FUNCTIONAL PROPERTIES OF WASTES FROM CABBAGE (*Brassica oleracea* L. var. *capitata)* AND CAPSICUM (*Capsicum annuum* L. var. *annum)*

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By

LIANG JIA LUN

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ABSTRACT

FUNCTIONAL PROPERTIES OF WASTES FROM CABBAGE (*Brassica oleracea* L. var. *capitata)* AND CAPSICUM (*Capsicum annuum* L. var. *annum)*

Liang Jia Lun

Waste generation by individual has become an irrefutable source of environmental issue at this modern era. Within 23 developing countries including Malaysia, every person could produce wastes up to 77 kg per day in which 45% is made up of food waste. At the same time, the threat from non-communicable diseases (NCDs) is emerging which eventually increases the financial burden of medical care. Overweight and obesity, are the risk factors of NCD that need to be coped with as highlighted by the Ministry of Health. Malaysia is claimed to be the country with the highest overweight and obese population in South-East Asia. Capsicum seed core and cabbage outer leaves are wastes that normally disposed from the food-related industry and households. We postulated that these wastes possess similar functional properties as the edible parts of the vegetables. We tested the *in-vitro* bile acid binding of the freeze-dried and pulverized vegetable wastes. We extracted crude phytochemicals from the wastes and analysed the antibacterial, antioxidant and anti-obesity activities. Two methods of extraction (solvent extraction, SE; and ultrasound-assisted solvent extraction, UASE) were used.

We applied Kirby-Bauer Assay, DPPH (α-Diphenyl-α-Picrylhydrazyl) radical scavenging activity and lipase inhibiting assay on the extracts. Both capsicum seed core and cabbage outer leaves that underwent UASE exhibited a higher reaction activity than that of SE. The extracts also showed concentration-dependant significant effect on most of the mentioned assays conducted. However, in terms of lipase inhibition assay, the negative effect shown in extract of cabbage outer leaves with solvent extraction (ScabL) suggested the possibilities of the presence of antagonists. The present study indicates that both capsicum seed core and cabbage outer leaves are potential sources of phytochemicals which could be useful ingredients for nutraceutical and pharmacognosy sectors.

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Last but not least, I would like to thank my friends for their laughter and companionship as well as support during the days in the laboratory.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Liang Jia Lun

APPROVAL SHEET

The project entitled "**FUNCTIONAL PROPERTIES OF WASTES FROM CABBAGE (*Brassica oleracea* L. var. *capitata*) AND CAPSICUM (*Capsicum annuum* L. var. *annum*)**" was prepared by LIANG JIA LUN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

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(Ms. Teo Kah Cheng)	(Dr. Chang Ying Ping)
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Date: ……

FACULTY OF SCIENCE

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Date: _____

PERMISSION SHEET

It is hereby certified that **LIANG JIA LUN** (ID No: **13ADB00032**) has completed this final year project entitled "FUNCTIONAL PROPERTIES OF WASTES FROM CABBAGE (*Brassica oleracea* L. var. *capitata)* AND CAPSICUM (*Capsicum annuum* L. var. *annum)*" supervised by MS. TEO KAH CHENG from the Department of Biomedical Science and DR. CHANG YING PING from the Department of Chemical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

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Yours truly,

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(LIANG JIA LUN)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

a.k.a.	Also known as
ATCC	American Type Culture Collection
BC	Before century
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CH_4	Methane
CI	Critical Index
CO_2	Carbon dioxide
d.b.	Dry basis
DCA	Deoxycholic acid
DF	Dietary fibre
DHA	Docosahexaenoic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Effective concentration
etc.	Etcetera
et al.	Et alia (and others)
f.w.	Food waste
GAE	Gallic acid equivalent
GDP	Gross domestic product
HCl	Hydrochloric acid
HTC	Hydrothermal carbonisation
H_2SO_4	Sulphuric acid
IC	Inhibitory concentration

IDF	Insoluble dietary fibre
i.e.	Id est (that is)
ITC	Isothiocynates
LDL	Low density lipoprotein
MANS	Malaysian Adults Nutrition Survey
MHA	Muelier Hinton agar
MIC	Minimum inhibitory concentration
MSW	Municipal solid waste
NA	Nutrient agar
NaOH	Sodium hydroxide
NCD	Non-communicable disease
NHMS	National Health and Morbidity Survey
No.	number
ORAC	Oxygen radical absorbance capacity
PL	Pancreatic lipase
p-NPP	Para-nitrophenyl palmitate
ppb	Parts per billion
r	Coefficient of correlation
R^2	Regression coefficient
ScabL	Solvent extracted cabbage outer leaves
ScapC	Solvent extracted capsicum seed cores
SDF	Soluble dietary fibre
SE	Solvent extraction
UASE	Ultrasound-assisted solvent extraction
UcabL	Ultrasound-assisted cabbage outer leaves

UcapC	Ultrasound-assisted solvent extracted capsicum seed cores
U.K.	United Kingdom
U.S.	United States
USP	Ubiquitin-specific protease
WHO	World Health Organization
WRC	Water retention capacity

CHAPTER 1

INTRODUCTION

Waste generation by individual has become an irrefutable source of environmental issue. Khairuddin et al. (2015) has been stated that within 23 developing countries including Malaysia, every person could produce wastes up to 77 kg per day and the upper limit is expanding up to now. With the tremendous increase of world population, it is also estimated that the amount of municipal solid waste (MSW) generated would be nine million tons annually by year 2020 (Troschinetz and Milhelcic, 2009). Although the average national recycling rate is about 3-5% per annum in each country, this sole number is insufficient to cover up the average waste producing rate from the citizens in Malaysia, which was recorded as 4.3% per year (Victor and Agamuthu, 2013).

From various researches done in studying the municipal solid wastes (MSWs) generation and population growth in Malaysia, It was reported that the average amount of MSW generated per capita per day has increased drastically from 0.5 – 0.8 kg (2003) to 0.5 – 2.5 kg (2014) (Johari et al., 2014). It was recorded also the total waste generated in peninsular Malaysia was 23000 tons/day in year 2010, 25000 tons/day in year 2012 and estimated to reach 30000 tons/day in year 2020 (Ministry of Housing and Local Government, 2010). From the total MSW generated, about 47% of the waste is made up of organic food

waste. As a result, accumulation of wastes and garbage has become a serious problem due to limited landfill for disposal, and also the enormous negative impacts exerted on living organisms globally. The problems include environmental pollutions, release of greenhouse gases, wastage of human resources and money, and others. (Abdullah et al., 2013; Khairuddin et al., 2015).

On the other hand, prevention and control of non-communicable diseases (NCDs) are always the focus of Ministry of Health Malaysia. From the annual report of NCD prevention and control 2011, from year 2006 to 2011, there were significant increases in prevalence of diabetes (11.6% to 15.2%), and hypercholesterolaemia (20.6% to 35.1%). Besides, from the total diabetes cases, about 39% of the cases were associated with hyperlipidaemia and about 4% were associated with obesity. Overweight and obesity are defined as excessive or even abnormal accumulation of fat that may harm normal body health (World Health Organization, 2015). Overweight or obesity is caused by imbalance between calories consumed and calories expended. Genetic factors may be a cause of this problem as well. WHO media centre stated that the worldwide obesity has increased for more than two folds since 1980. In the year 2014, about 39% of world population, which is more than 1.9 billion adults who aged 18 years old and above, were overweight. From this number, 600 million of them, i.e. 13%, were actually obese (WHO, 2015). Most importantly, the number of obese population is still increasing. Obesity leads to adverse health conditions including cardiovascular heart diseases and hypercholesterolaemia, the medical burden which needs to be borne by an

obese nation, is enormous. Finkelstein, Trogdon and Dietz (2009) found that obese people in United States spent about 40 billion U.S. dollars in their medical treatment, including $7 billion in prescription of drugs, in year 2006. This amount of medical burden was found to be one of the reasons to the rise in inflation-adjusted health spending in U.S.

In order to find out a possible resolution in mitigating the above stated problems, it was proposed that the usage of cabbage outer leaves and capsicum seed cores which normally thrown away by the farmers, could possess some health-promoting activities. The hypothesis statement was done by referring to the selected researches done on the edible parts of capsicum and cabbage on their functional properties. The researches carried out included antioxidant activities, antimicrobial effects, *in vitro* bile acids binding, total phytochemical content measurements, glucose retardation index, bacterial survival assay, etc. (Ayaz et al., 2008; Fuentes-Alventosa et al., 2009; Bajpal et al., 2011; Jaiswal, Abu-Ghanam and Gupta, 2011; Sotelo et al., 2015). Therefore, it is anticipated that the non-edible part (a.k.a. waste) of the cabbage and capsicum may contain the bioactive compounds that show the similar effects with that of the edible parts.

Capsicum (*Capsicum annuum*), or normally known as bell pepper, is widely cultivated in Africa, Mediterranean and Asian countries (Silva et al., 2013). *C. annuum* has been widely used in culinary decoration as well as spice and flavouring in food. Besides, capsicum has been recognised to be a rich source

of vitamins, minerals, capsaicinoids and alkaloids. This makes capsicum as one of the most important vegetables on the dining tables. Capsicum is present in different form of morphology due to their nutrient contents and genetic difference (Hill et al., 2013; Wahyuni, 2011). Among the colourful variety of the bell peppers, green and red bell peppers are the one frequently consumed. Depending on the texture, taste and customer requirements, their functions could be shifted in different occasions to become a spice, a colorant, and etc. Capsicum seed cores are one of the wastes or by-products of capsicum fruits. They were normally thrown away along with seeds as their texture and taste were not acceptable as a food material.

Cabbage (*Brassica oleracea*) is a common leafy vegetable which are cultivated worldwide. Fresh leaves of cabbage are always used as one of the ingredients in food especially in Chinese and French culinary. It has high environmental adaptability, nutritional values as well as easy to prepare and consume (Lisiewska et al., 2009). In addition, cabbage contains a rich resource of glucosinolates, phenolics, vitamins and minerals that has been proven to have significant health-promoting effects to human body (Zocca, Lomoloni and Lante, 2010; Banerjee, Renna and Variyar, 2015). Cabbage outer leaves are the initial by-products that normally trimmed by farmers before the cabbage is being marketed. The outer leaves are prone to be infested, discoloured due to chemical reactions, microbial decay, high exposure to pesticides as well as a hard texture to be consumed.

It was estimated that within the MSW produced, about 57% of the wastes were food waste, which includes meat, vegetables and fruits, no matter they were edible or non-edible (Khairuddin et al., 2015). The evaluation of the usable values in both capsicum seed core and cabbage outer leaves in this research could generally reduce the waste production at the same time save up part of the resources used to eliminate the wastes. The benefits go to the anti-obesity research also. There are some researches that focus on the genetic treatment of the obese people but failed to do so as genetic-caused obesity is due to multiple gene factors. Instead of targeting genetic defects, this research rather targeted on the potentially post-natal control of cholesterol deposition from the body emulsification process and inhibition of triglyceride digestion. In addition, other benefits of wastes usage such as antibacterial effects and antioxidant activities were also proposed in this research. These functional properties not only could allow the underutilization of selected plant wastes, but also raise their marketing value as a natural antibiotic alternatives or even becoming health-promoting supplements. The present study reports for the first time to evaluate the antibacterial, antioxidant and anti-obesity effects of capsicum seed cores and cabbage outer leaves.

There are five objectives in this research:

- To extract the secondary metabolites from capsicum seed cores and cabbage outer leaves through solvent extraction (SE) and ultrasound-assisted solvent extraction (UASE).

- To compare the activity of secondary metabolites extracted from SE and UASE.
- To determine the antibacterial effect of the sample extracts.
- To measure the antioxidant capacities of the sample extracts.
- To evaluate the anti-obesity effects of the samples through lipase inhibition and *in vitro* bile acids binding capacities.

CHAPTER 2

LITERATURE REVIEW

2.1 Rationale of Making Use of Wastes

As the number of global population increases drastically, economic development, along with the rapid industrialisation and urbanisation, has caused the ever changing consumption patterns of the citizens (Tan et al., 2015). This was shown apparently in food-related fields. From the food producing industry to the food servicing industry, the advancement of civilisation has brought us huge tonnes of municipal solid wastes (MSWs).

Waste was defined as the residues of low or no values that are being discarded after the desired products were processed. MSW, commonly known as rubbish or refuse, is normally produced from commercial, residential and institutional areas. Currently, MSW is generated at a rate which imposed a threat environment conservation due to it has exceeded the rate of disposal or treatment by the local municipal authorities or rate of natural decomposition (Tan et al., 2015).

Zaman (2016) has studied the municipal solid waste management system of 172 countries with a total population of 3.37 billion. It was indicated that the population generated about 1.47 billion tonnes (436 kg/cap/year) of municipal

solid waste each year and waste generation is increasing over time. Besides, the study also found that there was a positive correlation ($r = 0.539$, $p < 0.05$) between per capita income gross domestic product (GDP/capita/year) and per capita waste generation (kg/capita/year) and a similar correlation is also observed ($r = 0.653$, $p < 0.05$) between per capita income (GDP/year) and per capita resource recovery (kg/year) (Zaman, 2016).

Meantime, surveys showed that approximately 160 billion kg of edible food are available for annual human consumption in United States of America (U.S.). However, approximately 30% of the food will be wasted or lost due to disposal from farmers, retailers, markets, restaurants and consumers (Kosseva, 2011). The same goes to United Kingdom (U.K.) and Japan, who tossed away about 30%-40% of their food produced every year. While in Malaysia, the situation is worse due to the growth in economy and technology advancement. Data indicated that from year 1997 to 2007, the amount of MSW produced per year was increased from 5.6 million ton to 7.65 million ton, with an increase of 28%. In addition, almost half (47%) of the total MSW was made up of food waste (Noor et al., 2013).

2.1.1 Environmental Issues Caused by Wastes

Various field of industries especially food-related industry produces huge amount of wastes daily from the production, preparation and packaging process. These wastes require extra space and cost of management. To remove

the accumulated waste products, municipal authorities had come to some solutions including landfilling, anaerobic digesting, incinerating, animal feeding and etc. These approaches impose respective deleterious effect to the environment.

According to Abdullah et al. (2013), disposal of waste to the landfills has caused various problems like ground water pollution and easy putrefaction of other organic molecules due to large high-moisture content of the wastethat induce microbial growth. During waste degradation, at least 50% of the total carbon in MSW be converted into leachate and released as landfill or greenhouse gases that lead to global warming (Zhang, Yue and Nie, 2012). Huber-Humer, Kjeldsen and Spokas (2011) reported that the organic carbon within the MSW is partly degraded by microbes and resulting in the emissions of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4), albeit the amount is low if compare to the carbon dioxide released by power plants and transport.

Generally, approximately 90% of the carbon atom is converted into landfill gases in a bioreactor while the remaining 10% will remain in the leachate. Referring to Huber-Humer, Kjeldsen and Spokas (2011), though carbon dioxide will be released during waste degradation, the amount released into the atmosphere is negligible. Methane gas, is a powerful greenhouse gas as the warming potential of methane gas is 25 which the number is 25 times higher than the warming potential of carbon dioxide estimated, contributed by its

longer atmospheric residence time and strong molar absorption coefficient for infrared (Huber-Humer, Kjeldsen, and Spokas 2011). Research conducted by Solomon et al. (2007) also reported that the global atmospheric concentration of CH_4 has increased dramatically since 1990 to 2005, from 715 up to 1732 parts per billion (ppb), due to anthropogenic activities such as combustion, excavation, industrial revolution, intensive livestock farming and so on.

On the other hand, incineration also emits greenhouse gases as a result of extensive combustion at high temperature. While for anaerobic digestion, besides its greenhouse effect, the wastewater produced from the digestion process often causes eutrophication and acidification of local aquatic ecosystems, leading to the deaths or retarded growth of aquatic lives (Aparicio et al., 2016; Salemdeeb et al., 2016).

2.1.2 Significance of Waste Re-Usage

In recent years, there is a remarkable demand for appropriate nutritional standards on the quality of the bio-based products. This can be characterised by the decreasing availability of commercialised health-promoting products and the rising cost of raw materials together with great concern about environmental issues including pollution and wastage (Laufenberg, Kunz and Nystroem, 2003). As a result, studies that focus on the recovery, reuse and utilisation of waste are being emphasised. This approach is valid and workable for food wasted from food processing industry as the manufacturing practice

enable to the recovering or homogenous by-products and upgrade those by-products, residues or wastes into more useful products with marketable value.

It is anticipated that the study on the functional properties of waste could be potentially useful in contributing to its applications in pharmacognosy, agricultural, nutraceutical or even medicine fields. With the different chemical composition contained in the wastes, the possibility in discovering various functional ingredients would be greatly increased.

