UNIVERSITI PUTRA MALAYSIA

# PRODUCTION AND CHARACTERISATION OF LOW SODIUM HYDROLYSATES FROM DEFATTED SOY FLOUR [GLYCINE MAX (L.) MERR.] AND ROSELLE SEED FLOUR (*HIBISCUS SABDARIFFA* L.)

WONG KAM HUEI

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By

WONG KAM HUEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirement for the Degree of Doctor of Philosophy

June 2003

**Dedicated to:**

**My beloved and dearest**

**Husband and Son**

**Mum and Dad**

**Sisters and Brothers**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

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**Faculty: Food Science and Biotechnology**

The study investigated production and characterisation of low sodium hydrolysates from defatted soy flour (DSF) and roselle seed flour (RSF). The problem of high sodium content in acid hydrolysis was over-come by replacing partly sodium with other cations in the neutralisation process and using enzymatic hydrolysis method. The results showed that acid hydrolysates, neutralised with 75.0% sodium hydroxide, 12.5% calcium hydroxide, and 12.5% potassium hydroxide, were at an acceptable level.

The enzymatic hydrolysis of DSF and RSF by three different commercial enzymes- crude Bromelain, Flavourzyme and Protamex were studied. The enzyme decay experiment showed that all enzymes were inactivated at 100°C. They had both endopeptidase and exopeptidase activities, with Flavourzyme showing the most prominent exopeptidase properties. The $pK_a$ value for Bromelain with DSF (DB) was 7.39, while the optimum conditions were pH 6.5 at 55°C with 100 mg/ml enzyme at 3% protein substrate concentration. The $pK_a$ value for Flavourzyme with DSF

(DF) was 6.50, while the optimum conditions were pH 6.5, 50°C, with 200 mg/ml enzyme at 3% protein substrate concentration. For Protamex with DSF (DP), the $pK_a$ was 7.50, while the optimum conditions were pH 6.5, 60°C, with 150 mg/ml enzyme at 3% protein substrate concentration. RSF with Bromelain (RB) had a $pK_a$ value of 6.97, optimum conditions were pH 7.0, 55°C, with 100 mg/ml enzyme at 2.5% protein substrate concentration. Flavourzyme with RSF (RF) had a $pK_a$ value of 6.80 and optimum activity was at pH 6.5, 50°C, with 100 mg/ml enzyme at 3% protein substrate concentration. Protamex with RSF (RP) showed a $pK_a$ of 6.49, while optimum conditions were at pH 5.5, 55°C, with 100 mg/ml enzyme at 3.5% protein substrate concentration.

DB had the highest degree of hydrolysis (DH) whereas RF had the lowest DH among the hydrolysates. After 5-hour hydrolysis, DP and RF produced a small peptide of about 90 Dalton (Da), similar in size to alanine. Amino acids released were determined by reverse phase chromatography and were used to predict the possible flavour properties of the hydrolysates obtained by comparing the flavour notes of amino acids that had undergone Maillard reaction. Most of the produced hydrolysates contained high amount of cysteine and glutamic acid. Therefore they were evaluated for meaty flavour and umami taste through sensory evaluation. Sensory evaluation studies showed that DF and RF have good potential as flavour ingredients or enhancers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**PENGHASILAN DAN PENCIRIAN TERHADAP HIDROLISAT RENDAH NATRIUM DARIPADA TEPUNG SOYA TANPA LEMAK [*GLYCINE MAX* (L.) MERR.] DAN TEPUNG BIJI ROSELLE (*HIBISCUS SABDARIFFA* L.)**

Oleh

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Satu kajian telah dijalankan terhadap penghasilan dan pencirian tentang hidrolisat rendah natrium tepung soya tanpa lemak (DSF) dan tepung biji roselle (RSF). Masalah tentang kandungan natrium yang tinggi ini telah diatasi dengan menggantikan sebahagian daripada natrium tersebut dengan jenis kation yang lain dalam proses peneutralan dengan menggunakan kaedah hidrolisis berenzim. Keputusan menunjukkan bahawa hidrolisat asid yang dineutralkan dengan 75.0% natrium hidroksida, 12.5% kalsium hidroksida dan 12.5% kalium hidroksida adalah diterima.

