



UNIVERSITI PUTRA MALAYSIA

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

MOHD NAZRI ZAYAPOR.

FSTM 2005 7

**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**

November 2005



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*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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

By

MOHD NAZRI ZAYAPOR

November 2005

Chairperson: Professor Jamilah Bakar, Ph.D.

Faculty: Food Science and Technology

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 ($> 10 \text{ mgg}^{-1}$) and taste amino acids (glutamic acid, glycine and alanine) ($> 5 \text{ mgg}^{-1}$ 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 ($> 5 \text{ mgg}^{-1}$ in total) in all extracts. In general, longer simmering time significantly ($p < 0.05$) produced more bitter amino acids, whereas shorter time ($< 30 \text{ min}$) 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 monophosphate (UMP) and 5' cytidine monophosphate (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.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**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 – 95°C) perenehan, nisbah Sepit Merah - air (4 – 6), kepekatan garam (1 – 10%) dan dilakukan ke atas kepala, ekor, dan sepit secara berasingan. Asid amino jumlah ($> 10 \text{ mgg}^{-1}$) dan asid amino perisa ($> 5 \text{ mgg}^{-1}$ 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.

ACKNOWLEDGEMENTS

Praise to the Almighty for giving the strength, courage, patience, and capability to complete this project and thesis write up. Deepest thanks and appreciation to the Chairperson of the Supervisory Committee, Professor Dr. Jamilah Bakar for guidance, advice, encouragement, and patience throughout the project course. All valuable experiences and chances that gained toward the completion of the study are always remembered. This deepest gratitude also goes to co-supervisors, especially Dr. Nazimah Sheikh Abdul Hamid for the guidance in statistical analyses, and the financial support; Assoc. Prof. Dr. Russly Abd. Rahman and Mr. Shahrim Abd. Karim.

Great appreciations also go to dedicated technical staffs, En. Azman, En. Halim, En. Rosli, Kak Jem, Kak Sharul, En. Azhar and En. Razali. Deeply thanks to all friends: Kak Nani, Kak Ann, Kak Normah, Harvin, Wee Sim (Bibik Choo), Mani, Tan Kar Weng, Bapak Misnawi, Bapak Yusep, and Azli for sharing knowledge and brilliant ideas, and for the cooperation and supportive spirits throughout the years.

Bountiful appreciation to my beloved parents for the never-ending support, spiritually and financially. Sincere gratitude to the major financial support, IRPA fund, and KUB Holdings Sdn. Bhd. that was awarded to Prof. Dr. Jamilah Bakar. Thank you.

TABLE OF CONTENTS

	Page
DEDICATION	iii
ABSTRACT	iv
ABSTRAK	vii
ACKNOWLEDGMENT	x
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xviii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxiii
 CHAPTER	
 I INTRODUCTION	 1
 II LITERATURE REVIEW	 4
Redclaw (<i>Cherax quadricarinatus</i>) as a Potential Flavouring Source	4
Australian Redclaw Crayfish	4
Basic Anatomy and Physiology	7
Aquacultural Attributes and Market Aspect	10
Post-harvest Aspects and Flavour Characteristics	12
Market Trends in the Flavour Industry	14
Chemistry of Flavour Components	19
Non-Volatile Components and Its Contribution to Seafood Flavour Formations	19
Volatile Components in Seafood and Its Product Flavour Development	27
Flavour Development	50
Analyses of Flavour Components	59
Analyses of Taste Components	59
Analyses of Aromatic Flavour Components	68
Sensory Measurements	72
Principal Component Analysis (PCA)	77
Score Plot	78
X-Loading Plot	79
Principal Component-Models	80
Pre-Processing Data	81
Partial Least Square Regression (PLS-R)	81



III	SCREENING OF A SUITABLE CONDITIONS FOR REDCLAW (<i>Cherax quadricarinatus</i>) AQUEOUS EXTRACT	83
	Introduction	83
	Material and Methodology	86
	Size Measurement and Determination of Edible Tissue Yield	87
	Aqueous Extraction at Different Time, Temperature, Salt Concentration and Water Ratio	88
	Proximate Analyses of Redclaw Body parts	88
	Amino acid Analyses of Redclaw Body parts and Its Extract	90
	Statistical Analysis	91
	Results and Discussions	93
	Size of Redclaws and Yield of Its Edible Tissues	93
	Proximate Compositions	95
	Composition of Amino Acids (AAs) in Redclaw Head, Tail, and Claws	100
	Effect of Aqueous Extraction Variables on the Amino Acids Distribution in the Redclaw Extracts Produced from Different Body Parts	104
	PCA of Head	105
	PCA of Tail	109
	PCA of Claws	111
	Selection of Extraction Conditions for Production of Redclaw Flavour Extract	114
	Conclusion	116
IV	CHEMICAL AND TASTE ATTRIBUTES OF REDCLAW (<i>Cherax quadricarinatus</i>) AQUEOUS EXTRACT	118
	Introduction	118
	Materials	120
	Methodology	120
	Preparation of Redclaw Extract (EXT) and Redclaw Paste (RRC)	120
	Proximate Analyses	122
	Titratable Acidity and pH Measurements	123
	Amino acids Analysis	124
	5' Nucleotides Analysis	124
	Soluble Sugar Analysis	126
	Organic Acid Analysis	127
	Taste Evaluations of EXT and the Commercial Flavourings	128
	Statistical Analyses	129