2.1.3 Relevant Researches

There are many research as conducted successfully determining the functional properties of vegetable wastes. Genevois, Flores and de Escalada Pla (2016) used pumpkin by-products as a substrate for the growth of *Lactobacillus casei* even at low water content, making the pumpkin waste possible to become alternative material for producing growth media. Martinez et al. (2012) carried out research on the chemical, technological and *in vitro* antioxidant properties determination from fibres of various fruits wastes. Rakholiya, Kanerua and Chanda (2014) also reported that extracts from fruit and vegetable peels (*Citrus limon*, *Manikara zapota*, and *Carica papaya*) inhibit the growth of various types of microorganism, including bacteria and fungi.

Ma and Mu (2016) also reported that from the animal study conducted, both soluble and insoluble fibres from deoiled cumin waste give significant effects

on type 2 diabetes rats besides able to decrease hepatic lipogenesis. Soluble dietary fibres exhibited better effects in improving blood glucose and showed higher anti-diabetic activity.

2.2 Capsicum and Cabbage

Capsicum and cabbage are commonly found and consumed in Malaysia. They are easily to be cultivated in tropical-seasoned countries and widely used in a variety of fields.

2.2.1 Origin, Characteristics and Nutritional Values

Bell pepper or sweet pepper (*Capsicum annuum* L. var. *annum*) has been used in the human diet since 7500 BC and grown by Native Americans between 5200 to 3400 BC (El-Ghorab et al., 2013). It is a non-pungent type of pepper that found ubiquitously in India. *Capsicum annuum* is a kind of vegetable that has thick-walled fruits, non-pungent pericalp and placenta tissues. Bell peppers can be found in different colours which is the major factor contributed to customer purchasing decisions. Carotenoids and flavonoids are colorants in bell peppers that impart red and orange colours to the fruits. Green coloured pepper is mostly due to the composition of chlorophylls. Yellow pepper is formed due to the presence of zeaxanthin, lutein α- and β-carotene (Sun et al., 2007). The fruit can either be consumed in raw, cooked, matured or immature form. It also responsible to be the most important crop in India as well as the

world's second most important vegetable from *Solanaceae* family next to tomato (Vengaiah and Pandey, 2006).

Bell pepper has widely been used in India, whether it is used as medicine, food, chutney, or even in ritual ceremony (Vengaiah and Pandey, 2006). More often, peppers are utilised as a colouring agent, whether by the food or in cosmetic industry. Oleoresin is an ingredient inside Capsicum sp., which being extracted out from the fruits and marketed as varieties of commercial products, including liniments, bugs repellents and etc. (Vengaiah and Pandey, 2006; Silva et al., 2013).

Sweet pepper is an excellent source of vitamin A and C. The provitamin A and C contained inside the fruits will increase as the pepper becoming more mature (Vengaiah and Pandey, 2006; Topuz and Ozdemir, 2007). Besides, bell pepper also consists of variety of bioactive compounds including phenolic compound, flavonoids and carotenoids which well-known of antioxidant capability. In addition, these compounds are also proven to have therapeutic effects on human health, especially in the prevention of gastric ulcer and cardiovascular disease, simulation of the immune system and protection against age-related macular degeneration and cataracts (Howard et al., 2000; Materska and Perucka, 2005; Sun et al., 2007; Sgroppo and Pereyra, 2009; Silva et al., 2013; Materska, 2014).

Cabbage (*Brassica oleracea* L. var. *capitata*) is considered as the most widely grown and most important vegetable among *Brassicaceae* family that consumed worldwide. The centre for origin of cabbage is recognised at north European countries, around the Baltic Sea coast and the Mediterranean region (Kibar, Karaağaç and Kar, 2016). The high adaptability and ease in cultivation of the cabbage has made cabbage to become widely cultivated throughout the world.

Apart from their economic importance, cabbage is rich in vitamins, fibres and minerals (Šamec et al., 2011). Besides, it consists of high-levels of sulphur containing amino acids, antioxidants, and even anti carcinogenic compounds (Singh et al., 2010). Phytochemical-analysis has shown that white cabbage is rich in phenolic compounds, carotenoids and glucosinolates (Bhandari and Kwak, 2015; Kusznierewicz et al., 2008). This has made fresh cabbage, either prepared separately or mixed with other vegetables such as tomatoes and onions in the form of fresh juice or vegetable soup that often incorporated in commercial weight-loss diets (Greenly, 2004; Rokayya et al., 2013).

It was suggested that diets contain cabbage could be alternative therapies for cancer patients as well as to improve the bioavailable content of non-heme iron (Šamec et al., 2011). Since cabbage has been proven scientifically on its anti-inflammatory and antibacterial effects, it is not surprise to find its application in traditional medicine, such as treatment of minor cuts and wounds and mastitis, reducing serum LDL levels as well as in palliation of

disease symptoms that associated with gastrointestinal disorders (irritable bowel syndrome, gastritis, peptic and duodenal ulcers) (Šamec et al., 2011; Suido et al., 2002).

2.2.2 Generation and Post-Harvesting Handling of Wastes

According to Kiaya (2014), post-harvest lost refers to the degradation in both quantity and quality of a food production from initial harvest stage to consumption. Quality losses are loss of food due to lacking of nutrient/caloric composition, the acceptability, and the edibility of a given product, while quantity losses include any circumstances that will result in the loss of the amount of a product (Kiaya, 2014). According to Gustavsson, Cederberg, Sonesson (2011), the study suggested that roughly one third of food produced for human consumption is lost globally, recorded about 1.3 billion tons per year. Foods are wasted in consumption stages due to different consumer behaviour and a lack of coordination between different roles in the supply chain. In the early to middle stages of the food supply chain, food loss is due to managerial, financial and technical issues, including insufficient harvesting facilities, inadequate harvesting techniques as well as lacking information on climatic conditions and marketing systems (Gustavsson et al., 2011). Others causes of post-harvest lost includes lack of proper care, use of inappropriate harvesting equipment, disease, pest invade, and loss of interest in improving the quality of handling techniques (Kasso and Bekele, 2016).

The wastes produced from every stage of the food chain supply will either being disposed to dumping site or waste processing factory for further separation or post-harvest utilisation. In recent years, other than landfills, post-harvest handling of waste emphasises the re-usage of plant wastes to become feed sources for ruminants (silage) (Özkul, Kılıç and Polat, 2011). This is because most of the fruit and vegetable wastes have high moisture content that will rot in 3-4 days if left untreated. However, they are also full of soluble carbohydrates that could be easily eaten and digested by animals which made the ensilage to become a convenient and easy way for feeding. Besides, industries also convert wastes into biofuels using various methods, including transesterification of fats and oils from waste to produce biodiesel, anaerobic digestion to produce methane-rich gas (biogas), fermentation of carbohydrates to produce bio-alcohols, dark fermentation to produce hydrogen from carbohydrates, pyrolysis and gasification of wastes into liquid or gas fuels as well as hydrothermal carbonisation (HTC) of by-products into energy-rich source. Nevertheless, production of biomaterial through fermentation of carbohydrates from food wastes also being introduced as one of the ways in post-harvest waste handling (Girotto, Alibardi and Cossu, 2015).

2.2.2.1 Capsicum Seed Cores

Capsicum seed core refers to the fair placenta that located in the inner, centre part of a capsicum. It was formed by axile plancentation and normally compartmentalises the capsicum into three lobules. Since the capsicum seed core may contain up to 89% of alkaloid capsaicin, burning sensation could be

felt when touched. Besides large amount of seeds will be attached on the capsicum seed core, miniature sterile fruits will also developed from the placental wall (Tiwari et al., 2011).

Capsicum seed core are normally being discarded during processing to pre-consumption stage. This may be due to the pungent and spiciness of the core as well as the texture is not preferable by the consumers. Therefore, the seed cores are usually thrown away directly for landfilling, anaerobic digesting or incinerating. Animal feeding by using capsicum seed cores is prohibited as the pungency may cause gastrointestinal symptoms on the animals (Myers, Smith and Graham, 1987).

2.2.2.2 Cabbage Outer Leaves

Cabbage outer leave refers to the big, dark green coloured outer leaves that split opened from the centre core. Normally the outer leaves will be removed before selling at the market or further processing at the food-processing industry (Tanongkankit, Chiewchan and Devahastin, 2010). This is due to the evolution of customer consuming pattern that they prefer to consume the softer inner part of the cabbage but not the outer leaves that they might found the texture is hard to masticate and swallow.

Due to high nutrient contents such as anti-carcinogenic compounds, phenolic compounds, Vitamin C and high fibre content inside the cabbage outer leaves,

they are not discarded into landfill or incinerating, but they are normally used in animal feeding or fertilizer (Chaisamlitpol et al., 2014). Older generations will also tend to use the outer leaves to act as anti-inflammation drug to treat small wound and cut (Šamec et al., 2011).

2.2.3 Related Researches Conducted

Limited researches showed there are multiple nutrients and secondary metabolites inside cabbage outer leaves. Yet, the functional properties of cabbage outer leaves are still undiscovered. However, there are no researches had been done in investigating the nutrient content and functional properties of capsicum seed cores. We anticipated that both capsicum seed core and cabbage outer leaves may consist of similar functional properties with that of edible part of the plants. Therefore, literature search on both edible parts and wastes of cabbage and capsicum was carried out and reported in the following subtopics.

2.2.3.1 Compositional Analysis

There are numerous studies carried out on the composition of white cabbage (*Brassica oleracea* L. var. *capitata*) outer leaves. The moisture content determination of cabbage outer leaves was carried out by Chaisamlitpol et al. (2014) showed that the initial moisture content in the fresh leaves was about 10.20 ± 0.53 kg/ kg (dry basis). This figure is similar with that of research

conducted by Nilnakara, Chiewchan and Devahastin (2009), which recorded as 13.63 ± 2.61 kg/ kg (d.b.).

Chaisamlitpol et al. (2014) reported that in the microwave assisted extraction, the glucosinolate content of cabbage outer leaves was about 842.15 µmol/ 100 g (d.b.). It was reported that the total glucosinolates content decreased upon hot air drying above 60^{O}C due to the loss of function of myrosinase, which hydrolyse glucosinolates into sulforaphane. However, minimum activity of glucosinolates was detected suggested that the presence of activity at high temperature.

Chaisamlitpol et al. (2014) also reported that the total sulforaphane content of fresh outer leaves is 21.19 mg/ 100 (d.b.). This figure is much higher than those reported in the similar researches carried out by Lekcharoenkul et al. (2014) and Tanongkankit et al. (2011), recorded as 13.90 and 1.23 mg/ 100 g (d.b.). The drying process at temperature exceeding 60^{o}C recorded a decrease in sulforaphane activity.

Phenolics are polar compounds found in plant extract which normally associated with other bio-molecules such as chlorophyll, lipid, protein and inorganic compounds. Total phenolic content of fresh cabbage outer leaves was 1919.97 mg GAE/ 100 g (d.b) (Chaisamlitpol et al., 2014). The drying

process exceeding 60ºC significantly decreased the phenolic content of the dried cabbage outer leaves.

Flavonoids are less polar if compared to phenolics. They are found inside the vacuoles of the cells (Ksibi et al., 2015). Flavonoid aglycons were quantified by Howard et al. (2000) in capsicum species after acid hydrolysis with standard recoveries of 78 and 99% for quercetin and luteolin, which are antihistaminic flavonoids. It was detected that the total flavonoids decreased from 31.71 to 23.15 mg/ kg during maturation in plants investigated. This might due to metabolic conversion of flavonoids into other secondary metabolites or degradation via enzymatic actions (Howard et al., 2000). Howard and the team found out that the major flavonoids in the capsicum were β-cryptoxanthine, α- and β-carotenes, provitamin A, capxanthin, zeaxanthin and lutein. It was observed that the level of carotenoids increased exponentially when maturation but lutein level decreased to an undetectable level after the capsicum has fully matured.

According to Nilnakara, Chiewchan and Devahastin (2009), total vitamin C content of cabbage outer leaves was 31.97 ± 0.51mg/ 100 g (wet matter) or 532.85 ± 8.49 mg/ 100 g (dry matter). This figure was supported by Chu et al. (2002), which recorded 27.32 mg/ 100 g. Nilnakara and the team (2009) also suggested that vitamin C will be degraded at high temperature and very soluble in water. This is proven by the 35% loss of vitamin C during blanching.

In capsicum, total phenols, ascorbic acid, total chlorophyll, and sugar content were tested by Sgroppo and Pereyra (2009). According to them, one of the reasons that caused phenolic accumulation is due to the wounding of the plant. The phenolic compounds from the plants will normally be oxidised upon wounding and subsequently initiate repairing mechanism. The repairing process was reported that will affect the flavour quality and colour of the fruits. The reported concentration of total phenol inside capsicum was 0.890 ± 0.200 mg chlorogenic acid equivalent kg^{-1} (f.w.) (Sgroppo and Pereyra, 2009). The post-harvest heat treated bell peppers at 55 and $60^{O}C$ showed 24% increase in total phenolic content which might due to the heat inducing damage on the peppers.

Total ascorbic acid content of pepper was between 47.83 and 86.58 mg/ 100 g at pH 5.81 (Sgroppo and Pereyra, 2009). It was proved that ascorbic acid is more stable in acidic condition but a significant decay of ascorbic acid was observed when it was stored at $0^{O}C$ for about 10 days. The decay might due to accelerated enzymatic activity resulted from cellular disruption. Generally during maturation, the ascorbic acid content either increased or maintained (Howard et al., 2000).

Sugars including glucose, fructose and sucrose are mainly found in bell peppers, which gives the taste of vegetables. Study from Sgroppo and Pereyra (2009) showed that heat treatment of the capsicum did not affect the sugar content, which roughly recorded as 3.792 mg/ 100 g when stored at $4^{O}C$ and

2.87 mg/ 100g when stored at 10^{O}C. Chlorophyll is the major colour pigment in peppers especially green peppers which was not affected by heat treatment.

El-Ghorab et al. (2013) reported that the major volatile compounds identified in bell pepper include benzaldehyde, 2-methoxy-3-isobutyl-pyrazine, Z-β-ocimene, dimethylbenzene, and heptane-2-one, E-β-ocimene, linalool, nona-trans, cis-2,6-dienal, hexanal and biphenyl. On the other hand, the non-volatile compound (or as known as oleoresins) was determined through extraction by using various organic solvents. Since oleoresins contain high boiling non-volatile compounds including gums and resins that normally used as spices, they contribute to the aroma, flavours and pungency of the capsicums. Highest yield of extraction was done by using methanol while hexane yield the lowest non-volatile compounds. The major compounds found in the extracts were polyphenols, carotenoids and flavonoids.

2.2.3.2 Functional Properties

The functional properties discussed in this session were limited on the plant material interaction with human upon consumption. This session focused on researches carried out on the cabbage, cabbage outer leaves and capsicum. The researchers are mostly emphasizing the effects of post-harvest handling on the functional properties such as antioxidant capacity, antibacterial activity and anti-obesity effects under different conditions.

In cabbage outer leaves, colour measurement and total antioxidant activity were conducted. Tanongkankit, Chiewchan and Devahastin (2012) reported that the colour changes of dried cabbage outer leaves dietary fibre due to the fact that intracellular air will be forced out during blanching process and this may indirectly conserved the colour pigments inside the cabbage such as chlorophyll and carotenes. The total antioxidant activity of cabbage outer leaves detected in DPPH and ABTS assays decreased drastically when undergoing heat drying. This may be due to the degeneration of potent antioxidant i.e. phenolic compounds.

Brown and Morra (2009) studied the antifungal and antibacterial properties of glucosinolates, the main phytochemicals found in cabbage. They suggested that glucosinolates may greatly alter fungal and bacterial populations. They reported that the presence of myrosinase and several glcosinolates were cytotoxic towards *Salmonella typhimurium* while isothiocyanates (ITC) are the most potent inhibitors in prohibiting microbial growth. In fact, benzyl ITC, which can be found in cabbage, is used as antibiotic to treat respiratory and urinary infections (Dufour et al., 2013).

For capsicum, the antioxidant capacity, sensorial evaluation, cholesterol oxidation inhibition capacity, and DHA oxidation inhibition capacity were investigated in several researches. Sgroppo and Pereyra (2009) found out that in 4^{O}C, there was no significant difference of antioxidant capacity in capsicum. However, the antioxidant capacity of capsicum was decreased when stored in

higher temperature which believed could be eliminated by decreasing the storage temperature. The DPPH radical scavenging assay was carried out by Sun et al. (2007). A positive correlation (r = 0.72) was found in lipid peroxidation and phenolic content. Besides, the significant correlation of total flavonoids and carotenoids with antioxidant effects were also reported. Hence, Sun et al. (2007) concluded that phenolic content in the capsicums played important role in antioxidant activity. In the same research, it was reported that the antioxidant activity of green, red, orange and yellow peppers in oxygen radical absorbance capacity (ORAC) was 5.58, 9.01, 9.84 and 10.24 μmol Trolox equivalent/ g, respectively. The significant difference between red and green pepper could be explained by the difference in phenolic, flavonoid and carotenoid content (Sun et al., 2007).

