# CHARACTERIZATION AND PROCESS OPTIMIZATION OF *COLLOCALIA FUCIPHAGA* EXTRACT

NOOR SUZANA BINTI BAKAR

A thesis is submitted in fulfillment of the requirements

for the award of the degree of

Bachelor in Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

JANUARY 2012

# ABSTRACT

The purpose of this study is to characterize and investigate the optimum condition of temperature and liquid solid ratio (LSR) in *Collocalia fuciphaga* extract. The formation of functional group in the *Collocalia fuciphaga* was confirmed by fourier transform infrared spectroscopy (FTIR) analysis of the untreated and treated sample while inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the heavy metals contents inside the *Collocalia fuciphaga.* Water extraction method was employed as a function of temperature and LSR in order to identify their effects to the protein extract concentration from *Collocalia fuciphaga* and subsequently determine its optimum condition using response surface methodology (RSM). The FTIR spectrums of the untreated and treated sample resulted in the same trend of spectrum. This is because the functional group of the protein extract, O-H bond, N-H bond, C=O bond, and C-H bond, respectively did not change after the extraction process. From the analysis using the ICP-MS, it was clearly showed that concentration of aluminium, arsenic and lead in the *Collocalia fuciphaga* was 2.378 mg/L, 0.044mg/L and 0.125mg/L which is lower than the maximum concentration allowable of aluminium, arsenic and lead (7mg/L, 2mg/L and 3.402mg/L, respectively). The optimum condition of temperature and LSR were found to be $38^{O}$C and 42:1 while the protein extract concentration was 0.3477g/L. Increase in temperature after this optimum value resulted in decrease in protein extract concentration due to the destruction of protein structure at high temperature.

# ABSTRAK

Tujuan kajian ini adalah untuk mengenalpasti dan mengkaji keadaan optimum suhu dan nisbah cecair terhadap pepejal (LSR) bagi proses pengekstrakan protein daripada *Collocalia fuciphaga*. *Spektroskopi inframerah* transformasi *fourier* (FTIR) digunakan untuk menentukan kumpulan berfungsi sampel *Collocalia fuciphaga* yang tidak dirawat dan dirawat. Manakala *Spektrometer Jisim - Plasma Gandingan* Aruhan (ICP-MS) telah digunakan untuk menentukan kandungan logam berat di dalam *Collocalia fuciphaga.* Kaedah pengekstrakan menggunakan air telah digunakan dalam kajian ini. Faktor kesan suhu dan LSR terhadap kepekatan ekstrak protein daripada *Collocalia fuciphaga* telah dikaji dan seterusnya ditentukan keadaan optimum proses pengekstrakan ini dengan menggunakan kaedah tindak balas permukaan (RSM). Spektrum FTIR untuk sampel yang tidak dirawat dan dirawat menunujukkan bentuk graf yang sama. Ini adalah kerana ekstrak protein mempunyai kumpulan berfungsi O-H , N-H,C=O dan C-H tidak berubah selepas proses pengekstrakan. Daripada analisis menggunakan ICP-MS, ia jelas menunjukkan bahawa kandungan aluminium, arsenik dan plumbum dalam *Collocalia fuciphaga* adalah 2.378 mg/L, 0.044mg/L dan 0.125mg/L yang mana ianya kurang daripada kepekatan maksima yang dibenarkan iaitu 7mg/L, 2mg/L dan 3.402mg/L bagi kandungan aluminium, arsenik dan plumbum, masing-masing. Keadaan optima suhu dan LSR pada 38$^{o}$C dan 42:1 menghasilkan kepekatan protein ekstrak yang paling tinggi iaitu 0.3477g/L. Peningkatan suhu selepas nilai optima akan menyebabkan pengurangan kepekatan protein ekstrak kerana struktur protein akan terganggu pada suhu yang tinggi.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

The word `protein' is defined as any of a group of complex organic compounds, consisting essentially of combinations of amino acids in peptide linkages, that contain carbon, hydrogen, oxygen, nitrogen, and usually, sulfur (Yada, 2004). Protein molecules tend to unfold and even become fully denatured under unfavoured conditions, such as a high temperature, an acidified condition, a high pressure or even excessive shear. Denatured protein molecules will aggregate and/or crosslink to form larger clusters and, at high concentrations, will form a three-dimensional solid-like network (or a gel). Therefore, proteins are regarded as one of the main classes of building blocks used in many semi-solid foods for conferring mechanical properties (Lianqing *et al*., 2008).