Kaedah hidrolisis berenzim yang dilakukan terhadap DSF dan RSF dengan menggunakan tiga jenis enzim komersial- Bromelain kasar, Flavourzyme dan Protamex telah dikaji. Eksperimen degradasi enzim menunjukkan bahawa semua enzim dapat dinyahaktif pada 100°C. Eksperimen ini juga menunjukkan kedua-dua aktiviti endopeptidase dan eksopeptidase, dengan Flavourzyme menunjukkan ciri-ciri eksopeptidase yang paling utama. Nilai $pK_a$ untuk Bromelain dengan DSF (DB) adalah 7.39,

manakala keadaan optimum adalah pada pH 6.5, 55°C, 100 mg/ml enzim dan 3% kepekatan substrat protein. Nilai $pK_a$ untuk Flavourzyme dengan DSF (DF) adalah 6.50, dan keadaan optimum adalah pada pH 6.5, 50°C, 200 mg/ml enzim dan 3% kepekatan substrat protein. Bagi Protamex dengan DSF (DP), nilai $pK_a$ adalah 7.50, dan keadaan optimum adalah pada pH 6.5, 60°C, 150 mg/ml enzim dan 3% kepekatan substrat protein. RSF dengan Bromelain (RB) mempunyai nilai $pK_a$ sebanyak 6.97, dan keadaan optimum pada pH 7.0, 55°C, 100 mg/ml enzim dan 2.5% kepekatan substrat protein. Flavourzyme dengan RSF (RF) mempunyai nilai $pK_a$ sebanyak 6.80 dan aktiviti optimum pada pH 6.5, 50°C, 100 mg/ml enzim dan 3% kepekatan substrat protein. Protamex dengan RSF (RP) menunjukkan bahawa $pK_a$ adalah pada 6.49 dan keadaan optimum pada pH 5.5, 55°C, 100 mg/ml enzim dan 3.5% kepekatan substrat protein.

DB memberikan darjah hidrolisis (DH) yang paling tinggi dan RF mempunyai DH yang paling rendah di antara hidrolisat. Selepas hidrolisis untuk 5 jam, DP dan RF menghasilkan peptida yang halus kira-kira 90 Dalton (Da) dan ia adalah agak serupa dengan saiz alanin. Asid amino yang dibebaskan ditentukan dengan kromatografi fasa terbalik. Asid amino tersebut telah digunakan untuk meramalkan ciri-ciri perisa yang mungkin bagi hidrolisat yand dihasilkan dengan membandingkan perisa asid amino yang telah melalui reaksi 'Maillard'. Kebanyakan daripada hidrolisat yang dihasilkan adalah tinggi kandungan sistein dan asid glutamik. Oleh yang demikian hidrolisat tersebut telah dinilaikan terhadap perisa daging dan rasa

umami melalui penilaian rasa. Kajian penilaian rasa menunjukkan bahawa DF dan RF berpotensi sebagai bahan perisa ataupun penambah perisa.

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| | |
|---|---|
| AH | Amount of hydrolysis |
| AU | Anson Unit, a measure of proteolytic activity on denatures hemoglobin at pH 7.5 and 25°C |
| B | Volume of base consumed during the hydrolysis (L) |
| BCA | Bicinchoninic acid |
| BSA | Bovine serum albumin |
| Da | Dalton |
| DAC1 | Acid hydrolysate of DSF that neutralised with alkaline of composition C1 (Table 3.5) |
| DAC2 | Acid hydrolysate of DSF that neutralised with alkaline of composition C2 (Table 3.5) |
| DAC3 | Acid hydrolysate of DSF that neutralised with alkaline of composition C3 (Table 3.5) |
| DAC4 | Acid hydrolysate of DSF that neutralised with alkaline of composition C4 (Table 3.5) |
| DAN | Acid hydrolysate of DSF that neutralised with alkaline of composition N (Table 3.5) |
| DB | Enzymatic hydrolysate of DSF that hydrolysed by Bromelain |
| DF | Enzymatic hydrolysate of DSF that hydrolysed by Flavourzyme |
| DH | Degree of hydrolysis |
| DP | Enzymatic hydrolysate of DSF that hydrolysed by Protamex |
| DSF | Defatted soy flour |
| EVP | Enzymatically hydrolysed vegetable protein |
| $h$ | Hydrolysis equivalents, defined as equivalents of peptide bonds cleaved per kg protein |
| $h_{tot}$ | Total number of peptide bonds in a protein, expressed in the same unit as $h$ |
| HPA | Hide powder azure |

