NUTRITIONAL COMPOSITIONS, TOTAL PHENOLICS, ANTIOXIDANT CAPACITIES, AND STUDENTS' KNOWLEDGE LEVEL ABOUT PLANT-BASED MEAT ITEMS

By

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ABSTRACT

NUTRITIONAL COMPOSITIONS, TOTAL PHENOLICS, ANTIOXIDANT CAPACITIES, AND STUDENTS' KNOWLEDGE LEVEL ABOUT PLANT-BASED MEAT ITEMS

CHEN YU WEI

Plant-based meats (PBM) items are processed plant-based products intended to replicate the sensory characteristics of animal-based products. With increasing global health awareness, PBM items are gaining popularity owing to their superior nutritional and antioxidant characteristics, which make them potentially a healthier option than meat-based products. However, little is known about the nutritional and antioxidant information on ready-to-eat PBM items, particularly those marketed in Malaysia. Therefore, this study aims to determine the nutritional compositions (carbohydrate, protein, fat, fiber, ash and moisture) using proximal analyses and antioxidant properties (total phenolics content (TPC), total flavonoid content (TFC) and antioxidant capacities) using Folin-Ciocalteu, Aluminuium Chloride, DPPH and ABTS assays, for three PBM items: Kale Pizza (KP), Double Cheeseburger (DCB) and Spaghetti in Carbonara with Plant-Based Minced Meat (SC). One-way analysis of variance (ANOVA) and Tukey test were used to determine the significant differences of results at $p<0.05$ level, and Pearson correlation was performed to examine the

association between antioxidant parameters. Also, a cross-sectional survey was conducted to assess the consumers' knowledge of PBM items among Universiti Tunku Abdul Rahman (UTAR) undergraduate students. Questionnaires were distributed virtually to eligible participants throughout the data collection period. Based on the results, moisture (54–66%) constituted the largest proportion of all samples, followed by carbohydrates (28–33%), fat (3–6%), fiber (2–4%) and ash (1–3%), while protein (<l%) made up the least. KP and DCB had lower moisture content, higher ash, protein, fat and carbohydrate contents than SC. There were no significant differences in fiber content, TPC and TFC, while SC exhibited the lowest antioxidant capacity. Besides, TPC exhibited a strong association with antioxidant capacity ($r = 0.94$; $r = 0.90$), while TFC had a weak-to-moderate association ($r = 0.46$; $r = -0.31$). Overall, UTAR undergraduates revealed a low knowledge level (37%), particularly in terms of nutritional knowledge of PBM items, highlighting the necessity of educational efforts to improve their understanding in this aspect.

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DECLARATION

I hereby declare that this final year 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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APPROVAL SHEET

This final year project report entitled "**NUTRITIONAL COMPOSITIONS, TOTAL PHENOLICS, ANTIOXIDANT CAPACITIES, AND STUDENTS' KNOWLEDGE LEVEL ABOUT PLANT-BASED MEAT ITEMS**" was prepared by CHEN YU WEI and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Dietetics at Universiti Tunku Abdul Rahman.

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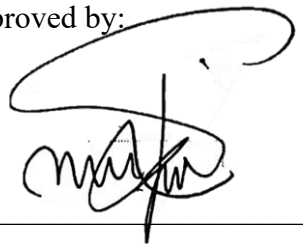

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I hereby give permission to the University to upload the softcopy of my final year project report in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(CHEN YU WEI)

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LIST OF ABBREVIATIONS

AA	Antioxidant activity
ABTS	2,2′-azinobis(3-ethyl benzothiazoline-6-sulfonate acid)
ANOVA	Analysis of variance
CAGR	Compound annual growth rate
CVD	Cardiovascular diseases
DCB	Double Cheeseburger
DPPH	2,2-diphenyl-1-picrylhydrazyl
F-C	Folin-Ciocalteu
GAE	Gallic acid equivalent
GI	Glycemic index
IC50	half-maximal inhibitory concentration
KP	Kale Pizza
MUFA	Monosaturated fats
NHMS	National Health and Morbidity Survey
NP	Nutrition professionals
PBM	Plant-based meat
PDCAAS	Protein Digestibility Corrected Amino Acid Score
QE	Quercetin equivalent
RNI	Recommended Nutrient Intakes
SC	Spaghetti in Carbonara with Plant-Based Minced Meat
TFC	Total flavonoids content
TPC	Total phenolics content
UTAR	Universiti Tunku Abdul Rahman

CHAPTER 1

INTRODUCTION

1.1 Research Background

Plant-based meat (PBM) items are processed foods made from an appropriate blend of plant-based or vegetable ingredients that are intended to replicate the sensory characteristics of animal-based products (McClements and Grossmann, 2021; Swing, et al., 2021; Huang, et al., 2022). Sometimes, they are also referred to as meat analogs, imitation meat, meat substitutes or faux meat (Abdullah, et al., 2022). Soy, wheat and pea proteins are commonly used as protein sources for PBM items (Huang, et al., 2022). The addition of plant-based lipids, flavorings, colorings, binding agents or other food additives in PBM items could help to enhance their similarity to meat and increase consumer acceptability (Kyriakopoulou, et al., 2018). They can be seen in the market in various forms, such as plant-based sausage, patties, nuggets, ham, etc. (Mohamed, et al., 2017).

The rising popularity of PBM items has been observed globally in recent times (Swing, et al., 2021). By 2030, the PBM sector is predicted to achieve a revenue of US $16.55 billion with a compound annual growth rate (CAGR) of 15.1% (Data Bridge Market Research, 2023). Western countries were the dominant market for selling PBM and its products (Choudhury, et al., 2020). Curtain and Grafenauer (2019) mentioned that there has been a five-fold surge in the number of PBM items in Australia over a period of four years. Other than that, Ismail,

Hwang and Joo (2020) pointed out that the emerging attentiveness and rising demand for PBM items in Asian countries will provide a significant opportunity for the PBM industry.

One of the main drivers for the transformation of PBM consumption was the growth of health concerns. Excessive intake of meat increases the risk of several diet-related chronic diseases, including cardiovascular diseases (CVD) and cancer, as high levels of cholesterol are found in meat (Aschemann-Witzel, et al., 2020; Jahn, Furchheim and Strässner, 2021). Apart from that, the population of vegetarians and flexitarians (semi-vegetarians) also increasing rapidly (Michel, Hartmann and Siegrist, 2021). PBM products targeted these populations to meet their nutritional needs while fulfilling their meat cravings (Safdar, et al., 2022). In addition to health reasons, Jahn, Furchheim and Strässner (2021) highlighted in their review that animal cruelty issues serve as a significant motivation for individuals to adopt plant-based diets. Also, PBM production could effectively mitigate the adverse impacts of animal agriculture, such as deforestation and can be friendly to the environment.

Despite the current efforts to develop PBM, there are several barriers as meats are generally viewed as high-quality protein providing all the essential amino acids (Aschemann-Witzel et al., 2020), and the principal sources of key micronutrients (Alessandrini, et al., 2021). Hence, some PBM items use complementary proteins to fulfill the amino acid requirements and are fortified with vitamins and minerals such as vitamin B12 and iron that are only found in

animal-based products (Kyriakopoulou, et al., 2018). Nevertheless, despite PBM items being structurally comparable to animal-meat products, they nonetheless differ nutritionally (Abdullah, et al., 2022). PBM items have the potential to serve as a sustainable alternative to animal meat owing to their superior nutritional characteristics (Singh, et al., 2022). Compared to animal meat, it is claimed to be lower in calories and total fats, no cholesterol, high dietary fiber (Alessandrini, et al., 2021), and many health-promoting phytochemicals (Kyriakopoulou, et al., 2018). However, as the ingredients used vary among different formulations of plant-based meat products, the nutritional compositions may also vary (Ketelings, et al., 2023).

There is a growing demand for plant-based protein products in Malaysia as influenced by rising trends in health consciousness worldwide. These modifications involve a shift towards nutritious diets, as well as the concept of vegetarianism and flexitarianism (Austrade, 2021). Also, abstaining from meat is also well known in the religious practices of Buddhism and Hinduism (Mohamed, et al., 2017). In Malaysia, regional plant-based food businesses have sprouted up, providing plant-based meals tailored to local flavor preferences (Austrade, 2021). Despite this, Malaysia still has the highest incidence of overweight and obesity among Southeast Asian countries (Tarmizi, Daudi and Rahman, 2020). According to the National Health and Morbidity Survey (NHMS) conducted in 2019, one in every two Malaysian adults has problems with overweight or obesity (Institute for Public Health, 2020). This is because poor dietary habits were observed in Malaysian adults with high intakes of animal-based protein and low intake of dietary fibers, all of which fall outside

of the recommended intake range suggested by Recommended Nutrient Intakes (RNI) for Malaysia (Lee and Muda, 2019). Not only that, the number of obesity and heart-related problems among Malaysians is still increasing (Mohamed, et al., 2017), suggesting there is a need to increase awareness of plant-based product intake in Malaysia, especially PBM items.