Results and Discussion	130
Proximate Compositions of Redclaw Extract and Selected Flavourings	130
Amino Acid Profiles of Uncooked Redclaw, Redclaw Extract and the Selected Commercial Flavourings	135
PCA of Amino Acid Compositions in EXT and the Selected Commercial Flavourings	140
Content of 5' Nucleotides in EXT and the Selected Commercial Flavouring	143
PCA of 5' Nucleotides Profiles in EXT and the Selected Commercial Flavourings	146
Soluble Sugars in EXT and the Selected Commercial Flavourings	149
PCA of Soluble Sugars in EXT and the Selected Commercial Flavourings	152
Organic Acid content in EXT and Selected Commercial Flavourings	155
PCA of Organic Acids in EXT and Selected Commercial Flavourings	157
PLS Analysis of Taste Profiling Data of EXT and the Selected Commercial Flavourings	159
Conclusion	163
VI AROMATIC FLAVOUR AND SENSORY PROFILE OF REDCLAW (<i>Cherax quadricarinatus</i>) AQUEOUS EXTRACT	165
Introduction	165
Materials	168
Methodology	168
Preparations of Redclaw Extract	168
Preparation of Redclaw Extract Concentrate (RCE)	169
Aroma Evaluation Training	169
Aroma Evaluation of RCE and Selected Commercial Flavourings	172
Extractions of Aromatic Compounds from RCE and Cooked Redclaw (CCH)	172
Gas Chromatography/ Mass Spectrometry (GC/MS) Analyses of RCE Extracted at Different pH Conditions and Cooked Redclaw (CCH)	173
Statistical Analyses	174

19	Screening design of aqueous extractions of Redclaw as generated by ECHIP software	89
20	Average length (cm), weight (g) and edible tissue yield of Redclaw crayfish	93
21	Proximate contents of 1 st and 2 nd batch harvest	96
22	Amino acids of uncooked Redclaw body parts	101
23	Physico-chemical composition of uncooked Redclaw (RRC), Redclaw extract (EXT) and selected commercial flavourings	131
24	Amino acid composition of RRC, EXT and the selected flavourings	136
25	5' mononucleotides contents in EXT and the selected commercial flavouring products	144
26	Soluble sugar contents in Redclaw samples and the selected commercial flavourings	150
27	Organic acids in Redclaw samples and selected commercial flavourings	156
28	Flavour vocabulary, references and intensities identified by the panellists	170
29	List of aromatic compounds for RCE extracted at pH 7.8 (RCE7.8)	185
30	List of aromatic compounds for RCE extracted at pH 6.4 (RCE6.4)	188
31	List of aromatic compounds for RCE extracted at pH 4.8 (RCE4.8)	190
32	Lists of aromatic compounds for cooked Redclaw at pH 6.4 (CCH)	192
33	List of aroma variables included in the data analysis	200

LIST OF FIGURES

Figure		Page
1	Classification of cultivable crustaceans	5
2	Redclaw distribution in its native habitat	7
3	Anatomical illustration of <i>Cherax quadricarinatus</i>	8
4	Crayfish product forms	13
5	The statistical demands for F&F	14
6	Percentage annual growth for global flavour industry	15
7	Fish or seafood aromas	28
8	Mechanism of 5,8,11-tetradecatrien-2-one formation via lipoxygenase action	34
9	Sulphur-containing volatiles	42
10	Ribose derived sulphur-containing compounds	44
11	Proposed degradation of eicosapentanoic acid to propanal	52
12	Complex pathways of the Maillard reactions	54
13	Derivatisation of amino acids with PITC	60
14	Liken-nickerson apparatus	69
15	The interaction of sensory science with all aspects of food manufacturing business	72
16	Sequential order of sensory evaluations	74
17	Data set represented as an X-matrix with n-objects of columns by p-variables of rows	77
18	X-loading plot of head extract with distribution of amino acids at different extraction conditions	106
19	Score plot of head extracts at different extraction conditions	107

20	PCA Bi-plot of tail extract produced at different extraction parameters	110
21	X-loading plot of claws extract with distribution of amino acids at different extraction conditions	112
22	Score plot of claws extract at different extraction conditions	113
23	Flow diagram of Redclaw extract processing	121
24	X-loading of amino acid compositions in EXT and the selected commercial flavouring products	141
25	Score plot of amino acids compositions in EXT and the selected commercial flavouring products	142
26	Bi-plot of the composition of 5'nucleotides in EXT and the selected commercial flavouring products	147
27	Bi-plot of soluble sugar compositions in EXT and the selected commercial flavouring products	153
28	Bi-plot of organic acids in EXT and the selected commercial flavourings	158
29	X-loading weights and Y-loading of PLS2 on the amino acids contents and sensory profiling data	160
30	Score plot of PLS2 on the amino acids contents and sensory profiling data	161
31	Measured vs. Y-prediction plot for umami perception of selected commercial flavourings and EXT	164
32	Quantitative descriptive analyses of EXT and selected commercial products	176
33	Comparison of the total content (% area) of different chemical classes in CCH and RCEs	176
34	Comparison of the total content (% area) of Sub-classes of nitrogen containing compounds in CCH and RCEs	184
35	Comparison of the total content (% area) of sub-classes of hydrocarbons in CCH and RCEs	184



36	X-loading plot for volatile compounds in RCE 4.8, RCE 6.4, RCE 7.8 and CCH	201
37	Score plot of Redclaw aroma compounds extracted at different pH conditions	203



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 achieved 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 *Parastadidae* 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 m³ (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.