Cholesterol and DHA oxidation inhibition assay was carried out by Sun et al. (2007). It was proven that capsicum has significant effects in preventing the oxidation of DHA, which approximately conserved about 50% of it. The same situation goes to cholesterol oxidation inhibition assay that the four kinds of peppers used showed conservation of cholesterol level ranged from 60 to 85%. The researchers found that the oxidation inhibition capabilities of capsicum were correlated with phenolic compounds found in the capsicum.

The quality of capsicum was tested in sensorial evaluation using heat treatment of 55 and 60^{O}C from Sgroppo and Pereyra (2009). It was reported that in both temperature used, heat treatment capsicums have a higher score

than experiment control. From the report, it was noticed that there was no microbial growth, capsicum was maintained in great firmness, fresh aroma and poor juice leakage. For the capsicum that sored in $10^{O}C$, it lost its quality faster in microbial growth and sensorial changes.

Table 2.1: Summary on the functional properties reported on cabbage, capsicum and cabbage outer leaves.

Sample	Functional properties	Reference
Cabbage outer leaves	Antioxidant capacity (DPPH and ABTS)	Tanongkankit, Chiewchan and Devahastin (2012)
Cabbage	Antifungal and antibacterial activity	Brown and Morra (2012)
Capsicum	Antioxidant capacities, sensorial evaluation	Sgroppo and Pereyra (2009)
	DPPH radical scavenging assay, cholesterol oxidation inhibition capacity, and DHA oxidation inhibition capacity	Sun et al., 2007

2.3 The Values of Vegetable Wastes

2.3.1 Dietary Fibre and Its Functional Properties

Most of the by-products from fruits and vegetables are promising source of functional compounds and dietary fibres (Abirami, Nagarani and Siddhuraju, 2014). Dietary fibre (DF) is a complex, natural carbohydrate polymer which consists of a variety of non-starch polysaccharides such as hemicellulose, cellulose, lignin, pectin, gum, β-glucans, and etc. (Liu et al., 2016). Investigation on the functional properties of dietary fibre conducted had proven that the consumption of DF has several health-promoting benefits including colon cancer prevention, serum lipid and cholesterol reduction, as well as delaying the absorption and digestion of carbohydrates. This leads to controlled body weight and postprandial glucose responses, which attributed to the hypoglycemic and hypocholesterolemic properties of DF.

Galisteo, Duarte and Zarzuelo (2008) and Peerajit, Chiewchan, and Devahastin (2012) also reported that soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) with high water swelling capacity (WSC) and water retention capacity (WRC) enhances satiety when consuming DF. Besides increasing faecal volume, the defecation time is shortened, hence reducing the risk of constipation, overweight, obesity and even colon cancer (Hong et al., 2012; Ma and Mu, 2016). On the other hand, several beneficial metabolic, physicochemical and functional properties of DF were proven to have positive physiological effects on human body, including prolongs satiety, regulates and improves the intestinal microflora growth, reduces the re-absorption of bile

acids, retard starch digestion, controls the intestinal absorption of glucose and LDL cholesterol, prevents cardiovascular diseases and diabetes (Abirami, Nagarani, and Siddhuraju, 2014; Galisteo et al., 2008; Peeranjit, Chiewchan and Devahastin, 2012).

Recent studies reported that DFs, especially those derived from by-products of fruits, vegetables, grains (Abdul-hamid and Luan, 2000; Fernando et al., 2005; Chau, Wang, and Wen, 2007), exerting different physicochemical and functional properties depending on the food sources, sample treatment, methods of extraction, chemical composition, physicochemical structure, modification process of fibre compositions and microstructures, as well as particle size of DF (Wuttipalakorn, Srichumpuang, and Chiewchan, 2009; Martínez et al., 2012; Peerajit, Chiewchan and Devahastin, 2012; Abirami, Nagarani, and Siddhuraju, 2014; Ma and Mu, 2016).

2.3.1.1 Effects of Dietary Fibres towards Bile Acids

The potential hypocholesterolemic properties of DF in managing non-communicable diseases such as cardiovascular disease, overweight and obesity have been well documented in various studies. Upon consumption, DFs swells inside the gastrointestinal tract to trap the bile acids secreted along with the water content absorbed. In addition, DFs might gel up in aqueous condition due to the presence of water and peristalsis action. This may help in minimising the contact of bile acids and dietary cholesterol (Zhou et al., 2005).

Through the retaining of bile acids inside DFs, bile acids reabsorption into the body is reduced and their faecal excretion will be increased instead. Eventually this could prevent the dietary cholesterol to be digested by bile acids but enable the body to utilise the endogenous cholesterol for daily energy usage. Hence, the body experiences an overall hypocholesterolemic effect resulted from consuming DFs (Gohil and Lele, 2014). However, the hypolipidemia and hypocholesterolemia effects of DF vary depending on the composition, viscosity, average molecular weight, treatment and preparation of the DF itself (Zhou et al., 2005). In addition, binding of bile acids also prevent them from recirculating inside human body, results in not only reduced cholesterol absorption, but also excretion of cancer causing toxic metabolites at the same time utilized the serum cholesterol in making more bile acids (Kahlon, Chapman and Smith, 2007). Therefore, investigation on various source of DF is essential in discovering potential material for applications in nutraceutical and pharmacognosy.

2.3.1.2 Mechanism of Bile Acids Binding in Relation to Weight Management and Cholesterol Lowering

There is an increase trend of overweight and obese population in Malaysia as shown by Figure 1.1 and 1.2.

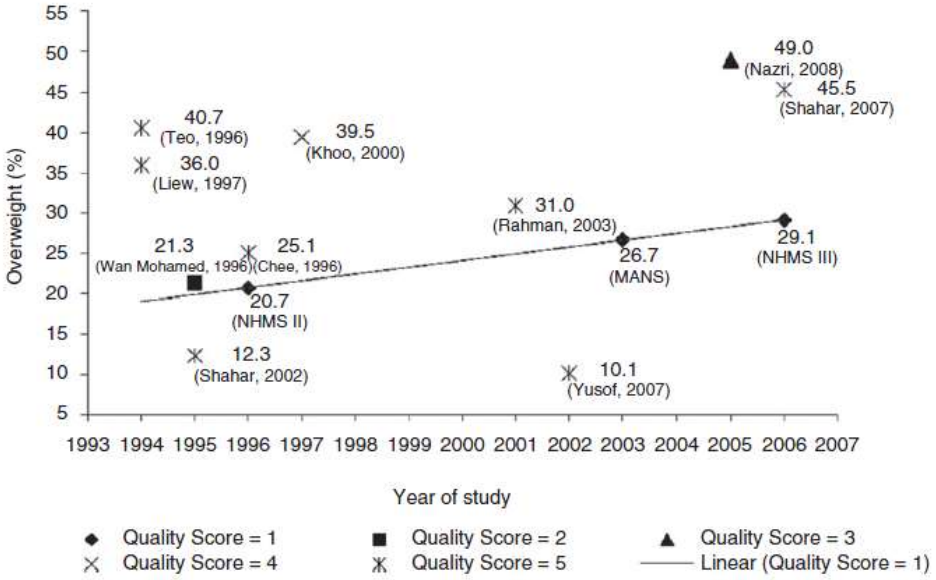


Figure 1.1: Prevalence of overweight in adults in Malaysia by year of study and quality score. The line represents the linear trend between national population based studies with the highest quality score. MANS, Malaysian Adults Nutrition Survey; NHMS, National Health and Morbidity Survey (Khambalia and Seen, 2009).

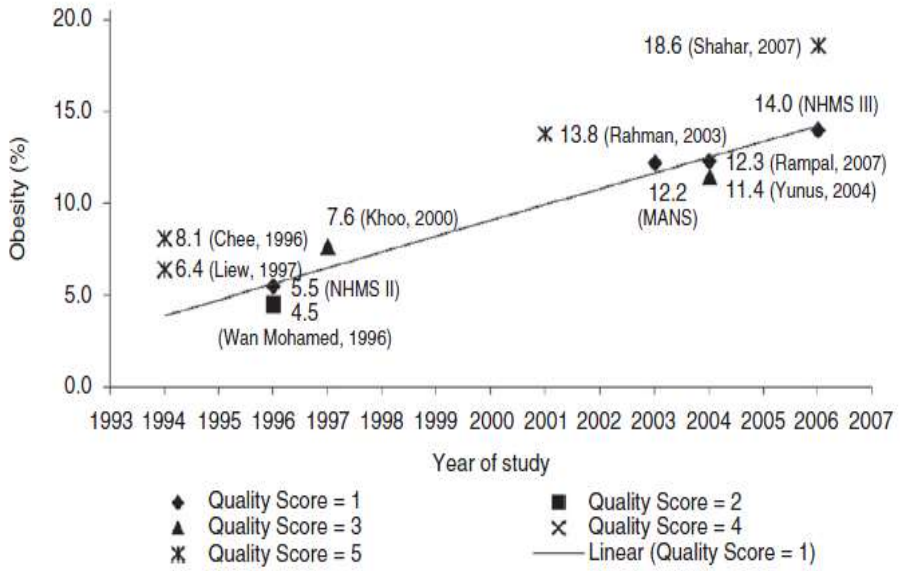


Figure 1.2: Prevalence of obesity in adults in Malaysia by year of study and quality score. The line represents the linear trend between national population based studies with the highest quality score. MANS, Malaysian Adults Nutrition Survey; NHMS, National Health and Morbidity Survey (Khambalia and Seen, 2009)

Khambalia and Seen (2009) reported that the increase of overweight and obese populations has brought the worldwide attention into a focus by indicating and linking the dramatically increased of both overweight and obesity populations with the worldwide increase of non-communicable diseases. In Malaysia, the most reliable estimates for the prevalence of overweight (25–29.9 kg m-2) and obesity (≥30 kg m-2) among adults in Malaysia is 29.1% (95% CI: 28.6%, 29.7%) and 14.0% (95% CI: 13.6, 14.5), respectively. From the figures shown above, there was a slight increase in the overweight population observed from year 1994 to 2007 but threefold increase in the obese populations.

To cope with this problem, the Ministry of Health Malaysia has organised various campaigns to all Malaysians on healthy eating habit and balanced diet besides avoiding sedentary lifestyle. (Abas, 2016; Augustin, 2016) More portions of fruit and vegetable in the diet is always recommended for weight-reduction. This is mainly due to lower calorific value and the rich amount of dietary fibre (DF) in fruit and vegetables. Zacherl, Eisner and Engel (2011) reported that the hypolipidemic and hypocholesterolemic effects of SDF are better than that of IDF. The mechanism of IDF (include lignin, cellulose and chitin) acting on bile acids is based on the direct binding action in the rigid structure together with limited amount of water molecules. The bile acids bound by IDF are excluded from the enterohepatic circulation and excreted out from the gastrointestinal tract. This mechanism allows the cholesterol emulsifier pass out more quickly and caused the bulky cholesterol left inside the bolus undigested. The undigested cholesterol will eventually pass out together with faeces (Zacherl, Eisner and Engel, 2011).

On the contrary, water-soluble dietary fibres such as β-glucan, pectin and psyllium reduce the serum cholesterol level in a different mechanism. Due to the solubility of DF in the presence of water, the binding of water in the chyme will cause the DF increase in viscosity and subsequently trap the bile acids inside. The high viscosity of the fibre-containing chyme reduces the diffusion rate of bile acids leading to reduced rate and amount of reabsorption by the body and finally excreted. There are some studies indicated that water-soluble dietary fibres may carry out direct binding action besides increase in viscosity, but the actual mechanism is still remain controversial and unclear (Zacherl, Eisner and Engel, 2011).

Through the binding action of DF, the large-sized cholesterol would not be absorbed by the body and pass along the gastrointestinal tract with the bolus. The mentioned mechanisms can bring anti-obesity effects due to several physiological changes in the body. The trapping and binding of bile acids allows the excess conversion and secretion of bile acids from cholesterol stored inside the body. Besides reducing the serum cholesterol level, the bile acid-cholesterol interaction also limits the cholesterol amount that accessible for the lipoprotein production as well as lipid storage. This probably leads to anti-obesity effects. Studies also showed that the presence of dietary fibres in the gut can inhibit the biosynthesis of endogenous cholesterol produced by colon bacteria during fermentation of soluble fibre, through the usage of short chain fatty acids. In addition, the binding of bile acids may also prevents their

biotransformation or metabolism into other compounds which is potentially harmful as some secondary bile acids like deoxycholic acids may possess mutagenous properties (Gorecka, Korczak and Flaczyk, 2003).

2.3.2 Plant Natural Products: Secondary Metabolites

Plants produce a diverse and a vast variety of organic compounds normally known as secondary metabolites which were not involved directly in the primary biochemical pathways including cell growth and development of the plants. Primary metabolites refer to the compounds that vital for the core housekeeping functions such as macromolecules production, cell division and growth, and energy generation. Generally, secondary metabolites are commonly known as phytochemicals, plant xenobiotics, secondary compounds, and antinutritional factors. These compounds are usually used in regulation of symbiosis, defence mechanism against consumers and pests, manipulation of seed germination, inhibition of competitive plant species and etc., which assist the plants to survive in an ever changing environment. Due to the presence of both toxic and beneficial secondary metabolites in plants, consumption of certain plants might cause negative physiological impacts such as gangrene, goitre, neurological dysfunctions, reproductive problems and even death. While some secondary metabolites in certain plant species are related with health-promoting effects, depending on the concentration and structure of the bioactive compounds during digestion process and metabolism. Makkar, Siddhuraju and Becker (2007) indicated the usefulness of plant

secondary metabolites in contributing to the human health, including antioxidant capacity, antimicrobial, antiviral and anticancer effects.

2.3.2.1 Types of Secondary Metabolites

Generally, there are three major groups of secondary metabolites i.e. phenylpropanoids, isoprenoids and alkaloids. Phenylpropanoids are derivatives of phenylalanine or tyrosine. In phenylpropanoids, it contains a diverse variety of phenolic derivatives, including flavonoids, coumarins, tannins, stilbenes, lignans, lignin, etc. These compounds were found to serve as antibiotics, fungicides, component of cell wall and antioxidants. In isoprenoids, or as known as terpenoids, they exist in all living organisms. In plants, there are more than 25,000 distinct isoprenoids were identified through studies. The examples of isoprenoids include linalool, pinene, isoprene, capsidiol, gibberellin, casbene, sitosterol, carotenoids and etc. Basically they are functioning as antibiotics to protect the plant from pathogens, attract insects for pollination, electron transport carriers, plant hormones and membrane constituents. Alkaloid is a nitrogen-containing ring structure that form from the plant metabolisms as metabolites. The examples of alkaloids include nicotine, cocaine, coniine, retrosine, lupinine, morphine, codeine, reserpine and so on. They serve as neurotransmitter agonists, neurotransmitter reuptake inhibitors, hypertensive drugs, antipsychotic drugs, antimalarial drugs, antineoplastic chemotherapeutic agents and even rodenticides (Buchanan, Gruissem and Jones, 2015).

Although secondary metabolites involved in diverse *in vivo* plant defence and survival activities, but the *in vitro* specific function most of the phenolics, terpenes, quinones, flavonoids, and etc. still remain unknown. Only limited amount of studies indicated that some secondary metabolites targeted molecules involved in bioprocesses, including mitochondrial-related biochemical processes and electron transport chain (Morrissey, 2009). However, with the risen of attention from researchers on the functional properties of secondary metabolites, a miscellaneous *in vitro* studies were conducted in discovering the hidden importance of those bioactive compounds leading to the current advancement of pharmacognosy and nutraceuticals. In the subsequent session, a brief description and explanation are given on the assays conducted in this study.

2.3.2.2 Antibacterial Activity of Secondary Metabolites

One of the best methods in investigating the beneficial effects of secondary metabolites is through the pathogen defencing or sensing and signalling activities, especially in antibacterial and antifungal testing. Although rapid technological advances has brought the introduction of various kinds of antibiotics, the development of resistance strains from bacteria have increased the level of challenges in discovery of alternative antibiotics. In addition, the cases of allergic towards synthetic antibiotics have made the task even harder. There are huge amount of studies related to this topic using different kinds of fruits, vegetables, medicinal plants and even wastes. Bakht, Syed and Shafi (2015) used disc diffusion method to investigate the antibacterial and

antifungal activities of traditional medicinal plant. Disc diffusion technique was used in the study with the rationale of low cost, more convenient to carry out and principle is easy to understand. The method utilises the presence of sample extract in inhibiting the growth of selected bacteria, followed by the measurement of degree of inhibition in millimetre using a ruler. Although the technique was rather inaccurate as compared to other methods such as microbroth dilution method, it is still the most popular technique chosen by the researchers to initially test out the effects of secondary metabolites (Bakht, Syed, and Shafi, 2015; Eloff, 1998). Though modifications of the experiment such as increase the repeats of the assay, the accuracy of minimum zone of inhibition (MIC) measured from selected bacteria may be improved and the errors caused by human could be minimised as well.