1.2 Problem Statements

To present, most studies in overseas have compared the nutritional compositions and antioxidant properties between plant-based meat (PBM) and animal-based meat. PBM is generally known to be a healthier option for meat due to its superior macronutrient and phytochemical profiles (Singh, et al., 2022). Nevertheless, these studies primarily concentrate on assessing the isolated food items. Little is known about the nutritional compositions and antioxidant properties of food in ready-to-eat forms, especially those PBM items marketed in Malaysian restaurants. Moreover, the actual nutrient intake of consumers cannot be accurately reflected by solely depending on data from isolated food items, because it ignores other food ingredients included in the meal (Salau and Hasan, 2014). Typically, a ready-to-eat cooked meal often comes with major sources of carbohydrates including wheat, rice and noodles (Tarmizi, Daudi and Rahman, 2020), implying that these additions might further influence the nutritional profiles of PBM items.

Furthermore, it is commonly known that plant protein products and local plant-based food companies are sprouting in Malaysia (Austrade, 2021). Despite this encouraging trend, several research had pointed out the possible obstacles in PBM marketing due to a lack of consumers' understanding regarding PBM (Wang, et al., 2023). In Malaysia, only a study was conducted exclusively targeting Chinese non-vegetarian customers to evaluate their opinion towards plant-based food and found that 58.6% of them had favorable perceptions (Mohamed, et al., 2017). However, the consumers' knowledge of PBM items among Malaysian undergraduate students was scarcely explored up to this point, which leaves a research gap. As such, this study is designed to assess the nutritional compositions and antioxidant properties of PBM items, considering their serving with other food ingredients, as well as the knowledge levels among undergraduate students towards PBM items.

1.3 Objectives

1.3.1 General Objective

To investigate the nutritional compositions, total phenolics, and antioxidant capacities of plant-based meat (PBM) items in relation to consumer knowledge of PBM items.

1.3.2 Specific Objectives

1) To determine the nutritional compositions (moisture, ash, fat, protein, carbohydrate and fiber contents) of PBM items.
2) To determine total phenolics content (TPC), total flavonoids content (TFC) and antioxidant capacities of PBM items.
3) To determine the correlation between TPC, TFC and antioxidant capacities of PBM items.
4) To assess consumers' knowledge towards PBM items among Universiti Tunku Abdul Rahman (UTAR) undergraduate students.

1.4 Hypothesis

1.4.1 Null Hypotheses, H_0

- There is no significant difference in moisture, ash, fat, protein, carbohydrate and fiber contents between PBM items.
- There is no significant difference in TPC, TFC and antioxidant capacities between PBM items.
- There is no correlation between TPC, TFC and antioxidant capacities.
- The level of knowledge of PBM items among UTAR undergraduate students is low.

1.4.2 Alternative Hypotheses, H_1

- There is a significant difference in moisture, ash, fat, protein, carbohydrate and fiber contents between PBM items.
- There is a significant difference in TPC, TFC and antioxidant capacities between PBM items.
- There is a correlation between TPC, TFC and antioxidant capacities.
- The level of knowledge of PBM items among UTAR undergraduate students is high.

1.5 Significance of Study

This study is designed to investigate the nutritional compositions, total phenolics and antioxidant capacities of PBM items in the menu offered by a plant-based meat restaurant. This assists in closing the knowledge gap and offers valuable insights into the nutritional compositions and antioxidant profiles of PBM items, which would support consumers in making better-informed decisions regarding their food choices. This study also intends to offer relevant insights to facilitate future product research and development.

Besides, this is a preliminary study on UTAR undergraduate students to investigate their level of knowledge of PBM products. The findings offer significant implications for raising their understanding, awareness and familiarity with the nutritional aspects of PBM items. This study also seeks to provide a better understanding of the existing knowledge pattern of PBM items among undergraduates, which could assist in the effectiveness of promotional efforts intended at raising PBM items consumption in university settings. Future researchers may explore the knowledge, attitudes and practices regarding PBM items among undergraduate students.

CHAPTER 2

LITERATURE REVIEW

2.1 Nutritional Composition of Plant-Based Meat (PBM) Products

2.1.1 Moisture

Water is a key component of the majority of food items. The stability, quality, texture, and physical appearance of foods were all influenced by their moisture level (Marshall, 2010). Several studies indicated that PBMs exhibited less moisture content than their respective conventional animal meat products (Bakhsh, et al., 2021a; Ghangale, et al., 2022). The decreased moisture loss in PBMs is probably attributed by the ability of dietary fibers to hold onto water during formulation processes (Ghangale, et al., 2022), particularly with the incorporation of methylcellulose (MC). In the thermal gelation of methylcellulose, a gel-like layer produced upon heating, serving as a barrier against moisture loss in PBM products (Bakhsh, et al., 2021a). Furthermore, raising in MC concentration from 1.5 to 4.0% resulted in a reduction of water holding capacity (Bakhsh, et al., 2021b), indicating a decline in moisture levels within PBM products.

2.1.2 Ash

When organic substances in food have totally oxidized or ignited, the resulting inorganic residue is known as ash. The amount of minerals in food can be determined by their ash content (Marshall, 2010). Few studies have compared the ash content of PBMs with animal-based meat. A higher ash content was found in PBM patties than in beef and pork patties (Bakhsh, et al., 2021a). Nevertheless, Ghangale, et al. (2022) reported that ash content was substantially greater in the control sample, when compared to PBM imitates formulated with 70% jackfruit (M1), as well as 20% jackfruit and 50% cashew (M2). The result may be due to the addition of jackfruit to PBM imitates resulted in an increase in ash level, which was attributed to extra minerals, starch and fiber. In short, the choice of raw materials used can account for differences in ash concentration.

2.1.3 Fat

Fat contributes significantly to the nutritional value and sensory characteristics of meat and its alternatives (Kołodziejczak et al., 2021). Fat could enhance the sensory profiles of PBM items, including texture, flavour and tenderness (Kyriakopoulou, et al., 2018). In terms of nutrition, the use of vegetable fats in PBM items is more advantageous for health as they contain less saturated fat and without cholesterol (Fresán, et al., 2019; Kołodziejczak, et al., 2021). PBMs are typically composed of 0 to 15% of vegetable oils (Huang, et al., 2022), which is often derived from sunflower oil, rapeseed oil, canola oil, maize oil, coconut oil and soya oil (Kyriakopoulou et al., 2018).

Nonetheless, most studies reported a similar finding which PBMs were generally lower in total fat and saturated fat than their respective animal-meat products (Kyriakopoulou, et al., 2018; Curtain and Grafenauer, 2019; Alessandrini, et al., 2021; Bakhsh, et al., 2021a; Safefood, 2021). The reduced fat content in PBMs might be ascribed to the use of defatted materials and the presence of lipids may impact the fibrous structures development in PBMs production (Kyriakopoulou et al., 2018). Moreover, Bakhsh, et al. (2021a) highlighted the inclusion of non-polar hexane during the high-pressure extrusion process, which probably eliminated part of the fat in PBM patties.

Besides, Cutroneo, et al. (2022) revealed that, apart from white meat, all meat controls exhibited higher saturated fat levels, and Alessandrini et al. (2021) published similar finding, stating that animal meat products contained double the amounts of saturated fat than PBM alternatives. They also noted an association between decreased fat content and lower calorie levels in PBMs. Hence, PBM has the potential to assist individuals in preventing excessive intake of calories and saturated fat, which are linked to obesity and cardiovascular diseases (CVD), respectively (Alessandrini et al., 2021). This suggested PBMs offer a better nutritional profile and health benefits than animal meat products.

Additionally, the quantity of fat in PBM products is influenced by the raw materials used in the formulation. According to Fresán, et al., (2019), nut-based meat analogues had significantly higher levels of total fat and monosaturated

fats (MUFA) compared to wheat- and soy-based products. Ghangale et al. (2022) also reported the greatest fat content in the sample formulated with cashew nut flour (M2).

2.1.4 Protein

The primary sources of protein for PBM products were soybean protein, wheat gluten and pea protein (Kołodziejczak, et al., 2021; Huang, et al., 2022). The favored protein option in PBM items was soy because it has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of approximately 1.00, meaning that it is a complete protein offering all the amino acids (Cutroneo, et al., 2022) and is more affordable. Additionally, the manufacturing of PBM has also progressively incorporated alternative protein sources derived from oilseed crops and fermentation, as well as microorganisms (such as fungi) (Kyriakopoulou et al., 2018).

In general, PBM items are marketed as foods that are rich in protein (Safefood, 2021). However, when in comparison to animal-meat products, the majority of PBM items were generally less protein-rich (Curtain and Grafenauer, 2019; Alessandrini, et al., 2021; Bakhsh, et al., 2021a; Safefood, 2021; Harnack, et al., 2021; Cutroneo, et al., 2022). Nevertheless, Ghangale et al. (2022) found that the PBM analogues had more protein than the control, with sample M2 having a considerably higher protein level. This difference was demonstrated by the fact that sample M2 was primarily made with protein-rich ingredients like cashew nut flour, whereas M1 contained mainly jackfruit, which is protein-

deficient in nature. Similarly, a study in Greece pointed out that all meat substitutes were rich in protein to their animal-based products (Katidi, et al., 2023). In addition, they also found that only the protein content of sausage substitutes varied significantly compared to other meat substitutes, with wheat-based sausages having the highest protein content followed by soy-based ones. Comparable to the findings of Fresán, et al., (2019), soy-based PBM products had the highest protein level among all PBMs.

