0.06 unit transglutaminase.

APPENDIX B

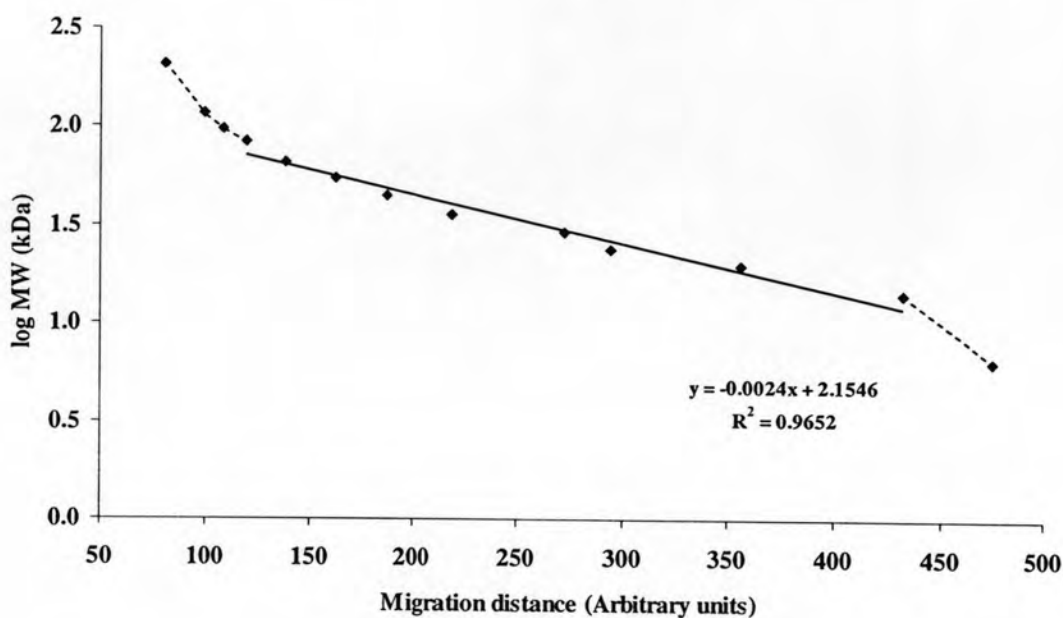


Figure B1 Calibration curve of standard marker proteins on SDS-PAGE at 12.5% gel. Data was taken from the gel in Figure 4.1.

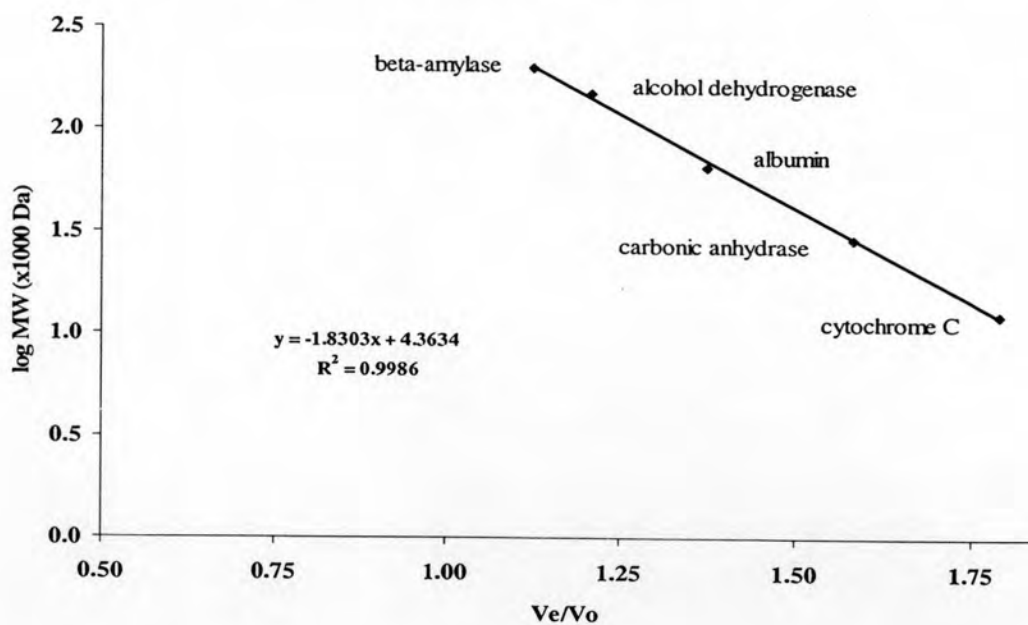


Figure B2 Gel filtration calibration curve from a column (16/70 cm) of Sephacryl S-200 HR, flow rate 0.5 mL/min, fraction volume 2.5 mL. The elution was performed by 0.05 M phosphate buffer, pH 7.0, containing 0.15 M NaCl in refrigerator (4°C).

