



UNIVERSITI PUTRA MALAYSIA

***PROXIMATE COMPOSITION, TOTAL PHENOLIC CONTENT AND
TOTAL FLAVONOID CONTENT OF PASTA MADE FROM
COMPOSITE SOY AND WHEAT FLOUR***

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TABLE OF CONTENT

TITLE.....	i
SUPERVISOR'S SIGNATURE.....	ii
ACKNOWLEDGEMENT.....	iii
TABLE OF CONTENT.....	iv
LIST OF TABLES	viii
ABSTRACT.....	x
ABSTRAK.....	xi
CHAPTER 1.....	1
INTRODUCTION.....	1
1.1 Background.....	1
1.2 Problem Statement.....	3
1.3 Significance of the study.....	4
1.4 Objectives.....	5
1.4.1 General Objective	5
1.4.2 Specific Objectives	5
1.5 Null hypothesis	5
CHAPTER 2.....	6
LITERATURE REVIEW	6
2.1 Soybean	6
2.1.1 Overview of soybean.....	6
2.1.2 Soy fortified products	7

2.1.3 Soybean and obesity	7
2.1.4 Soybean and coronary heart disease.....	8
2.1.5 Soybean and cancer	9
2.1.6 Soybean and osteoporosis.....	9
2.2 Pasta	10
2.3 Proximate analysis	11
2.3.1 Moisture content.....	11
2.3.2 Ash.....	12
2.3.3 Protein	13
2.3.4 Fat.....	15
2.3.5 Carbohydrate.....	16
2.4 Phenolic compounds	17
2.5 Flavonoid compounds	18
CHAPTER 3	20
METHODOLOGY	20
3.1 Sample selection and sampling method	20
3.2 Reagents and chemicals	20
3.3 Experimental design	21
3.4 Processing of soy flour.....	22
3.5 Preparation of pasta.....	22
3.6 Cooking process.....	23
3.7 Proximate analysis	23
3.7.1 Determination of moisture content.....	23

3.7.2 Determination of ash content.....	24
3.7.3 Determination of protein content.....	24
3.7.4 Determination of fat content.....	25
3.7.5 Determination of total carbohydrate content	26
3.8 Extraction method	26
3.8.1 Aqueous extraction	26
3.9 Determination of Total Phenolic Content	27
3.10 Determination of Total Flavonoid Content	28
3.11 Sensory evaluation.....	28
3.12 Statistical analysis	29
CHAPTER 4	30
RESULTS AND DISCUSSIONS.....	30
4.1 Proximate analysis	30
4.1.1 Moisture content.....	30
4.1.2 Ash content	32
4.1.3 Protein content.....	35
4.1.4 Fat content.....	38
4.1.5 Total Carbohydrate Content.....	40
4. 2 Total phenolic content	44
4.3 Total flavonoid content.....	46
4.4 Sensory evaluation.....	48
CHAPTER 5.....	51
CONCLUSION, LIMITATIONS AND RECOMMENDATIONS	51

5.1 Conclusion 51

5.2 Limitations and recommendations..... 52

REFERENCES..... 54

APPENDICES 58



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LIST OF TABLES

Table 1: Moisture content of wheat flour and soy flour	30
Table 2: Previous study of moisture content of wheat flour and soy flour	31
Table 3: Moisture content of wheat pasta and soy-wheat composite pasta	32
Table 4: Ash content of wheat flour and soy flour.....	32
Table 5: Previous study of ash content of wheat flour and soy flour	33
Table 6: Ash content of wheat pasta and soy-wheat composite pasta.....	34
Table 7: Previous study of ash content of wheat pasta and soy-wheat composite pasta	34
Table 8: Ash content between flour and pasta.....	35
Table 9: Protein content of wheat flour and soy flour.....	35
Table 10: Previous study of protein content of wheat flour and soy flour	36
Table 11: Protein content of wheat pasta and wheat-soy composite pasta	37
Table 12: Previous study of protein content of wheat pasta and soy-wheat composite pasta.....	37
Table 13: Protein content between flour and pasta	38
Table 14: Fat content of wheat flour and soy flour	39
Table 15: Previous study of fat content of wheat flour and soy flour	39
Table 16: Fat content of wheat pasta and soy-wheat composite pasta	40
Table 17: Total carbohydrate content of wheat flour and soy flour	41
Table 18: Previous study of total carbohydrate content of wheat flour and soy flour.....	41
Table 19: Total carbohydrate content of wheat pasta and soy-wheat composite pasta	42
Table 20: Previous study of total carbohydrate content of wheat pasta and soy composite pasta.....	43
Table 21: Total carbohydrate content between flour and pasta.....	43

Table 22: Total phenolic content of wheat flour and soy flour..... 44

**Table 23: Total phenolic content of wheat pasta and soy-wheat composite pasta
..... 45**

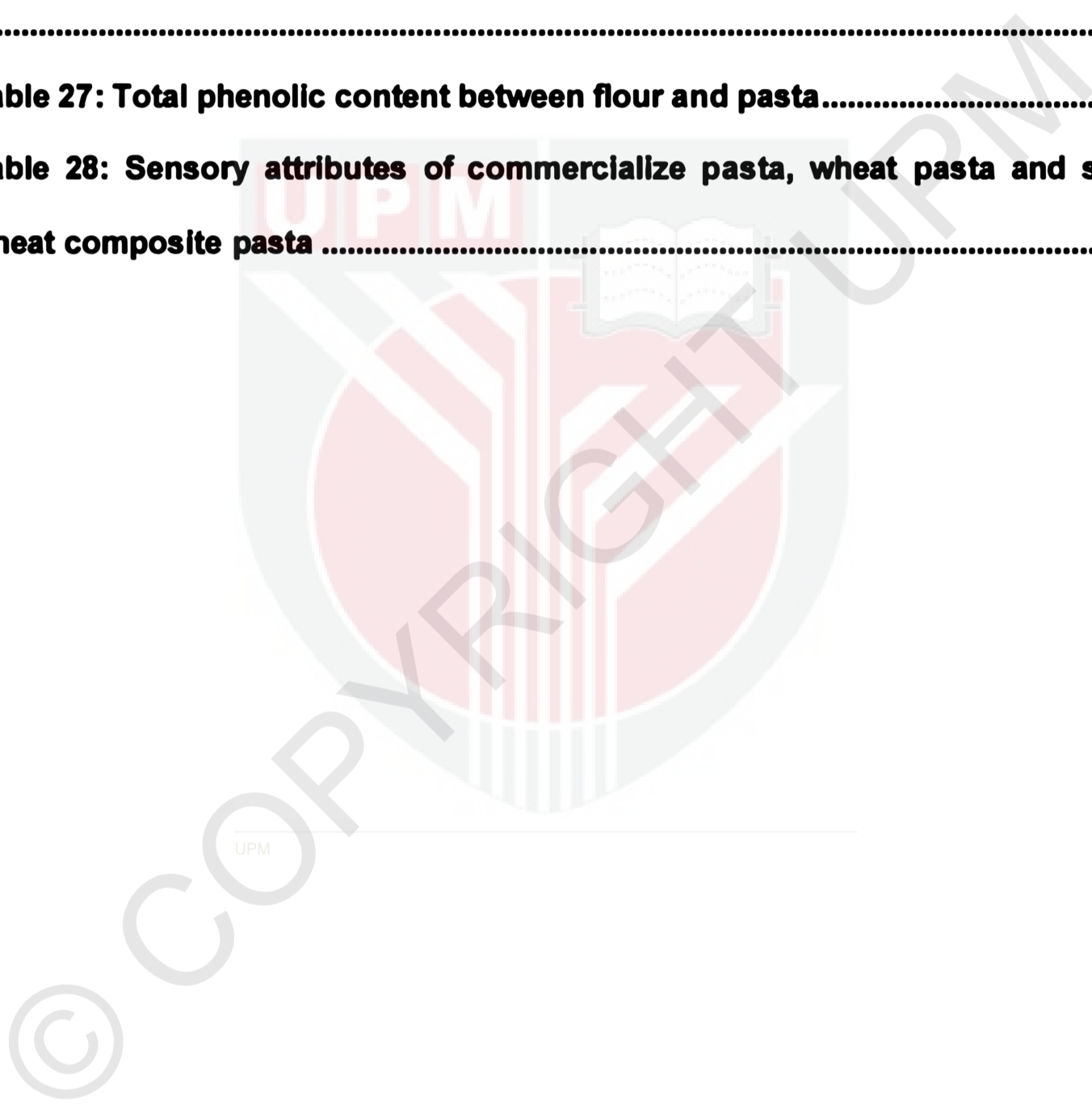
Table 24: Total phenolic content between flour and pasta..... 46

Table 25: Total flavonoid content of wheat flour and soy flour 46

**Table 26: Total phenolic content of wheat pasta and soy-wheat composite pasta
..... 47**

Table 27: Total phenolic content between flour and pasta..... 48

**Table 28: Sensory attributes of commercialize pasta, wheat pasta and soy-
wheat composite pasta 50**



ABSTRACT

PROXIMATE COMPOSITION, TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT OF PASTA MADE FROM WHEAT FLOUR AND COMPOSITE SOY FLOUR

Fauzeen Atira Mohd Roseli

Pasta is one of the most consumed cereal-based food products worldwide. It is made from refined wheat which most of refined grain foods contain high energy and low in nutrient content which can lead to several non-communicable diseases. Hence, there is consumer demand on improving pasta and noodles nutritional properties, texture and appearance by using natural food additive. Many researches showed that the components in soybean can prevent and treat chronic diseases like cardiovascular disease, obesity, diabetes mellitus and cancer. In order to improve the nutritional properties and prepare a nutritious pasta, wheat flour was fortified with 25% of soy flour. Therefore, the purpose of this study is to determine the nutrient composition, total phenolic content and total flavonoid content in pasta made from wheat flour and composite soy flour. Sensory evaluations were also determined for the appearance, aroma, colour, firmness, taste and overall acceptability of the pasta samples. The proximate analysis was conducted using AOAC International methods while total phenolic content and total flavonoid content were conducted using Folin-Ciocalteu method and Aluminium Chloride Colorimetric method, respectively. Sensory evaluation was involved with 30 untrained panellists from University Putra Malaysia's students will be involved. The results showed that soy pasta contain higher moisture, ash, protein and fat content ($p < 0.05$). Meanwhile, wheat pasta contains higher total carbohydrate content ($p < 0.05$). Soy-wheat composite pasta presented higher total phenolic content and total phenolic content compared to wheat pasta ($p < 0.05$). For the sensory evaluation, it revealed that commercialize pasta have higher overall acceptability followed by soy-wheat composite pasta and wheat pasta, respectively. Overall, this study showed that soy-composite pasta had superior nutritional values compared to wheat pasta (not durum). Panellists preferred commercialized pasta; however, soy-composite pasta was accepted better than wheat pasta. Therefore, soy pasta could be one of the pasta variants that could potentially give health benefit to the consumer. This soy pasta can be used as a nutritious food for low income group in developing countries and as natural supplement to those who intended to take high protein food.