In summary, the protein content of PBM items could be different based on the selection of ingredients (Katidi, et al., 2023). For example, Safefood (2021) categorized plant-based burgers based on their ingredients and found that burgers made from wheat, pea or soy proteins and mycoprotein had comparable protein content to chicken burgers, whereas those made from vegetables or beans contained less protein. As a result, it indicated that the former group of ingredients can be considered good sources of protein, while the latter group of ingredients contains a lower protein level.

2.1.5 Carbohydrate

The carbohydrate sources in PBM products generally come from cereal grains, legume flours and modified functional polysaccharides such as starch, fiber, gum and modified cellulose (Huang, et al., 2022). They were usually characterized as stabilizers, gelling agents, and thickeners (Safefood, 2021) to create a texture that was comparable to animal-meat products (Huang, et al., 2022). Additionally, due to the gelatinization-inducing properties of starches,

they are incorporated into PBM products to enhance the texture, shelf life, cohesion, and elasticity **(**Katidi, et al., 2023).

PBM had a significantly elevated total carbohydrate content compared with animal-meat products (Curtain and Grafenauer, 2019; Cutroneo, et al., 2021; Safefood, 2021). The amount of carbohydrates of PBM products ranged from 7.9 to 16.7% (Curtain and Grafenauer, 2019). Furthermore, the values may vary depending on the source of the substituted carbohydrates used. For instance, cereal-based meat alternatives contained more carbohydrates and sugars than the corresponding animal-meat products. This also implied that carbohydrates and sugars are also introduced to the diet when attempting to substitute meat (Cutroneo et al., 2022).

2.1.6 Dietary fiber

Fiber is a plant-based component with anti-enzymatic digestive properties that occurs naturally in grains, fruits, vegetables and nuts. It can be grouped into two main categories: water-soluble fiber (including pectin, gums and mucilages), and water-insoluble fiber (including cellulose, hemicellulose and lignin). Enzymic gravimetric and enzymic-chemical methods are the typical methods for determining dietary fiber content in foods (Dhingra, et al., 2012). Notably, methylcellulose (MC) is a fiber-rich thickener that is frequently used in PBM manufacture. In addition to this, maize and barley malt are often used as high-fiber carbohydrate sources (Safefood, 2021).

A number of studies have shown that PBM is substantially higher in dietary fiber compared to animal meat products (Curtain and Grafenauer, 2019; Alessandrini et al., 2021; Bakhsh, et al., 2021a, Cutroneo, et al., 2021; Safefood, 2021; Ghangale, et al., 2022; Katidi, et al., 2023). Due to the addition of carbohydrate ingredients, the dietary fiber content of PBM is beyond that of animal meat equivalents, which are deficient in fibers in their natural state (Safefood, 2021). Besides, Bakhsh, et al. (2021a) mentioned that adding plants and polysaccharides to plant-patties formulation could improve their textural attributes and fiber content in the final product. Apart from that, the greater fiber content in PBM might assist consumers to reach their recommended fiber intake (Alessandrini, et al., 2021).

In addition, Fresán, et al. (2019) assessed the nutritional values of PBM products that were grouped accordingly to their primary constituents. Soy-based products had the greatest levels of carbohydrates and dietary fiber, followed by wheat- and nut-based products. This may be due to the significant amount of water-soluble dietary fiber contained in soybean (Kyriakopoulou, et al., 2018).

2.2 Total Phenolics, Flavonoids and Antioxidant Capacities of PBM products

The term "phenolic" or "polyphenol" can be used to define compounds containing at least one aromatic ring connected with one or more hydroxyl functional groups (Zhou, et al., 2016). Polyphenols are a kind of bioactive compound (Ho, 1992) found naturally as secondary metabolites in a broad variety of plants including fruits, vegetables, whole grains and tea (Blainski, Lopes and Mello, 2013). The plant polyphenols primarily function as phytoalexins, pollinators attraction, antioxidants, pigmentation and UV light blockers (Blainski, Lopes and Mello, 2013).

The classification of polyphenols can be divided into flavonoids and non-flavonoids (Singla, et al., 2019). Flavonoids, referred to as vitamin P, are plant secondary metabolites which are essential for the formation of yellow and other pigments in plants (Rebaya, et al., 2015). Flavonoids account for 60% of all polyphenols, whereas phenolic acids, the dominant category of non-flavonoid compounds, account for 30% of all polyphenols (Zhou, et al., 2016; Singla, et al., 2019).

Besides, polyphenols also play a significant role in determining the antioxidant capacity of botanical sources (Fadly, Purwayantie and Arundhana, 2020). Antioxidant capacities can be described as the potential of compounds in foods and biological systems to neutralize free radicals (Floegel, et al., 2011; Martinez-Morales, et al., 2020). According to Aryal, et al. (2019), a positive

relationship was found between total phenolics (phenolics and flavonoids) and antioxidant capacity. This is due to the presence of hydroxyl groups in polyphenols, which allows them to efficiently squelch these damaging free radicals by supplying hydrogen atoms and thus exhibiting powerful antioxidant characteristics. Moreover, the antioxidant characteristics offered may lower the likelihood of getting non-communicable diseases in humans including cancers, cardiovascular diseases (CVD), diabetes, as well as skeletal and neurological disorders (Kupina, et al., 2018). Certain polyphenols can also promote the cells to produce antioxidants endogenously (Aryal, et al., 2019).

Other than that, the total phenolics content and antioxidant capacities of PBM items and animal-meat products have been compared in a few prior studies. A study revealed that the plant-based substitutes were shown to include a broader range and higher quantity of phenolic compounds than beef, which could be better for human wellness by minimizing inflammation and oxidative stress (van Vliet, et al., 2021). Similar work has also been pursued by Abdullah, et al. (2022), who concluded that PBM products had better antioxidant profiles as compared to animal meat products.

Furthermore, the antioxidant properties among PBM items were also investigated. The study by Abdullah, et al. (2022) stated that Hungarian sausages had the highest polyphenol content and antioxidant capacity values across all PBM samples owing to the use of oat flour in products. Oat flour contains phenolic compounds called avenanthramides, which have 10- to 30-

fold the antioxidant activity of other phenolic compounds. Another study noted that PBM burger patty with higher maize proportions had the highest phenol value and antioxidant activity, suggesting that maize might have a greater impact on antioxidant activity than oyster mushrooms or kidney beans (Fadly, Purwayantie and Arundhana, 2020). In short, different ingredients used in the production of PBM items contain different amounts of bioactive compounds, which can result in varying levels of phenolic content and antioxidant capacity.

2.3 Antioxidant Assays

2.3.1 Folin–Ciocalteu (F-C) Assay

The Folin-Ciocalteu (F-C) colorimetric assay has been extensively used for quantifying the total phenolic contents of plant and food sample extracts since it is a simple and time-saving method (Blainski, Lopes and Mello, 2013; Cao, et al., 2020). In the presence of alkaline, the F-C reagent, a combination of phosphotungstic acid and phosphomolybdic acid, will cause oxidation of the phenolic compounds in the crude extract (Manessis, et al., 2020). When the F-C reagent is reduced, a blue mixture of tungsten and molybdenum oxides is obtained (Ahmed and Iqbal, 2018), with an absorbance of 765 nm. The degree of blue color formation increases with phenolic concentration (Kapina, et al., 2018).

2.3.2 Aluminium Chloride Assay

The flavonoid contents were measured employing the aluminium chloride colorimetric assay. When the aluminium chloride is added to an alkaline solution of sodium nitrate, the aluminium ions bind to the keto- and hydroxyl-groups of the flavonoids to create acid-labile compounds (Ahmed and Iqbal, 2018), resulting in a yellow-colored solution. Following the introduction of sodium hydroxide, the solution becomes red with an absorbance of 510 nm for the determination of the flavonoid content (Pękal and Pyrzynska, 2014).

2.3.3 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant capacities of extracts can be determined using the DPPH assay (Manessis, et al., 2020). DPPH is a chemical containing free radicals with stability (Aryal, et al., 2019) and can be dissolved in an organic solvent (Manessis, et al., 2020). The antioxidants present in the extracts reduce the DPPH molecules by donating hydrogen atoms to generate a stable form of DPPH-H. Upon exposure to antioxidants, the DPPH molecules are reduced, resulting in a dark purple to yellowish discoloration. The unreduced DPPH allowed for the spectrophotometric detection of the antioxidant capability at 517 nm (Asadujjaman, Hossain and Karmakar, 2013). In general, the results were presented as half maximal inhibitory concentration (IC_{50}), referring to the quantity of potential antioxidants necessary to scavenge the free radicals by half (Martinez-Morales, et al., 2020). DPPH assay is a prevalent method because of its simplicity, rapidity and applicability with hydrophobic antioxidants (Floegel, et al., 2011). Nonetheless, it is unable to independently reveal the actual

sensitivity of the antioxidants as it depends on the response time and antioxidant/DPPH ratios **(**Amorati and Valgimigli, 2015**)**.