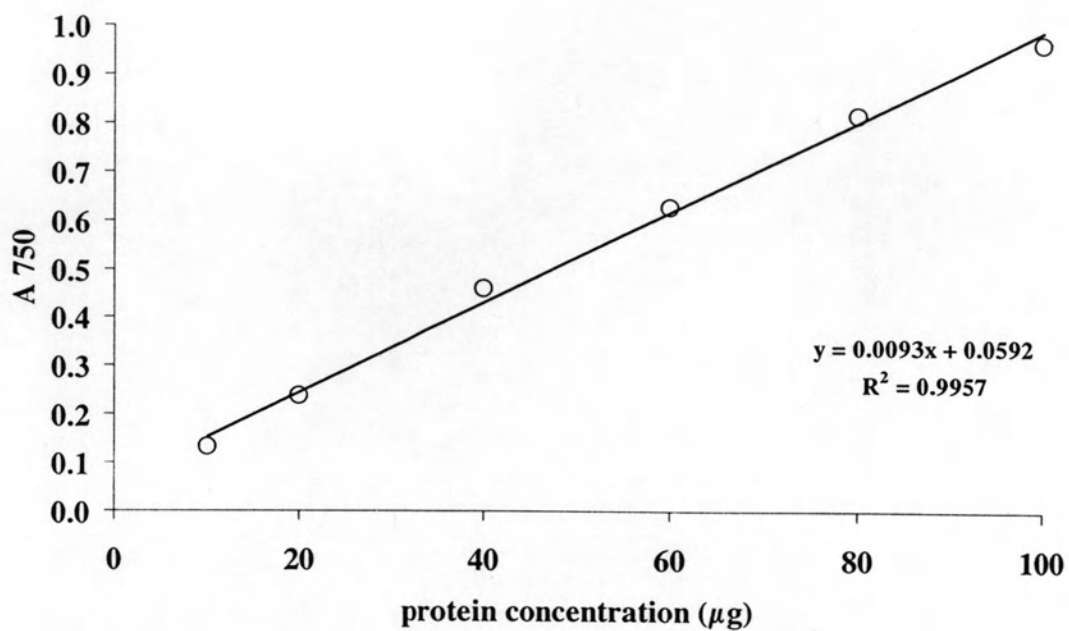


Figure B3 The Lowry calibration graph using bovine serum albumin as reference protein.

APPENDIX C

Table C1 Fraction of TBSP on gel filtration

Peak No.	Fraction No.	V_e	V_e/V_o	log MW (kDa)	MW (kDa)
1	25	62.5	1.04	2.4568	286
<u>Molecular weight and peak data for standards</u>					
Blue dextran	24	60.0(V_o)	-	-	2000
Beta-amylase	27	67.5	1.1250	2.3010	200
Alcohol dehydrogenase	29	72.5	1.2083	2.1461	150
Albumin	33	82.5	1.3750	1.8195	66
Carbonic anhydrase	38	95.0	1.5833	1.4624	29
Cytochrome C	43	107.5	1.7917	1.0934	12.4

Note: Gel filtration was performed on a Sephacryl S-200 HR (16/70) flow rate 0.5 ml/min, fraction volume = 2.5 ml/tube. The column 16/70, $V_o = 60$. The elution was performed by 0.05 M phosphate buffer, pH 7.0, containing 0.15 M NaCl in refrigerator (4°C). See section 3.1.8 for further details.

Table C2 pH profiles of solubility of TBSP and trypsin modified TBSP

pH	Sample solubility (Relative Scale)		
	No trypsin	+trypsin (1h)	+trypsin (24h)
2	0.8	2.1	0.6
3	0.5	0.8	0.1
4	0.3	0.6	0.1
5	0.5	0.8	0.2
6	0.6	1.1	0.5
7*	1.0*	1.3	0.6
8	0.9	1.5	0.6
9	0.8	1.8	0.6

Note: * reference sample relative solubility value = 1.0 (pH 7). All samples were incubated at 55°C.