Proteins are also recognised as one of the main classes of surface-active agents in liquid foods for stabilising dispersed particles and fat droplets, due to the polarised distribution of hydrophobic and hydrophilic groups along the back bone. Protein molecules adsorb at the oil–water interface to lower the interfacial tension and, therefore, make such thermodynamically less favourable dispersed systems stable for an extended shelf life. The importance of protein application in foods can also be seen in many other aspects. For example, it was reported that, in combination with polysaccharides and starches, proteins could be applied as a meat alternative, as a fat replacer or a filler in manufacturing healthier foods. Proteins also have special uses as foaming agents or as functional ingredients for nutrient delivery in foods (Roger, 2001).

The diversity of the structural role of proteins in various food raw materials is illustrated by comparing protein structures in the muscle tissues of meat, fish and squid, the protein bodies of plant tissues such as cereals, legumes, oilseeds and shell (nut) fruits, and the casein micelle structure of bovine milk. Interactions of proteins with other components are exemplified in protein-starch interactions observed during dough processing and baking, protein-hydrocolloid interactions in dairy proteins in food processing products, protein-fat interactions in comminuted meat emulsions, mayonnaise and cheese, protein-water as well as protein-protein matrix interactions in fish surimi gels, yogurt and cheese (Yada, 2004).

According to Ebru *et al* (2010), the amount of protein was quantified using the Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as the standard. The extraction method was assessed by the protein yield or the extraction rate, which was defined as the percentage ratio of the protein quantity extracted to the total amount of the protein in the tea leaf. A discontinuous SDS gel electrophoresis of 15% acrylamide was performed using a vertical mini-gel system of 0.75 mm thickness. The gel was prepared according to the method described by Laemmli (1970). Proteins of different molecular weights, b-lactoglobulin (18.4 kDa), lactate dehydrogenase (35.0 kDa), ovalbumin (45.0 kDa), bovine serum albumin (66.2 kDa) and galactosidase (116.0 kDa), were used as markers. The molecular weight of a tea protein was determined by comparing its migration distance with the standard markers. The amino acid analysis of tea protein was performed using a Hitachi 835-50 Amino Acid Analyser (Hitachi, Japan).

Besides that, other widely method use for the protein contain determination is The Lowry method. However, Pierce, 1996 (from the instruction manual) has developed a modified cupric sulphate-tartrate reagent that places two of the three reagents in the original established Lowry method with one stable reagent, thus, avoiding the necessity to prepare the fresh reagents daily. There is nearly a 100% correlation of the colour response curves with various proteins between the Pierce modified Lowry protein assay reagent and the original Lowry method.

Before protein assay were made, the Modified Lowry and Folin-Ciocalteu reagents must be prepared. The Modified Lowry reagent was prepared by adding the Reagent A (20 g $Na_2CO_3$ + 4 g NaOH dissolved in 1 liter distilled water) and Reagent B (2.5 g $CuSO_4.5H_2O$ + 5 g sodium citrate dissolved in 1 liter distilled water) in the proportion of 50:1. While the Folin-Ciocalteu reagent was prepared by diluting the supplied 2X reagent (2N) 1:1 with distilled water.

In this research, *Collocalia fuciphaga* is use as the raw material. *C. fuciphaga* species is popularly known as the White Nest or House Nest swiftlet. *C. fuciphaga* measures about 12 cm in its entire length and weighs about 15 to 18 gm. In recent years, hormone-like substances such as mitogen and avian epithelial growth factor have been found in *C. fuciphaga* (Jie *et al*., 2009).