2.3.4 2,2′-azinobis(3-ethyl benzothiazoline-6-sulfonate acid) (ABTS) Radical Scavenging Assay

The ABTS assay can also be used for determining an extract's antioxidant capacity. Potassium persulfate is required for the production of the ABTS radical chromophore (Manessis, et al., 2020), which has a maximum absorbance of 734 nm (Amorati and Valgimigli, 2015). Following the addition of hydrogen-donating antioxidants, the ABTS radicals are reduced, which results in discoloration from blue-green to colorless and changes in the absorption band (Floegel, et al., 2011). Depending on the activity and quantity of the respective antioxidants, the degree of decolorization will vary. The ABTS assay is simple to carry out, appropriate for hydrophilic and lipophilic antioxidants and pH-independent, nevertheless, releasing of radicals necessitates a further process, and the radicals degrade with time (Manessis, et al., 2020).

2.3 Consumers' Perceptions Towards PBM Products

Theretofore, studies on consumer perception of PBM products have been done in several countries, with varied responses documented due to cultural variations. In general, consumers' acceptability of meat substitutes, including PBMs, has been limited compared to traditional meat (Onwezen, et al., 2021). A comparable result was found among German consumers, who have only a

minimal consumption of PBMs and rate them negatively, whereas meat is rated favorably (Michel, Hartmann and Siegrist, 2021). Yet, Dutch consumers deemed meat alternatives offered more health advantages than traditional meat (Ketelings, et al., 2023). In a selection experiment, the United States (US) showed considerably greater meat attachment and lower levels of familiarity and acceptance of PBMs in contrast to China and India (Bryant, et al., 2019).

Besides, consumers' food preferences can be influenced by a multitude of factors. According to research on Swedish adults, the adoption of meat alternatives and veganism is more popular among females, youth and those with tertiary education. The primary drivers of meat alternatives adoption were the awareness of environmental and well-being, as well as familiarity with meat alternatives (Carlsson, Kataria and Lampi, 2022). Moreover, Estell, Hughes and Grafenauer (2021) examined perceptions of PBMs between consumers and nutrition professionals (NP). They indicated that 74% of respondents had attempted PBM alternatives, with NPs motivated by health concerns and consumers motivated by ethics. Another study of Midwest university students stated that 55% had attempted PBM alternatives. Still, contradictory findings proposed that there was no gender variation in taking PBM alternatives and the choice of PBM alternatives was mainly motivated by engagement in new meals and social influences aside from environmental and well-being considerations (Davitt, et al., 2021).

Furthermore, taste is the main obstacle to the consumption of PBM alternatives for Swedish consumers, as well as Australian consumers and NPs (Estell, Hughes and Grafenauer, 2021; Carlsson, Kataria and Lampi, 2022). Similarly, consumers would prefer commercial beef burgers over plant-based burgers even if the burgers tasted the same, but only 8% of them were fully convinced by this statement. Also, it was claimed that just 21% of them would choose plant-based burgers if they cost identical to beef burgers (Slade, 2018), suggesting that price is an influencing factor in PBM alternatives intakes. Ketelings, et al. (2023) attributed poor buying intention for PBM alternatives is hampered by their expensiveness and poor palatability compared to meat, apart from familiarity. Hence, there is potential for PBM alternatives to successfully replace meat if offered at a comparable taste and cost to meat (Michel, Hartmann and Siegrist, 2021).

In accordance with Aurier and Ngobo (1999), consumers' knowledge can be identified in two basic categories: familiarity and product knowledge. Familiarity refers to cumulative consumer experiences, while product knowledge is described as the consumers' understanding of specific information regarding a product (Shen and Chen, 2020). Consumers' knowledge corresponds positively with purchasing intentions (Ateke, Walter and Didia, 2018). Consumers with an advanced level of knowledge can assess the characteristics and quality of products via internal clues. Nonetheless, Taiwanese research revealed no connection between consumers' knowledge and buying intentions for PBM alternatives (Shen and Chen, 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Framework

The research was conducted according to the flowchart in Figure 3.1 and Figure 3.2.

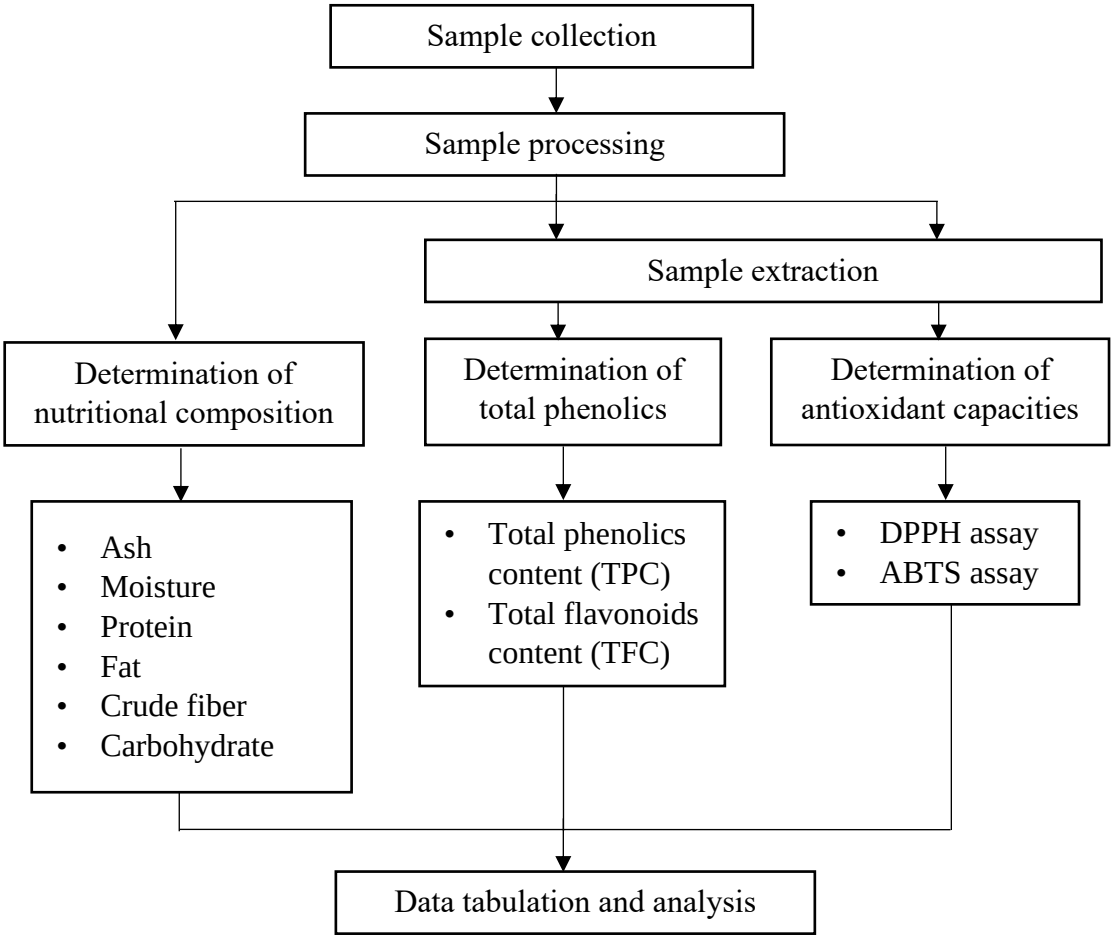

Figure 3.1: An overview of research framework for laboratory work

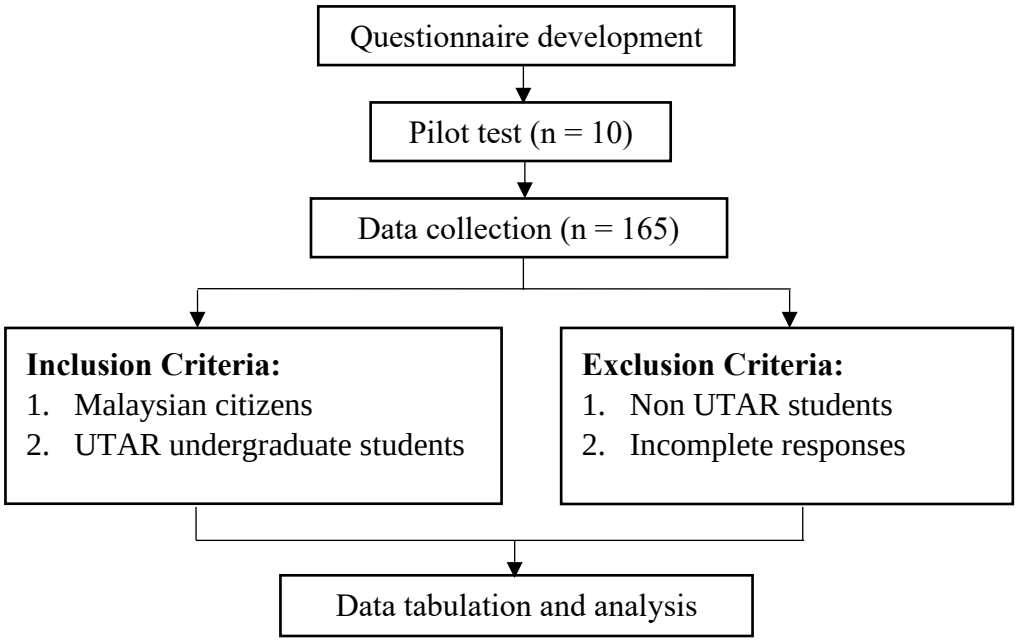

Figure 3.2: An overview of research framework for survey study

3.2 Materials

Sulphuric acid (H_2SO_4) solution, potassium sulphate (K_2SO_4) powder, copper sulphate ($CuSO_4$) powder, distilled water, sodium hydroxide (NaOH) solution, boric acid (H_3BO_3) solution, bromocresol green/methyl red indicator solution, hydrochloric acid (HCl) solution, petroleum ether 40/60, acetone, sodium carbonate (Na_2CO_3) solution, gallic acid solution, aluminium chloride hexahydrate ($AlCl3 \cdot 6H_2O$) solution, sodium nitrite ($NaNO_2$) solution, quercetin solution, DPPH free radical solution, ABTS free radical solution, trolox solution and methanol.