APPENDIX D

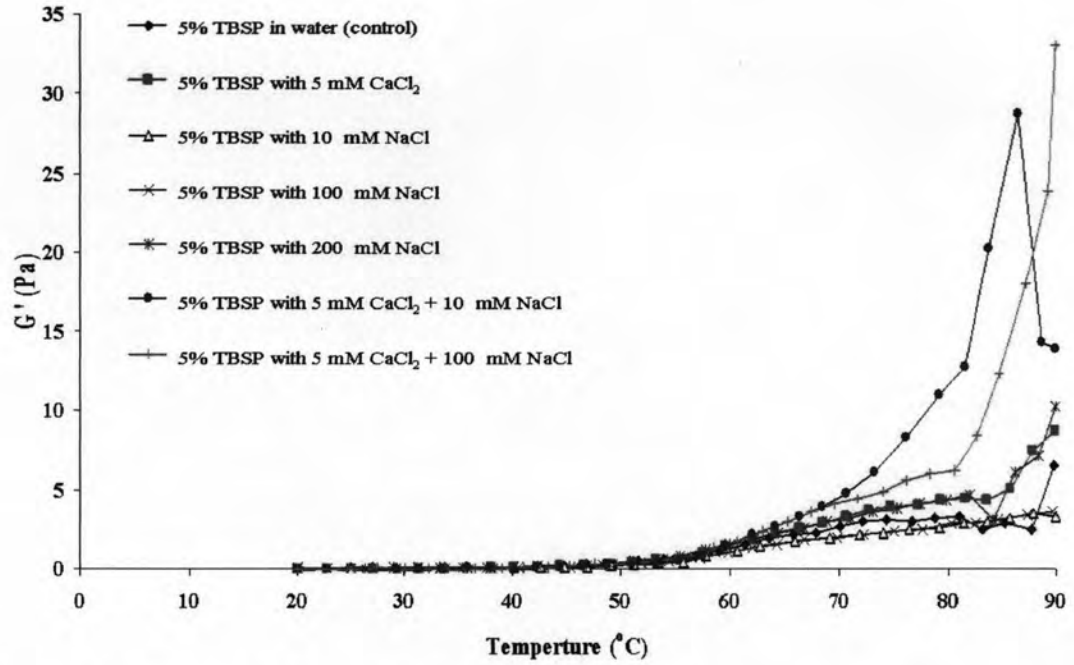


Figure D1 Effect of CaCl₂ and NaCl on G' of 5% TBSP.