ABSTRAK

PROKSIMAT ANALISIS, JUMLAH KANDUNGAN FENOLIK DAN JUMLAH KANDUNGAN FLAVONOID PASTA DARIPADA TEPUNG GANDUM DAN KOMPOSIT TEPUNG SOYA

Fauzeen Atira Mohd Roseli

Pasta adalah salah satu produk makanan berasaskan bijirin yang paling banyak digunakan di seluruh dunia. Pasta ini diperbuat daripada tepung gandum halus. Kebanyakan produk bijirin yang diproses mengandungi kandungan tenaga yang tinggi dan rendah nutrient. Hal ini boleh menyebabkan penyakit tidak berjangkit. Oleh itu, terdapat peningkatan permintaan daripada pengguna untuk meningkatkan nutrisi, tekstur dan rupa pasta dengan menambah bahan semula jadi. Banyak kajian telah dijalankan menunjukkan komponen dalam kacang soya dapat menghalang dan merawat penyakit kronik seperti penyakit kardiovaskular, obesiti, kencing manis dan kanser. Bagi meningkatkan nutrisi makanan dan menyediakan pasta yang berkhasiat, 25% tepung gandum telah digantikan dengan tepung soya. Oleh itu, tujuan kajian ini adalah untuk menentukan komposisi nutrien, jumlah kandungan fenolik dan jumlah kandungan flavonoid dalam pasta yang diperbuat daripada tepung gandum dan tepung soya komposit. Penilaian sensori juga ditentukan untuk rupa, aroma, warna, kekenyalan, rasa dan penerimaan keseluruhan pasta. Proksimat analisis telah dijalankan dengan menggunakan kaedah *AOAC International* manakala jumlah kandungan fenolik dan jumlah kandungan flavonoid telah dijalankan menggunakan kaedah *Folin-Ciocalteu* dan kaedah *Aluminium Chloride Colorimetric*. Penilaian sensori melibatkan 30 ahli panel tidak terlatih daripada pelajar Universiti Putra Malaysia. Keputusan menunjukkan pasta soya mengandungi kelembapan, abu, kandungan protein dan lemak yang tinggi ($p < 0.05$). Sementara itu, pasta gandum mengandungi kandungan karbohidrat yang tinggi ($p < 0.05$). Pasta soya mengandungi jumlah kandungan fenolik dan flavonoid yang tinggi berbanding pasta gandum ($p < 0.05$). Untuk penilaian sensori, komersial pasta mempunyai penerimaan yang lebih tinggi secara keseluruhan diikuti oleh pasta soya dan pasta gandum. Secara keseluruhannya, kajian ini menunjukkan pasta soya mengandungi kandungan nutrisi yang lebih tinggi daripada pasta gandum. Keseluruhan panelis memilih pasta yang dikomersialkan, walau bagaimanapun, pasta soya diterima lebih baik daripada pasta gandum. Kajian ini mencadangkan penggunaan gandum durum untuk meningkatkan penerimaan keseluruhan pasta dan mendapatkan pasta yang lebih berkhasiat. Oleh itu, pasta berpotensi memberi manfaat kesihatan kepada pengguna. Pasta soya ini boleh digunakan sebagai makanan berkhasiat untuk kumpulan berpendapatan rendah di negara-negara membangun dan menjadi suplemen semulajadi bagi mereka yang berhasrat untuk mengambil makanan protein yang tinggi.

CHAPTER 1

INTRODUCTION

1.1 Background

Pasta is one of the most consumed cereal-based food products worldwide. It is a staple food of Italian cuisine. Main ingredients of pasta are wheat flour and water (Giacco et al., 2015). Milling, mixing, extrusion and drying are the basic steps in production of pasta (Giannetti, Mariani, & Mannino, 2013). Pasta can be the basis of many cuisines due to its taste and easy to cook (Czaja et al., 2018). Camillo and Karim (2014) found that there is an increase in number of Malaysian's consumer towards western cuisine due to change of urbanization.

Functional food consists of food components that provide nutritional value that can reduce the risk of certain disease and enhancing overall health (Ross, 2000). Nowadays, people are interested in functional food because they believe there is correlation between diet and healthy living. Functional food with healthy lifestyle can prevent non-communicable disease (Hasler et al., 2009).

Wheat is a major cereal crop in many parts of the world. It is also a staple food of many diets worldwide. Wheat belongs to *Triticum* genus (McKevith, 2004). It has two main types of wheat which are common wheat (*Triticum aestivum*), known as bread wheat and durum (*Triticum durum*), known as pasta wheat (FAO, 1994). Common wheat is the most widely planted over the world. Wheat consists of three parts; bran, germ and endosperm. Whereas, refined wheat only consists of

endosperm while the germ and bran had been removed during processing. (Emalaku et al., 2017).

From the statistic reported by Food and Agriculture Organization Statistic (2019), the largest production of wheat worldwide is China followed by India. Rapid economic growth and modernization led to high demand on wheat (Bautista, 2017). Increase in westernize diet in Asia change the dietary pattern from the consumption of rice to the consumption of wheat and wheat-based products (Pingali & Rosegrant, 1998).

Wheat is the main source of carbohydrate and also contain other important nutrients like protein, fat, minerals and vitamins (Shewry & Hey, 2015). However, wheat contains low protein quality as the concentration of amino acids in wheat is lower than animal foods (Young & Pellett, 1985). It can be improved by combining it with other protein foods like legumes.

Soybean is a type of legumes and main source of vegetable oil and protein (Jones & Liu, 2009). It is originated from China and brought to the United States in the late 1800s (Wang et al., 2006). Soybean can be considered to be an excellent health food as it contains good quality protein with minimal saturated fat and moderate amount of carbohydrates (Farzana & Mohajan, 2015).

Jooyandeh (2011) also claims that soybean is one type of functional foods because it contains isoflavone and several phytochemicals that can give benefit to health. This study found that there is correlation between consumption of soy with the reduction of cholesterol level and depletion risk of non-communicable disease like cancer and heart disease. The phytochemicals have special properties such as antioxidative and anticarcinogenic activities that help in diminish the exposure of coronary heart disease and cancer.

The purpose of this study is to determine the proximate composition, total phenolic content and total flavonoid content in pasta made from composite soy and wheat flour. The proximate analysis will be conducted, which includes determination

of moisture, ash, protein, fat, carbohydrate content, total phenolic content and total flavonoid content in pasta samples.

1.2 Problem Statement

Pasta is made from refined wheat. Most of foods from refined grains contains high energy and low in nutrient content (Steffen et al., 2003). Overconsumption of refined grain foods may result in overweight and obesity, type 2 diabetes mellitus as well as increase risk of the atherosclerosis (Liu et al., 2000; Brouns et al., 2013).

According to statistic provided by World Health Organization (2018), it stated that 41 million people die from the non-communicable disease (NCD). Out of 41 million, 17.9 million people die due to cardiovascular disease, 9.0 million people die because of cancer, 3.9 million people die from respiratory disease and 1.6 million from diabetes. Factors that lead to NCD are tobacco use, physical inactivity, harmful use of alcohol and unhealthy diet (World Health Organization, 2018).

Based on the National Health and Morbidity Survey (NHMS), the prevalence of diabetes mellitus increases from 15.2% in 2011 to 17.5% in 2015. Statistic provided by WHO (2016), 9.8% of Malaysians are having diabetes, 37.3% are overweight and 12.9% are obese. Based on statistic of global status report from WHO (2010), ASEAN country have higher prevalence of high blood pressure, overweight and obesity in adults. It indicates that Malaysia have higher chance expose to the risk of non-communicable disease.

By using the United States Department of Agriculture National Nutrient Database for Standard Reference, the nutritional value per 100g of wheat flour for carbohydrate is 74.00g, fat is 1.00g, protein is 9.00g and fiber is 13.00g. While the nutritional value per 100g of soy flour for carbohydrate is 33.92g, fat is 1.22g, protein is 51.46g and fiber is 17.5g. From the data given, it shows that fiber in soy flour is slightly higher compared to wheat flour. High fiber intake will increase satiety that

made consumer reduce their food intake (Slavin, 2005). It can reduce the risk of overweight and obesity that may prevent non-communicable diseases.

Besides, soybean can be one of the mediums in order to reduce the risk of non-communicable disease. It is a type of legumes that rich in protein which help in improving health (Mani & Ming, 2016). Chatterjee et al. (2018) found that soy consumption can reduce the risk of non-communicable disease. Food and Drug Administration (1999) approved the health claim on soy protein that it can reduce coronary heart disease. Huang et al. (2016) found that soy food can reduce the pathogenic population in gut. Soy protein can overcome obesity by reducing the plasma lipid and fat accumulation that can prevent the development of atherosclerosis and lipotoxicity as well (Velasquez & Bhathena, 2007).

1.3 Significance of the study

People nowadays are concern towards their health. They believe diet plays important roles in preventing non-communicable diseases. They will likely to choose nutrient-dense food compared to energy-dense food. This study can give additional knowledge on nutrition. Besides, comparison data on nutritional composition of pasta made from soy flour and wheat flour will help in giving factual input which contain higher nutritional value, hence will help consumer in making better choice.

A study conducted by Li et. al (2014) found that consumers demand improve pasta and noodles in terms of nutritional properties, texture and appearance by using natural food additive. Therefore, this present study will modify the ingredient of pasta which include a composite of soy flour as additional ingredient.

Malaysian Food Composition Database contain data on nutritional values of food and beverages that are common in Malaysia. Nutrient data obtained can be updated in the Malaysian Food Composition Database. The nutrient data can help health professionals for dietary assessment in the future study.

1.4 Objectives

1.4.1 General Objective

To investigate the proximate compositions, total phenolic content and total flavonoid of wheat flour, soy flour, pasta made from wheat flour and composite soy flour and sensory evaluation of pasta made from wheat flour and composite soy flour.

1.4.2 Specific Objectives

- i. To determine and compare the proximate composition (moisture, ash, protein, fat and carbohydrate) between wheat flour and soy flour.
- ii. To determine and compare the proximate composition (moisture, ash, protein, fat and carbohydrate) between pasta made from wheat flour and composite soy flour.
- iii. To determine and compare the total phenolic content and total flavonoid content between wheat flour and soy flour.
- iv. To determine and compare the total phenolic content and total flavonoid content between pasta made from wheat flour and composite soy flour.
- v. To determine the sensory evaluation between commercialize pasta, pasta made of wheat flour and pasta made of composite soy flour.

1.5 Null hypothesis

There is no significant difference in the proximate compositions, total phenolic content and total flavonoid of wheat flour, soy flour, pasta made from wheat flour and composite soy flour and sensory evaluation of commercialize pasta, pasta made from wheat flour and pasta made from composite soy flour.

CHAPTER 2

LITERATURE REVIEW

2.1 Soybean

2.1.1 Overview of soybean

Glycine max, the soybean is belonging to the family *Leguminosae* that originated in southeastern Asia and was introduced to the USA in 1765. It is one of the important crops in the ancient time. The soybean plant is bushy with hairy stems and leaves. It grows 0.5m to 1.5m tall. Soybean pod consists of one to three seeds. The shape of soybean seeds is varying from almost spherical to long oval. The size can be smaller than a pea or as large as a kidney bean. The color of the seed mostly is yellow but some are green, and the hilum may be black, brown, buff or light yellow (Hauze et al., 2017).