3.3 Sample Preparation

3.3.1 Sample Collection

A total of three plant-based meat (PBM) food samples in ready-to-eat forms were collected from a local vegan restaurant in Ipoh, Perak. All ingredients of the samples were packed in individual packets in a vacuum and frozen state. Table 3.1 shows the name of the food samples and their ingredients list.

Table 3.1: The food samples and its ingredients.

Food samples	Ingredients
Kale Pizza	Kale, white button mushroom, green vegetable paste, mozzarella cheese
Double Cheeseburger	Bun, V plant-based meat patty, tomato, fresh coral, pickled cucumber, cheddar cheese, mayo sauce
Spaghetti in Carbonara with Plant-Based Minced Meat	Spaghetti, shimeji mushroom in creamy sauce, V minced meat

3.3.2 Sample Processing and Storage

The ingredients of food samples in individual packets were defrosted in a water bath using tap water for around 30 minutes. For sample homogenization, all the ingredients of each food sample were gathered and blended using a countertop blender, Series 5000 Blender Core (PHILIPS, Netherlands) until a paste-like form was obtained. The homogenized food samples were kept in storage bags with zippers and stored in a chest freezer at -20 ^{o}C for further use.

3.3.3 Sample Extraction

Sample extraction was required to obtain sample extracts for the determination of total phenolics, flavonoids and antioxidant capacities. The method was adopted from Wong, et al. (2014) with minor modifications. As a standard stock solution, 3 g of homogenized sample was extracted with 30 mL of 80% methanol in a ratio of 1:10. The sample was first agitated with an orbital shaker incubator (Protech) at 150 rpm for 1 hour at room temperature (25 ºC). Then, the sample was centrifuged at 4500 rpm for 15 minutes at room temperature by a refrigerated benchtop centrifuge 3-18KS (Sigma, United Kingdom). The top fraction of the centrifuge tube, known as supernatant, was collected and stored in capped bottles at -20 °C until further use.

3.4 Proximate Analysis

The nutritional compositions of PBM items were determined by adopting the methods from the Association of Official Analytical Chemists (AOAC, 1995), including ash, moisture, protein, fat and crude fiber analyses. All the analyses were measured in duplicate.

3.4.1 Determination of Moisture Content

The moisture contents of the food samples were determined using moisture analyzer MX-50 (A&D Company Limited, Japan). 10 g of food sample was weighed directly on the sample pan and spread evenly in order to obtain a

reliable result. The sample was dried at a drying temperature of 105 ºC. Lastly, the value displayed on the analyzer was read and recorded.

3.4.2 Determination of Ash Content

The ash contents of the food samples were determined using the dry-ashing method. First, the empty crucibles and lids were ignited in the muffle furnace (Nabertherm, Germany) for 15 minutes at 550 ºC and cooled in a desiccator to achieve room temperature. The weight of empty crucibles and lids (M1) was obtained. Next, 4 g of samples were weighed and recorded as M0. The samples were heated on the hotplate until completely charred in the fume hood. Then, the crucibles with charred samples were ignited in the furnace for 8 hours at 550 ºC until a light grey to white ash was obtained. Lastly, the crucibles containing ash were cooled in a desiccator and the weight of the crucible with lids and ash (M2) was obtained. The percentage of ash content of the food sample was determined by the following formula:

$$\mathrm{A}(\%) = \frac{\mathrm{M2} - \mathrm{M1}}{\mathrm{M0}} \times 100\%$$

Where,
A = Percentage of ash content (%)
M0 = Weight of food sample (g)
M1 = Weight of empty crucible and lid (g)
M2 =Weight of crucible and lid with ash (g)

3.4.3 Determination of Protein Content

The protein contents of food samples were determined by the Kjeldahl method. This method can be subdivided into three different processes, namely digestion, distillation and titration. The Speed Digester K-436 (BÜCHI Labortechnik, Switzerland) was used for the digestion of food samples. The digester was first preheated for at least 15 minutes at 470 ºC. Meanwhile, 2 g of food samples were weighed and placed into each digestion tube. Following the catalyst, 7 g of potassium sulphate and 0.8 g of copper sulphate, as well as 20 mL of concentrated sulphuric acid (98%) were added to each digestion tube. A blank without food samples was prepared with only sulphuric acid and catalysts. Then, the prepared digestion tubes in the digestion rack were loaded into the preheated digestion block and the exhaust system was attached to the tubes. The samples were digested until all of them were shown a clear green or blue solution, which indicated the end of digestion. The rack of tubes was removed from the digester and was cooled for approximately 30 minutes and proceeded to further procedures.

The Distillation Unit K-355 (BÜCHI Labortechnik, Switzerland) was used for distillation. 25 mL of 4% boric acid and a few drops of color indicator or bromocresol green/methyl red indicator solution were added to a conical flask. Then, the cooled digestion tube and conical flask were inserted into the distillation unit accordingly. The cooled digest was diluted with 40 mL of distilled water and 60 mL of 32% sodium hydroxide solution. The samples were

distilled for 4 minutes until the solution color in the conical flask turned from slightly pink to blue.

Lastly, the distillate in the conical flask was titrated with 0.1 M of hydrochloric acid until an end-point was achieved where blue turns to slightly pink. The volume of acid used for titration was read and recorded. The percentage of nitrogen and protein contents of food samples were calculated by following formulas:

$$N(\%) = \frac{[V(1) - V\,(B1)] \times f \times 1 \times 0.1 \times 14.007}{m \times 1000} \times 100\%$$

$$P(\%) = N(\%) \times PF$$

Where,
N = Percentage of nitrogen content (%)
P = Percentage of protein content (%)
V(1) = Titration volume for sample (mL)
V(B1) = Titration volume for blank (mL)
f = Factor of titrant
1 = Molar reaction factor of titrant, HCl
0.1 = Normality of titrant, HCl (mol/L)
14.007 = Molecular weight of nitrogen (g/mol)
m = weight of food sample (g)
1000 = Conversion factor of mL to L
PF = Protein factor

3.4.4 Determination of Fat Content

The fat contents of food samples were determined using Soxtherm® rapid extraction system (C. Gerhardt GmbH & Co. KG, Germany). First, the extraction beakers with three pieces of boiling stones were preheated in drying oven at 105 °C for 1 hour and then cooled in a desiccator for 30 minutes. The

weight of each extraction beaker was measured as M1 value. Next, 4.5 g of food samples were weighed (M0) and wrapped in filter paper. The samples were inserted into an extraction thimble and placed into the preheated extraction beakers. Approximately 90 mL of 40/60 petroleum ether was poured into each beaker in the fume hood. The beakers were then inserted into Soxtherm® extraction unit and all parameters for the analysis was preset on Soxtherm® Manager program. The sample extraction was carried out automatically for 150 minutes by the instrument. After that, the extraction beakers containing sample residue and boiling stones were heated in a drying oven at 105°C for 1 hour and cooled in a desiccator. Lastly, the weight of extraction beakers was recorded as M2 value. The percentage of fat contents of the food samples can be obtained by using the following formula:

$$\mathrm{F}(\%) = \frac{\mathrm{M2} - \mathrm{M1}}{\mathrm{M0}} \times 100\%$$

Where,
F = Percentage of fat content (%)
M0 = Weight of food sample (g)
M1 = Weight of extraction beaker with boiling stones before extraction (g)
M2 = Weight of extraction beaker with boiling stones and fat after extraction (g)

3.4.5 Determination of Crude Fiber Content

The crude fiber contents of food samples were done by the gravimetric method using Gerhardt Fiber Bag-System, FBS6 (C. Gerhardt GmbH & Co. KG, Germany). The crucibles were first incinerated in the muffle furnace at 600 °C for 30 minutes, then cooled in a drying oven at 105 °C for 30 minutes and desiccator for another 30 minutes, accordingly. Meanwhile, the empty fiber bags

were pre-dried in a drying oven at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. The weight of fiber bags for food samples was recorded as M1, while the weight of fiber bag for blank was recorded as B1. 2 g of food samples (M2) were placed into each fiber bag except for the blank. Next, the glass spacers were fitted with fiber bags and loaded into carousel in a beaker. The food samples with more than 10% fat content were defatted by multiple rinses of petroleum ether and allowed air-dried in the fume hood.