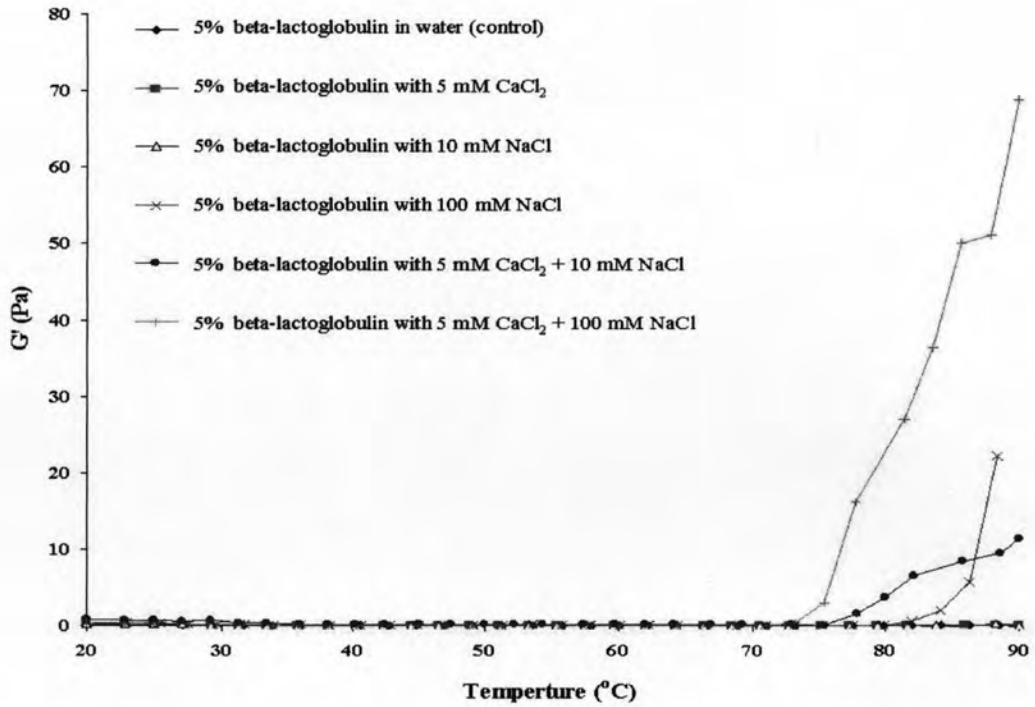


Figure D2 Effect of CaCl₂ and NaCl on G' of 5% beta-lactoglobulin.

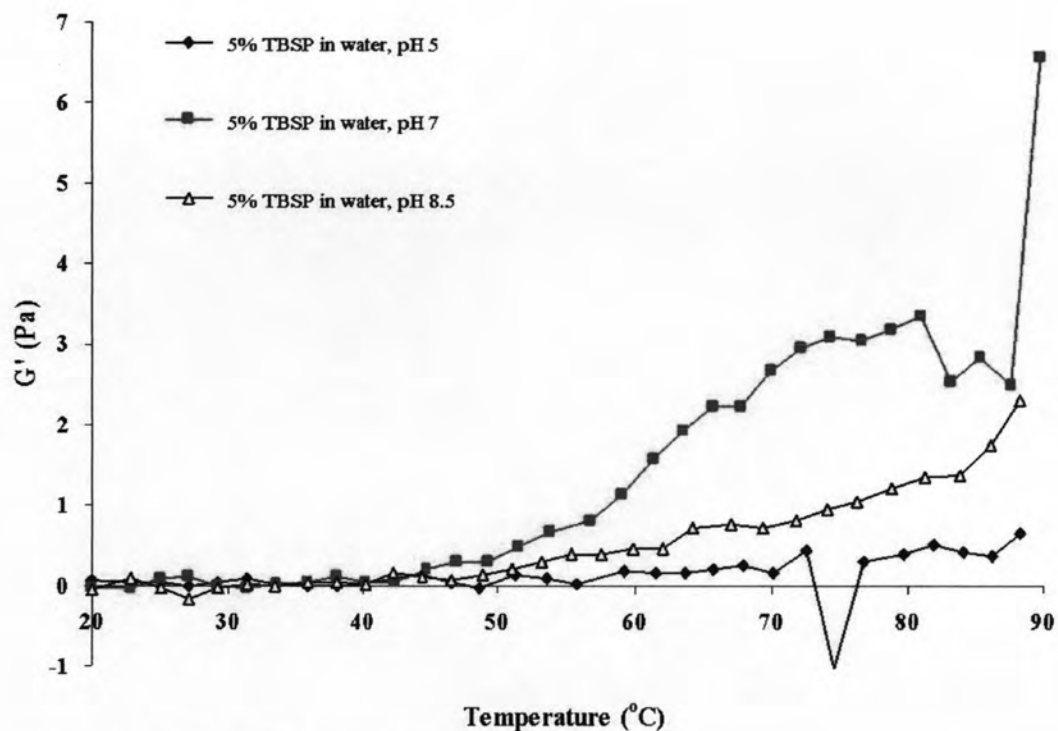


Figure D3 Effect of pH on G' of 5% TBSP.