The nests are cleaned by soaking them in water until the nest cement is softened and the tightly bound laminae partially loosen. Small feathers and fine plumage are then manually removed with tweezers with the cleaned strands subsequently being re-arranged and molded into chips of various shapes, air-dried, and packaged for sale around the world (Massimo, 2005). *C. fuciphaga* helps to stimulate cell division and growth and can enhance tissue growth and regeneration. They strengthen the body, moisturize the skin, maintain beauty, provide energy and enhance the metabolism of fat. The *C. fuciphaga* is adaptable for either sex or any age group (Jie *et al*., 2009).

It has been used for centuries whether as a tonic or a health food. However, its usage and benefits are mainly based on historical, anecdotal and observational reports. In Traditional Chinese Medicine (TCM), the nest is believe to offer good effect for treating consumptive diseases, curing tuberculosis, dry coughs, suppressing cough and phlegm-dyspnea (difficult breathing), alleviating asthma, hemoptysis (coughing blood), improving the voice, asthenia, stomach ulcer, relieving gastric troubles, and general weakness of bronchial ailments. It is also traditionally used to nourish the kidneys, lungs heart and stomach to aid renal functions, raise libido, strengthen the immune system, promote growth, enhance the immune system, improve concentration, increase energy and metabolism, and regulate circulation (Kong *et al*., 1987; Chan 2006).

Malaysia is currently the third largest producer of edible birds' nests (7% of gross supply value) in the world, behind Indonesia (60%) and Thailand (20%). A kilogram of unprocessed white edible birds' nests (around 90 to 120 nests) is able to fetch production level prices of RM$4500 to RM$6000 in 2006, with supply of white edible birds' nests being severely tight as compared to ever increasing levels of demand from consumer countries all over the world.

A kilogram of processed white edible birds' nests is able to fetch retail level prices of RM$15000 to RM$25000 in 2006 in Hong Kong and China. It has been hypothesized in their above publication that due to the following enduring qualitative reasons that the:

1. Consumption of edible birds' nests is considered as a status symbol;
2. The health giving properties of consuming edible birds' nests;
3. Strong economic growth rates experienced by Hong Kong, China and Taiwan.
4. Potential of edible birds' nests as a base mineral to be used in the production of herbal and vitamin supplements; the international market for edible birds' nests will continue to grow at double-digit rates for the next 2 decades or so.

## 1.2 PROBLEM STATEMENTS

Taking care in a lifestyle is one of the important steps that need to concern in the daily life. Everyone has a different perspective on what a healthy lifestyle is, but it really comes down to practicing good health habits and giving up harmful ones.

With a healthy lifestyle, people can only get positive reinforcements out of it, such as feeling good; will have more energy, sleep better and be more relaxed, looking good; will have a nice toned body, strong muscles, bright eyes healthy hair and skin, and most important people will be happy and will have a better outlook on life.

Nowadays, there are some people that allergic with the protein extracted from the marine life and animal because it generally associated with high fat content and because of that, when consumed in large amounts, it attributed to high risks of disease, including high blood pressure and heart diseases. In an addition, there are many of the protein extracted are came from the porcine sources that cannot be used as a component of some foods, due to religious barriers.

Besides that, there is no research have been focus on the optimum value of temperature and liquid solid ratio of protein concentration from the *C.fuciphaga*.

## 1.3 OBJECTIVES

- The main objective of this research is to characterize and optimize protein extraction yield from *C.fuciphaga*.

  .
- In this research also have a few specific objectives. The specific objectives are:

  - To establish how temperature influence extraction protein yield
  - To establish how liquid solid ratio influence extraction protein yield
  - To optimize the extraction condition to produce higher extraction protein yield

## 1.4 SCOPES OF THE STUDY

- This research is focusing on the characterization of *C. fuciphaga* using the Fourier Transform Infrared Spectroscopy (FTIR) and inductively coupled plasma (ICP).
- Study the effect of two parameters, ratio of the C. *fuciphaga* with the distilled water (LSR) from 10:1 to 50:1 (v/w) and the temperature of the extraction from 25- 65$^{o}$C.