Moreover, 360 mL of 0.13 mol/L sulphuric acid was added into the beaker and gently mixed with the samples by rotating the carousel for 1 minute. The samples were first boiled for 3 to 5 minutes, then brought to a gentle simmer for 30 minutes. Subsequently, the carousel with samples was rinsed with several changes of hot water to remove acid residue. The steps were repeated by replacing 360 mL of 0.23 mol/L of sodium hydroxide. After digestion had been completed, the fiber bags containing digested samples were placed into the pre-incinerated crucibles and dried in a drying oven at 105 °C for 4 hours. After cooling in the desiccator for 30 minutes, the crucibles with fiber bags and dried digested samples were weighed and recorded as M3, while the crucible with a blank fiber bag was recorded as B3.

Finally, the crucibles with fiber bags were incinerated in a muffle furnace at 600 °C for 4 hours. The crucibles with ash were allowed to cool in the drying oven and desiccator accordingly. The weight of crucibles containing sample ash was recorded as M4, while the weight of crucibles containing the ash blank fiber

bag was recorded as B4. The percentage of crude fiber content for each food sample was calculated using the following formula:

$$\mathrm{CF}(\%) = \frac{[(\mathrm{M3} - \mathrm{M1} - \mathrm{M4}) - (\mathrm{B3} - \mathrm{B1} - \mathrm{B4})]}{\mathrm{M2}} \times 100\%$$

Where,
CF = Percentage of crude fiber content (%)
M1 = Weight of empty fiber bag (g)
M2 = Weight of food sample (g)
M3 = Weight of crucible and dried fiber bag (g)
M4 = Weight of crucible and ash (g)
B1 = Weight of fiber bag, blank value (g)
B3 = Weight of crucible and dried fiber bag, blank value (g)
B4 = Weight of crucible and ash, blank value (g)

3.4.6 Determination of Carbohydrate Content

The percentage of carbohydrate contents in food samples were calculated using the formula below (Singh Gaur and Kaliyadan, 2022):

$$\mathrm{C}(\%) = 100\% - (\mathrm{A}\% + \mathrm{M}\% + \mathrm{F}\% + \mathrm{P}\% + \mathrm{CF}\%)$$

Where,
C = Percentage of carbohydrate content (%)
A = Percentage of ash content (%)
M = Percentage of moisture content (%)
F = Percentage of fat content (%)
P = Percentage of protein content (%)
CF = Percentage of crude fiber content (%)

3.5 Determination of Antioxidant Properties

The total phenolics, including total phenolics content (TPC), total flavonoids content (TFC), as well as antioxidant capacities, including DPPH and ABTS radical scavenging assays, were performed to study the antioxidant properties of the food samples. All the assays were carried out in duplicate.

3.5.1 Determination of Total Phenolics Content (TPC)

The Folin-Ciocalteu (F-C) assay was performed following a modified method outlined by Wong, et al. (2014) to determine the total phenolics content of food samples. First, 100 µL of sample extracts were added with 200 µL of F-C reagent and incubated at dark for 10 minutes. The mixture was subsequently mixed with 1 mL of 7% sodium carbonate and incubated in the dark for another 30 minutes. A UV-vis spectrophotometer GENESYS 20 (Thermo Scientific, United States) was used to measure the mixture's absorbance at a wavelength of 765 nm. A blank containing simply distilled water was assayed. The standard curve was plotted using gallic acid at concentrations of 0, 20, 40, 60, 80 and 100 µg/mL in distilled water. The phenolic contents of food samples were presented in mg of gallic acid equivalent (GAE) per g of dry sample.

3.5.2 Determination of Total Flavonoids Content (TFC)

The flavonoid contents of food samples were determined using aluminium chloride assay described by Ee, et al. (2018) with minor modifications. First, 250 µL of sample extract was mixed with 75 µL of 5% sodium nitrite and 1.25 mL of distilled water, followed by 6 minutes of incubation in the dark. Subsequently, 0.3 mL of 10% aluminum chloride hexahydrate ($AlCl3 \cdot 6H_2O$) was added and mixed thoroughly using a vortex mixer and incubated at dark for another 6 minutes. After that, 1 mL of 1 M sodium hydroxide was added to the mixture and incubated for 10 minutes. A wavelength of 510 nm was applied to read the mixture absorbance. 80% methanol was used as a blank and quercetin at concentrations from 0 to 100 µg/mL in 80% methanol was used to plot the standard curve. The flavonoid contents were expressed as mg of quercetin equivalent (QE) per g of dry sample.

3.5.3 DPPH Free Radical Scavenging Assay

The method for DPPH assay was adopted from Ee, et al. (2018) with slight modifications. 1 mL of sample extract (20 to 100 mg/mL) and 1.4 mL of newly made 0.1 mM DPPH free radical solution were mixed and incubated for 20 minutes in the dark at room temperature. All sample tubes were wrapped with aluminum foil. Then, the mixture absorbance was read at a wavelength of 517 nm. The standard curve was generated using Trolox with concentrations ranging from 0 to 16 µg/mL in 80% methanol, and the blank was used with 80% methanol.

3.5.4 ABTS Free Radical Scavenging Assay

The ABTS assay was conducted based on a method described by Wong, et al. (2014). 100 µL of sample extract (20 to 100 mg/mL) was combined with 1 mL of diluted ABTS free radical solution. The mixture was mixed thoroughly and incubated for 6 minutes in the dark at room temperature. The mixture absorbance was assayed at 734 nm using UV-visible spectrophotometer GENESYS 20 (Thermo Scientific, United States) against distilled water blank. The standard curve was generated using Trolox with concentrations ranging from 0 to 20 µg/mL in 80% methanol.

For both DPPH and ABTS assays, the results were expressed in antioxidant activity percentage (AA%) using the following formula and half maximal inhibitory concentration (IC_{50}) was obtained by referring to the regression line of the graph of AA% against sample concentrations.

AA (%) = [(A0 - A1)/A0] x 100%

Where,
AA = radical scavenging activity (%)
A0 = absorbance of control
A1 = absorbance of sample extract

3.6 Survey Development Procedures

3.6.1 Study Design

A cross-sectional study was designed for this research. A non-probability sampling method of convenient sampling was employed for data collection in the period from March to June 2023. Prior to data collection, ethical approval had been requested and was permitted by the UTAR Scientific and Ethical Review Committee (Appendix A) to acknowledge that this research was conducted in accordance with Personal Data Protection Act 2010.

3.6.2 Inclusion and Exclusion Criteria

The target respondents of this study were Malaysian adults who are currently undergoing undergraduate programmes at Universiti Tunku Abdul Rahman (UTAR), in both Kampar and Sungai Long campuses. Foundation and postgraduate students, and staffs were not eligible to participate in this study. Non-UTAR students, international students and incomplete responses were excluded from this study as well.

3.6.3 Sample Size

The sample size was calculated using Daniel (1999) single population formula,

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

Where
n = sample size
Z = statistic for a level of confidence
P = expected prevalence
d = precision

The expected prevalence rate used in the calculation was 11% (Rakuten Insight, 2021). With 95% level of confidence (Z = 1.96) and 5% of precision (d = 0.05), the sample size was calculated as

$$n = \frac{1.96^2(0.11)(1-0.11)}{0.05^2}$$

$$= 150.43 \approx 150$$

With 10% of non-response rate, n = 150 x 1.1

= 165 respondents

After considering a 10% non-response rate, the final sample size for this study reached 165 respondents.

3.6.4 Research Instrument

An online self-administered questionnaire (Appendix B) was used to evaluate the level of knowledge of consumers towards PBM items among UTAR undergraduate students. The questionnaire was developed using Google Form in English language and it took around 2 to 5 minutes to complete.

On the first page of questionnaire, the purpose of this study, the inclusion criteria of respondents and the requirement of consent from the respondents before they answered the questionnaire were included. This questionnaire consists of 15 questions divided into two sections – Section A and Section B. A total of five questions are in Section A to collect the sociodemographic profiles of respondents, including gender, course, year of study, religion and diet pattern. Section B consists of a total of 10 closed-ended questions subdivided into two parts. Part 1 comprised four questions to assess the general knowledge of consumers towards PBM items, while Part 2 encompassed six questions to assess the nutrition-related knowledge of PBM items in comparison to animal-based meat. The items in Section B were in the form of "Yes", "Not sure" and "No", respondents were required to choose their answer based on their current knowledge. The survey questions were developed using various articles as sources (He, et al., 2020; Alessandrini, et al., 2021; Kyriakopoulou, Keppler and van der Goot, 2021; Abdullah, et al., 2022; Safdar, et al., 2022; Wang, et al., 2022). Each correct answer was rewarded 1 mark, conversely, 0 mark was given to the false answer. Hence, the maximum score of Section B was 10.

3.6.5 Pilot Test

Consequently, a pilot test was performed to determine the understanding of respondents to this questionnaire and the duration of filling up the questionnaire to verify the validity and reliability of the developed questionnaire before it is distributed to a larger sample. 10 eligible respondents were involved in the pilot study, and their feedbacks were collected. The questionnaire was revised based on the comments given to improve the quality of the question. As a result, the respondents managed to understand and answer the questions accordingly.

3.6.6 Data Collection

The final questionnaire was distributed virtually among eligible participants via Microsoft Teams, and social media platforms such as WhatsApp and Instagram throughout the data collection period.