2.3.2.3 Antioxidant Effects of Secondary Metabolites

Besides antibacterial activities, oxidative stress has been a wide and major research that attracted the attention of various researchers. Either directly or indirectly, the ability of certain secondary metabolites to cope with oxidative stress could implicate its therapeutic potential on human diseases including cancer, brain dysfunction, cardiovascular diseases and allergy. Recent studies showed that there has been a huge increase in scientific interest on the secondary metabolites which possess antioxidant activities (Cartea et al., 2011; Bansal et al., 2013). Among the various antioxidant evaluations, DPPH free radicals scavenging method was popular due to its rapidness, ease of performance, reproducibility and usefulness of the assay at ambient

temperature to preventing thermal degradation of the tested compounds (Villaño et al., 2007; Kedare and Singh, 2011). This assay utilise the reduction of cell damaging, unstable free radicals that would change the violet DPPH into yellow colour in the presence of antioxidant compounds and subsequently quantified the changes spectrophotometrically (Tan and Lim, 2015). However, the DPPH assay comes in several weaknesses including the inconsistence of DPPH concentrations, sample concentration and volume, pH and incubation times.

2.3.2.4 Lipase Inhibition of Secondary Metabolites

Lipases are enzymes that normally used in digesting fats, including phospholipids and triglycerides. There are numerous kinds of lipase secreted by human body at different part of the gastrointestinal tract such as pancreatic, lingual, gastric, hepatic and lipoprotein lipases. Among the secreted lipases, pancreatic lipase (PL) plays a vital role in digestion of triglycerides effectively through the removal of fatty acids at α- and α'- positions of substrate, yielding β-monoglycerides and fatty acid chains (Mukherjee, 2003; Shi and Burn 2004). Studies showed that phytochemicals from traditional medicinal plants exhibit PL inhibition properties which could lead to decrease in dietary triglycerides absorption from the body and eventually leading to net loss of serum triglyceride level (Birari and Bhutani, 2007). Referring to the same authors, since pancreatic lipase is the major triglycerides digesting enzymes, the ability of secondary metabolites to bind with the enzyme may cause the inhibition of enzymatic activity of pancreatic lipase, leaving the triglycerides undigested or

partly digested. This may interferes the absorption of 2-monoglycerides and fatty acids into the body and interrupts the production of micelles. The method utilised the colour changing of the substrate in the presence of PL and sample extract under basic condition to detect the degree of PL inhibition spectrophotometrially (Jang et al., 2008; Kim et al., 2010; Chanmee, Chaicharoenpong and Petsom, 2013; Marrelli et al., 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Equipment

Chemicals and reagents used are listed at below along with their manufacture and section(s) of usage.

Table 3.1: List of chemicals and reagents with their manufacture sources and section(s) of usage.

Chemicals/ reagents	Manufacturer	Concentration	Section(s)
Ascorbic acid	HmbG Chemicals	0.50-6.00 mg/ ml	Section 3.8.1, 3.8.2
Bromine water	QRëC	1.0%	Section 3.6.3
Chenodeoxycholic acid	CALBIOCHEM	0.70 µg/ ml	Section 3.4.1, 3.4.2
Chloroform	QRëC	99.9%	Section 3.6.8
Cholic acid	Alfa Aesar	0.70 µg/ ml	Section 3.4.1, 3.4.2
Ciprofloxacin	USP	5.00 µg/ ml	Section 3.7.2, 3.7.3
Deoxycholic acid	EMD Millipore Corp.	0.70 µg/ ml	Section 3.4.1, 3.4.2
Di-Sodium hydrogen phosphate anhydrous	QRëC	14.2 mg/ ml	Section 3.4.1, 3.4.2
DPPH (α-Diphenyl-α-Picrylhydrazyl)	ALDRICH	0.15 mM	Section 3.8.2
Ferric chloride	FRIENDEMANN SCHMIDT	0.05 g/ ml	Section 3.6.4
Furfural solution	Alfa Aesar	0.25%	Section 3.4.3
Gelatin	nacalai tesque	0.01 g/ ml	Section 3.6.7
Hydrochloric acid	Fisher Scientific	0.01 M	Section 3.4.1, 3.4.2
		0.10 M	Section 3.4.2
Iodine solution	Fisher Scientific	12.7 mg/ ml	Section 3.6.1
Liquid nitrogen	THE LINDE GROUP	100%	Section 3.2.2

Methanol	Reagent DUKSAN	60%	Section 3.5.1, 3.5.2, 3.7.3
		95%	Section 3.8.1, 3.8.2
Mueller Hinton agar	HIMEDIA	38.00 mg/ ml	Section 3.7.3
Nutrient agar	MERCK	2.00 mg/ ml	Section 3.7.1
Pancreatin	Puritan's Pride PREMIUM	0.10 mg/ ml	Section 3.4.2
Potassium iodide	GENE Chemicals	0.02 g/ ml	Section 3.6.1
Sodium chloride	Fisher Scientific	0.85%	Section 3.7.1
Sodium hydroxide	DAEJUNG	0.10 M	Section 3.4.2
Sulphuric acid	QRëC	70%	Section 3.4.3

Equipment used was listed at below along with their manufacturer and section(s) of usage.

Table 3.2: List of equipment used with their manufacture sources and section(s) of usage.

Equipment	Manufacturer
Autoclaved machine	HIRAYAMA
Blender	WARING LABORATORY
Centrifuge machine	eppendorf Centrifuge 5702
Freeze drying machine	SCANVAC CoolSafe
Incubator	memmert
Lamina Flow Cabinet	ESCO
Moisture analyser	AND MX-50
Microtiterplate reader	BMG LABTECH FLUO star Omega
pH meter	Sartorious PB-10
Rotary evaporator	BUCHI
Shaking incubator	YIH DER LM-450D
Siever	Retsch AS 200
Sonicator	BRANSON 5510
Spectrophotometer	Thermo Scientific Genesys 20
Vacuum pump filtrator	CORNING 431118
Vortex mixer	K.K. VM-300
Water bath	memmert
Weighing machine	KRN ABJ

3.2 Sample Preparations

3.2.1 Sample Collection

Fresh capsicum and cabbage wastes were obtained from Veg Station (M) Sdn. Bhd. Faulty samples including rotten samples and foreign matters were selected and separated.

3.2.2 Sample Processing and Treatment

After washing and air drying of both sample wastes, cabbage outer leaves were cut manually into small pieces while capsicum placenta were separated from seed, sterile secondary pepper, pericarp and stem, followed by mashing of cores into small pieces by using a knife. Both types of wastes were weighed, and pre-treated with liquid nitrogen in 600 ml ilShinBioBase freeze drying flasks. Immediately, the flasks were plugged into a freeze drying machine for freezing process. Weights of the flasks were recorded daily until the decreases of the weights were within 0.01g.

3.2.3 Sample Pulverization and Storage

Dried samples were pulverized into powder form using a mortar and pestle. They are then being sieved through two sizes, i.e. 500 µm and 250 µm. Samples with different particle sizes were placed into different Schott bottles sealed with parafilm. They are stored in room temperature prior to extraction for further analysis.

3.3 Determination of Moisture Content

Moisture content of sample powders was measured using an AND MX-50 Moisture Analyzer. The mode of the moisture analyser was set as "Constant Temperature" with the temperature of 133^{O}C. About 1 g of sample was put on the sample pan and the accuracy of the moisture content was selected as "Low". The analyser was started to heat the sample powders after the lid was closed. The moisture contents of samples were recorded in percentage.

3.4 *In Vitro* Bile Acids Binding

The hypocholesterolemic effect of capsicum seed cores and cabbage outer leaves were analysed based on the procedures of Kahlon and Chow (2000); Kahlon, Chapman, and Smith (2007); and Rubio-Senent et al. (2015) with slight modifications. Generally, sample powder was firstly undergone acid digestion, mixed with bile acids, followed by pancreatin enzymatic digestion, and the change in the free bile acids was measured using a spectrophotometer. Individual bile acids include cholic acid (CA), deoxycholic acid (DA), and chenodeoxycholic acid (CDCA). Triplicate of the assay were carried out to ensure the reproducibility.

3.4.1 Chemicals and Bile Acids Preparation

Using aqueous dilution method, some chemicals were prepared including 0.01 M hydrochloric acid (HCl), 0.10 M HCl, 0.1 M sodium hydroxide (NaOH), and 70% sulphuric acid (H_2SO_4), 0.25% furfural solution, and 0.10 M

disodium hydrogen phosphate. Phosphate buffer (0.10 M, pH 7) was prepared by mixing 244 ml of 0.10 M HCl and 756 ml of 0.10 M di-sodium hydrogen phosphate. Re-confirmation of the buffer pH was carried out by using a Sartorious PB-10 pH meter. Other chemicals including 0.7 µmol/ ml bile acids and 10 mg/ ml pancreatin were prepared using 0.10 M, pH 7.0 phosphate buffer as the diluent.

3.4.*2 In Vitro* Digestion

One hundred miligrams of capsicum seed cores and cabbage outer leaves powder were weighed respectively in test tubes. A sample blank, a 100% BA tube and negative controls for each respecting acids were prepared as well. To simulate the human digestion system, samples were digested by 1 ml of HCl in the test tubes. The tubes were incubated at 37^{O}C in a water bath for an hour. Next, 120 µl of 0.1 M NaOH was added to neutralise the pH to 7. Immediately, 4 ml of bile acids were added, followed by 5 ml of porcine pancreatin (activity equivalent ranged between 6× to 12× USP). For the tubes of negative control and sample blank, bile acids were replaced by 0.1 M phosphate buffer while mixture contained bile acids without sample was set as positive control. The tubes were incubated at 37^{O}C in a water bath for another hour. The supernatant of each sample was recovered after centrifugation at 1120×g at room temperature for 10 minutes using an Eppendorf Centrifuge 5702. The total contents of each respective tube were summarised at below:

Table 3.3: The contents of the tubes in *in vitro* digestion process.

Tube name	Sample powder (mg)	0.01 M HCl (ml)	0.10 M NaOH (ml)	0.7 µmol/ ml Bile acids (ml)	0.10 M Phosphate buffer (ml)	10 mg/ ml Pancreatin (ml)
100% BA	-	1.00	0.12	4.00	-	5.00
Negative controls	100.00	1.00	0.12	-	4.00	5.00
Sample blank	-	1.00	0.12	-	4.00	5.00
Sample tubes	100.00	1.00	0.12	4.00	-	5.00

3.4.3 Bile Acids Analysis

Free bile acids were measured using colorimetric approach as described by Rubio-Senent et al. (2015) with modifications. About 100 µl of supernatant obtained from every tube were added with 5 ml of 70% H_2SO_4 in new tubes. The tubes were then heated at 90^{O}C in a water bath for an hour. After cooling for 10 minutes, 1 ml of freshly prepared 0.25% furfural solution was added into the tubes and mixed thoroughly. They were left in a dark condition for another one hour to allow the development of pink colour. Absorbance of each tube was measured at 490 nm using a spectrophotometer (Thermo Scientific Genesys 20).

3.5 Phytochemical Extraction of Capsicum Seed Cores and Cabbage Outer Leaves

The procedures used in phytochemical extractions were adapted from Pandhair and Sharma (2008), Cox, Abu-Ghannam and Gupta (2010) as well as Jaiswal, Abu-Ghannam and Gupta (2011) with modifications. The sample powders obtained from section 3.2.3 were used in two types of extraction in order to obtain the respective primary crude extracts containing the phytochemicals.

3.5.1 Solvent Extraction (SE)

Approximately 5 g of capsicum seed cores and cabbage outer leaves sample powders were measured and put into different 250 ml conical flasks. The powders were soaked with 100 ml of methanol (60% v/v) and shaken at 250 rpm for 1 hour at 37^{O}C in a YIH DER LM-450D shaking incubator (ratio of sample : solvent = 1:20). After incubation, the suspensions were filtered through three layers of cheese cloth and a layer of No. 1 Whatman filter paper with the help of a vacuum pump filtrator (CORNING 431118). The respective filtrates of the samples were then transferred into different 500 ml Schott bottles with labels. The soaking, incubation, filtration and transferring processes were repeated again to increase the volume of suspensions obtained. The methanol in both suspension mixtures was evaporated using a rotary evaporator from BUCHI with a pressure of 337 Pa at 40^{O}C. The crude extract obtained was distributed into three different specimen tubes and the respective initial weight was recorded. The methanol residue in the crude extract was

allowed to further evaporate at 37^{O}C in an incubator. The crude extract was weighed daily until the decreases of the weights were within 0.01g. To store the crude extracts, the specimen tubes were closed with caps, sealed with parafilms and stored in 4^{O}C refrigerator prior to further analysis.

3.5.2 Ultrasound-Assisted Solvent Extraction (UASE)

About 5 g of capsicum seed cores and cabbage outer leaves sample powders were measured and put into different 250 ml conical flasks. The powders were soaked with 100 ml of 60% methanol solution and put into a BRANSON 5510 sonicator for an hour (ratio of sample :solvent = 1:20). After incubation, the suspensions were filtered through 3 layers of cheese cloth and a layer of No. 1 Whatman filter paper with a vacuum pump filtrator (CORNING 431118). The respective filtrate of different samples was then transferred into different 500 ml Schott bottles with labels. The soaking, sonication, filtration and transferring processes were repeated to obtain sufficient filtrate for further analysis. The methanol in both suspension mixtures were then underwent evaporation using rotary evaporator from BUCHI at 40^{O}C under the pressure of 337 Pa. The crude extract was weighed daily until the decreases of the weights were within 0.01g. To store the crude extracts, the specimen tubes were closed with caps, sealed with parafilms and stored in 4^{O}C refrigerator prior to further analysis.

3.6 Preliminary Qualitative Screening of Phytochemical Compounds

There were four crude extracts produced from the extraction processes, i.e. solvent extract of cabbage outer leaves (ScabL), solvent extract of capsicum seed cores (ScapC), ultrasound-assisted solvent extract of cabbage outer leaves (UcabL) and ultrasound-assisted solvent extract of capsicum seed cores (UcapC). They were screened using different phytochemical detection tests on eight types of compound based on the method of Ugochukwu, Uche, and Ifeanyi (2013). For all the tests carried out, the concentration of crude extracts used was 10 mg/ ml.

3.6.1 Test for Alkaloids

Wagner's reagent was prepared by mixing 0.127 g of iodine powder and 0.2 g of potassium iodide in 10 ml of water. Three to five drops of Wagner's reagent were added into 2 ml of crude extracts. Any reddish brown colour precipitate formed indicates the presence of alkaloids.

3.6.2 Test for Flavonoids

Approximately 2 ml of crude extract was added into different test tubes. Then, a few drops of 20% sodium hydroxide were then added into each tube. An intense yellow colouration will be formed immediately. This was followed by the addition of few drops of 0.01M hydrochloric acid. The discolouration of the yellow colour indicates the presence of flavonoids.

3.6.3 Test for Glycosides

Approximately 1 ml of crude extract was added with a few drops of 1% bromine water. Any yellow precipitate observed indicates the presence of glycosides.

3.6.4 Test for Phenols

A few drops of ferric chloride solution (5% w/v) were added into test tubes containing 2 ml of crude extracts. The formation of a deep blue or black colour indicates the presence of phenols.

3.6.5 Test for Quinoles

About 1 ml of crude extracts was added into different test tubes. Few drops of concentrated hydrochloric acid (1.0 M) were then added into each tube. A yellow colour precipitation formed indicates the presence of quinoles.

3.6.6 Test for Saponins

In each test tube, 6 ml of distilled water was added to 2 ml of crude extracts. After the tubes were closed with stoppers, they were shaken manually and vigorously. The formation of persistent foam indicates the presence of saponins.

3.6.7 Test for Tannins

Few drops of gelatin solution (1% w/v) were added into test tubes containing 1 ml of crude extracts. The formation of a white colour precipitate indicates the presence of tannins.

3.6.8 Test for Terpenoids

In each test tube, 2 ml of crude extract was added followed by 1 ml of 99.9% chloroform. After mixing the solutions thoroughly, a few drops of sulphuric acid (1.0 M) were added. The immediate formation of a reddish brown precipitate indicates the presence of terpenoids.

3.7 Antibacterial Activity

Kirby-Bauer assay (a.k.a. discs diffusion assay) was carried out by using ciprofloxacin as the positive control and 60% methanol (which is the solvent of crude extracts) as negative control. Preliminary screening of various concentration of methanol solution (0% - 60% v/v) was carried out in the form of discs diffusion assay on all selected bacteria but no noticeable effects observed and this was supported by Tanner and Wilson (1943). The method of Kirby-Bauer assay described was adapted from CLSI (2012); and Delva and Goodrich-Schneider (2013) with slight modifications. Triplicates of the assay were carried out for each type of crude extracts.

