UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF EXTRACTION METHODS AND FLAVOUR PROFILING OF REDCLAW (CHERAX QUADRICARINATUS) CRAYFISH

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OPTIMISATION OF EXTRACTION METHODS AND FLAVOUR PROFILING OF REDCLAW (*Cherax quadricarinatus*) CRAYFISH

By

MOHD NAZRI ZAYAPOR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the Requirements for the Degree of Master Science

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To my family,

My parent ~ Rosminah & Mohd Zapar Zayapor

Brother & sister ~ Najib & Nadrah

Niece & Nephew ~ Nana & Wawan

To my friends,

Kak Nani, Kak Ann, Kak Normah, Harvin, Wee Sim (Bibik Choo), Mani, Tan Kar Weng, Bapak Misnawi, Bapak Yusep, Azli and all who knows me

To dedicated and helpful staffs,

En. Azman, En. Halim, En. Rosli, Kak Jem, Kak Sharul, En. Azhar and En. Razali

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Chairperson: Professor Jamilah Bakar, Ph.D.

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Redclaw crayfish (*Cherax quadricarinatus*) is native to Australia and was introduced to Malaysia in the past few years. This study on the Redclaw crayfish flavour extract was carried out at three different stages with the respective objectives: 1) screening stage; to determine a suitable extraction condition, 2) flavour profile comparison with the commercial flavourings; to find the similarities of flavour profile of Redclaw extract with the commercial flavourings, and 3) identification of aromatic compounds; to determine the aroma profile in Redclaw aqueous extract and cooked crayfish. At the screening stage, different extraction conditions vis. simmering time (10 – 40 min) and temperature (65 - 95°C), Redclaw-water ratio (4 – 6), and salt concentration (1 – 10%) was carried out for head, tail and claws. Total amino acids (> 10 mgg^{-1}) and taste amino acids (glutamic acid, glycine and alanine) (> 5 mgg^{-1} of the total three amino acids) were chosen as the indicators of the flavour quality. Principal Component Analysis (PCA) was used to study the pattern of amino acid distributions. It was found that extraction conditions at salt concentration of less than 5.5%; water ratio of less than 5; simmering

time and temperature of less than 30 min and 80°C, respectively produced more taste amino acids (> 5 mgg^{-1} in total) in all extracts. In general, longer simmering time significantly ($p<0.05$) produced more bitter amino acids, whereas shorter time (<30 min) resulted in higher distribution of sweet and umami-precursor amino acids. Bitter amino acids increased as the temperature increased above 90°C. Salt concentration and Redclaw-water ratio (w/v) did not give significant affect on the distribution of amino acids in any body-part.

At the comparison stage, amino acids, 5' mononucleotides, soluble sugars, and organic acids were chosen as the taste-active component quality indicators. PCA was also used to determine the similarities of Redclaw extract (EXT) with the selected commercial flavourings. EXT produced at 85°C for 30 min with 5.0% salt and Redclaw-water ratio of 1:2 (w/v) was used in this study. It had relatively lower taste-active components than the selected commercial flavourings. PCA showed that EXT has similar amino acid profiles to shrimp paste and fish sauce in particular that of glutamic acid and was also similar to fish sauce in its 5' uridine monophospate (UMP) and 5' cytidine monophospate (CMP) content. Redclaw extract concentrate (RCE) produced has an earthy and cooked cabbage-like, however, less fishy and seafood-like aroma than all commercial flavourings.

Aroma identification of aqueous extract was carried out at pH 7.8, 6.4 and 4.6; while cooked Redclaw was maintained at its original pH to

determine the effect of pH on aroma profile. At pH 7.8, 6.4 and 4.6, a total of 82, 44 and 46 aromatic compounds were identified in the extract, respectively. However at pH 6.4, cooked Redclaw contained 73 positively identified aromatic compounds and did not show similar aroma profile to RCE extracted at the same pH condition. Major aromatic compounds were hydrocarbons (61 – 82%), comprising of alkanes (8 – 19%), alkenes (0.1-1.8%), cyclic hydrocarbons (0.3 – 1.5%) and others hydrocarbons (49 – 64%). Butylated hydroxytoluene (BHT) was the dominant compound in all extracts and cooked Redclaw studied.

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PENGOPTIMAAN KAEDAH EKTRAKSI DAN PEMPROFILAN PERISA UDANG KRAI SEPIT MERAH (*Cherax quadricarinatus*)

Oleh

MOHD NAZRI ZAYAPOR

November 2005

Pengerusi: Professor Jamilah Bakar, Ph.D.