Soybean known as nutritional powerhouse. It declared as functional foods because soybean has components that have many health promoting effects. The major components in soybean is lipid and protein. Many researches had been conducted either *in-vitro* and *in-vivo*, with animals and human subjects show the components in soybean can prevent and treat chronic diseases like cardiovascular disease, obesity, diabetes mellitus and cancer. The bioactive components of soybean that responsible of these effects are isoflavones, lecithin, saponins, lectins, oligosaccharides, and trypsin inhibitors (Chen et al., 2012; Liu et al., 2004).

2.1.2 Soy fortified products

There have several research studies have been conducted on several products made from cereal based enriched with soy flour in order to improve the nutritional value. Composition of soy flour in product is not only beneficial for vegetarian but for the diet therapy as well as isoflavone in soy delivers positive effects on human health (Keefe et al., 2015). A study conducted by Aleem et al. (2012), it shows that there is significant increase of the protein and fiber while decrease in fat and carbohydrate value with 20 percent of defatted soy flour was comparable to control (wheat flour) biscuit. Bashir et al. (2012) conducted a study on fortified pasta with 10% of defatted soy flour and 14% of chickpea flour results in increasing the value of protein, fiber and ash content of the pasta maintaining the fat at ideal level compared to control (100% wheat flour). Cookies, macaroni and burger prepared from flour mixtures using wheat, soy and defatted sesame flour have been enhanced the overall acceptability and physical quality characteristics of the products (Marques et al., 2000).

2.1.3 Soybean and obesity

Obesity and diabetes mellitus are two major concern of nutritional disorder in Malaysia. Both are well-known as the major risk factor of non-communicable disease like cardiovascular disease. Obesity happened due to imbalance of energy intake and energy expenditure. It often coexists with insulin resistance, hyperinsulinemia and abnormal lipid metabolism and further will develop diabetes mellitus, atherosclerosis and cardiovascular disease (Bhathena & Velasquez, 2002). Several studies claim that soy protein can help in managing obesity and diabetes mellitus. Goodman-gruen & Kritz-silverstein (2001) in a study on postmenopausal woman who usually consumed soy proteins result in reducing body mass index, elevating HDL cholesterol and

reducing fasting insulin level. Similarly, Mezei et al. (2003) conducting an experiment on obese Zucker rats that has been fed with high-isoflavone soy protein diet shows the significant improvement in glucose tolerance and lipid metabolism.

2.1.4 Soybean and coronary heart disease

According to Department of Statistic Malaysia, 2017, coronary heart disease such as cardiovascular disease is the leading cause of death. Dietary intake is one of the factors that lead to coronary heart disease (Frank & Hu, 2009). Frank et al. (1998) suggest that higher intake in saturated fat and trans-unsaturated fat as well as cholesterol can develop this disease. Accumulation of excess saturated fat and cholesterol in blood vessel may cause high blood pressure, which later on will lead to stroke eventually death (Bronner, Kanter & Manson, 1995). A research conducted by Baum et al. (1998) found that continuous consumption of soy protein can decrease the low-density lipoprotein (LDL) cholesterol as well as reduce the risk of cardiovascular disease. It is also proven by Khosla, Samman, & Carroll (1991) where they conducted an experiment on rabbits consumed soy protein derived from soybean and results in lowering the LDL cholesterol level. There also clinical trials on human which regularly consumed soy protein (beverage powder) can reduce plasma total and LDL cholesterol concentrations (Taku et al., 2007; Wangen et al., 2001). Isoflavones in soy protein can reduce the LDL cholesterol level by 0.18 mmol/L (7.0 mg/dL or 4.98 %) (Taku et al., 2007). Food and Drug Administration (1999) approved the health claims on soy protein consumption can reduce the risk of coronary heart disease. As a result, isoflavone can prevent the coronary heart disease by lowering the LDL cholesterol level.

2.1.5 Soybean and cancer

Some researchers prove that daily consumption of soybean can reduce the hormone related cancers like breast cancer and prostate cancer. Soybean contains phytoestrogen which also known as isoflavone that plays an important role in cancer protection. (Umphress et al., 2005). Western people had higher mortality rate of breast cancer and prostate cancer compared to Asian especially Japan that might be due to dietary intake (Nomura et al., 1978; Li et al., 2013). Traditional Japanese cuisine mostly are soy-based products like tofu, natto and miso soup. A case-control study conducted by Wu et al., 1996 found that higher intake of tofu among Asian-American woman reduce the occurrence of breast cancer. A cohort study by Kurahashi et al., (2007) relation on prostate cancer in Japanese men shows there is significant correlation higher intake of soy-based products and lower risk of prostate cancer. An experiment on animals shows that there is inhibition of tumor activity by consumed the soy isoflavone (Kim, Jeong, & Kim, 2008). However, De Souza et al., (2010) claims that soybean cannot be recommend yet as a prevention and treatment of prostate cancer.

2.1.6 Soybean and osteoporosis

Osteoporosis is usually occurred in post-menopausal women due to decline in estrogen. It will result in loss of bone density and poor bone quality (Meunier et al., 1999). One of the therapies that can overcome the risk is Hormone Replace Therapy (HRT). However, most women refused to use the treatment because it can elevate the risk of breast cancer (Zaheer & Humayoun Akhtar, 2017). Soy foods can be an alternative to HRT since numerous of studies found the beneficial effect of soybean isoflavone on bone health. A cohort study by Zhang et al. (2005) claim that

consumption of soy foods can lower the risk of fracture in postmenopausal women. Isoflavone act in overcoming the bone resorption and trigger the bone formations (Arjmandi & Smith, 2002). Potter et al. (1998) found that after 6 months, there is significantly increased bone mineral content and bone mineral density of the lumbar spine in postmenopausal women who consumed 90 mg isoflavones. Overall, long term intake of soybean is recommended in reducing the risk of osteoporosis.

2.2 Pasta

Pasta is a staple food of Italian cuisine and widely consumed around the world (Martinez et al., 2007). In Malaysia, there is a study conducted by Camillo and Karim (2014) found that there is an increase in number of Malaysian's consumer towards western cuisine including pasta. Pasta is accepted by consumer worldwide as it is easy to handle, cook, store and its taste can be blended with any cuisine (Czaja et al., 2018). It is mainly made from wheat flour and water (Giacco et al., 2015). Durum wheat is used in pasta because of its features that can give unique color, flavor and texture of pasta (Feillet & Dexter, 1996). However, most of pasta products use common wheat as it is easy to access and the price is much cheaper (Fuad & Prabhasankar, 2010). The steps of making pasta involves milling, mixing, extrusion and drying (Giannetti et al., 2013). Usually 1-cup serving cooked pasta contains 200 calories, 7 grams of proteins and 40 grams carbohydrates, including 2 grams of fiber (Bruso & Jessica, nd).

In some countries in Africa, Asia and United States, most of the low-income people depends on pasta and breads made from low-protein grains that lead to protein deficiency (Shogren, Hareland, & Wu, 2006). Common pasta usually made from durum wheat flour that is rich in carbohydrate but deficit in essential nutrients.

Thus, many researches have been conducted in improving the protein content in pasta such as inclusion of soy protein. A study conducted by Bashir et al. (2012) found that pasta containing 14% chickpea flour and 10% defatted soy flour have better quality and nutritious. Another study from Haber et al. (1978), suggested that high-protein of soy increase dough strength but have low quality of pasta. As indicated by Bahnasser and Khan (1986), fortified pasta with 10% of legume flour met the Food and Drug Administration (FDA) specification and have acceptable beany taste. Modification of high-protein pasta with 50% of soy flour have beany and bitter flavor while pasta with 35% of soy flour have greater acceptability of flavor and texture (Shogren, Hareland, & Wu, 2006).

2.3 Proximate analysis

In general, every food contains macronutrients and micronutrients. Macronutrients are nutrient required by human in large quantity. It acts as fuel energy to sustain body functions and perform daily activity. These nutrients include carbohydrate, protein and fat. While micronutrients are nutrients need in small quantity. It includes vitamins and minerals which play role in ensure normal metabolism and functioning of the body to maintain health (Food and Agriculture Organization (FAO), 2014).

2.3.1 Moisture content

Moisture content is the quantity of water contain in food. This is one of the vital procedures on food sample and quite challenging to get accurate and precise data. Total moisture correlate with water activity measurement in food that plays important

role to ensure the stability and quality of food products (McClement, 2003). Moisture content in foods varies, however water is a fundamental constituent of many foods (Bradley, 2010). One of the keys determining factor for structure and texture properties of food products is the moisture content (Chen & Rosenthal, 2015). It also can be the parameter to food safety. Hence, moisture control in food product is important to slow down the microbial growth (Zoecklein, 2010).

Evaporated method, physical method and chemical method are several methods used to determine the moisture content. Selection of method depends on the form of water present in the food and accuracy of the results desire because different methods have different level of accuracy, speed and sensitivity (Nielson, 2017). Evaporated method (oven drying) was chosen in this study. For this method, sample is heated under particular conditions and moisture content will be calculated based on the weight loss of the sample. Type of oven used, conditions within the oven, and the time and temperature of drying influence the amount of the moisture (Bradley, 2010). The advantages of this method are cost effective, quick and easy to measure the moisture content in food as it fully evaporated. However, if the time and temperature of heating is extended, it will cause decomposition (Kelly, 2004).

2.3.2 Ash

Ash is one of the parts in proximate analysis in order to determine the total amount of mineral content in the food. Ash represents the inorganic residue remaining after the complete oxidation of organic substances in food. Minerals can be differentiated from other food components as it cannot be destroyed by heating and have low volatility (Marshall, 2010). Determination in ash content is crucial in nutrition labelling, food quality (concentration and type of minerals contain, taste, appearance,

texture and stability), microbial stability as high minerals content may retard the microbial growth, nutrition and processing (McClement, 2003).

There are two main types of ashing; dry ashing method and wet ashing method. Type of method chosen based on the purpose and type of analyzing as well as the availability of the equipment (McClement, 2003). Both methods used to determine the proximate composition analysis and specific mineral, however, wet ashing method is preparation for specific mineral like Fe, Cu, Zn and P, metallic poison and usually add with acids. A study by Ali et al. (1988) on comparison of both method for elemental analysis of peat proved that wet ashing have higher value for Zn, Cu and Fe while other elements are constantly higher by using dry ashing.

In this study, dry ashing method was applied. Ash content is determined by using high temperature muffle furnace that can keep temperature between 500° C and 600°C for 24 hours (McClement, 2003). Dry ashing is much simpler, safer and economic compare to wet ashing since it probably used carcinogenic or explosive material (Andrejko et al. 1983). However, there have high tendency in losing volatile elements like Cu, Fe, Ni, P and Zn due to high temperature and long heating time (Marshall, 2010).

2.3.3 Protein

Protein is a crucial part of body tissue and important nutrient for growth. It acts in making enzymes, hormones and immunoprotein as well as transport protein. It is made from element carbon, hydrogen, oxygen and nitrogen atom which arrange together into amino acids and link in a chain (RNI, 2017). There are 20 different kinds of amino acids which 9 of them are essential amino acids (need to obtain from foods)

and the rest of them are non-essential amino acids (can be synthesized by body). Protein source mainly from animals and plants. Animal protein has higher quality of protein compared to plant protein as animal protein consists of all essential amino acids while plant protein lacks one or more essential amino acids (Hoffman & Falvo, 2005).