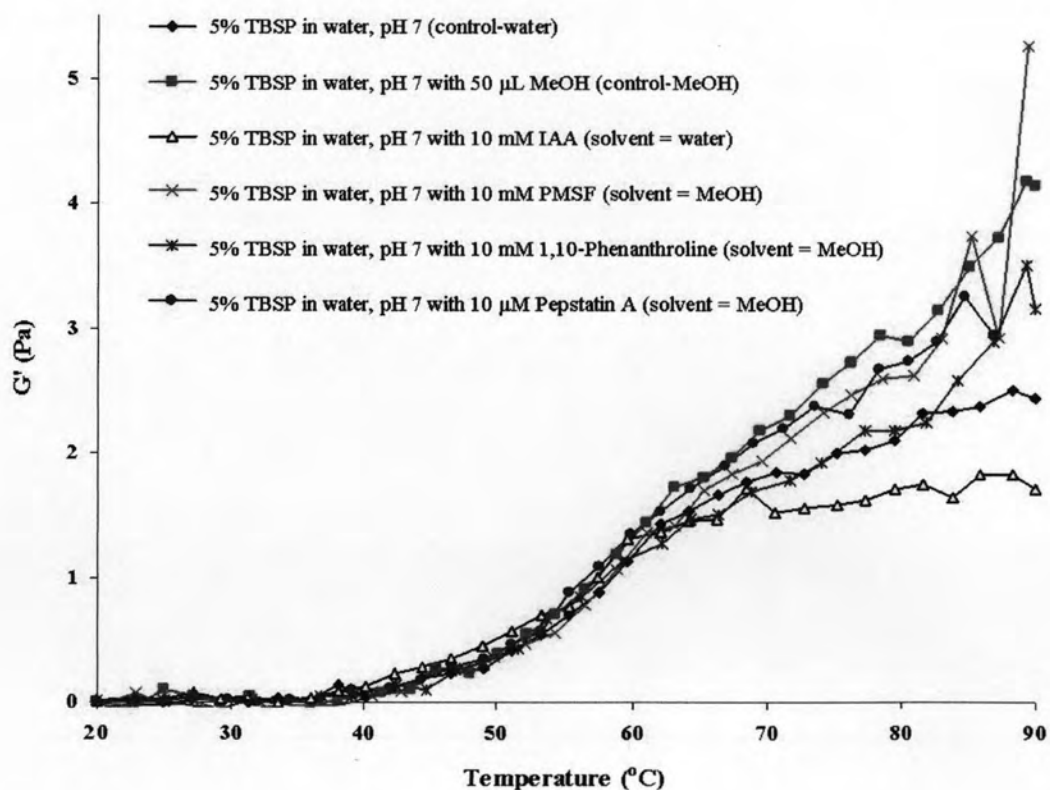


Figure D4 Effect of inhibitors on G' of 5% TBSP.

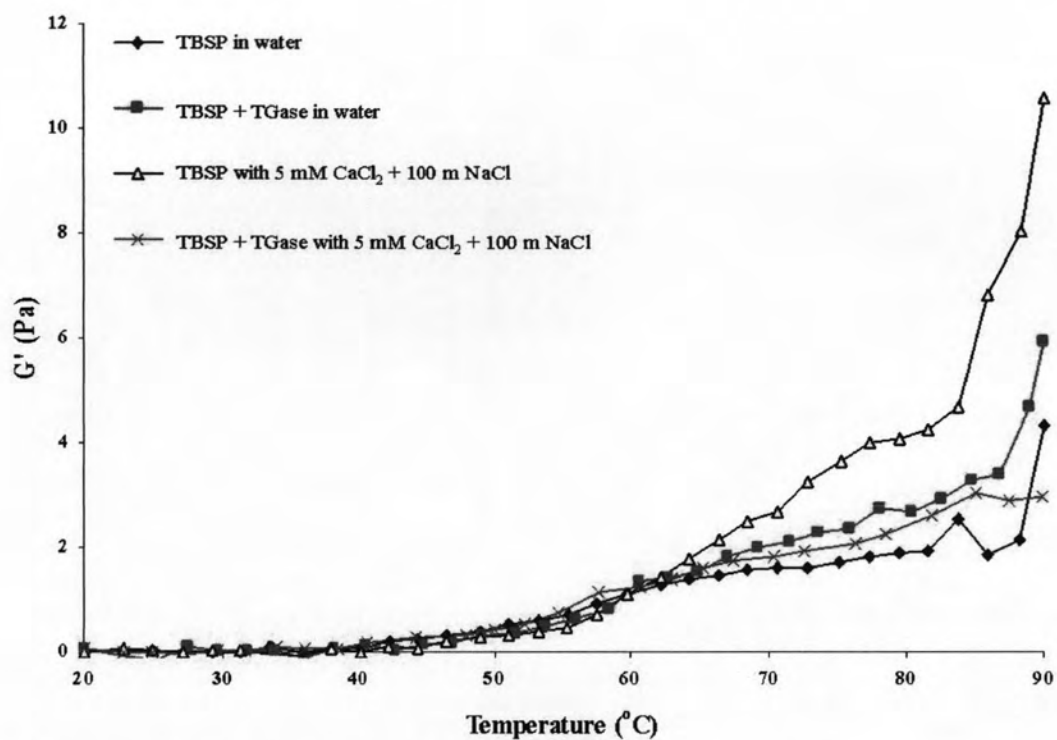


Figure D5 Effect of TGase on G' of 5% TBSP.