Fakulti: Sains dan Teknologi Makanan

Udang krai Sepit Merah (*Cherax quadricarinatus*) berasal dari Australia dan telah diperkenalkan di Malaysia beberapa tahun lalu. Kajian ke atas ekstrak akueus udang krai Sepit Merah ini dilakukan pada tiga peringkat yang berlainan dengan objektif berikut: 1) peringkat penyaringan; untuk menentukan keadaan pengekstrakan yang sesuai, 2) perbandingan profil perisa ekstrak Sepit Merah dengan perIsa komersil yang terpilih; untuk melihat persamaan profil perisa di antara ekstrak Sepit Merah dengan perIsa komersil, dan 3) pengenalpastian profil aroma ekstrak akueus Sepit Merah dan Sepit Merah yang telah direbus. Pada peringkat penyaringan, keadaan pengekstrakan adalah seperti berikut: masa (10 – 40 min) dan suhu (65 - 95ºC) perenehan, nisbah Sepit Merah - air (4 – 6), kepekatan garam (1 – 10%) dan dilakukan ke atas kepala, ekor, dan sepit secara berasingan. Asid amino jumlah (> 10 mgg^{-1}) dan asid amino perisa (> 5 mgg^{-1} berdasarkan jumlah) telah dipilih sebagai penunjuk kepada kualiti perisa. Analisis Komponen Utama (*Principal Component Analysis, PCA*) telah digunakan

untuk mengkaji corak taburan asid amino. Pada keadaan pengekstrakan garam <5.5%, nisbah Sepit Merah - air < 5, masa dan suhu perenehan < 30 min dan < 80°C, telah menghasilkan lebih banyak asid amino perisa (> 5 mgg^{-1} bagi jumlah keseluruhan) bagi kesemua ekstrak. Secara umumnya, lebih lama perenehan lebih banyak asid amino pahit yang terhasil, dimana, perenehan singkat (<30 min) menghasilkan taburan asid amino manis dan pelopor umami yang tinggi ($p<0.05$). Asid amino pahit bertambah dengan peningkatan suhu perenehan lebih daripada 90°C. Kepekatan garam dan nisbah Sepit Merah - air tidak memberi kesan signifikan kepada taburan asid amino ($p>0.05$) di dalam kesemua ekstrak.

Pada peringkat perbandingan, asid amino, nukleotida 5', gula terlarut, dan asid organik telah dipilih sebagai penunjuk komponen aktif-rasa. PCA juga telah digunakan untuk melihat persamaan ekstrak Sepit Merah (EXT) dengan perisa komersial yang terpilih. EXT yang terhasil pada 85°C untuk 30 min dengan 5.0% garam dan nisbah Sepit Merah-air 1:2 (w/v), telah digunakan untuk tujuan perbandingan. Ia didapati mengandungi lebih rendah komponen aktif-rasa berbanding perisa komersial yang dipilih. PCA menunjukkan EXT mempunyai persamaan dengan otak udang dan kicap ikan dalam profil asid amino terutamanya dalam kandungan asid glutamik. Ia juga mempunyai persamaan dengan kicap ikan dalam kandungan 5'UMP dan 5'CMP.

Pekatan ekstrak Sepit Merah (RCE) yang terhasil mempunyai bau tanah dan kubis masak, tetapi, kurang berbau ikan dan makanan laut

berbanding perisa komersial. Pengenalpastian bahan aromatik ke atas ekstrak cecair dijalankan pada pH 7.8, 6.4, dan 4.8, manakala bagi udang krai rebus, pHnya dikekalkan pada pH asal, 6.4 untuk menentukan perbezaan kesan pH ke atas profil bahan aromatik. Pada pH 7.8, 6.4, dan 4.8, sejumlah 82, 44 dan 46 bahan aromatik telah dikenalpasti di dalam ekstrak akues, mengikut urutan. Walaubagaimanapun pada pH 6.4, udang krai rebus mengandungi 73 bahan aromatik yang dikenalpasti dan tidak menunjukkan persamaan profil bahan aromatik dengan ekstrak yang ditentukan pada pH yang sama. Bahan aromatik utama adalah hidrokarbon (61 – 82%) yang terdiri daripada alkana (8 – 19%), alkena (0.1- 1.8%), hidrokarbon siklik (0.3 – 1.5%) dan lain-lain hidrokarbon (49 – 64%). Hidroksitoluena terbutil (BHT) adalah bahan aromatik yang dominan didalam kesemua ekstrak dan Sepit Merah rebus.