The recommended intake of protein is 20% from total energy intake. Lack of protein rarely happens as protein can be found in most of the foods. People who are at risk of protein deficiency are those on a nutrient-poor diet like strict vegan diet, allergies and metabolism disorders that limit the absorption of protein (RNI, 2017). It will cause growth retardation, muscle wasting, low immunity, anemia and slow healing process (World Health Organization (WHO), 2007).

In order to determine protein content in food, the Kjeldahl method and Dumas method are the common methods used in nutrition labelling and quality control. Jung et al. (2003) conducted a study on comparing the Kjeldahl method and Dumas method for determining protein contents of soybean products. The result shows that the Dumas method provides a higher value of protein determination compared to the Kjeldahl method. This is because the Dumas method tends to measure all forms of nitrogen including organic and inorganic which are not measured by the Kjeldahl method (McClement, 2010). However, due to the availability of equipment, the Kjeldahl method will be used in this study. The Kjeldahl method undergoes three main steps which are digestion, neutralization and distillation followed by titration (Chang, 2010). The nitrogen content represents the protein content.

2.3.4 Fat

Fat is one of the macronutrients together with carbohydrate and proteins. It plays important role in physiological function as well as growth and development. Apart of that, it acts as carrier and absorption of fat-soluble vitamin (vitamin A, D, E and K) and also protect vital organ from injury like lung and preserve body heat to keep warm. Fat provides higher source of energy which is 9kcal/g. However, fat is second major source of energy after carbohydrate. Excess fat will store in adipose tissue. When there is no availability of carbohydrate, this excess fat will be used as energy (RNI, 2017). Overconsumption of fat usually happen. The excess fat will deposit in body and accumulate that can lead to overweight and obesity later on will develop non-communicable disease like hypertension, atherosclerosis and diabetes mellitus. Contrarily, there is rare case on fat deficiency except for individual that have malabsorption problems (NHS, 2011).

Soxhlet method is used in this study to determine the crude fat content of the food sample. This method is official by AOAC and widely used in many experiments (Min & Ellefson, 2010). Preparation of sample for extraction involve solvent selection, drying sample, particle size reduction and acid hydrolysis. Petroleum ether, hexane and ethyl ether are solvent commonly used in fat extraction. These solvents cannot easily penetrate into foods containing water, hence dry sampling must be used in this method to get efficient extraction (McClements, 2003). Drying sample suggested to use vacuum oven drying method because it used low temperature as high temperature will cause lipid bound to carbohydrate and protein (Dekker, 2014). Soxhlet method have many advantages as it can maintain relatively high extraction temperature, easy to conduct and inexpensive equipment (Luque de Castro & GarGarcóÁa-Ayuso, 1998). The fat in the sample will be extracted by using the

Soxhlet extractor and measured by weight loss of the sample or by weight of the fat removed (Min & Ellefson, 2010).

2.3.5 Carbohydrate

Carbohydrate is one of the basic food groups that play an important role in human health. It is also the main source of energy to the human body before protein and fat. There are three major types of carbohydrate which are starch which also known as complex carbohydrate, sugars and fiber (Wheeler & Pi-Sunyer, 2008). Dietary guideline for Americans (2010) stated that fiber is non-digestible kind of carbohydrate whereas starch and sugar will be digest and absorb in small intestine. Carbohydrates will break down into simplest form which is monosaccharide (glucose, sucrose and galactose) to make it easier for absorption.

Main role of carbohydrate is to smoothen the metabolism process in the body by providing the energy to the body cells (Mul et al., 2015). It also plays an important role in maintaining the glycemic homeostasis (FAO), 1997). Besides that, in food production, carbohydrate aid in appearance of the food, textural, characteristic as well as give sweetness (McClement, 2003).

Carbohydrate provide 4kcal per gram of energy. The recommended intake for complex carbohydrate intake is around 50 to 70% of the total energy intake (RNI, 2017). Excess sugar intake will store in liver and muscle. Overconsumption of carbohydrate lead to overweight and obesity as carbohydrate will convert into energy. Excess energy accumulate in the body later on will convert into fat and stored in adipose tissue (Van Dam & Seidell, 2007).

In determination of carbohydrate content, starch, sugar and fiber are known as total carbohydrate (American Diabetes Association, 2015). In this study, it is to determine the available carbohydrate content which only include sugars and starch. Based on previous studies, there are variety of methods used to determine total carbohydrate. There have varieties way to determine it. One of the methods is the method by difference which has been used widely for nutrition labelling purpose. According to Nantel (1999), this method is the simplest as it only uses formula by the subtraction of the sums of moisture, ash, protein, fat and fiber from the total weight of sample. However, lab method is more precise compared to difference method.

2.4 Phenolic compounds

Phenolic compounds are secondary metabolite in plants that are derived from pentose phosphate, shikimate and phenylpropanoid pathway (Randhir, Lin, & Shetty, 2004). It is composing from one or more aromatic ring bearing one or more hydroxyl groups (Balasundram, Sundram, & Samman, 2006). Simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids are group of phenolic compounds that usually found in foods (M.-T. Huang & Ferraro, 1992). Since it aids in plant defense mechanism against pathogens, parasites and predator (Báidez et al., 2007, it also provides color of plants. Lima et al., (2014) reported that phenolic compounds found abundantly in fruits and vegetables.

Polyphenol become a popular topic to be studied as the ability to provide positive health effect by several mechanisms. It plays role in many biological actions including reducing the low-density lipoproteins oxidation, scavenge free radicals and regulate the vascular tone (Rodrigo & Bosco, 2006). Besides, it consists of favorable characteristics like antioxidant, immune regulatory actions, anti-cancer as well as anti-

bacterial activity and some of study found that it can improved wound healing (Małgorzata Paszkiewicz et al., 2011). Vijayalaxmi et al., (2015) found similar outcome that polyphenol help in reducing risk of chronic disease and cancers, maintain normal blood pressure and enhance endothelial function. In this study, Folin-Ciocalteu method was used to determine total phenolic content in the sample.

2.5 Flavonoid compounds

Flavonoids are a class of phenolic compounds which synthesized by phenylpropanoid pathway (Panche, Diwan, & Chandra, 2016). It is widespread in many parts of plants including seeds, bark, leaves and flower and more than 4000 various flavonoids have been identified (Kumar & Pandey, 1997). In dietary source, flavonoid can be consumed from fruits, vegetables and some beverages like tea, cocoa and wines (Heim et al., 2002). Flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols and chalcones are major classes of flavonoids (Cook & Human, 1996). The carbon atom is form in two aromatic rings which connected by three-carbon "bridge" and the classification is depends on the oxidation state of the central carbon (Barve et al., 2006).

Flavonoid act as secondary antioxidant defense system as well as gives scents and flavor to plants which can attract animals. Besides, anthocyanin in flavonoid plays role in fruits and vegetable by providing the red, purple and blue colors (Khoo et al., 2017). In population studies, they found that flavonoids are well known with their health benefits properties especially antioxidant and chelating abilities (Symonowicz & Kolanek, 2012). Antioxidant is determined by the number of hydroxyl group, as the higher the number of hydroxyl group, the greater the antioxidant activity (Cao, Sofic, & Prior, 1997). Peluso (2006) indicates that consumption of flavonoid

inversely correlated with mortality due to cardiovascular disease as it can overcome atherosclerosis by lowering lipoprotein oxidation, reduce platelet aggregability, improve vascular reactivity and aid in regulate plasma lipid and glucose homeostasis. Kumar & Pandey (2013) also suggests that flavonoid can provide protective effects that against many infectious and degenerative diseases such as heart diseases, cancers and age-related diseases.



CHAPTER 3

METHODOLOGY

3.1 Sample selection and sampling method

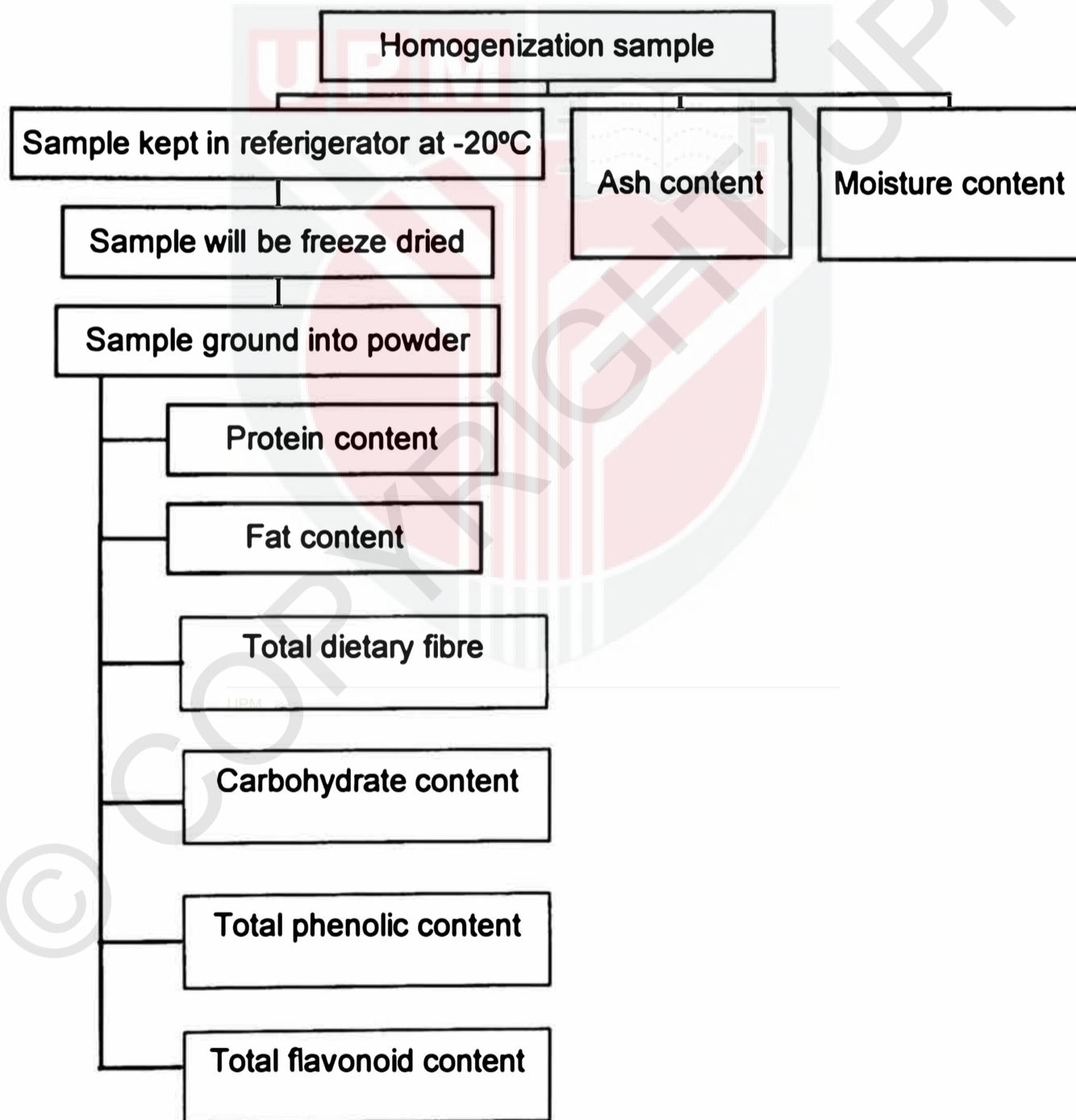
Pasta made from wheat flour and composite soy flour were analyzed in this study. Wheat flour and soy bean were purchased from Tesco, IOI City Mall. Pasta had been prepared in laboratory and it was freeze dried for further used in analysis.