- Response surface methodology (RSM) is uses to determine the optimal condition of the extraction temperature and the ratio between distilled water and the *C.fuciphaga* for the protein extraction.

## 1.5 RATIONALE AND SIGNIFICANCE

Protein bioseperation, which refers to the recovery and purification of protein products from various biological feed streams, is considered to be one of the most important unit operations in the bioprocess industry. This is mainly because of the phenomenal development in the field of modern biotechnology and the increasing demand for more and more protein products to be purified in larger quantities (Daliya *et al*., 2007).

Previous studies by many researchers extract protein using pigeon pea (Ivone *et al*., 2000), germinant pumpkin seeds (Li *et* al., 2005), red pepper seed (*Capsicum frutescens*) (Ebru *et al*., 2010) and soybean (Rosenthal *et al*., 2001). There is no prior research that has been study about the optimum condition of extraction temperature and liquid solid ratio for the protein extracted from *C. fuciphaga*. Besides that, the sample is not from the marine life and animal source so it do not causes allergic to people and associated with high fat content. Therefore it is suitable for all ages including the pregnancy mother and the people that allergic with the protein extracted from the marine life. Besides that, this material is a halal product. So, it is suitable for Muslim and Vegetarian people.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 SWIFTLET FARMING INDUSTRIES IN MALAYSIA

The past 5 years ago, there is a new developments in the swiftlet farming industry and the industry is new in Malaysia as compared to other central and long-standing industries such as palm oil, oil and gas, timber, rubber and financial services. The swiftlet farming industry in Malaysia is a sudden becomes broader after the Asian Economic Crisis of 1997-1998. Many businesses, especially small to medium sized businesses, at that time experienced hard times and some of the business was close down. The places where the businesses reside were left clear due to the fact that no other businesses had sprung up to take their place as a result of the depressed economic environment at that time. Malaysia is currently the third largest producer of edible birds' nests (7% of gross supply value) in the world, behind Indonesia (60%) and Thailand (20%). (Hameed, 2007).

The swiftlet farming industry has a potential to bloom into a multi-million ringgit industry due to the industry's relatively profitable risk-return profile as well as a continuously growing request for edible birds' nests by the overseas countries. There is also a discernable world-wide trend pursued by international as well as home grown pharmaceutical and herbal products companies in using edible birds' nests as base materials for producing natural and organic health supplement products for local and overseas consumption. Currently, the business of swiftlet farming essentially involves

the conversion of people-centric buildings into buildings used to house and protect a certain species of swiftlets (i.e. the white edible birds' nests swiftlets or the Aerodramus Fuciphagus species of swiftlets) that can only be found in the South East Asian region as well as the design and construction of purpose-build buildings for the purposes of accommodating such swiftlet populations as well (Hameed, 2007).

A continuous vocalization of swiftlet chirps and a mating sound are performed using speakers and audio systems installed every day within such buildings in order to lure the swiftlets that are flying overhead to fly into the said buildings to mate and make the buildings their new home. Almost 99% of all swiftlet farms in Malaysia are geared towards the production of white edible birds' nests (Hameed, 2007).

The swiftlet farming industry in Malaysia only started to gather momentum after the Asian Economic Crisis of 1997-1998. During that period, many businesses, especially small to medium sized businesses, experienced hard times and a great number of them closed down throughout the country. The premises that these businesses were located in were left empty due to the fact that no other businesses had sprung up to take their place as a result of the depressed economic environment at that time. Rather than leave their properties idle, quite a number of the landlords for these properties then had decided to convert their untenanted properties into swiftlet farms. At that time, there was only one research and development company specializing in the establishment of swiftlet farms in Malaysia and had almost single-handedly aided and helped grow the industry into becoming what it is today (Ibrahim *et al*., 2009).