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LIST OF ABBREVIATIONS

SDE	Simultaneous Steam Distillation / Exctraction
PCA	Principal Component Analysis
PLS – R	Partial Least Square - Regression
PC	Principal Component
PITC	Phenylisothiocynate
RRC	Uncooked Redclaw
EXT	Redclaw Extract
RCE	Redclaw extract concentrate
CCH	Cooked Redclaw
OYST	Oyster sauce
FS	Fish sauce
CS	Chicken stock
OTK	"Otak udang" shrimp paste
HPLC	High Performance Liquid Chromatography
GC/MS	Gas chromatography / Mass spectrometry
RNA	Ribonucleic acid
F&F	Flavour and Frangrance
MSG	Monosodium glutamate
IMP	Inosine 5' monophosphate
GMP	Guanosine 5' monophosphate
AMP	Adenosine 5' monophosphate
CMP	Cytidine 5' monophosphate
UMP	Uridine 5' monophosphate

XMP	Xenosine monophosphate
FAAs	Free amino acids
AAs	Amino acids
Asp	Aspartic acid
Glu	Glutamic acid
Ala	Alanine
Gly	Glycine
Ser	Serine
Thr	Threonine
Lys	Lysine
Pro	Proline
His	Histadine
Met	Methionine
Val	Valine
Arg	Arginine
Iso	Isoleucine
Phe	Phenylalanine
Try	Tryptophan
Leu	Leucine
Try	Tyrosine
Cys	Cysteine
JSL	Japanese Spiny Lobster
SNL	Shovel – nosed Lobster
NPS	Northern Pink Shrimp
SS	Spotted Shrimp

CHAPTER 1

INTRODUCTION

At the end of last century, the global flavour market size was roughly estimated in the range of USD 4 - 5 billion (Zick, 1999). With an increase in world travel, availability of ethnic ingredients and influx of immigrants, the global trend towards multiethnic tastes and unique flavour has increased the demand for more flavourful food versions (Anonymous A).

Recovery of natural flavours can be achieved through distillation of essential oil, concentration of fruit and vegetable juices, fermentation and enzymatic modification of animal/plant proteins, and extraction of animal and vegetable juices (Gatfield, 1996). Supercritical fluid or carbon dioxide and water (aqueous) extractions are the common flavour extraction methods. However, the latter is cheaper and can provide higher original flavour retention (Ochi, 1980).

Seafood particularly crustacean serves as a wide source of natural tasty flavour. Japan has led the world in exploiting seafood as the major source of seafood flavour compounds; glutamate and inosinate salt for food processors (Motozki, 1969). Baek and Cadwallader (1996) have shown that wastes from crustacean by-processings can be completely utilised to obtain flavour and their precursors prior to pigment and chitin extractions. The by-

product of crustacean processing has also shown to be a potential flavourant in oriental prawn cracker (Teerasuntonwat and Raksakulthi, 1995).

Utilisation of seafood processing by-products as potential condiments had been acheived through enzymatic modification (Jao and Ko, 2002; Imm and Lee, 1999; and Kim et al., 1997), fermentation and autolysis (Funatsu et al., 2000a and b; Morioka et al., 1999; and Hayashi et al., 1993), and membrane treatment (Jao and Ko, 2002; and No and Meyers, 1989). The advantages conferred through the utilisation of seafood by products include: (1) having the status of a 'natural' substance, (2) reduction in waste product formation and (3) improvement in environmental control.

Australian Redclaw crayfish (*Cherax quadricarinatus*) that belongs to the *Crustacean* group and the *Parastadicidae* family is found in the tropical river systems in the Southern Hemisphere. Its cultivation has been attempted in Johore, a southern state of Malaysia Peninsular. The production of Redclaw in Malaysia was reported to about 12 tonnes per year of 40-50 m^3 (Chang, 2001). Redclaw aqua cultural attributes include the ease of reproduction, relatively fast growth, large size, non-aggressive nature, and high marketability. The marketable size of the crayfish is in the range of 50 to 100 g.

Jones (1989) stated that the flavour of smaller (<100 g) crayfish was more popular, regardless of the sex differences and claw meat was described as having sweeter flavour than the tail meat. Konosu and

Yamaguchi (1990) reported that glycine, alanine, proline and serine are the major amino acids responsible for the sweet taste in crustacean. They also concluded that glycine, glutamic acid and the buffering capacity of whole amino acids also contributed to the crustacean flavour.

Although Redclaw has a unique flavour, the report on its flavour profile is limited. This study was conducted with the objectives of (1) to determine the effect of extraction parameters on the amino acids profile of the extracts, (2) to determine non-volatile and volatile flavour components of the extract, and (3) to compare the Redclaw aqueous extract with the selected commercial flavouring products.