3.2 Reagents and chemicals

Sodium Hydroxide solution, 40%(w/v), Catalyst tablet (3.5g K₂SO₄, 0.4g anhydrous CuSO₄), Methyl red indicator (Dissolve 100 mg methyl red in 100 ml 95% ethanol), Bromocresol green indicator (Dissolve 100 mg bromocresol green in 100 ml 95% ethanol), 2% Boric Acid solution, Concentrated sulphuric acid (H₂SO₄) 98%. 0.1M hydrochloric acid, phosphate buffer, α -amylase, protease, amyloglucosidase, Folin-Ciocalteu reagent, sodium carbonate, 5 % sodium nitrate, 10% aluminum chloride hexahydrate, sodium hydroxide

3.3 Experimental design

The sample were prepared and kept in the refrigerator at -20 °C to preserve the nutrient content in the sample. Then, the sample was divided into two portion which one portion for determination of moisture and ash and the other portion undergo freeze dried for determination of protein, fat, dietary fiber, carbohydrate, total phenolic content and total flavonoid content.



3.4 Processing of soy flour

Processing soy flour from soybean was using the method by Tyug et al. (2010) with some modification. Soybean was washed and soak for 8 hours to eliminate the inedible soy husk. Then it was roasted in the oven at 230°C/110°C until it completely dried (estimated time was around 1 hour 20 minutes). The dried soybeans were collected and ground into powder by using grinder. The coarse powder was sieve by passed through the 1.0 mm sifter until the consistency of flour was reached. The flour was packed in the airtight container and stored at 5 °C as recommended by Larrosa et al. (2013) for further use.

3.5 Preparation of pasta

The pasta was prepared following the method by Lorenzo et al. (2008) with some modification. Wheat flour and soy flour were prepared in the ratio of 100:0 and 75:25, respectively. A study conducted by Baiano et al. (2009) found that by incorporating more than 25% of soy flour in making pasta will cause the dough brittle as lack of gluten content to make it elastic. Flour, egg powder and salt were mixed together using the laboratory scale high speed blender for 1 minute at 400 rpm. Then, sunflower oil was added gradually to the mixture and mixed for 1 minute. Lastly, water was added and blended until the dough became consistent at speed 2. The dough kept in the sealed plastic bag and place in the refrigerator for one day. This relaxation process made the dough become more consistent as it allows the starch granules to be hydrated (Larrosa et al., 2013). The dough was divided into small size and rolled to make the dough into sheet. Then, pasta was made with 2 mm thickness and 30 cm length by using hand-operated noodle machine. The prepared pasta was wrapped in the sealed plastic bag and placed in refrigerator at -20°C until further analysis

3.6 Cooking process

The cooking process of pasta was followed the method by Foo et al. (2011) with some modification. 100g of distilled water was boiled in a pot. Then, 5.0g of pasta was added in boiling water for 10 minutes. The pasta was stirred to prevent from clumping. After that, pasta was taken out and rinse with cold water and drained for 10 minutes and proceed to further analysis (Foo et al., 2011).

3.7 Proximate analysis

3.7.1 Determination of moisture content

Moisture content of cooked pasta was determined by using direct heating method also known as drying method (AOAC, 2006) with minor modification. A clean aluminum dish with cover was dried by placing it in the oven for 4 hours at 105 °C. Then, cooled inside the desiccator to room temperature and weighed. 10 g of sample was added to the aluminum dish and placed in the oven at 105°C overnight. The aluminum dish was cooled by placing it in desiccator. Once reached the room temperature, the weight of the aluminum dish was weighed. The process was performed in triplicate for each sample. The moisture content of the sample was calculated as below:

$$\% \text{ of moisture} = \frac{\text{Loss of weight of the sample (g)}}{\text{Weight of the sample taken (g)}} \times 100\%$$

3.7.2 Determination of ash content

The ash content was determined by using dry ashing method (AOAC, 2006) with some modification. The crucible placed inside a muffle furnace at 550 °C for 15 minutes. The crucible was cooled and weighed. 5g of each sample was added into the crucible and placed in the muffle furnace overnight at 550 °C. Then, the crucible was placed in the desiccator to cool down to room temperature and weighed. The experiment for each sample was done in triplicate. Ash content was calculated by using equation (Marshall, 2010):

$$\% \text{ of ash} = \frac{\text{Weight of the sample weight of sample (g)} - \text{weight of empty crucible (g)}}{\text{Weight of crucible and ash (g)}} \times 100\%$$

3.7.3 Determination of protein content

The protein content was determined by using kjedahl method (AOAC International, 2006). Kjedahl method consisted of three steps which were digestion, distillation and titration. Firstly, 1g of dried sample was weighed and transferred into kjedahl flask with two tablets of catalyst and 15ml of concentrated of sulphuric acid. The mixture was shaken gently. Digestion was started by heating the flask at 420 °C until the liquid became clear and blue-green solution was obtained. The digestion was diluted with water and sodium hydroxide. After the digestion process was completed, the flask was allowed to cool to room temperature then 50ml of distilled water was added. Then, receiver solution was prepared by adding 10 ml of 2% boric acid, 10 ml/L bromocresol green indicator and 7 ml/L methyl red indicator in conical flask. Lastly, the content of conical flask was titrated with 0.1M hydrochloric acid until the content change from green to purple. The experiment was done in triplicate. Protein content was calculated as below:

$$\% \text{ of Nitrogen} = \frac{\text{Corrected volume of acid} \times 0.2 \text{ (N of HCl)} \times 14}{\text{weight of sample}} \times 100\%$$

$$\text{Protein content (\% by weight)} = \text{nitrogen content} \times 6.25$$

Where:

N HCl = normality of HCl, in mol/1000ml

Corrected acid volume = (ml std. acid for sample) – (ml std. acid for blank)

14 = atomic weight of nitrogen

Most protein contains 16% N, so the conversion factor is 6.25 (100/16=6.25)

% protein = % N × 6.25

3.7.4 Determination of fat content

The fat content was determined by using Soxhlet method. Firstly, the round bottom flask was weighed and added with 60ml of petroleum ether. 2 g of dried sample was weighed and added into extraction thimble. The extraction thimble was placed into the extractor. The extraction flask was connected to the extractor that contain the thimble and the extractor was connected to the reflux condenser. Then, the flask was heated for approximately 8 hours. After extraction was completed, the flask was dried in oven at 100°C for 30 minutes. The flask then was cooled down to room temperature in the desiccator and weighed. The experiment conducted in triplicate. Fat content was calculated as below:

$$\% \text{ of Fat} = \frac{\text{weight of fat in sample}}{\text{weight of dried sample}} \times 100\%$$

Where:

Weight of fat in sample = weight of (flask + fat) – weight of flask

3.7.5 Determination of total carbohydrate content

The total carbohydrate content was determined by using Anthrone method. Firstly, extraction was prepared by adding 1g of sample with 10ml of water and 13 ml of 52% perchloric acid. The mixture was stirred with glass rod for 20 minutes then diluted it until 100ml and filtered into 250ml volumetric flask. After that, 1ml of the sample extract and glucose standard working solution were diluted with water until 100ml. 1ml of diluted sample extract was pipetted into the test tube and 5ml of anthrone reagent (0.1 % in conc H₂SO₄) was added to each test tube.

All the content of the test tube was mixed and bluish-green solutions were formed. Then, placed it in water bath for 12 minutes and cooled to the room temperature. Optical density was measured at 620nm by using spectrophotometer. Calibration curve was construct on a graph paper, by plotting the glucose concentration (0-0.15mg/ml) on x-axis and absorbance at 620nm on the y-axis. From the graph, the amount of carbohydrate present in the sample tube was calculated. The experiment was conducted in triplicate. Total carbohydrate content was calculated as below:

$$\text{Total carbohydrate content} = \frac{\text{Absorbance of unknown}}{\text{Concentration of unknown}} \times \frac{\text{Absorbance of standard}}{\text{Concentration of standard}}$$

3.8 Extraction method

3.8.1 Aqueous extraction

Aqueous extraction was determined using a method from (Lante et al., 2018) with some modification. 10g of dried sample was weighed and 50mL of deionized water was added. Mixture was stirred with vertical stirrer for 0.1 hour. The sediment was filtered through Whatman paper No. 1 while the supernatant was freeze dried and stored at -20°C to be used in further analysis.

3.9 Determination of Total Phenolic Content

Total phenolic content of pasta was determined by using Folin-Ciocalteu method by (Gull et al. (2018) with some modification. 0.5ml of aqueous extraction was transferred into a test tube with 5 ml Folin-Ciocalteu reagent at 0.2 N. The mixture then let to stand at room temperature for 5 minutes. After that, it neutralized with 4.0 mL aqueous solution of sodium carbonate (75 mg mL⁻¹) and incubated for two hours at room temperature. The absorbance of the mixture was measured with spectrophotometer at 760 nm. Triplicate sample was prepared.

Total phenolic content was determined by using gallic acid standard curve (Akillioglu and Karakaya, 2010). The absorbance of the different concentrations (0.00, 0.25, 0.50, 0.75 and 1.00 mM) of Gallic acid were used to plot the standard curve (Noreen, Semmar, Farman, & McCullagh, 2017). It can be determined using calculation as below:

$$\text{Total Phenolic Content (mg GAE/g)} = cV/m$$

*c – Concentration of gallic acid or samples obtained from calibration curve in mg/ml

V – Volume of extract in ml

m – Mass of extract in gram

3.10 Determination of Total Flavonoid Content

Total flavonoid content was determined by using aluminum chloride colorimetric by Liu et al. (2008) with some modification. 50µl of sample from aqueous extraction was added into a tube with 4mL of distilled water. Then, added another 0.3ml of sodium nitrate solution. The mixture was left for 5 minutes at the room temperature. After that, the mixture was added with aluminum chloride hexahydrate solution and it was left for another 6 minutes at the room temperature. Next, 2ml of sodium hydroxide was added to the mixture and fill the mixture with distilled water until reach the volume 10mL. Then, left the mixture for 15 minutes and immediately read the absorbance using spectrophotometer at 510 nm. Quercetin with different concentration (200 – 1000 µg/ml) was used in order to create the calibration curve. The total flavonoid content was quantified by the calibration curve. The formula used to determine total flavonoid content as below:

$$\text{Total Flavonoid Content (mg QE/g)} = cV/M$$

*c – Concentration of quercetin or samples obtained from calibration curve in mg/ml

V – Volume of extract in ml

m – Mass of extract in gram

3.11 Sensory evaluation

To further evaluate the pasta, sensory evaluation was conducted by using method from Padalino et al. (2015) with some modification. The purpose of sensory

evaluation was to determine the preferable choice between commercial pasta and pasta made from composite soy and wheat flour. 30 untrained panelists from University Putra Malaysia's students were involved. During sensory evaluation, panelists rate the product on scale based on their response. The scale ranges from "1" (extremely unpleasant) to "9" (extremely pleasant). The evaluation included appearance, aroma, color, firmness, taste and overall acceptability.