Over the last 20 years, the swiftlet farming industry in the country basically grew through mostly private funding and operational initiatives and without any help whatsoever from the public sector. The major swiftlet farming areas are located mostly in secondary and tertiary townships where food source is in abundance and pollution levels are at their relative minimum. These secondary and tertiary townships include Kampong Tebing, Kampong Tasoh, Kampong Banat Bawah, Jampong Bakan, Kuala Nerang, Pokok Sena, Kampong Tanjung Radin, Kuala Ketil, Lunas, Kulim, Sungai Petani, Jitra, Bukit Mertajam, Nibong Tebal, Kepala Batas, Cangkat Kledang, Legong, Jelai, Cangkat Jering, Bruas, Pantai Remis, Lumut, Teluk Intan, Setiawan, Bagan Serai,

Parit Buntar, Selama, Tanjung Malim, Kuala Kubu Bahru, Rawang, Kepong, Cheras, Slim River, Kulai, Kanpong Bahru Paroi, Alor Gajah, Ayer Pasir, Durian Tunggal, Tangkok, Pagoh, Bukit Pasir, Kampong Machap, Ulu Tiram, Tai Hong Village, Senai, Pontian Kecil, Jemaluang, Kampong Seri Pantai, Mersing, Kampong Sawah Datuk, Kampong Air Papan, Kuala Besut, Tok Soboh, Kampong Pinang, Rompin, Pekan, Kuala Terengganu and Pasir Mas (Hameed, 2007).

In its essence, a swiftlet farm is a place in which edible white nests swiftlets mate, build their nests, raise their younglings and live in. Swiftlets had traditionally lived in caves. With their migration into the city and town centres through the years, these swiftlets will find places to live that are not dissimilar to that of their natural cave environment. Therefore, all swiftlet farmers have endeavored to design, construct and renovate their swiftlet farms in ways which will control the light intensities, humidity levels, air flow standards, pressure levels, safety perceptions, heat standards, odors and smells and swiftlet flight-paths in order to mimic swiftlet cave environments so as to encourage swiftlets to nest within the said farms.

Once a swiftlet farm has been completely constructed, swiftlet mating sounds and swiftlet chirps are played using audio systems through tweeters in order to ‘advertise’ to swiftlets flying above the new swiftlet farm that there is a new place for them to stay. These new swiftlets will then nest on wooden planks and lay eggs. Throughout the last 20 years or so, many technological advances, swiftlet farm design leaps as well as improvements in the behavioral characteristics of edible nests swiftlets have been made by the participants of the swiftlet farming industry in the areas of swiftlet farm design and construction, audio systems, mating and chirping sound identification and modulation, swiftlet flight-paths within farms and tweeter design (Hameed, 2007).

Many owners of swiftlet farms whose farms are located in close proximity to each other (within a 5km radius) are constantly trying to outdo their neighborly competition by implementing more and more scientifically researched and developed swiftlet farming products within their swiftlet farms in order to attract swiftlets from the surrounding competing farms into theirs. A stage has almost been reached whereby it is

now becoming more of a scientific endeavor of luring swiftlets to nest within a swiftlet farm as compared to the more unsuccessful and traditional 'hit-or-miss' method of swiftlet farming.

## 2.2 *COLLOCALIA FUCIPHAGA*

These birds (Figure 2.1) are found only in the Southeast Asian region. The nests are built almost exclusively by the 7–20 g male swiftlet over a period of approximately 35 days during breeding season. The salivary nest cement is the most important ingredient in the edible bird's nest and is undoubtedly one of the most expensive food ingredients in the world. They construct their nests with a glutinous nest-cement produced by a pair of large, lobed salivary glands under the tongue. It is this nest-cement that constitutes the raw material of bird's nest soup and renders the nest its commercial importance (Massimo, 2005).

**Figure 2.1:** Edible-nest Swiftlet

The nests are composed mainly of glycoprotein. The carbohydrate component consists of 9% sialic acid, 7.2% galactosamine, 5.3% glucosamine, 16.9% galactose,

and 0.7% fucose. The most abundant amino acids present are serine, threonine, aspartic acid, glutamic acid, proline, and valine. One study has shown the presence of a glycoprotein capable of promoting cell division, and another has demonstrated the presence of an epidermal growth factor–like protein (Denise *et al*., 2001).