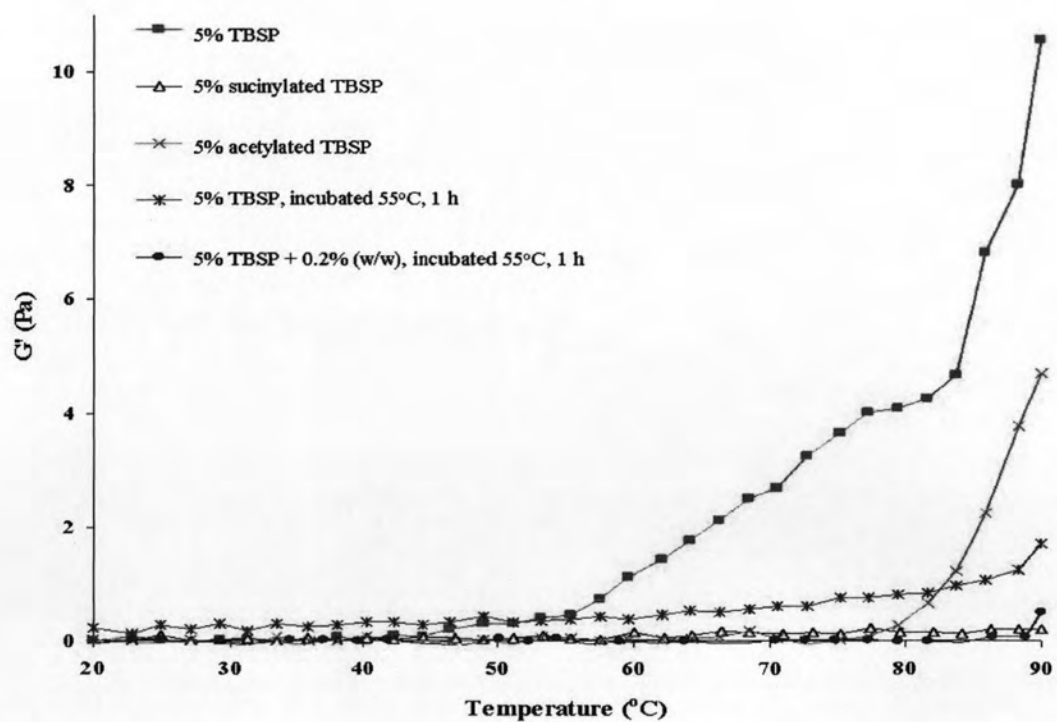


Figure D6 Effect of modification on G' of modified TBSP in 5 mM CaCl₂ with 100 mM NaCl solution.



VITA

Miss Nisanarth Krasaechol was born on June 29th, 1971 in Chonburi, Thailand. She obtained a B.Sc. degree in Food Science and Nutrition from Burapha University in 1994 and a M.Sc. degree in Food Technology from Chulalongkorn University in 1998. After graduating in 1998 she worked as an instructor for Department of Food Science, Faculty of Science, Burapha University, Chonburi, Thailand. In 2001, she received a scholarship from Ministry of Royal Thai government to extend her Ph.D. at the Department of Food Technology, Faculty of Science, Chulalongkorn University. During this time she also got a scholarship for 1 year to conduct her research at Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, Pennsylvania, USA. After her Ph.D. degree, she will join the Department of Food Science, Faculty of Science, Burapha University, Chonburi, Thailand.

Publications:

- Krasaechol, N., Sanguandeeikul, R., Duangmal, K. and Owusu-Apenten, R.K. 2005. Characteristics and functional properties of sarcoplasmic proteins from threadfin bream (*Nemipterus hexodon*). Session 71-B-14. Poster. IFT Annual Meeting, July 15-20, New Orleans, Louisiana.
- Krasaechol, N., Sanguandeeikul, R., Duangmal, K. and Owusu-Apenten, R.K. 2006. Characteristics and functional properties of sarcoplasmic proteins from threadfin bream (*Nemipterus hexodon*). Journal of Food Science. (submitted).