3.12 Statistical analysis

The data was analyzed by using IBM SPSS Version 21. Independent t-test was used to determine the mean and standard deviation of proximate analysis, total phenolic content and total flavonoid content. While, ANOVA was conducted to determine the mean and standard deviation of sensory evaluation. The significance $p < 0.05$ was used in this study.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Proximate analysis

4.1.1 Moisture content

Moisture content was determined by measuring the mass of the sample before and after water is removed by evaporation. Fresh samples were used in this experiment. An independent sample t-test was conducted and it showed that the moisture content of wheat flour was significantly higher than soy flour ($p < 0.001$). The moisture content of wheat flour and soy flour were 17.50g/100g and 13.600g/100g on average of wet weight basis, respectively.

Table 1: Moisture content of wheat flour and soy flour

Sample	Mean (g/100g) \pm SD	p-value
Wheat flour	17.501 \pm 0.211	<0.001
Soy flour	13.600 \pm 0.476	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p < 0.05$ level

Based on the results, moisture content in wheat flour and soy flour are comparable with previous study by Tharise et al (2014) and Senthil et al. (2002). The results are similar with the present study which moisture content in wheat flour is

higher than soy flour (Table 2). The contrasting results between previous and present studies that may be due to different cultivars and type of wheat and soybean used in respective studies.

Table 2: Previous study of moisture content of wheat flour and soy flour

References	Flour	Results (g/100g)
Tharise et al (2014)	Wheat	13.32
	Soy	6.63
Senthil et al. (2002)	Wheat	12.68
	Soy	5.84

There are two main reasons to identify the moisture content in the flour. First, the amount of total dry solid in flour. Based on the results, it shows that soy flour contains low moisture content compare to wheat flour. Its indicate that soy flour consists of greater amount of total dry solid compare to wheat flour (Farzana & Mohajan, 2015). Secondly, high moisture in flour will let the growth of organisms and produce off odors and flavors. The recommended of moisture content to prevent the microbial growth and chemical changes during storage is below than 14% (Shahzadi, Butt, Rehman, & Sharif, 2005). The moisture content for both flours are below the recommendation and safe for storage. A lower amount of water in food can prolong the storage life of a product as it provides unfavorable condition for the growth of bacteria. The availability of water is one of the most favorable conditions for bacteria growth.

Meanwhile, the moisture content of wheat pasta and soy-wheat composite pasta were 14.315g/100g and 14.839, respectively. There was no significant difference in the moisture content of wheat pasta and soy-wheat composite pasta ($p=0.677$). Soy-wheat composite pasta had higher moisture content than wheat pasta. This result is in agreement with the study of Shogren, Hareland, & Wu (2006).

Table 3: Moisture content of wheat pasta and soy-wheat composite pasta

Sample	Mean (g/100g) ± SD	p-value
Wheat pasta	14.315 ± 0.332	0.677
Soy-wheat composite pasta	14.839 ± 1.868	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

4.1.2 Ash content

Ash content was determined by using dry ashing method which the sample has been heated in muffle furnace at 550°C for overnight until white ash obtained. The ash content has been calculated by weighing the ash in the crucible after the heating was complete. Fresh sample were used in this analysis. Ash content was determined to identify the level of mineral in the sample. It was positively correlated with the mineral content (Marshall, 2010). Ash content of wheat flour and soy flour were 1.728g/100g and 7.597g/100g, respectively. The ash content of soy flour was significantly higher than wheat flour ($p < 0.001$).

Table 4: Ash content of wheat flour and soy flour

Sample	Mean (g/100g) ± SD	p-value
Wheat flour	1.728 ± 0.408	<0.001
Soy flour	7.597 ± 0.281	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

There are some studies done on the ash content of the wheat flour and soy flour as shown in Table 5. The studies are aligned with present study. It shows soy flour

has a significantly higher ash content compared to wheat flour. Processing technique and methodology used in conducting the experiment influence the differences of results between previous and present studies.

Table 5: Previous study of ash content of wheat flour and soy flour

References	Flour	Results (g/100g)
Senthil et al. (2002)	Wheat	0.60
	Soy	6.76
Bashir (2012)	Wheat	0.80
	Soy	5.93

Soy flour has higher ash content compared to wheat flour. One of the main reasons is wheat flour has been refined while soy flour is unrefined. The bran and germ of refined wheat flour were removed during processing while unrefined soy flour contain all the kernel parts; bran, germ and endosperm. The non-endosperm (germ and bran) consist greater portion of minerals compared to endosperm (Posner & Hibbs, 2005). Hence, soy flour has higher ash due to the non-endosperm parts.

Besides, soy bean is belonging to the family of *Leguminosae*. According to Farzana & Mohajan (2015), legumes is a good source of ash. It has been reported by Onyeka and Dibia (2002) that soybean contain of huge amount of minerals. The higher amount of mineral in soybean is phosphorus (695.20 mg/100 g) followed by calcium (300.36 mg/100 g), magnesium (258.24 mg/100 g), iron (16.4 mg/100 g), sodium (3.0 mg/100 g) and zinc (2.7 mg/100 g) while cadmium is below detectable range (Etiosa, Chika, & Benedicta, 2018).

Correspondingly, the ash content of wheat pasta and soy-wheat composite pasta were 1.145g/100g and 3.052, respectively. There was a significant difference in the ash content of wheat pasta and soy-wheat composite pasta ($p=0.002$). Soy-

wheat composite pasta had higher ash content than wheat pasta and this might represent higher mineral content.

Table 6: Ash content of wheat pasta and soy-wheat composite pasta

Sample	Mean (g/100g) ± SD	p-value
Wheat pasta	1.145 ± 0.174	0.002
Soy-wheat composite pasta	3.052 ± 0.414	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

These results are similar from previous studies stated in the Table 7 shows the increases ash content with the fortification of soy flour. The results obtained has huge differences between previous and presents studies. These can be explained by the type of wheat and soy used in respective studies.

Table 7: Previous study of ash content of wheat pasta and soy-wheat composite pasta

References	Pasta	Results (g/100g)
Shogren et al. (2006)	Wheat	9.74
	Soy-wheat composite	9.83
(Bashir, 2012)	Wheat	9.17
	Soy-wheat composite	11.33

The increase in ash content in soy-wheat composite pasta may be due to higher content of ash in the soy flour than in the wheat flour. There was a significantly difference between flour and pasta for both wheat and soy ($p < 0.001$). The pasta samples contained lower ash content compared to the flour may be due to the losing of ash during the processing of pasta.

Table 8: Ash content between flour and pasta

Sample	Mean (g/100g) ± SD	p-value
Wheat flour	1.728 ± 0.408	<0.001
Wheat pasta	1.145 ± 0.174	
Soy flour	7.597 ± 0.281	<0.001
Soy-wheat composite pasta	3.052 ± 0.414	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

4.1.3 Protein content

Protein content was determined using the Kjeldhal method which the samples undergo three crucial steps (digestion, distillation and titration). The protein content in the sample was calculated from the amount of acid used to change the color of the solution during the titration. The protein content of wheat flour was 14.413 g/100g of dry weight. Meanwhile, protein content of soy-wheat composite flour was 56.805 g/100g of dry weight. Soy flour had significantly higher protein content compared to wheat flour ($p < 0.001$).

Table 9: Protein content of wheat flour and soy flour

Sample	Mean (g/100g) ± SD	p-value
Wheat flour	14.413 ± 0.439	<0.001
Soy flour	56.805 ± 0.177	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

According to nutrition information panel (NIP) from the packaging, soybean shows higher protein content than wheat flour. A study on protein content of wheat flour and soy flour by Senthil et al. (2002) also revealed the same results as shown in Table 10. Methodology used in determining the protein content and some errors during the experiment may influence the differences of the results in respective studies.

Table 10: Previous study of protein content of wheat flour and soy flour

References	Flour	Results (g/100g)
NIP	Wheat	9.00
	Soy	51.46
Senthil et al. (2002)	Wheat	10.50
	Soy	52.050

Soybean is generally rich with protein content. According to FAO (1970), amino acid profile in soybean is the best among other plant protein. It also known as a complete protein since it consists of all the essential amino acids that required for human health (Thrane et al., 2017). The nine essential amino acids that found in soybean are similar in many animal foods including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Goldflus et al., 2006). Nowadays, the uses of soy protein in production was elevated due to its functional properties and it is an economic source of dietary protein (Hawa et al., 2018).

In the contrary, wheat have low concentration of amino acids compare to animal foods. It contains low lysine and threonine as well as tryptophan. Inadequate intake of lysine and other amino acids can lead to chronic health problem like stunted growth. However, when wheat is combine with other food proteins such as legumes, oil seeds

or animal products, the proteins of wheat demonstrate excellent nutritional complementarity (Young & Pellett, 1985).

Meanwhile, the protein content of wheat pasta and soy-wheat composite pasta were 12.723g/100g and 23.717, respectively. There was a significant difference in the protein content of wheat pasta and soy-wheat composite pasta ($p < 0.001$). Soy-wheat composite pasta had higher protein content than wheat pasta.

Table 11: Protein content of wheat pasta and wheat-soy composite pasta

Sample	Mean (g/100g) \pm SD	p-value
Wheat pasta	12.723 \pm 0.200	<0.001
Soy-wheat composite pasta	23.717 \pm 0.332	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p < 0.05$ level

In comparison of protein content of wheat pasta and soy-wheat composite pasta with previous study, there is slightly difference results as listed in Table 12. Different cooking time during the pasta production could possibly affect the protein content in the samples.

Table 12: Previous study of protein content of wheat pasta and soy-wheat composite pasta

References	Pasta	Results (g/100g)
Shogren et al. (2006)	Wheat	15.36
	Soy-wheat composite	25.08
(Bashir, 2012)	Wheat	11.97
	Soy-wheat composite	18.13

The increase in protein content in soy-wheat composite pasta could be due to higher content of protein in the soy flour than in the wheat flour. Since soybean is a high-protein legume, Friedman and Brandon (2001) suggested it can be an excellent complement to lysine-limited cereal protein. Soy flour can be the basis of protein supplement in biscuit, bread, pasta, and other cereal products as many studies reveal that there are increase in protein content as increasing the soy flour fortification (Hegstad, 2008). There was a significant difference between flour and pasta for both wheat ($p=0.004$) and soy ($p<0.001$). The pasta samples contained lower protein content compared to the flour that might be due to the losing of protein during the cooking process of pasta.

Table 13: Protein content between flour and pasta

Sample	Mean (g/100g) \pm SD	<i>p</i> -value
Wheat flour	14.413 \pm 0.439	0.004
Wheat pasta	12.723 \pm 0.200	
Soy flour	56.805 \pm 0.177	<0.001
Soy-wheat composite pasta	23.717 \pm 0.332	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p<0.05$ level

4.1.4 Fat content

Fat content was determined by using Soxhlet extraction method. Solvent used was petroleum ether. The amount of fat accumulated in the flask was calculated by measuring the weight of volumetric flask before and after the extraction. Fat content of wheat flour and soy flour were 0.933/100g and 1.728g/100g, respectively. The fat content of soy flour was significantly higher than wheat flour ($p=0.044$).

Table 14: Fat content of wheat flour and soy flour

Sample	Mean (g/100g) ± SD	p-value
Wheat flour	0.933 ± 0.185	0.044
Soy flour	1.728 ± 0.437	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

Based on nutrition information panels (NIP) of respective flours, soy flour showed higher fat content compared to wheat flour. Similar result is reported by Senthil et al. (2002) as shown in Table 15. The results are slightly different for respective studies may be due to the different wheat and soybean samples cultivated and agricultural practiced in respective studies.