**Figure 2.2:** *Collocalia Fuciphaga*

Harvesting of the edible bird's nest for human consumption is a painstaking and often times dangerous operation for local collectors. Most nests (Figure 2.2) are built hundreds of feet up on cave walls and require the use of temporary scaffolding made of locally collected bamboo or ironwood. After collection, the tedious process of cleaning approximately 10 nests takes a person approximately 8 h (Koon *et al.,* 2002).

Bird's nest is rich in protein. Protein is vital for tissue growth, maintenance and repair, muscle contraction and oxidation functions. Proteins are composed of 20 amino acids, 11 of which can be synthesized by human body (non-essential), and 9 have to be obtained through food (essential) (Table 2.2). Bird's nest contains 18 amino acids, including ALL 8 essential amino acids (Massimo, 2005). Practitioners of traditional Chinese medicine have consistently indicated that the consumption of birds' nest soup

is beneficial for the treatment of a variety of health problems. It is often administered to the elderly and very young alike who are recovering from various types of infections (Koon *et al.*, 2002).

**Table 2.1: Nutritional Contents of Bird's Nest**

| ***Amino Acids in Bird's Nest*** (source: Massimo, 2005) | |
|---|---|
| **Essential** | **Non-Essential** |
| · Isoleucine | · Alanine |
| · Leucine | · Arginine |
| · Lysine | · Aspartic acid |
| · Methionine | · Cysteine |
| · Phenylalanine | · Glutamic Acid |
| · Threonine | · Glycine |
| · Tryptophan | · Histidine |
| · Valine | · Proline |
| | · Serine |
| | · Tyrosine |
| | · Asparagine |

Due to the highly evaluated function both nutritiously (water-soluble protein, carbohydrate, iron, inorganic salt and fiber) and medically (anti-aging, anti-cancer, immunity-enhancing, etc). It was even referred as "Caviar of the East". Current scientific study confirmed that *C.fuciphaga* has haemagglutination inhibiting activity against the influenza virus and contained avian epidermal growth factor (Marcone, 2005; Lin et al., 2006). Nowadays with the help of modern commercialization and technology, *C.fuciphaga* was developed into various food products including drink, food additive, and even cosmetic ingredient. At present *C.fuciphaga* raw material mainly comes from Asian countries, such as China, Malaysia, Indonesia, Vietnam, Thailand, Philippine and most *C.fuciphaga* products were consumed in these areas as well as in North America. Trade scale of global market has been going up for decades. In order to improve the

quantity and quality of *C.fuciphaga* and to protect the natural environment of swiftlet residence, man-made bird house was built widely (Yajun *et al*., 2010).

It is considered as a great delicacy and effective medicine as well as beauty enhancer within the Chinese community throughout the world. The earliest history of recorded edible-bird nest trading can be traced back to the year 1589 (Yeap, 2002). Admiral Cheng Ho sailed to South East Asia and brought back edible-bird nests from Indonesia as a gift to the Ming Dynasty's Emperor which opened up the trade of this valuable nest. Some researchers have even stated that the trade can be traced back 1000 years ago during the Tang Dynasty (A.D. 618-907).

## 2.3 PROTEIN EXTRACTION

Proteins which have found applications in food and pharmaceutical industry may be produced as protein isolate or as protein concentrate. After many separation steps in aqueous extraction process, the bulk of proteins may be recovered as concentrate in the solid phase or as isolate in the aqueous phase depending on many parameters of the extraction medium(Tiezheng *et al*.,2010). It has been reported that the low extraction yields of aqueous processes can be overcome by using enzymes that hydrolyse the structural polysaccharides forming the cell wall of oilseeds, or that hydrolyse the proteins which form the cell and lipid body membranes(Rosenthal *et al*.,2001). Protein molecules tend to unfold and even become fully denatured under unfavoured conditions, such as a high temperature, an acidified condition, a high pressure or even excessive shear. Denatured protein molecules will aggregate and/or crosslink to form larger clusters and, at high concentrations, will form a three-dimensional solid-like network (or a gel). Therefore, proteins are regarded as one of the main classes of building blocks used in many semi-solid foods for conferring mechanical properties (Dickinson, 1997).