| | |
|---|---|
| HPLC | High performance liquid chromatography |
| HVP | Hydrolysed vegetable protein |
| 3-MCPD | 3-Monochloro-propane-1,2-diol |
| MP | Mass of protein |
| MSG | Monosodium glutamate |
| $N_b$ | Normality of base in protein hydrolysis experiments |
| RAC1 | Acid hydrolysate of RSF that neutralised with alkaline of composition C1 (Table 3.5) |
| RAC2 | Acid hydrolysate of RSF that neutralised with alkaline of composition C2 (Table 3.5) |
| RAC3 | Acid hydrolysate of RSF that neutralised with alkaline of composition C3 (Table 3.5) |
| RAC4 | Acid hydrolysate of RSF that neutralised with alkaline of composition C4 (Table 3.5) |
| RAN | Acid hydrolysate of RSF that neutralised with alkaline of composition N (Table 3.5) |
| RB | Enzymatic hydrolysate of RSF that hydrolysed by Bromelain |
| RF | Enzymatic hydrolysate of RSF that hydrolysed by Flavourzyme |
| RP | Enzymatic hydrolysate of RSF that hydrolysed by Protamex |
| RSF | Roselle seed flour |
| TNBS | Trinitrobenzensulphonic acid |
| UV | Ultraviolet |
| $V_{max}$ | Maximum velocity |
| $\alpha$ | Degree of dissociation of the $\alpha$-amino group |

# CHAPTER 1

## INTRODUCTION

Chemical and biological methods are widely used for protein hydrolysis. Chemical hydrolysis involving acid or alkali is more common in industrial practices. However, occasionally hydrolysis by chemical reagents is believed to produce potentially hazardous by-products (Kristinsson and Rasco, 2000) such as the recently reported 3-monochloropropane-1,2-diol (3-MCPD) compound (Anon., 2001a; 2001b; Hodgson, 2001). However, the extent of usage of an acid hydrolysate as a flavour enhancer or ingredient in food is limited. According to Nagodawithana (1994), a high level of sodium salt is also a major concern. Against these concerns, the production of low sodium hydrolysates through acid and enzymatic hydrolysis processes was studied in this work.

Enzymatic hydrolysates are relatively new products and differ from acid hydrolysates. For processing, they need a more neutral pH and a lower temperature (Aaslyng *et al.*, 1998). Consequently, they are more promising for the food industry because it results in products of high functionality and nutritive value (Kristinsson and Rasco, 2000). There are indications that enzymatically hydrolysed vegetable proteins (EVPs) are becoming the interest for production of processed meat flavours (Aaslyng *et al.*, 1999). Moreover, EVP can be produced in a few hours, compared with the

traditional method of producing soy sauce which needs a fermentation period of several months (Weir, 1986).

Plant proteins are finding increasing commercial application in a number of formulated foods as an alternative to proteins from animal sources (Clemente *et al.*, 1999). Among plant proteins, soybean and wheat are the high protein sources most widely used for obtaining protein hydrolysates, followed by other sources such as peas (Periago *et al.*, 1998), chickpeas (George *et al.*, 1997) and also by-products of the oil industry, such as sunflower (Villanueva *et al.*, 1999) and rapeseed (Vioque *et al.*, 2000). Roselle seed, a by-product of the food industry, was also found to be a high protein source by Morton (1987). The factors that dictate the selection of raw materials for the production of hydrolysed vegetable proteins (HVPs) are price and the chemical, physical, organoleptic and toxicology properties of the finished product (Olsman, 1979). Thus, low priced plant proteins which are easily obtained from the market were used as the substrates of the hydrolysis processes in the study.

HVPs have long been used as flavouring agents. Generally, the flavour of HVPs is a result of the presence of free amino acids, smaller peptides, salt and various volatile compounds. The free amino acids have distinctive taste profiles, especially glutamic acid which is very important because of its umami taste (Aaslyng *et al.*, 1998). Glutamate, as part of a protein, is not a flavour enhancer, but when it is bound into a peptide structure, it may have the flavour enhancing properties of free form (Hamada