3.7.1 Bacterial Source and Maintenance of Culture

There were 6 kinds of bacteria were used in this assay, including 3 Gram positive bacteria [*Bacillus cereus* (ATCC: 13061), *Bacillus subtilis spp. spizizenni* (ATCC: 6633), *Staphylococcus aureus* (ATCC: 6538)] and 3 Gram negative bacteria [*Escherichia coli* (ATCC: 25922), *Pseudomonas aeruginosa* (ATCC: 27853), *Salmonella typhimurium* (ATCC: 14028)]. The bacteria growth was maintained through sub-culturing weekly or fortnightly on Nutrient Agar (NA). Glycerol stocks of bacteria were stored as well in order to prevent some undesirable conditions on bacteria growth conditions. One day before Kirby-Bauer assay was carried out, the bacteria will be sub-cultured, left overnight in 37^{O}C incubator, and diluted using 0.85% sodium chloride. The absorbance of bacteria culture will be adjusted within an absorbance range of 0.08 to 0.10 at a wavelength of 625 nm which is equivalent to the 0.5 McFarland standard. It was noted that the bacteria solution will be used within 15 minutes after the adjustment for standardisation.

3.7.2 Crude Extracts and Antibiotic Preparation

About 10 ml of 0.5 mg/ ml ciprofloxacin was prepared as the stock of future usage. From the stock, about 5 ml of 5 µg/ ml ciprofloxacin working solution was freshly prepared. On the other hand, about 0.1 g of crude extract obtained from section 3.5 was measured in 4 different falcon tubes. The crude extract was diluted with 4 ml of autoclaved distilled water and mixed thoroughly until the extract was fully dissolved. Then, 6 ml of pure methanol was added to make the total volume to become 10 ml. The diluted extracts were filtered into

micro-centrifuge tubes with a 1 ml syringes and 0.20 µm filter prior to the assay.

3.7.3 Kirby-Bauer Assay

The commercial antibiotic, sample extract solutions and diluted bacteria culture were prepared from 3.7.2 was respectively inoculated into 12 properly labelled agar plates. About 100 µl of diluted bacteria culture was pipetted onto a labelled agar plate, followed by swabbing the bacteria using a sterile cotton swab. The swabbing was done horizontally and then vertically to distribute the bacteria inoculum evenly. The agar plates with bacteria inoculum were covered and left standing for 10 minutes prior to insertion of discs. Next, empty paper disc (6 mm diameter) was dipped into positive control, negative control and sample solutions, respectively and placed on allocated positions on the agar. A sterile forceps were used to tap on the discs gently to ensure complete contact between discs and agar surface. The plates were left standing in sterile condition at upright position for 15 minutes to allow the solutions to be absorbed by the agar. Lastly, the plates were placed in 37^{o}C incubator for overnight at an inverted position.

3.7.4 Determination of Zones of Inhibition

A ruler was used to measure the diameter of zones of inhibition (if any). Three measurements were taken for each disc and their average values were tabulated.

3.8 Antioxidant Capacity Determination

DPPH radical scavenging assay of capsicum seed cores and cabbage outer leaves were carried out based on the procedures described by Siow and Hui (2013) with slight modifications. Triplicate of the assay were carried out to ensure the reproducibility of the results.

3.8.1 Preparation of Sample Extracts

Crude extract from section 3.5 was used to make a 10 mg/ ml stock solution. Then, various concentrations of sample extract were prepared from this stock solution. Ascorbic acid was used as the positive control while methanol (99.9%) was used as the negative control. The amount of stock solutions and solvent were described as below:

Table 3.4: Preparation of sample extracts with various concentrations.

Tube	1	2	3	4	5	6	7	8
Sample extract stock (10 mg/ ml) (ml)	0.05	0.10	0.15	0.20	0.30	0.40	0.50	0.60
Methanol (99.9%) (ml)	0.95	0.90	0.85	0.80	0.70	0.60	0.50	0.40
Final concentration (mg/ ml)	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0

Table 3.5: Preparation of ascorbic acid solutions with various concentrations.

Tube	9	10	11	12	13	14	15	16
Ascorbic acid stock (10 mg/ ml) (ml)	0.05	0.10	0.15	0.20	0.30	0.40	0.50	0.60
Methanol (99.9%) (ml)	0.95	0.90	0.85	0.80	0.70	0.60	0.50	0.40
Final concentration (mg/ ml)	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0

3.8.2 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity

For each tube, an aliquot of 2 ml of DPPH solution (0.15 mM) was added and the tube was left for 30 minutes in a dark condition. Then, the absorbance of the mixture was measured at 517 nm with methanol (99.9%) as blank. Radical scavenging activity of the tubes was calculated by using Equation 3.1 stated at below. Then, EC_{50} and IC_{50} values of samples were determined through the linear regression analysis from the DPPH radical scavenging activity graph.

Equation 3.1:

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

3.9 Ester Hydrolysis Capacity

Lipase inhibiting assay of capsicum seed cores and cabbage outer leaves was conducted based on the protocol described by Reshmi (2009). Lipase is the main enzyme used in hydrolysing ester bonds in triacylglycerols into fatty acids before they are being absorbed into the body. It is proven that the inhibition of lipase either chemically or physiologically could remain the triglycerides in bulky form and difficult to be absorbed. Basically, the ester substrate was mixed with lipase inhibitor followed by lipase. By comparing to a positive and a negative control, the percentage of lipase inhibition activity was examined colorimetrically using a spectrophotometer.

3.9.1 Preparation of Sample Extracts and Chemicals

The stock substrate solution of para-nitrophenol palmitate (*p*-NPP) (16.5mM) was prepared by dissolving in isopropanol. To prepare substrate working solution, 1 ml of the stock substrate solution was added with 9 ml of phosphate buffer (0.01 M, pH 8.0), containing Triton X-100 (4% w/v) and Arabic gum (0.1% w/v). Lipase (5 mg/ ml) and sample extracts (2.5, 5.0, 10 mg/ ml) were prepared by dissolving in phosphate buffer (0.01 M, pH 8.0) and methanol (99.9%), respectively. Orlistat (1 mg/ ml) was weighed and dissolved in methanol (99.9%) as positive control.

3.9.2 Lipase Inhibiting Assay

Approximately 900 µL of *p*-NPP working solution was added into different test tubes. Then, 100 µL of various concentrations of sample extracts were added into respective tubes and mixed well. Finally, 100 µL of lipase (5 mg/ ml) was added into the test tubes and mixed well again. Plant extract solution was replaced by same amount of orlistat (1 mg/ ml) in positive control while lipase was replaced by phosphate buffer (0.01 M, pH 8.0) in negative control. The test tubes were incubated at 37^{O}C in a water bath for 25 minutes. Different yellow colour intensity generated in each tube was measured by using a spectrophotometer (Thermo Scientific Genesys 20) at a wavelength of 400 nm. Blank of each tube was recorded at the first moment when the chemicals were mixed and the percentage of lipase inhibition was calculated by using the formula expressed below:

Percentage of porcine pancreatic lipase inhibition

$$= \quad 1 - \frac{Absorbance_{sample} - Absorbance_{inhibition\ control}}{Absorbance_{lipase\ control} - Absorbance_{inhibition\ control}} \times 100$$

CHAPTER 4

RESULTS

4.1 Changes in Physical Appearance Observed.

Figure 4.1: The cut sample of capsicum seed cores (left) and cabbage outer leaves (right).

There was about 7 kg of fresh sample (capsicum seed cores and cabbage outer leaves) obtained. The first batch of the sample was obtained on 3rd February 2016 while second and third batch of the sample was obtained during 22nd Feb and 7th March of the same year, respectively. The fresh sample of capsicum seed cores were obtained in rough, large and attached with seeds, sterile secondary peppers, pericarps, stems and some of the flesh. For cabbage outer leaves, the fragments of cabbage leaves were obtained. Dirt was observed inside the sample. The cut samples were shown in Figure 4.1.

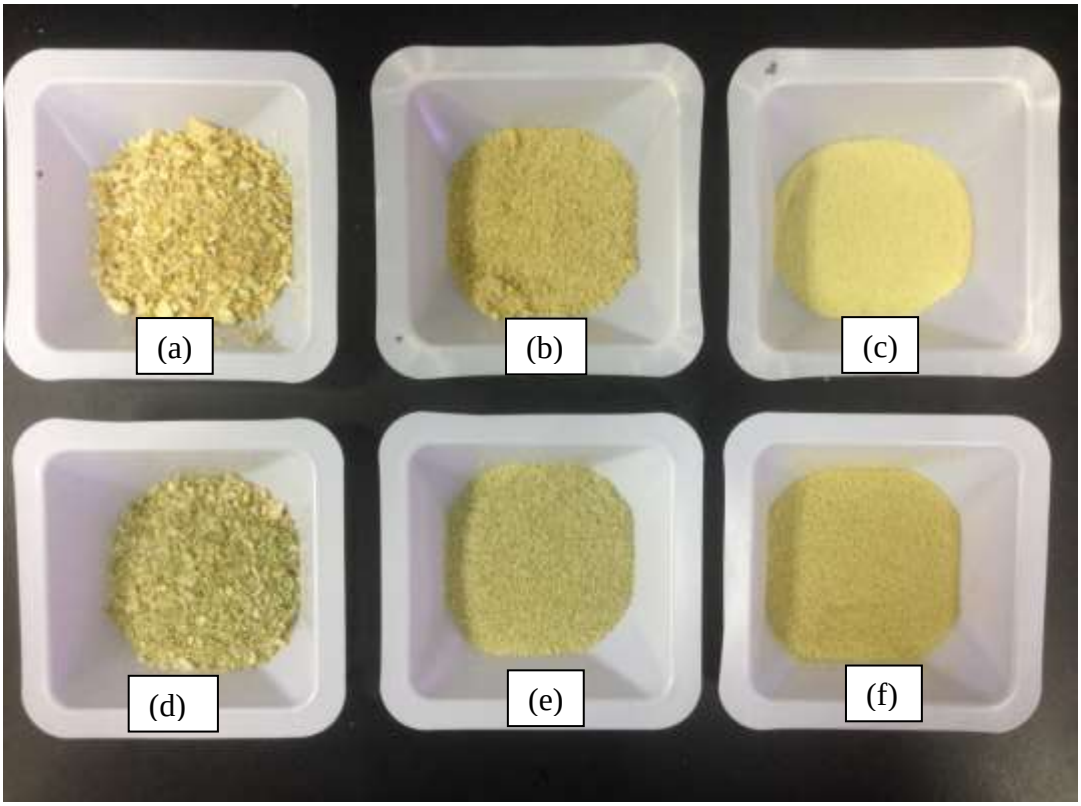


Figure 4.2: Dried capsicum seed core powder (a) 500 µm and above (b) 250 to 500 µm (c) 250 µm and lower as well as dried cabbage outer leave powder (a) 500 µm and above (b) 250 to 500 µm (c) 250 µm and lower.

After pre-treated with liquid nitrogen and pulverized, the sample underwent freeze drying treatment and sieved into 3 sizes i.e. 500 µm, 250 µm and < 250 µm. Generally the colour of each dried sample was relatively the same for all of the sizes. However, the colour of the capsicum seed core powder was dark green while cabbage outer leave powder was greenish-yellow. The physical appearance of the dried powder was shown in Figure 4.2.

4.2 Moisture Content

The three batches of the samples were mixed before sending for moisture content analysis. The tabulated data showed the measurements of fresh and dried weight, moisture content of samples and the yield of sample freeze drying.

Table 4.1: The fresh weight, dried weight, freeze drying yield and moisture content of samples.

	Cabbage	Capsicum
Fresh weight (g)	2370.18	1831.64
Dried weight (g)	133.75	116.66
Freeze drying yield (%)	5.64	6.37
Moisture content* (%)	4.68	4.75

*Results were based on the figure shown on the moisture analyser.

Based on Table 4.1, the decreased weight of the capsicum seed cores and cabbage outer leaves were calculated using mathematical equation:

$$\text{Weight loss (\%)} = \frac{\text{(Fresh weight of sample – Dried weight of sample)}}{\text{Dried weight of sample}} \times 100\%$$

From the calculation, the weight loss of cabbage outer leaves was 94.36% while capsicum seed cores had lost 93.63% of the weight due to freeze drying. By comparison, the water content inside cabbage waste is slightly higher than that of the capsicum waste. Besides, the moisture content of cabbage outer leaves was 4.68%, which slightly lower than that of the capsicum seed cores, which recorded as 4.75%. Similarly, the yield of cabbage waste (5.64%) also scored lower than that of capsicum waste (6.37%).

4.3 *In Vitro* Bile Acids Binding

The comparison was done by using the mixture with 100% bile acids without sample as positive control and 0% bile acids without bile acids as negative control. Generally, the bile acids binding capacities of both capsicum and cabbage powders scored in between 65% to 80%. Cabbage outer leaves bind to the three types of bile acids slightly higher than that of the capsicum seed cores. It was noticed that both sample powder bound highest to the CA, followed by CDCA and finally DCA. In CA, cabbage outer leaves bounded about 78.39% of it, which 4-5% more than that of the capsicum seed core powder, scored with 73.72%. In CDCA, the differences between two samples were closed to each other, recorded as 69.34% in cabbage outer leaves powder and 67.96% in capsicum seed core powder. The binding force of both samples with DCA is the lowest, but the percentage of binding was close to the percentage of CDCA bounded. Cabbage waste powder bounded DCA with 67.70%, which slightly more than that of the capsicum waste binding capacity recorded (65.44%).

Table 4.2: *In vitro* bile acids binding of waste samples with standard deviations.

Types of bile acids	Cabbage outer leaves	Capsicum seed cores
CA	78.39 ± 3.09	73.72 ± 2.94
DCA	67.70 ± 5.53	65.44 ± 4.60
CDCA	69.34 ± 4.77	67.96 ± 3.00

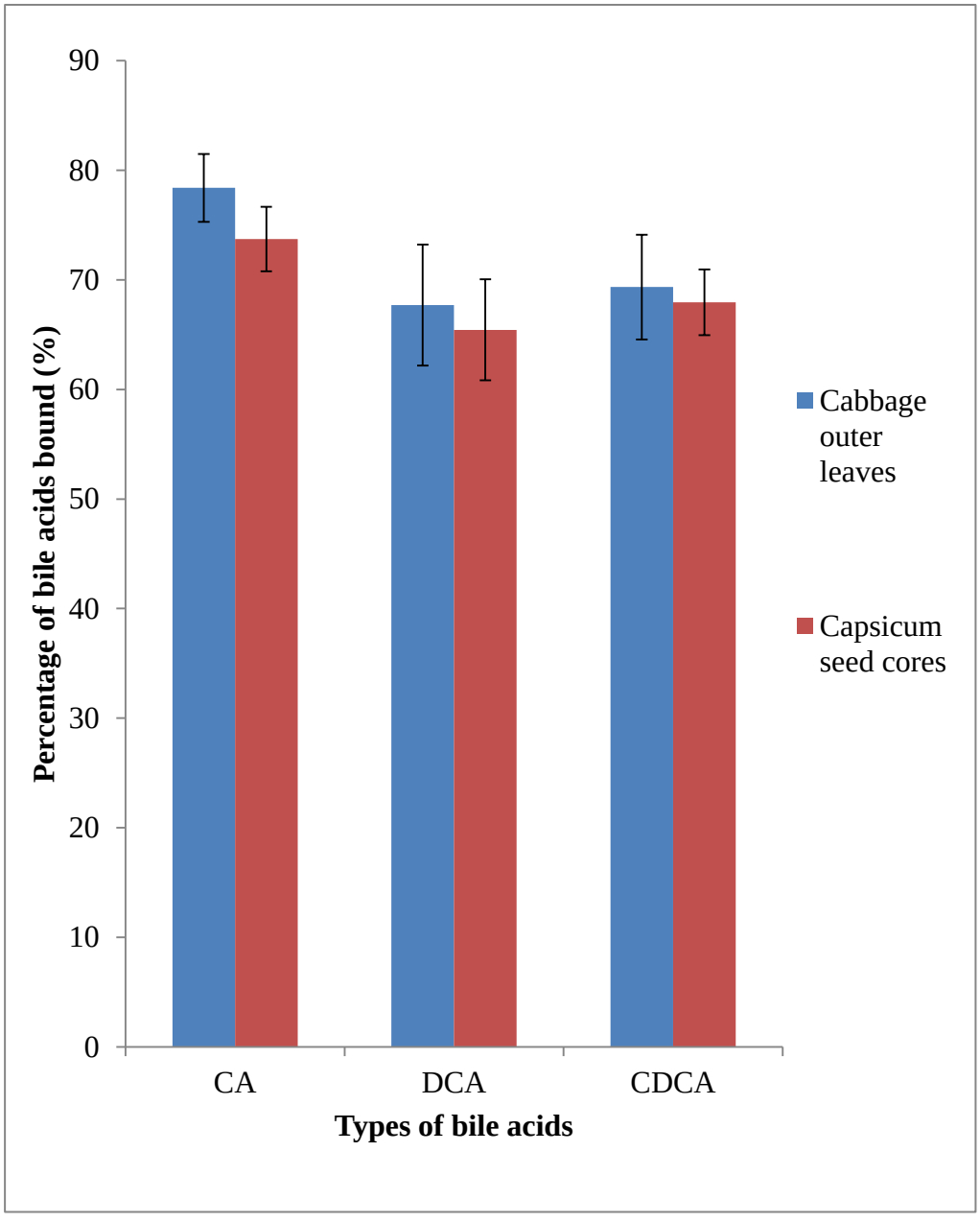


Figure 4.3: Percentage of bile acids bound by sample powder.