Table 15: Previous study of fat content of wheat flour and soy flour

References	Flour	Results (g/100g)
NIP	Wheat	1.00
	Soy	1.22
Senthil et al. (2002)	Wheat	1.24
	Soy	2.18

Soybean is globally known as an edible oil source (Farzana & Mohajan, 2015). It has the second highest oil content among the legumes after peanut (Thrane et al., 2017). According to Reddy (2004), 61% of soybean oil are polyunsaturated fat and 24% are monounsaturated fat which comparable to the unsaturated fat of other vegetable oils (85%). Soybean is indeed rich in polyunsaturated fats included two essential fatty acids which are linoleic (omega-6) and alpha-linolenic acids (omega-3) (Friedman & Brandon, 2001).

Both essential fatty acids can only be obtained from diet as it cannot be produced in body. The recommended ratio of omega-6 to omega-3 fatty acids in the diet is 4:1. Omega 3 fatty acids are important components of human cell membranes. It has anti-inflammatory effects which can protect against fatal heart disease. It also has important benefits for brain and metabolism (Simopoulos, 2008). Whereas, omega-6 fats are essential fats that are an important source of energy for the body.

The fat content in soy-wheat composite pasta was higher (0.745 g/100g) than the wheat pasta (0.726 g/100g). In addition, the mean difference of fat contents was not significantly different between both samples ($p=0.832$). This results in agreement with the study of Shogren, Hareland, & Wu (2006).

Table 16: Fat content of wheat pasta and soy-wheat composite pasta

Sample	Mean (g/100g) \pm SD	p-value
Wheat pasta	0.726 \pm 0.138	0.832
Soy-wheat composite pasta	0.745 \pm 0.047	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p<0.05$ level

4.1.5 Total Carbohydrate Content

Total carbohydrate content was determined by using the colorimetric clegg-anthrone method. Standard curve of glucose with the concentration ranging from 0.3 – 0.21 mg/ml was used to calculate the total carbohydrate content. From that, a linear calibration curve was plotted with equation of $y= 5.9901x + 0.1533$ ($R^2= 0.994$) as showed on Appendix 1. From the results, total available carbohydrate content of wheat flour and soy flour were 72.631g/100g and 29.278g/100g, respectively. The

total carbohydrate content of wheat flour was significantly higher than soy flour ($p < 0.001$).

Table 17: Total carbohydrate content of wheat flour and soy flour

Sample	Mean (g/100g) \pm SD	p-value
Wheat flour	72.631 \pm 1.474	<0.001
Soy flour	29.278 \pm 0.219	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p < 0.05$ level

Based on nutrition information panels (NIP) of respective flours, wheat flour showed higher total carbohydrate content compared to soy flour. Similar result is reported by Serna-Saldivar et al. (2019) as shown on Table 18. The results are slightly different for respective studies may be due to different cultivars and methodology used.

Table 18: Previous study of total carbohydrate content of wheat flour and soy flour

References	Flour	Results (g/100g)
NIP ^{UPM}	Wheat	74.00
	Soy	33.92
Serna-Saldivar et al. (2019)	Wheat	70.3
	Soy	25.4

According to Shewry and Hey (2015), the main dietary contribution of wheat are carbohydrate. Hence, wheat is a major source of starch and energy. The refined wheat removes the germ and bran while retains the endosperm which is mainly made up from carbohydrate (Emalaku et al., 2017). It indicates that wheat flour is mainly

composed by carbohydrate. As for soybean, there is a finding from Karr-Lilienthal et al. (2005) reported that it contain 35% of carbohydrate consists of digestible sugars, starch and non-digestible oligosaccharide.

Furthermore, the total carbohydrate content of wheat pasta and soy-wheat composite pasta were 69.493 g/100g and 64.315 g/100g, respectively. There was a significant difference between total carbohydrate content of wheat pasta and soy-wheat composite pasta ($p=0.002$). Wheat pasta had higher total carbohydrate content than soy-wheat composite pasta.

Table 19: Total carbohydrate content of wheat pasta and soy-wheat composite pasta

Sample	Mean (g/100g) \pm SD	p-value
Wheat pasta	69.493 \pm 0.596	0.002
Soy-wheat composite pasta	64.315 \pm 1.105	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p<0.05$ level

The total carbohydrate content of wheat pasta and soy-wheat pasta in the study showed similarity with the study conducted by Adegunwa et al. (2015) and Bashir (2012) as shown on Table 20. The wheat pasta was a significantly higher total carbohydrate content compared to soy-wheat composite pasta. There were slightly difference results from respective study as the production region and the cultivar could affect the nutrient content of the samples.

Table 20: Previous study of total carbohydrate content of wheat pasta and soy composite pasta

References	Pasta	Results (g/100g)
Adegunwa et al. (2015)	Wheat	65.12
	Soy-wheat composite	63.11
Bashir (2012)	Wheat	76.29
	Soy-wheat composite	67.31

The decreasing of total carbohydrate in the soy-wheat composite pasta could be due to the low carbohydrate content of added soy flour. There was a significantly difference between flour and pasta of total carbohydrate content for both wheat ($p=0.027$) and soy ($p<0.001$). The pasta samples contained lower carbohydrate content compared to the flour might be due to the additional water in the ingredients.

Table 21: Total carbohydrate content between flour and pasta

Sample	Mean (g/100g) \pm SD	p -value
Wheat flour	72.631 \pm 1.474	0.027
Wheat pasta	69.493 \pm 0.596	
Soy flour	29.278 \pm 0.219	<0.001
Soy-wheat composite pasta	64.315 \pm 1.105	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p<0.05$ level

4. 2 Total phenolic content

Total phenolic content was determined by using the standard curve of gallic acid with the concentration ranging from 0 – 0.50 mg/ml. From that, a linear calibration curve was plotted with the equation of $y = 3.9849x + 0.1103$ ($R^2 = 0.9801$) as shown in Appendix 2. The phenolic content in the samples were expressed as mg gallic acid equivalent (GAE)/100g dry weight.

Total phenolic content of wheat flour was 79.883 mg GAE/100g of dry weight while total phenolic content of soy flour was 175.074 mg GAE/100g dry weight. The results showed that total phenolic content in soy flour was significantly higher than in wheat flour ($p < 0.001$).

Table 22: Total phenolic content of wheat flour and soy flour

Sample	Mean (GAE/100g) ± SD	p-value
Wheat flour	79.883 ± 5.036	<0.001
Soy flour	175.074 ± 3.242	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

Most of plant-based food contains variety of phenolic compounds. Phenolic compounds have special properties like antioxidative and anticarcinogenic activities in fruits, vegetables and grains that can reduce the risk of coronary heart disease and cancer (Emmons & Peterson, 2001). It also act as plant resistance as it against the microbial infection in plant (Grassmann et al., 2002). Therefore, both wheat and soy have plenty of phenolic compounds.

However, soy flour has higher phenolic compound than wheat flour. Refined wheat flour has been used in this study which already remove the bran and germ part

of wheat while soy flour still retains all the part of kernel. According to the Xu and Chang (2008), non-endosperm parts of the seeds usually consisted of phenolics and other antioxidative components. Therefore, soy flour has higher phenolic compound compared to wheat flour. Genetics, environment and processing technique used influence the variability of phenolic compounds in the soybean (Sharma et al., 2018). Yet, several phenolic compounds like isoflavone, *p*-hydroxybenzoic acid, salicylic acid, *p*-coumaric acid and ferulic acid were detected in all soybean cultivars (Cheynier, 2012).

Whereas, the total phenolic content of wheat pasta and soy-wheat composite pasta were 56.977 GAE/100g and 78.204 GAE/100g, respectively. There was a significant difference in the total phenolic content of wheat pasta and soy-wheat composite pasta ($p=0.002$). Soy-wheat composite pasta had higher phenolic content than wheat pasta. The increase in phenolic content in soy pasta could be due to higher phenolic content in the soy flour compared to the phenolic content in the wheat flour.

Table 23: Total phenolic content of wheat pasta and soy-wheat composite pasta

Sample	Mean (GAE/100g) \pm SD	<i>p</i>-value
Wheat pasta	56.977 \pm 4.357	0.002
Soy-wheat composite pasta	78.204 \pm 3.224	

The values are expressed as the mean of three replicate samples \pm standard deviation.

* significant at the $p<0.05$ level

There were significant differences between flour and pasta of both wheat and soy ($p<0.001$). The phenolic content in flour was higher than in the pasta. The cooking process is one of the factors decrease in antioxidant activity due to leaching in the cooking water (Baiano et al., 2009).

Table 24: Total phenolic content between flour and pasta

Sample	Mean (GAE/100g) ± SD	p-value
Wheat flour	79.883 ± 5.036	<0.001
Wheat pasta	56.977 ± 4.357	
Soy flour	175.074 ± 3.242	<0.001
Soy-wheat composite pasta	78.204 ± 3.224	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

4.3 Total flavonoid content

Total flavonoid content was determined by using the quercetin standard curve with the concentration ranging from 0 – 0.1 mg/ml. From that, a linear calibration curve was plotted with the equation of $y = 6.5852x + 0.0061$ ($R^2 = 0.9978$) as shown in Appendix 3. The flavonoid content in the samples were expressed as mg quercetin equivalent (QE) per gram of sample dry weight.

The total flavonoid content of wheat flour was 61.400mg QE/g. Whereas, total flavonoid content of soy flour was 170.897mg QE/g. The results displayed that total flavonoid content of soy flour was significantly higher than wheat flour ($p < 0.001$).

Table 25: Total flavonoid content of wheat flour and soy flour

Sample	Mean (mg QE/g) ± SD	p-value
Wheat flour	61.400 ± 4.881	<0.001

Soy flour

170.897 ± 1.694

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

Generally, soybean or soy products have been known to contain high isoflavone. Isoflavone is a group of flavonoid compounds that are abundantly found in some of plant foods. Malonyl genistein, genistein, malonyl daidzein and daidzein are the main isoflavone forms of soybean. In addition, genistein and daidzein are the strongest isoflavones in term of antioxidant activity (Xu & Chang, 2008). Soy isoflavones have special properties that can promote health such as antioxidant, inhibitory on cancer cell proliferation, anti-inflammatory and preventive of coronary heart disease and osteoporosis (Wang et al., 2013).

In addition, the total flavonoid content of wheat pasta and soy-wheat composite pasta were 43.523mg QE/g and 60.882mg QE/g, respectively. There was a significant difference in total flavonoid content between wheat pasta and soy-wheat composite pasta ($p < 0.001$). Soy-wheat composite pasta had higher flavonoid content than wheat pasta. The increase in flavonoid content in soy pasta could be due to higher flavonoid content in the soy flour.

Table 26: Total phenolic content of wheat pasta and soy-wheat composite pasta

Sample	Mean (mg QE/g) ± SD	p-value
Wheat pasta	43.523 ± 1.475	<0.001
Soy-wheat composite pasta	60.882 ± 1.537	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the $p < 0.05$ level

There was significantly different between wheat flour and wheat pasta as well as soy flour and soy-wheat composite pasta ($p < 0.001$). The flavonoid content in the

flour was higher than in the pasta. During the cooking process, pasta was introduced to the boiling water that make the nutrient content in the pasta migrate to the water.