3.7 Statistical Analysis

The collected data was analyzed using Statistical Package for the Social Sciences (SPSS) version 27 with the aid of Microsoft Office Excel 2019. Food samples data were presented in the form of mean ± standard deviation and a statistical significance level of $p < 0.05$ was set. One-way analysis of variance (ANOVA), followed by Tukey test as the post-hoc test was performed to analyze the significant difference in nutritional compositions, total phenolics and antioxidant capacities of PBM items. The association between TPC, TFC and antioxidant capacities were determined using Pearson correlation.

Besides, the descriptive data obtained from the survey, including sociodemographic variables and knowledge variables were presented in frequency (n) and percentage (%). The total knowledge scores were summed up from the questions in Section B and further categorized as low, moderate and high knowledge levels using Bloom's cutoff categories (Alzahrani, et al., 2021), as shown in Table 3.2.

Table 3.2: Classification of knowledge levels using Bloom's cutoff categories.

	Categories	**Scores (%)**
Knowledges	High level	80-100
	Moderate level	60-79
	Low level	<60

CHAPTER 4

RESULTS

4.1 Proximate Analysis of PBM Items

The nutritional compositions for each plant-based meat (PBM) item (n = 3) were summarized in Table 4.1. Nutrient components, including moisture, ash, fat, protein, and carbohydrate contents differed significantly ($p < 0.05$) among the samples, while no significant difference ($p > 0.05$) in fiber content. In general, moisture (54 – 66%) comprised the largest proportion of all samples, followed by carbohydrates (28 – 33%), fat (3 – 6%), fiber (2 – 4%) and ash (1 – 3%), while protein (< l%) made up the smallest proportion.

Table 4.1: Nutritional compositions of plant-based meat (PBM) items

Nutritional composition (%)	**Food samples**		
	KP	**DCB**	**SC**
Moisture	55.79 ± 0.01^{b}	54.02 ± 0.22^{c}	66.03 ± 0.30^{a}
Ash	2.75 ± 0.00^{a}	2.60 ± 0.13^{a}	1.00 ± 0.00^{b}
Fat	5.67 ± 0.78^{a}	3.89 ± 0.16^{ab}	2.56 ± 0.47^{b}
Protein	0.53 ± 0.06^{a}	0.66 ± 0.06^{a}	0.24 ± 0.03^{b}
Crude fiber	2.00 ± 0.00^{a}	3.75 ± 1.06^{a}	2.00 ± 0.71^{a}
Carbohydrate	33.27 ± 0.74^{a}	33.35 ± 1.01^{a}	28.18 ± 0.86^{b}

* All data are shown as mean ± standard deviation of samples (n = 3).
* $^{a\text{-}c}$Different superscripts in the same row denote significant differences ($p < 0.05$) whereas the same superscripts denote no significant differences ($p > 0.05$) between samples.
* KP: Kale pizza; DCB: Double cheeseburger; SC: Spaghetti in carbonara with plant-based minced meat.

4.1.1 Moisture Content

The moisture content of samples varied from 54 to 66%, with significant differences ($p < 0.05$) found across all samples. SC contained the highest moisture content (66.03 ± 0.30%), followed by KP (55.79 ± 0.01%), while DCB had the least (54.02 ± 0.22%).

4.1.2 Ash Content

There was a statistically significant difference ($p < 0.05$) in ash content between the samples. SC had the lowest ash content (1.00 ± 0.00%), which differed from DCB (2.60 ± 0.13%) and KP (2.75 ± 0.00%). However, there was no difference between DCB and KP.

4.1.3 Fat Content

The greatest fat content was found in the KP (5.67 ± 0.78%), followed by the DCB (3.89 ± 0.16%) and SC (2.56 ± 0.47%). The difference between KP and SC was significant ($p < 0.05$), while the DCB revealed no difference between the two.

4.1.4 Protein content

The difference in protein content between samples was statistically significant ($p < 0.05$). The protein content did not differ between the DCB (0.66 ± 0.06%) and KP (0.53 ± 0.06%), however, both were statistically different from SC (0.24 ± 0.03%).

4.1.5 Crude Fiber Content

The crude fiber content of the samples was comparable and did not differ from one another ($p > 0.05$). DCB had the highest crude fiber content (3.75 ± 1.06%), followed by SC (2.00 ± 0.71%) and KP (2.00 ± 0.00%).

4.1.6 Carbohydrate Content

Carbohydrate content varied significantly ($p < 0.05$) amongst samples, ranging from 28 to 33%. DCB was determined to have the most carbohydrate content (33.35 ± 1.01%), and it differed significantly from SC but not from KP (33.27 ± 0.74%). SC with the lowest carbohydrate content (28.18 ± 0.86%) differed significantly from the other samples.

4.2 Antioxidant Properties of PBM Items

The total phenolic content (TPC) and total flavonoid content (TFC) for each PBM item ($n = 3$) were summarized in Table 4.2. There are no significant differences found ($p > 0.05$) in TPC and TFC between samples. Table 4.3 showed the antioxidant capacities of PBM items with both DPPH and ABTS assays, with significant differences found ($p < 0.05$) between samples.

Table 4.2: Total phenolics content (TPC) and total flavonoids content (TFC) of PBM items

Food samples	**TPC** (mg GAE/g sample)	**TFC** (mg QE/g sample)
KP	1.94 ± 0.24^{a}	1.19 ± 0.24^{a}
DCB	1.69 ± 0.11^{a}	0.96 ± 0.24^{a}
SC	1.36 ± 0.35^{a}	1.14 ± 0.16^{a}

* All data are shown as mean ± standard deviation of samples (n = 3).
* [a.]Same superscripts in the same column denote no significant differences ($p > 0.05$) between samples.
* KP: Kale pizza; DCB: Double cheeseburger; SC: Spaghetti in carbonara with plant-based minced meat; TPC: Total phenolic content; TFC: Total flavonoid content.

Table 4.3: Antioxidant capacities of PBM items

Food samples	**DPPH**		**ABTS**	
	AA (%)	IC_{50} (mg/mL)	AA (%)	IC_{50} (mg/mL)
KP	47.47 ± 2.04^{a}	0.89 ± 0.39^{a}	53.14 ± 4.14^{a}	5.29 ± 0.03^{a}
DCB	30.36 ± 4.00^{b}	1.60 ± 0.04^{a}	53.21 ± 1.41^{a}	6.98 ± 0.90^{a}
SC	24.18 ± 0.83^{b}	3.35 ± 0.04^{b}	36.59 ± 1.32^{b}	9.97 ± 0.34^{b}
Trolox		9.49 µg/mL		9.90 µg/mL

* All data are shown as mean ± standard deviation of samples (n = 3).
* [a-b.]Different superscripts in the same row denote significant differences ($p < 0.05$) whereas the same superscripts denote no significant differences ($p > 0.05$) between samples.
* KP: Kale pizza; DCB: Double cheeseburger; SC: Spaghetti in carbonara with plant-based minced meat; AA: Antioxidant activity; IC_{50}*:* Half-maximal inhibitory concentration.

4.2.1 Total Phenolics Content (TPC)

Figure 4.1 showed the standard curve of gallic acid for determining the TPC of PBM items. A linear equation of y = 0.0166x + 0.0322 with a regression correlation coefficient of R^2 = 0.9989 was obtained using gallic acid from concentrations of 0 to 100 µg/mL. The TPC was calculated and shown in Table 4.2. The TPC of samples varied from 1.36 to 1.94 mg GAE/g sample with no significant differences ($p > 0.05$) across all samples. KP contained the highest TPC, followed by DCB and SC.

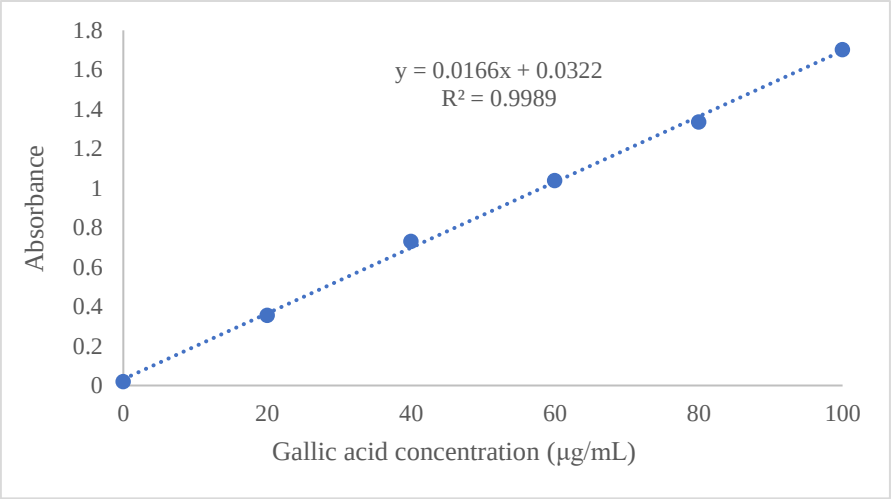

Figure 4.1: Standard curve of absorbance against gallic acid concentration for TPC

4.2.2 Total Flavonoids Content (TFC)

Figure 4.2 showed the standard curve of quercetin for determining the TFC of PBM items. A linear equation of y = 0.0007x + 0.0211 with a regression correlation coefficient of R^2 = 0.9944 was obtained using quercetin concentrations ranging from 0 to 100 µg/mL. The TFC was calculated and shown in Table 4.2. The TFC of samples varied from 0.96 to 1.19 mg QE/g sample with no significant differences ($p > 0.05$) across all samples. KP contained the highest TFC, followed by SC and DCB.