4.4 Yield of Extraction

The cabbage and capsicum sample powders were underwent two types of extraction prior to the phytochemical detection and subsequent assays, i.e. solvent extraction method (SE) and ultrasound-assisted solvent extraction method (UASE).

Table 4.3: The extraction yield of cabbage and capsicum wastes through SE and UASE.

	ScabL	UcabL	ScapC	UcapC
Extraction yield (%)	33.0	47.6	37.0	42.0

The extraction yield was calculated based on the dried weight basis of the samples by using the formula stated below:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of sample (g)} - \text{Weight of specimen tube (g)}}{\text{Weight of sample powder (g)}} \times 100\%$$

Generally in both samples, the yield of extraction from UASE was higher than conventional SE.

4.5 Phytochemical Detection

The phytochemical testing was done for the detection of eight selected types of compounds. For each phytochemical test, 10 mg/ ml of the extracts were prepared as the testing material. The observations are shown in Figure 4.4 and Table 4.4.

(a) Test for Alkaloids

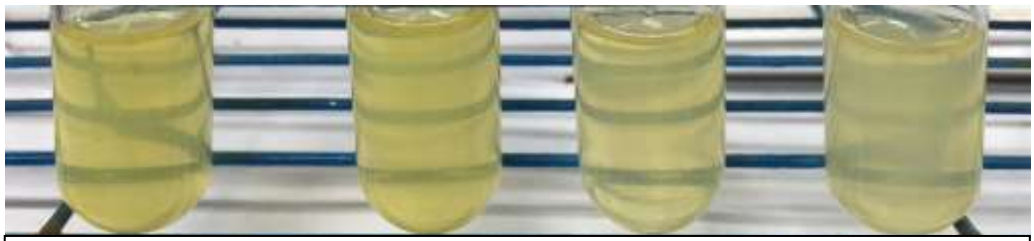
ScabL UcabL ScapC UcapC

(b) Test for Flavonoids

(i) After the addition of 20% NaOH:

ScabL UcabL ScapC UcapC

(ii) After the addition of diluted HCl:



ScabL UcabL ScapC UcapC

(c) Test for Glycosides

ScabL UcabL ScapC UcapC

(d) Test for Phenols

ScabL UcabL ScapC UcapC

(e) Test for Quinoles

ScabL UcabL ScapC UcapC

(f) Test for Saponins

ScabL UcabL ScapC UcapC

(g) Test for Tannins

ScabL UcabL ScapC UcapC

Figure 4.4 (Cont.): The observations on different sample extracts upon phytochemical tests.

Table 4.4: Phytochemical testing on selected compounds and the concentration observed.

Phytochemical compounds	ScabL	UcabL	ScapC	UcapC
Alkaloids	+++	++++	++	++++
Flavonoids	++	+++	++++	++++
Glycosides	++	+++	+++	++++
Phenols	-	-	-	-
Quinoles	++++	++++	++	+++
Saponins	-	-	+++	++
Tannins	++	+++	++	+++
Terpenoids	+	++	+++	+++

Symbols: ++++ implies highest positive indication of colour changes; +++ implies higher positive indication of colour changes; ++ implies medium positive indication of colour changes; + implies low positive indication of colour changes; - implies negative indication of colour changes.

From the Table 4.4, there were 6 phytochemicals detected among the 8 selected compounds. It was observed that capsicum extracts gave higher signals or indications on the presence of phytochemical compounds generally, including alkaloids, flavonoids, glycosides, saponins and terpenoids. On the other hand, cabbage waste showed strong positive sign of quinoles presence when comparing to that of the capsicum. All of the extracts showed negative indication towards phenol but relatively the same sign shown for tannins testing. However, brownish yellow colouration was observed in all of the tubes with a layer of white precipitate floating on the mixtures. By comparing

the effects of different extraction method, almost all of the testing indicated that UASE method yield more intense sign of phytochemicals' presence except for saponin detection. Capsicum extracts, ScapC was observed to yield 2 times more effervescence than UcapC in saponin detection (Refer to Figure 4.4).

4.6 Antioxidant Capacity

DPPH radical scavenging activity for the entire sample extracts were expressed in percentage (%) as shown in Figure 4.6 and Table 4.5. Almost all the extracts exceeded 90% radical scavenging rate at about 4 mg/ ml except for ScapC, which scavenge 90% of radicals at about 5 mg/ ml. The effective concentration of the sample extracts to scavenge half of the free radicals in the mixture (EC_{50}) was calculated by using the linear regression equation (Figure 4.5). All the sample extracts exhibited high antioxidant activity against free radicals, scoring in between 1.94 to 2.20 mg/ ml (Table 4.5).

Generally, UcabL exhibited the highest antioxidant activity, followed by UcapC, ScabL and finally ScapC. Both cabbage extracts apparently exhibited higher antioxidant activity than that of capsicum extracts. Ultrasound-assisted extracts possessed higher yield of antioxidant compound that gave higher scavenging activity.

Table 4.5: DPPH radical scavenging activity of sample extracts with standard deviations.

Concentration (mg/ml)	DPPH radical scavenging activity (%)								
	ScabL		UcabL		ScapC		UcapC		Ascorbic acid
0	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00
0.5	9.95 ± 2.33		21.1 ± 2.27		10.5 ± 3.80		15.4 ± 2.37		96.6 ± 0.00
1	28.7 ± 3.29		34.5 ± 1.17		39.6 ± 5.49		24.8 ± 0.80		96.4 ± 0.27
1.5	43.4 ± 2.68		44.8 ± 2.16		37.1 ± 2.01		38.4 ± 0.44		96.6 ± 0.88
1	56.4 ± 1.64		51.2 ± 2.27		47.4 ± 2.93		53.2 ± 0.27		96.4 ± 0.41
3	71.2± 3.08		80.9 ± 1.73		66.5 ± 5.67		81.4 ± 3.54		99.0 ± 0.07
4	92.7 ± 0.69		94.4 ± 0.86		85.6 ± 1.31		91.3 ± 0.91		99.1 ± 0.24
5	93.9 ± 1.46		95.3 ± 0.82		90.0 ± 1.06		93.5 ± 0.89		98.7 ± 0.38
6	93.7 ± 1.28		95.1 ± 0.67		88.8 ± 4.18		93.4 ± 0.36		98.8 ± 0.12

Table 4.6: EC_{50} values of the sample extracts.

Sample extracts	ScabL	UcabL	ScapC	UcapC
EC_{50} (mg/ ml)	2.04	1.95	2.20	2.01

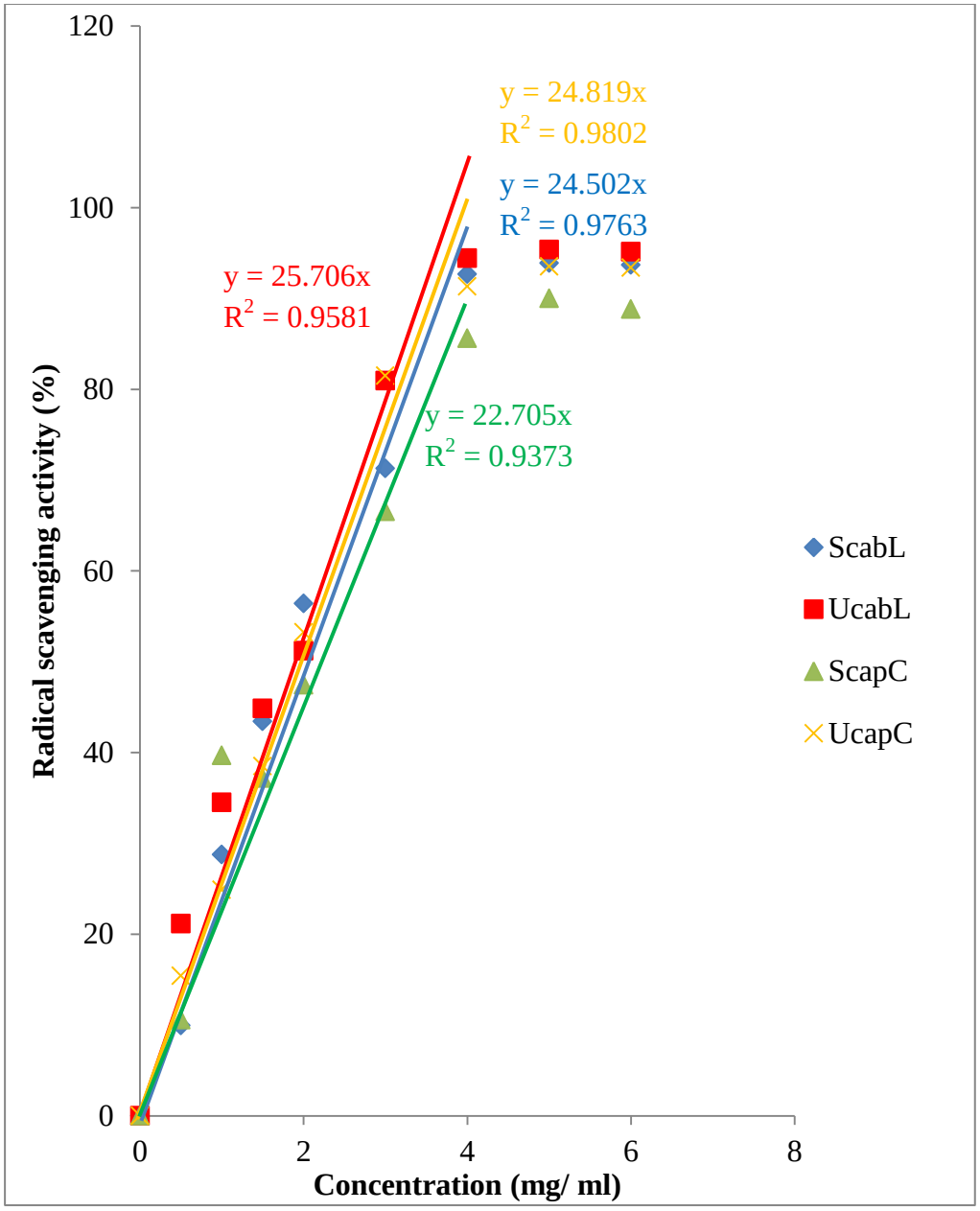


Figure 4.5: DPPH radical scavenging activity of cabbage and capsicum wastes extracts with linear regression lines and equations.

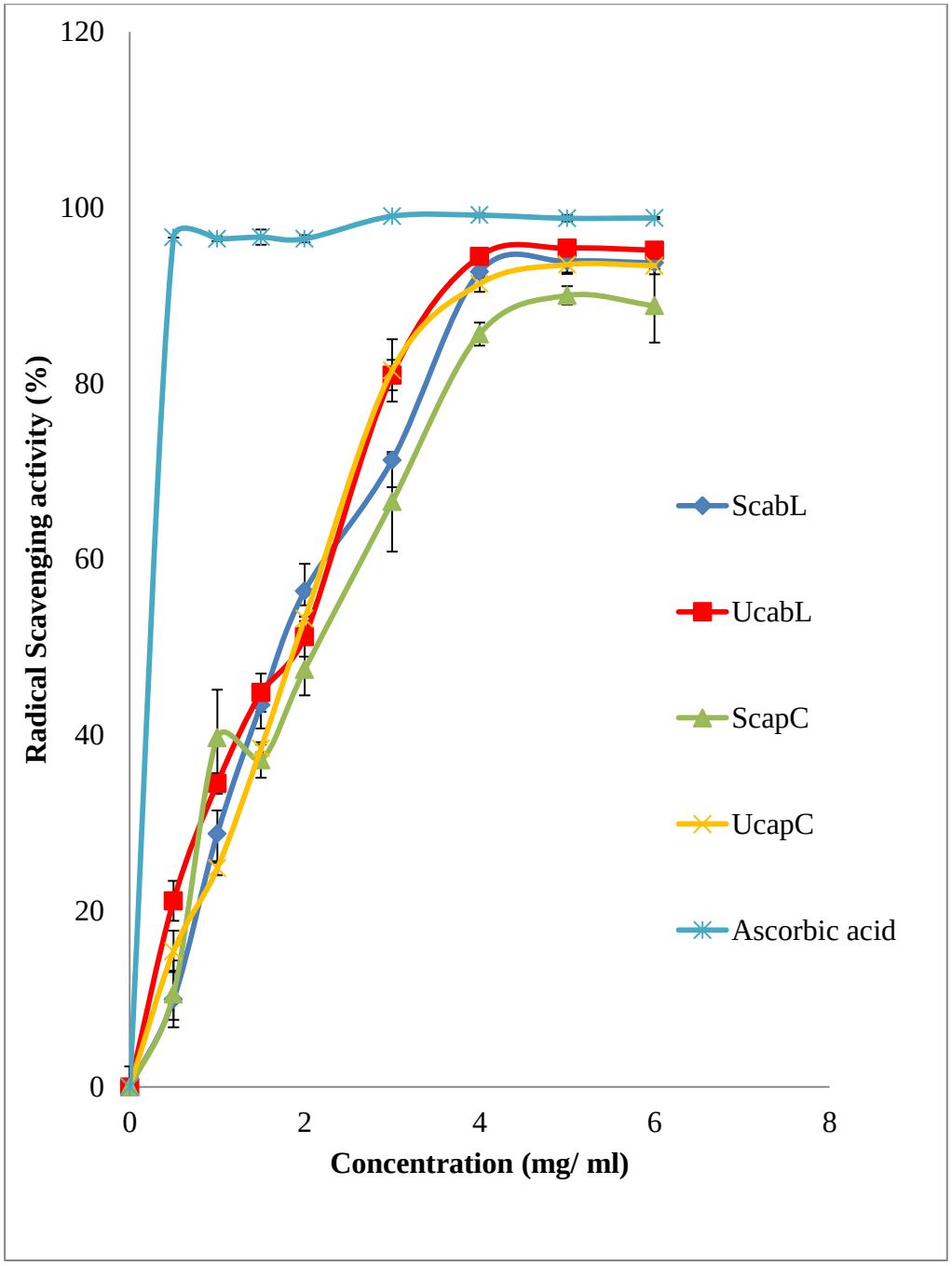


Figure 4.6: DPPH radical scavenging activity of cabbage and capsicum wastes extracts.

4.7 Antibacterial Activities

Before the commencement of the actual Kirby-Bauer assay, we conducted a preliminary screening by using various concentration of methanol on the bacteria tested (Refer to Appendix A, Figure 1). There were no inhibition effects by methanol (the solvent in our extraction) on bacteria growth. Table 4.7 tabulates the zone of inhibition on petri dishes, exerted by the sample extracts on bacteria growth. Among the bacteria tested, only half of them were susceptible to the mild growth inhibition from sample extracts, i.e. *B. cereus*, *B. subtilis*, and *P. aeruginosa*. Other bacteria showed undetectable zones of inhibitions. It was noticed that ScapC was the only extract that unable to inhibit the growth of *B. cereus*. The bacteria which were not inhibited by the extracts were not included in Figure 4.7 and 4.8. Basically with cabbage outer leaves and capsicum seed core extracts, UASE extracted samples were exhibited higher antibacterial activities as compared to the SE.

Table 4.7: Minimum zones of inhibition (mm) from sample extracts with standard deviations.

Bacteria	Negative control	Positive control	Cabbage Outer Leaves		Capsicum Seed Cores	
			SE	UASE	SE	UASE
B. cereus	N.D.	12.17 ± 2.46	8.00 ± 0.00	8.50 ± 0.50	N.D.	7.67 ± 0.29
B. subtilis	N.D.	18.17 ± 2.48	7.17 ± 0.76	9.17 ± 1.61	7.67 ± 1.15	7.67 ± 0.58
P. aeruginosa	N.D.	9.67 ± 1.83	7.33 ± 0.29	7.83 ± 0.29	7.00 ± 0.00	7.83 ± 0.29
S. aureus	N.D.	9.33 ± 1.21	N.D.	N.D.	N.D.	N.D.
E. coli	N.D.	24.83 ± 1.57	N.D.	N.D.	N.D.	N.D.
S. typhimurium	N.D.	21.50 ± 1.79	N.D.	N.D.	N.D.	N.D.