Table 27: Total phenolic content between flour and pasta

Sample	Mean (mg QE/g) ± SD	p-value
Wheat flour	61.400 ± 4.881	<0.001
Wheat pasta	43.523 ± 1.475	
Soy flour	170.897 ± 1.694	<0.001
Soy-wheat composite pasta	60.882 ± 1.537	

The values are expressed as the mean of three replicate samples ± standard deviation.

* significant at the p<0.05 level

4.4 Sensory evaluation

During the sensory evaluation, 30 untrained panelists from Universiti Putra Malaysia students were involved. Commercialize pasta, wheat pasta and soy-wheat composite pasta were evaluated on appearance, aroma, color, firmness, taste and overall acceptability. Panelists filled the form in Appendix 4 based on their preference for each attribute. Analysis of variance (ANOVA) showed that there were significant differences between commercialize pasta and soy-wheat composite pasta as well as commercialize pasta and wheat pasta for all the attributes. Whereas, there was no significant difference between soy-wheat composite pasta and wheat pasta for all the attributes as shown in the Table 28. Based on the sensory evaluation results, showed that commercialize pasta had the most preference of all the attributes followed by soy-wheat composite pasta and wheat pasta.

The taste is the most important factor that influence the overall acceptability of food products. This factor is the major concern in order to market the products. The score taste for control pasta (7.867) had higher preference compared to home-made pasta; soy-wheat composite pasta and wheat pasta. Meanwhile, the score for home-made pasta had been slightly increase to 5.933 from 5.533 with the fortification of soy flour. Wheat pasta was rated poorest in taste. The beany taste in soy-wheat composite pasta are in acceptable level (Akubor and Ukwuru, 2005). This results contradict with the study conducted by Farzana and Mohajan (2015) that found out low in preference as the increase in the level of substitution of soy flour.

The appearance was related to the texture of pasta. The control (commercial pasta) had the highest mean value (8.233). In comparison of home-made pasta, the mean scores for appearance of the pasta was higher in soy-wheat composite pasta (5.767) than wheat pasta (5.600). In the case of aroma, a similar trend had been displayed in the results. The control had the highest mean value (7.367) followed by soy-wheat composite pasta (6.200) and wheat pasta was rated poorest for aroma (5.867). There is no study conducted in comparing pasta made from wheat flour.

The control pasta had the highest mean score (7.967) for color followed by soy-wheat composite pasta (6.033) and wheat pasta (5.600). The durum wheat gives the yellow intense color to the commercialize pasta while soybean give the pale-yellow color to the soy-wheat composite pasta. Whereas, the wheat pasta has the white color from the wheat flour.

As for the firmness, the control pasta had the highest mean score (8.133) followed by soy-wheat composite pasta (7.400) and wheat pasta (5.300). Production of pasta involve the milling, mixing, extrusion and drying process (Giannetti, Mariani, & Mannino, 2013). This influence the firmness of the pasta. Kneading the dough is another important step to get the firmness of the pasta as it can helps in developing the gluten which can strengthen the dough. For commercialize pasta, the kneading process more uniform and faster compared to home-made pasta. Besides, the durum

wheat flour in commercialize pasta and soy flour have higher protein content than wheat flour, which produces the gluten necessary to strengthen the dough of pasta.

Overall acceptability was influenced by many aspects especially the taste. The commercialize pasta has the highest mean value (8.233) followed by soy-wheat composite pasta (6.233) and wheat pasta (5.900) for the overall acceptability.

Table 28: Sensory attributes of commercialize pasta, wheat pasta and soy-wheat composite pasta

Sample	Commercialize Pasta	Soy-wheat composite Pasta	Wheat Pasta
Appearance	8.233 ± 0.774	5.767 ± 1.524 ^a	5.600 ± 1.453 ^a
Aroma	7.367 ± 1.377	6.200 ± 1.540 ^a	5.867 ± 1.655 ^a
Color	7.967 ± 1.273	6.033 ± 1.377 ^a	5.600 ± 1.354 ^a
Firmness	8.033 ± 1.167	7.400 ± 1.567 ^a	5.300 ± 1.601 ^a
Taste	7.867 ± 1.106	5.933 ± 1.741 ^a	5.533 ± 1.950 ^a
Overall Acceptability	8.233 ± 1.104	6.233 ± 1.568 ^a	5.900 ± 1.626 ^a

Values are expressed as means ± standard deviation. Values with the same superscript in a row are not significantly different (P > 0.05).

CHAPTER 5

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 Conclusion

It can be concluded that the ash, protein and fat content of soy flour are higher than wheat flour. Whereas, the moisture and carbohydrate content of wheat flour is higher than soy flour. As for the total phenolic and total flavonoid content, both contents are higher in soy flour compared to wheat flour. The moisture, ash, protein, fat and total carbohydrate content were significantly different between wheat flour and soy flour. The total phenolic and total flavonoid content as well showed the same results.

The moisture, ash, protein and fat content of soy-wheat composite pasta is higher compared to wheat pasta. Meanwhile, only carbohydrate content is higher in wheat pasta compared to soy-wheat composite pasta. The total phenolic and flavonoid content also higher in soy-wheat composite pasta than wheat pasta. The ash, protein and total carbohydrate content were significantly different between wheat pasta and soy-wheat composite pasta. However, the moisture and fat content were not significantly different between wheat pasta and soy-wheat composite pasta.

The moisture, ash, protein, fat and total carbohydrate content in flour are higher compared to pasta. Similarly, total phenolic and total flavonoid content in flour are higher compared to pasta. This might be due to leaching of the nutrient content during the cooking process into the boiling water or loss the nutrient during the preparation of pasta.

For the sensory evaluation, most of the panelists preferred commercialize pasta over home-made pasta followed by soy-wheat composite pasta and wheat pasta. Its might be due to the different technique used in making the pasta. Besides that, this present study used refined wheat flour in order to make pasta while commercialize pasta used durum wheat flour. This influence the appearance, texture, color and firmness of the pasta as durum wheat has special characteristic that can give unique color, flavor and texture to the pasta. Hence, future study can replace the refined wheat flour to durum wheat flour to improve the overall acceptability of the pasta.

Overall, soy-composite pasta had superior nutritional values that potentially give health benefit to consumers. This soy pasta can be used as a nutritious food for low income group in developing countries and act as natural supplement to those who intended to take high protein intake. Besides, soy-composite pasta rich in antioxidant that can prevent cancer.

5.2 Limitations and recommendations

One of the limitations of this study is using only water as the solvent in the extraction. Solvent with different polarity should be used in future study to optimize the extraction of bioactive compound in the sample. Methanol extraction and ethanol extraction are mostly used in several other studies in order to optimize the extraction from wheat and soy sample.

Other than that, present study only obtained the nutritional value and preference of 25% of soy-wheat composite pasta. Future work can increase the percentage of soy flour substitution from 0 to 50%. As for the sensory evaluation, it only involved the

untrained panelist. In order to get more reliable and precise results, future study should involve the trained panelist.

Furthermore, future study can conduct the antioxidant assay as this study only determined the total phenolic and total flavonoid content of the samples. Last but not least, in this study refined wheat flour have been used to make pasta. Hence, future study can use durum wheat flour to improve the nutrient content and overall acceptability of the pasta.



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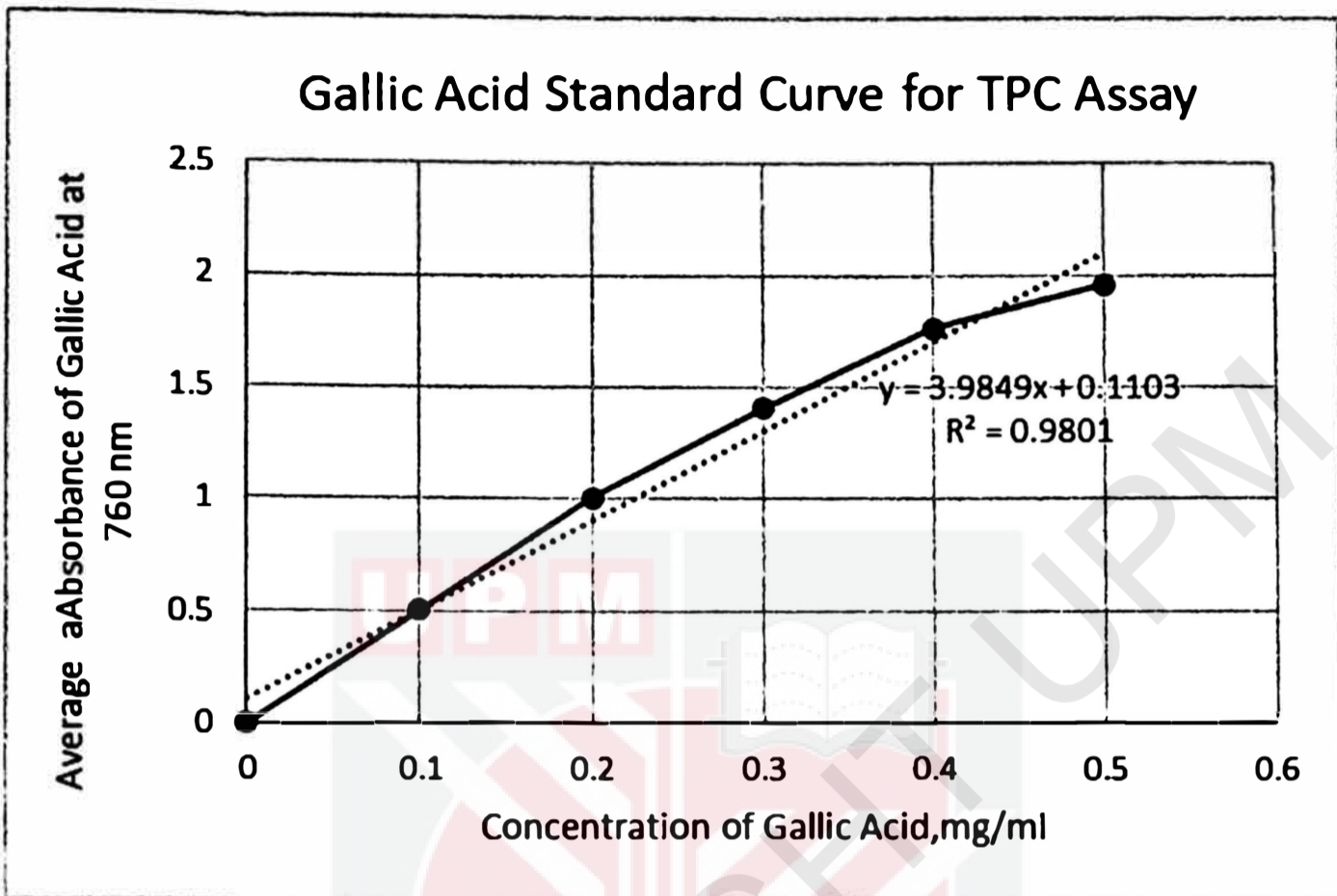
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APPENDICES



Standard curve of gallic acid for Folin-Ciocalteu analysis

(Total phenolic content)



Standard curve of quercetin for aluminum chloride colorimetric method

(Total flavonoid content)