Protein molecules tend to unfold and even become fully denatured under unfavoured conditions, such as a high temperature, an acidified condition, a high pressure or even excessive shear. Denatured protein molecules will aggregate and/or crosslink to form larger clusters and, at high concentrations, will form a three-dimensional solid-like network (or a gel). Therefore, proteins are regarded as one of the

main classes of building blocks used in many semi-solid foods for conferring mechanical properties. Proteins are also recognised as one of the main classes of surface-active agents in liquid foods for stabilising dispersed particles and fat droplets, due to the polarised distribution of hydrophobic and hydrophilic groups along the back bone. Protein molecules adsorb at the oil–water interface to lower the interfacial tension and, therefore, make such thermodynamically less favourable dispersed systems stable for an extended shelf life. The importance of protein application in foods can also be seen in many other aspects. For example, it was reported that, in combination with polysaccharides and starches, proteins could be applied as a meat alternative, as a fat replacer or a filler in manufacturing healthier food (Lianqing *et al*., 2008)

Proteins are also recognised as one of the main classes of surface-active agents in liquid foods for stabilising dispersedparticles and fat droplets (Dalgleish, 1997), due to the polarised distribution of hydrophobic and hydrophilic groups along the back bone. Protein molecules adsorb at the oil–water interface to lower the interfacial tension and, therefore, make such thermodynamically less favourable dispersed systems stable for an extended shelf life. The importance of protein application in foods can also be seen in many other aspects. For example, it was reported that, in combination with polysaccharides and starches, proteins could be applied as a meat alternative, as a fat replacer or a filler in manufacturing healthier foods (Roger, 2001).

Huge efforts have been made in extracting proteins from various sources for food applications. So far, proteins from two major sources (milk proteins and soy protein) are most widely used in the food industry, either as a general nutrients supply or as functional ingredients.Milk proteins (e.g., whey proteins or caseins) are probably the most commonly used proteins in all major types of food products. As from non-animal source, soy protein becomes increasingly used in food products because of its health benefits and characteristic physico-chemical properties (Endres, 2001). Proteins from other sources have also been studied for their functionality and potential food applications. Examples include corn protein (Myers *et al*., 1994), wheat protein (Hettiarachch *et al*., 1996), rice protein (Morita *et al*., 1996), seaweed protein (Fleurence, 1999), protein from yeast (Ganeva *et al*., 1999), fish protein (Afonso *et al*.,

2004), etc. However, the applications of these proteins were still very limited, either due to the limited source of supply or the non-satisfactory functionality.

Proteins also have special uses as foaming agents or as functional ingredients for nutrient delivery in foods (Chen *et al*., 2004). The ongoing development in protein extraction, purification and identification methods has significantly advanced our ability to address an increasing number of biological questions using proteomic approaches. Two-dimensional gel electrophoresis (2- DE) is one of the most widely used techniques for resolving complex protein extracts. The extraction of comprehensive protein populations from plants is particularly challenging due to the metabolic and structural characteristics of plant tissues, including the plant cell wall matrix. With the exception of mature and dormant structures, i.e., seeds and pollen, most plant cells have relatively low protein content, and are also rich in proteases and oxidative enzymes (Inder *et al*., 2009). Protein extraction from oat bran will increase the concentration of B-glucan or soluble fibre and in turn the value of oat by-product. The alkali method is reported to be the most commonly used procedure for protein extraction from oat flour (Xiao *et al*., 2008).

## 2.4 TYPES OF PROTEIN EXTRACTION

There are two types extraction used in the extraction of protein. There are the extraction using chemicals and enzymes.

### 2.4.1 Chemicals

The use of chemical has been widely recognised as a feasible method for protein extraction from plant sources. However, its effectiveness depends highly on the extraction conditions such as, agent concentration, extraction temperature, extraction time, volume–weight ratio between the extraction solvent and the raw material. Table 2.2 show the examples of chemicals used in the protein extraction.