N.D. signifies no zones of inhibitions detected.

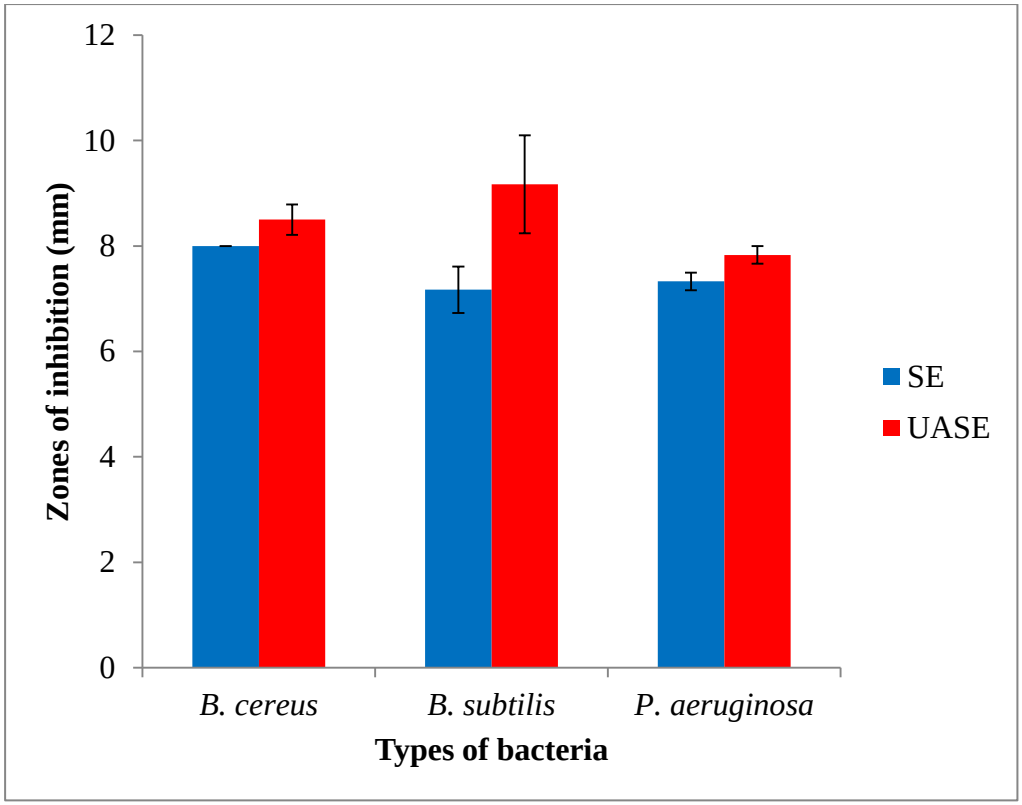


Figure 4.7: Averaged zones of inhibition from cabbage outer leaves extracts.

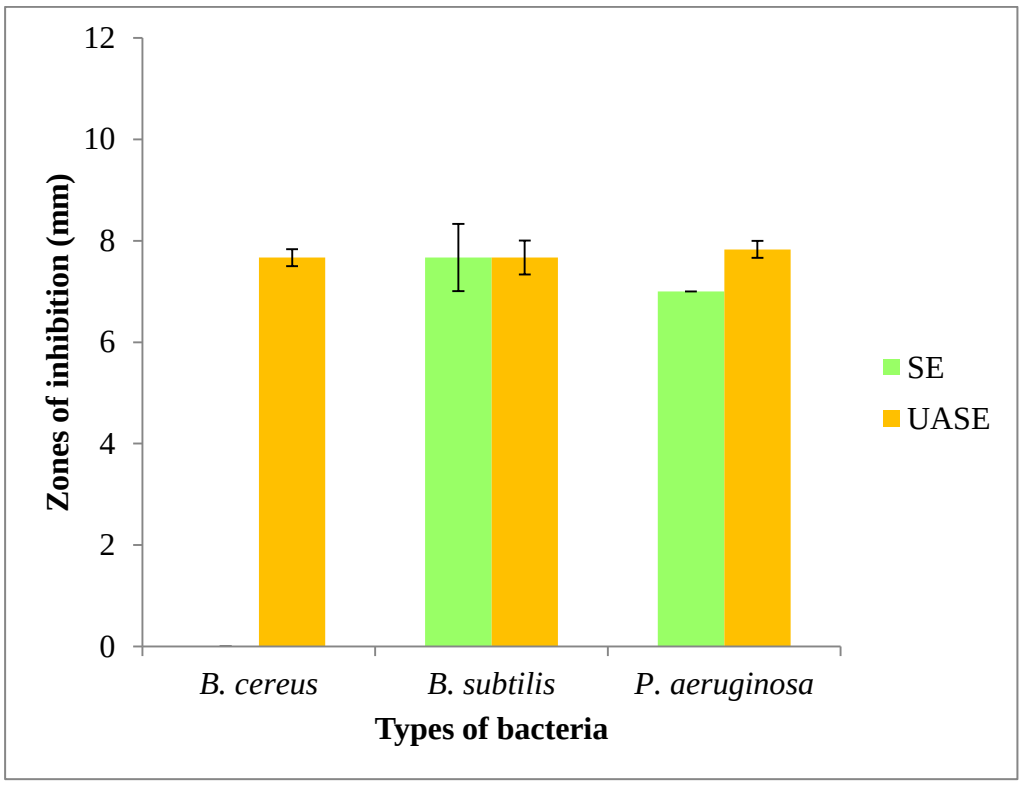


Figure 4.8: Averaged zones of inhibition from capsicum seed cores extracts.

4.8 Lipase Inhibition Assay

Different concentrations of sample extracts were used in the investigation of lipase inhibition capacity with utilizing 1 mg/ ml of orlistat as the positive control and mixture without inhibitors (either sample or orlistat) as the negative control. With the usage of porcine pancreatic lipase and para-nitrophenyl palmitate as substrate, inhibition of lipase activities were detected in 3 extracts except for ScabL. Generally, the higher the concentration of the extracts, the higher the inhibition activity towards porcine pancreatic lipase will be observed (Figure 4.9). ScabL showed zero inhibition while UcabL showed minimum inhibition on the pancreatic lipase, ranged from 6.21% (2.5 mg/ ml) to 27.66% (10 mg/ ml). On the other hand, capsicum extracts successfully inhibit the lipase enzyme effectively, ranged from 70.00% (2.5mg/ ml) to 75.98% (10 mg/ ml) for ScapC; and from 81.91% (2.5 mg/ ml) to 88.39% (10 mg/ ml) for UcapC. It was noticed that the absorbance values for both positive and negative control were assumed to be 100% and 0% lipase inhibition respectively. From the results shown, it was indicated that the effects of UASE is greater than that of the SE with higher percentage of lipase inhibition property.

Table 4.8: Averaged porcine pancreatic lipase inhibition of sample extracts at different concentration with standard deviations.

Concentration (mg/ ml)	Percentage of porcine pancreatic lipase inhibition (%)			
	ScabL	UcabL	ScapC	UcapC
2.5	0.00 ± 0.00	6.21 ± 6.98	61.00 ± 3.49	81.91 ± 4.80
5.0	0.00 ± 0.00	14.98 ± 8.95	64.64 ± 6.19	84.35 ± 3.76
10.0	0.00 ± 0.00	27.66 ± 2.23	75.98 ± 3.44	88.39 ± 3.67

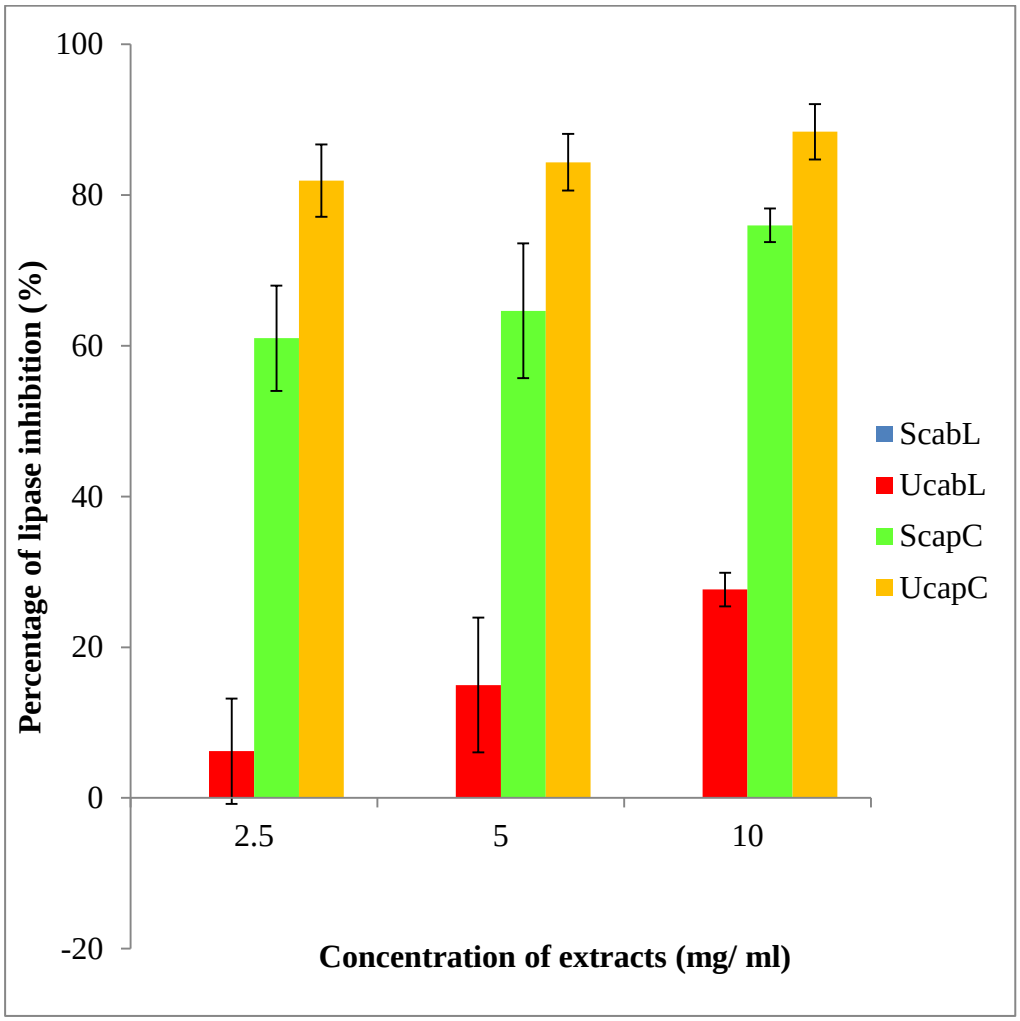


Figure 4.9: Lipase inhibition capacity from sample extracts at different concentrations.

CHAPTER 5

DISCUSSION

5.1 Possible Features of Physical Changes

In this study, freeze dried cabbage outer leaves and capsicum seed cores were used directly or underwent extraction for further compositional and functional investigations. They were cut and then mashed before they were sent for freeze drying process. This would allow the moisture content of each bit of the samples to become similar to each other without great differences. Besides, the small and equal size of the sample allows the shorter drying time as compared to larger sample sizes (Jomlapelatikul et al., 2016). Although the cutting had caused the changing of the size of the sample, but the colour of them were remained the same after cutting. The colour of the sample started to change after the freeze drying treatment. The colour of cabbage outer leaves has changed from greenish yellow into light yellow in colour while the colour of capsicum seed cores had changed from greenish white into dark greenish yellow. The changes of the colours were probably due to the excessive loss of water content, causing the macromolecules become more concentrated inside the sample. Besides, the drying process is usually time consuming which allows the innate metabolic process to continue, causing changes of colour and loss of ingredients (Yabar et al., 2011; Yuan et al., 2015).

Freeze drying was chosen because of its robustness, easy to manipulate, rapid drying, and able to prevent the degradation of heat sensitive compounds. After freeze drying, the texture of the samples became softer, drier and more crumbly, which were very suitable to be pulverized. The pulverization is to make sure the uniform size of the sample and maximise the surface area of the powder during further analysis.

5.2 Moisture Content

At the moisture content measuring stage, it was known that the water is the vital compound towards the life of plants and as solvent for dissolving or synthesis of minerals and nutrients, transportation agents for equilibrium or homeostasis maintaining, materials needed for photosynthesis, temperature regulator, structural agents, and etc. Thus the decreasing of the moisture content inside a plant cell may cause the impairment of above mentioned functions hence causing inactivation or conservation of functions of macromolecules inside, especially proteins like enzymes and bioactive compounds. This was supported by studies conducted which indicated that drying process is considered as the crucial step in the post-harvesting processes in limiting microbial growth and enzymatic degradation at the same time preserving the plant activities (Li et al., 2012; Yuan et al., 2015; Zhu et al., 2014). The moisture content observed for cabbage outer leaves and capsicum seed cores were 4.68% and 4.75% respectively. This indicated that there was slightly higher water content in capsicum seed cores than that of cabbage outer leaves after freeze drying process. The greater water loss by

cabbage samples has resulted in lower yield obtained in cabbage outer leaves as depicted in Table 4.1. In further study, investigations on the dietary fibres of cabbage outer leaves and capsicum seed cores should be carried out to clearly understand and differentiate the textures of waste from their fruits as well as to conduct more compositional analysis.

5.3 *In Vitro* Bile Acids Binding

In the investigation of bile acids binding from waste samples products, both cabbage outer leaves and capsicum seed cores showed positive results in binding of the three types of selected bile acids. Results showed that generally, cabbage outer leaves bound to the bile acids with slightly higher affinity than that of the capsicum seed cores. This might be due to the higher dietary fibre content of cabbage than that of the capsicum. The differences in bile acids binding between capsicum and cabbage also might be due to the differences in phytochemical compounds, physical binding sites and hydrophobicity of the samples (Kahlon, Chapman and Smith, 2007). The phytochemicals that might aid in the bile acids binding process are flavonoids, sulforaphane, tannins, isothiocynates, etc. Besides from chemical composition of the sample mentioned above, adsorption force, porosity and surface area also contributed to the higher bile acids binding of the cabbage waste powder (Cornfine et al., 2010; Górecka, Dziedzic, and Hęś, 2014). In addition, under the condition that both sample sizes were relatively the same (250 µm and lower), it was proposed that cabbage outer leaves have higher lignin fraction with lesser hydroxyl groups connected to the steroid rings, which makes it to achieve

greater bile acids binding affinity through sorption force and hydrophobic interaction, respectively. Lower degree of acetylation and methyl esterification on the cabbage outer leaves might also cause in the higher bile acids binding percentage compared to capsicum seed cores powder (Rubio-Senent et al., 2015). The difference in percentage of both samples binding with the selected bile acids might be due to the different conjugation of the acids which interferes the DF and phytochemicals to be associated with the bile acids. Figure 4.3 also indicated that the higher the affinity of the sample in binding with CA, the lower the affinity it will bind to the DCA, in which was tally with the study carried out by Camire and Dougherty (2003) and Zhou et al. (2005). For further studying the significance of the selected waste products towards hypocholesterolemic effects, other investigations including viscosity testing, binding capacity tests of samples under different conditions and pH, as well as heat treatment of the samples in enhancing solubility and viscosity of DFs should be carried out.

5.4 Yield of Extraction

There were four different extracts yielded from SE and UASE, i.e. ScabL, UcabL, ScapC and UcapC, with extraction yield of 33.0, 47.6, 37.0 and 42.0 respectively. It was noticed that both extracts from UASE have a higher yield than that of the extracts from SE. Except for the functioning of the solvent used, this might be due to the release of frequency and vibration from sonicator that cause the effective breaking of cell wall of vegetable wastes and cause more phytochemicals released from the plant cells. It was suggested that

the high frequency released from the sonicator not only could break the cell wall of selected vegetables wastes, but also would separate the essential bioactive phytochemicals that might associated with the inner part on the cell wall or even macromolecules. Since it was hypothesise that the higher yield of the extracts will contain higher amount of phytochemicals, hence there will be higher efficacy or potency in the subsequent assays tested. It was tally with the study carried out by Pico (2012) which stated that UASE extraction method will always better than that of the SE with higher physiological activities observed, cheaper, easier and produce higher quality yield. By comparing between extracts of cabbage outer leaves and capsicum seed cores, the higher yield of ScapC than ScabL in SE extraction method and greater yield of UcabL than UcapC in UASE extraction method indicated that SE is more effective in extracting phytochemicals inside capsicum waste and UASE was capable in producing higher amount of yield in cabbage wastes. Purification of extracts by using thin layer chromatography or liquid chromatography-mass spectrometer should be carried out in further study to achieve a higher efficacy and potency in assays conducted.

5.5 Phytochemical Detection

Based on Table 4.4, UASE extracted samples showed higher indications on the presence of the phytochemicals. This may be caused by the effectiveness of the extraction method in assembling essential phytochemicals through high frequency of wavelength. It was surprise to find that saponins detected in ScapC has higher intensity than that of UcapC while there was undetectable

saponin level in cabbage waste extracts. Besides the possible low initial content of saponins in cabbage outer leaves, the lower saponin level in UcapC might be caused by the sensitivity of saponins towards high frequency used during UASE extraction. It was noticed that there were variations of phytochemicals intensity observed while phenols showed negative indication for all of the extracts. However, there were colour changes of all of the tubes containing different samples, from light yellow to dark golden brown. Since the phenols inside the sample mixtures were unable to change the colour of the mixtures into dark blue or black colour, it was assumed that the screening test was not sensitive to detect the amount of the phenols extracted. This statement is further supported by studies carried out by Hutzler et al. in 1998 which the researchers discovered that there were phytochemicals associated with various cell organelles. To improve the phytochemical contents inside the extracts, optimization of the extraction may be carried out in order to extract different kinds of secondary metabolites maximally; no matter they are volatile, non-volatile, hydrophilic or hydrophobic phytochemicals. The optimisation may include the modification on the conditions applied on extraction methods, extraction duration, solvent concentration and etc.

5.6 DPPH Radical Scavenging Capacity

According to Table 4.5, the radical scavenging activity of the sample extracts were quite close to each other with fluctuating results recorded. This might be due to the primary crude extracts from the samples that contain high amount of other substances that interferes the action of bioactive secondary

metabolites. However, from the antioxidant effects calculated, the highest antioxidant potency was exhibited by UcapL, then UcapC, ScabL and finally ScapC. It was noticed that both UASE extracts from different samples exerted higher antioxidant effects than that of the SE extracts. This indicated that UASE extraction method was effective in extracting out phytochemicals with antioxidant properties, such as phenolics, alkaloids and carotenoids. Due to the extracts obtained were still primary crude extracts, the EC_{50} values of the extracts can be further lowered down if purifications were done. Within cabbage waste extracts, in most of the concentrations, radical scavenging activity of UcabL were higher than that of the ScabL except at the concentration of 2 mg/ ml. The same situation happened to capsicum extracts that in the category of 1 mg/ ml, ScapC had a higher positive antioxidant activity than UcapC. On the other hand, the radical scavenging activity of ascorbic acid had exceeded 96% at the concentration of 0.5 mg/ ml. This showed that ascorbic acid is a great antioxidant compound that could exert highest efficacy and potency of radical scavenging effects in the assay conducted.

5.7 Kirby-Bauer Assay

In the preliminary screening of antibacterial activities of methanol solution, the results showed no zones of inhibitions by various concentrations of methanol solutions ranged from 0% to 60%. It had proven that 60% methanol solution will not affect the growth of selected bacteria and suitable to be used as the negative control of the assay carried out. This might be due to the fast volatility rate of the methanol whose effect will lose gradually that led the

active dividing bacteria to continue replicating and eventually able to colonise into the zones of inhibition after methanol is evaporated. Since methanol solutions will only exert superficial drying effects on the bacteria, the moisture level lost in the bacteria content will be subsequently compensated or restored by the nurture of agar which contained a lot of water. This observation was in agreement with the results of Tanner and Wilson (1943) in which the bactericidal effect of methanol solutions on various bacteria was not noticeable. The same authors also reported that the shorter the carbon chain of alcohol, there will be weaker effects in antibacterial activities. While for the positive control, a working solution of 5 µg/ ml of ciprofloxacin were used which the concentration used was similar to the universal concentration of antibiotic recommended by Clinical & Laboratory Standards Institute (CLSI) (CLSI, 2012).

Besides, the wide-ranged spectrum of the antibiotic ciprofloxacin that could target and bind to the DNA gyrase and topoisomerase IV of bacteria and eventually inhibits the DNA replication of them. Generally, UASE extracted samples can exhibit greater antioxidant activities than that of the SE extracted samples. This is an indication that UASE extracted sample possess higher amount of antioxidative phytochemicals, but also able to assemble antibacterial secondary metabolites, such as flavonoids and terpenoids in targeting various kind of bacteria which led to growth inhibition. This finding was supported by the results observed from phytochemical detection in Table 4.4. ScapC was not able to cause any inhibition effects on *B. cereus* (Table 4.7 and Figure 4.8). The possible reason for this observation could be due to

insufficient in extraction duration that caused the low solvent penetration power into the plant cells to extract high amount of antibacterial phytochemicals. While in Figure 4.8, both UASE and SE extracted capsicum sample have the same degree of inhibition on *B. subtilis*. This might be due to the relatively similar amount of antibacterial phytochemicals being extracted and targeted on the bacteria. To further study in the antibacterial effects of extracts from cabbage outer leaves and capsicum seed cores, more strains of bacteria should be used. In addition, micro broth dilution assay should also be carried out in order to quantitatively measure the exact concentration needed to achieve the antibacterial effects.

5.8 Lipase Inhibition Assay

In porcine pancreatic lipase inhibition activity testing on the extracts, it was noticed that ScabL did not show the positive results. Besides lack of extraction duration and low solvent penetration power, this might be due to the possible antagonism effects from other secondary metabolites which cancel off the effects of lipase inhibition effects (Milugo et al., 2013). Although UcabL showed a positive lipase inhibition effects, but the inhibition effect was mild, scored from 6.21% (2.5 mg/ ml) to 14.98% (5.0 mg/ ml) and 27.66% (10 mg/ ml). Unlike cabbage waste extracts, capsicum seed cores extracts showed moderate to high pancreatic lipase inhibition actions, ranged between 60 to 90%. In every concentration used, UcapC always displayed higher inhibition effects than that of the ScapC, which contributed to evidence that UASE extracted sample can perform better than SE extracted samples. In general, with the increase of the concentration of the extract, the higher the lipase

inhibition effect was observed. Further study on the relativism which including synergism and antagonism among secondary metabolites should be investigated in order to make sure the maximum positive effects could be exerted by each of the extracts.

CHAPTER 6

CONCLUSION

In conclusion, our study revealed that both cabbage outer leaves and capsicum seed cores potentially possess relatively significant amount of dietary fibres and secondary metabolites in exerting antibacterial, antioxidant and anti-obesity effects. The smallest size of pulverized sample (250 μm and lower) was used to make sure the consistency and reproducibility of the results obtained. Colour changes on the samples were indicated by the increasing concentration of macromolecules inside the plant cells. The moisture content of both cabbage and capsicum wastes were 4.68% and 4.75% respectively, which allowed them to bind with water along with bile acids effectively. As for *in vitro* bile acids binding, both sample powders bound to CA, DCA and CDCA at different rates but in general, cabbage outer leaves had a higher affinity to bind with bile acids than that of capsicum seed cores.

Higher yield of extractions were observed by using ultrasound-assisted solvent extraction (UASE) as compared to solvent extraction (SE) method which may be due to the frequency released from the sonicator that able to break the plant cell more effectively at the same time separate the associated phytochemicals. The extraction yield of ScabL, UcabL, ScapC and UcapC were 33.0%, 47.6%, 37.0% and 42.0% respectively. In phytochemical screening of the extracts, generally more phytochemicals were detected in UASE than that of SE which

indicated that UASE is more effective extraction method than SE. The statement was supported by other researches as well as other assays conducted in our study. The absence of the phenols in all of the extracts might be due to insufficient duration of extractions that caused low amount of phenols to be extracted out.

In the rest of the assays conducted, i.e., DPPH radical scavenging capacity, Kirby-Bauer assay and lipase inhibition assay, UASE extracted samples possessed higher positive results than that of the SE extracted samples, in terms of potency and efficacy. The EC_{50} values of ScabL, UcabL, ScapC and UcapC were 2.04, 1.95, 2.20, 2.01 mg/ ml respectively. Although only *B. cereus*, *B. subtilis*, and *P. aeruginosa* were mildly susceptible towards extracts used, UASE extracted samples showed higher zones of inhibition (7.70 to 9.20 mm) than that of the SE extracted samples (7.20 to 8.00 mm). In lipase inhibition assay, ScabL showed zero inhibition while UcabL showed minimum inhibition on the pancreatic lipase, ranged from 6.21% (2.5 mg/ ml) to 27.66% (10 mg/ ml). Capsicum extracts successfully inhibit the lipase enzyme effectively, ranged from 70.00% (2.5mg/ ml) to 75.98% (10 mg/ ml) for ScapC; and from 81.91% (2.5 mg/ ml) to 88.39% (10 mg/ ml) for UcapC.

Further studies can be done on the purification of the samples, investigations on the total dietary fibres and phytochemicals content inside the samples quantitatively and relativism testing among phytochemicals. Other in vitro

studies are also required to further discover the functional properties of cabbage outer leaves and capsicum seed cores.

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APPENDICES

APPENDIX A

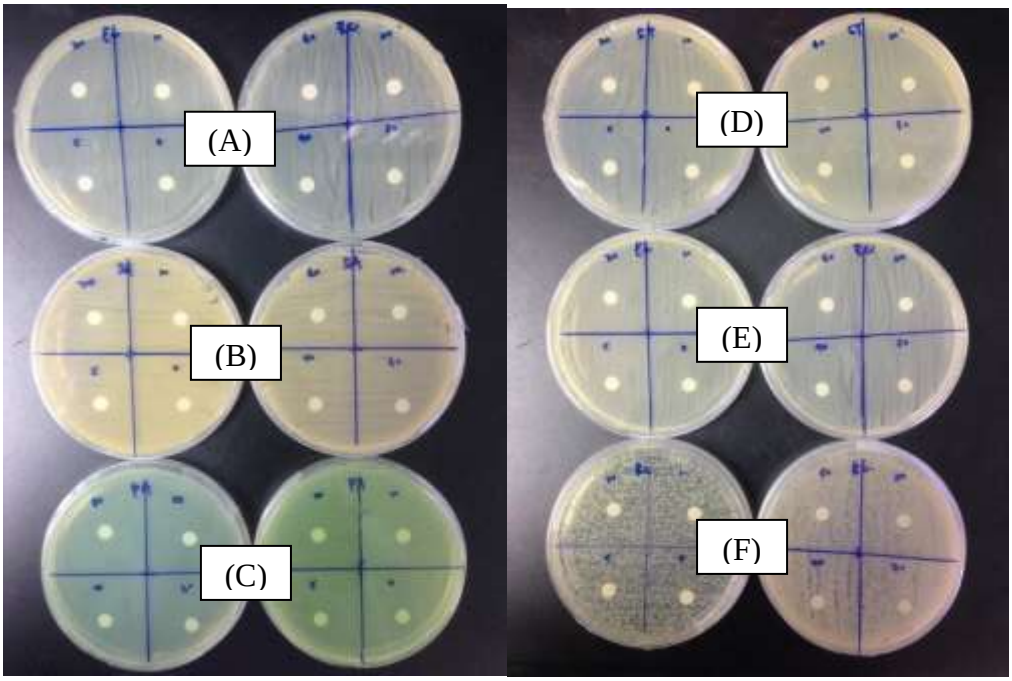

Figure 1: Preliminary screening of antibacterial effect using various concentrations of methanol solutions (0-60 mg/ ml) on (A), *Escherichia coli*; (B), *Staphylococcus aureus*; (C), *Pseudomonas aeruginosa*; (D), *salmonella typhimurium*; (E), *Bacillus subtilis*; (F), *Bacillus cereus,* after 18 hours incubation at 37ºC.

Table 1: Raw data of Kirby-Bauer assay.

	Sample	Zone of inhibition (mm)					
		BC	BS	SA	PA	EC	ST
	ScabL	8	8	N.D.	7.5	N.D.	N.D.
		8	6.5	N.D.	7	N.D.	N.D.
		8	7	N.D.	7.5	N.D.	N.D.
Mean		8	7.17	0	7.33	0	0
Standard deviation		0	0.76	0	0.29	0	0
Standard error		0	0.44	0	0.17	0	0
	UcabL	8	11	N.D.	8	N.D.	N.D.
		9	8.5	N.D.	7.5	N.D.	N.D.
		8.5	8	N.D.	8	N.D.	N.D.
Mean		8.5	9.17	0	7.83	0	0
Standard deviation		0.5	1.61	0	0.29	0	0
Standard error		0.29	0.93	0	0.17	0	0
	ScapC	N.D.	7	N.D.	7	N.D.	N.D.
		N.D.	9	N.D.	7	N.D.	N.D.
		N.D.	7	N.D.	7	N.D.	N.D.
Mean		0	7.67	0	7	0	0
Standard deviation		0	1.15	0	0	0	0
Standard error		0	0.67	0	0	0	0
	UcapC	7.5	7	N.D.	8	N.D.	N.D.
		7.5	8	N.D.	8	N.D.	N.D.
		8	8	N.D.	7.5	N.D.	N.D.
Mean		7.67	7.67	0	7.83	0	0
Standard deviation		0.29	0.58	0	0.29	0	0
Standard error		0.17	0.33	0	0.17	0	0
	Positive control	11	16	10	9	24	22
		13.5	17	8	13	27.5	22.5
		13.5	23	11	9	25	24
		12	18	10	10	23	19
		15	17	8	9.5	24	21.5
		8	18	9	7.5	25.5	20
Mean		12.17	18.17	9.33	9.67	24.83	21.5
Standard deviation		2.46	2.48	1.21	1.83	1.57	1.79
Standard error		1.01	1.01	0.49	0.75	0.64	0.73

Table 2: Raw data on absorbances of *in vitro* bile acids binding from cabbage outer leaves and capsicum seed cores.

		Absorbance (490 nm)		
	Bile acids	Cabbage Outer Leaves	Capsicum Seed Cores	Positive Control
	CA	0.088	0.118	0.399
		0.109	0.113	0.446
		0.098	0.128	0.535
Mean		0.098	0.120	0.460
Standard deviation		0.011	0.008	0.070
Standard error		0.006	0.004	0.040
	DCA	0.174	0.165	0.424
		0.163	0.18	0.452
		0.136	0.164	0.524
Mean		0.158	0.170	0.467
Standard deviation		0.0196	0.009	0.052
Standard error		0.0113	0.005	0.030
	CDCA	0.151	0.165	0.476
		0.115	0.130	0.452
		0.166	0.156	0.477
mean		0.144	0.150	0.468
stdev		0.026	0.018	0.014
std error		0.015	0.010	0.008

Table 3: Raw data on absorbance of DPPH radical scavenging capacity from different extracts.

Concentration	Absorbance (517 nm)							
(mg/ml)	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0
ScabL	12.6	32.5	46.4	56.9	74.8	91.9	95.1	92.3
	8.67	26.6	42.9	54.6	69.4	93.2	94.4	94.7
	8.55	27.2	41.1	57.7	69.6	93.0	92.3	94.1
UcabL	23.8	35.8	45.0	48.6	82.9	95.4	96.2	95.9
	19.7	33.6	47.0	52.6	79.6	94.1	95.3	95.0
	20.0	34.1	42.6	52.5	80.3	93.8	94.6	94.6
ScapC	14.4	45.6	36.9	50.8	73.1	87.1	91.1	91.9
	6.79	38.9	39.3	46.1	63.8	85.1	89.9	84.1
	10.5	34.7	35.4	45.4	62.8	84.7	89.0	90.5
UcapC	18.2	24.2	38.2	53.4	84.3	90.9	94.5	93.8
	14.1	25.8	38.3	53.4	82.7	92.4	93.3	93.3
	14.1	24.6	39.0	52.9	77.5	90.7	92.7	93.1
Ascorbic acid	96.6	96.6	96.6	96.5	99.0	99.4	99.1	98.9
	96.6	96.6	97.5	96.9	99.0	99.1	98.9	98.7
	96.6	96.1	95.8	96.0	99.1	98.9	98.4	98.8

Table 4: Raw data on absorbance of lipase inhibition assay from different extracts.

Sample	Concentration (mg/ ml)	Absorbance (517nm)		
ScabL	2.5	0.416	0.427	0.329
	5.0	0.304	0.324	0.314
	10.0	0.301	0.286	0.339
UcabL	2.5	0.274	0.279	0.299
	5.0	0.246	0.271	0.27
	10.0	0.237	0.229	0.227
ScapC	2.5	0.148	0.148	0.15
	5.0	0.139	0.149	0.131
	10.0	0.117	0.097	0.121
UcapC	2.5	0.113	0.075	0.103
	5.0	0.092	0.093	0.088
	10.0	0.08	0.081	0.082