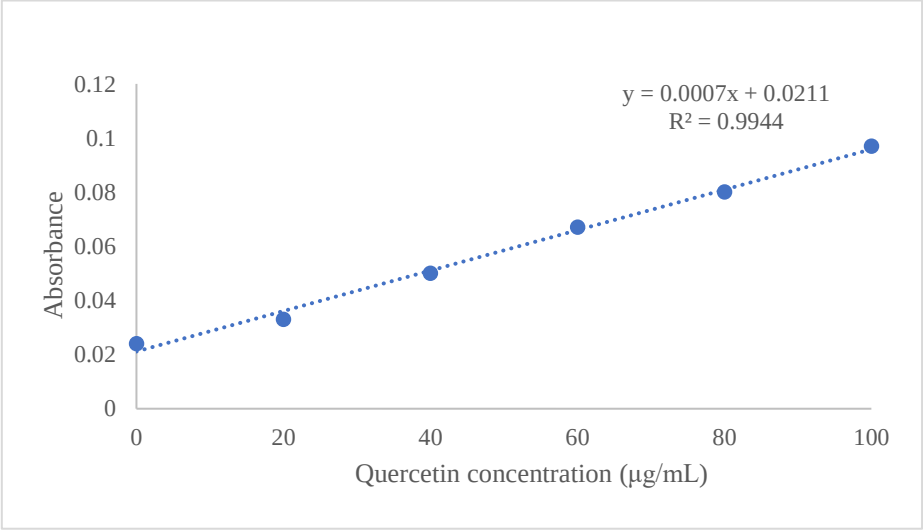

Figure 4.2: Standard curve of absorbance against quercetin concentration for TFC

4.2.3 DPPH Assay

The DPPH activities of PBM items were expressed in terms of antioxidant activity percentage (AA%) and IC_{50} in Table 4.3. The AA values varied from 24.18 to 47.47%, with KP having the highest AA% with a significant difference ($p < 0.05$). In addition, IC_{50} values were ranged from 0.89 to 3.35 mg/mL. SC was determined to differ significantly ($p = 0.00$) from both DCB and KP. However, IC_{50} value did not differ ($p > 0.05$) between the DCB and KP. Based on Figure 4.3, in comparison to Trolox as a positive control, the antioxidant capacities based on IC_{50} value in descending order were Trolox, KP, DCB and SC. The IC_{50} value of Trolox in DPPH assay was 9.49 µg/mL.

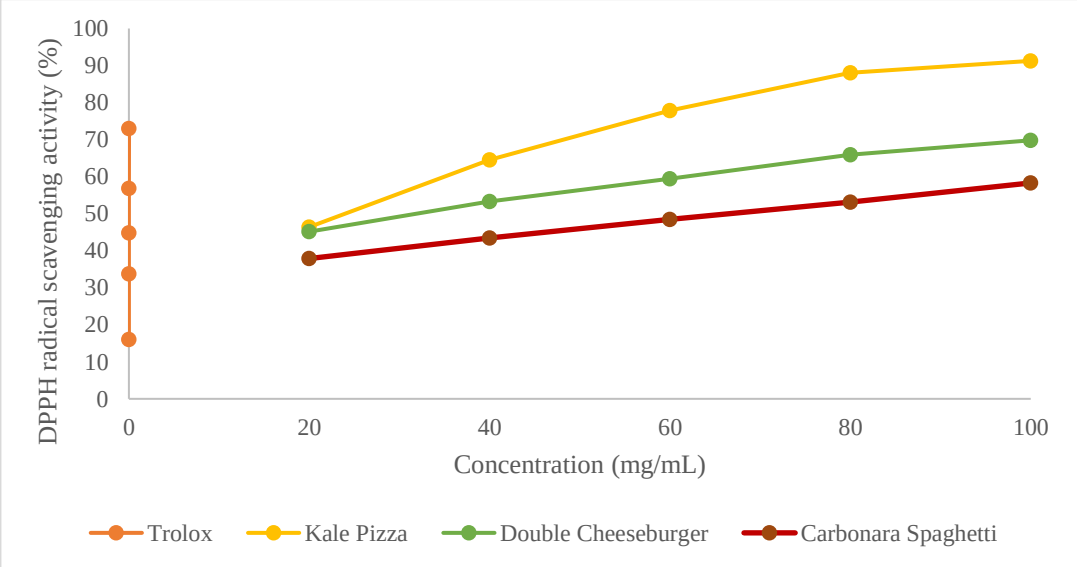

Figure 4.3: DPPH radical scavenging activity of PBM items with Trolox as comparison

4.2.4 ABTS Assay

The ABTS activities of PBM items were shown in terms of antioxidant activity percentage (AA%) and IC_{50} in Table 4.3. The AA% ranged from 53.21 to 36.59%, while the IC_{50} value ranged from 5.29 to 9.97 mg/mL. A similar trend was observed in AA and IC_{50} of ABTS assay, in which SC had the lowest values and differed from both DCB and KP significantly ($p < 0.05$). However, no significant difference ($p > 0.05$) was found in AA% and IC_{50} values of DCB and KP. Based on Figure 4.4, in comparison to Trolox as a positive control, the antioxidant capacities based on IC_{50} value in descending order were Trolox, Kale Pizza, DCB and SC. The IC_{50} value of Trolox in ABTS assay was 9.90 µg/mL.

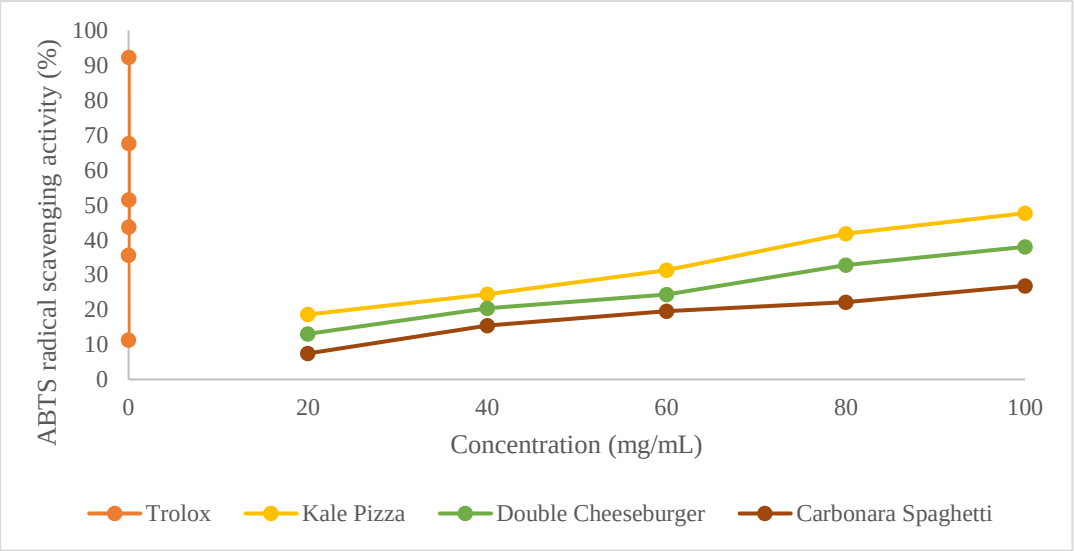

Figure 4.4: ABTS radical scavenging activity of PBM items with Trolox as comparison

4.3 Correlation Between TPC, TFC and Antioxidant Capacity Assays

The correlation between TPC and TFC and their corresponding AA% values for DPPH and ABTS scavenging activities is shown in Table 4.4. TPC demonstrated a strong positive correlation with DPPH (r = 0.94) and ABTS (r = 0.90). However, the data revealed a moderate positive correlation between TFC and DPPH (r = 0.46) and a weak inversely correlation with ABTS (r = -0.31).

Table 4.4: Pearson's correlation coefficient of TPC and TFC with AA% of DPPH and ABTS scavenging assays

		Pearson's correlation	
		TPC	**TFC**
AA%	DPPH	0.94	0.46
	ABTS	0.90	-0.31

4.4 Consumers' Knowledge Towards PBM Items Among UTAR Undergraduate Students

4.4.1 Sociodemographic Characteristics of Respondents

Table 4.5 showed the sociodemographic characteristics of the respondents. A total of 165 respondents participated in this study, the majority of whom were female (72.1%) and science students (66.1%), with just 27.9% and 33.9% being male and studying non-science students, respectively. In terms of education level, most of the respondents (34.5%) were in their first year of study, followed

by the third year (31.5%) and second year (27.3%). Besides, 81.8% of them were Buddhists, subsequent to Christians (9.7%) and other religions (6.7%), with Muslims accounting for only 1.8%. The diet patterns for nearly all respondents (96.4%) were non-vegetarians, with only 3.6% practicing vegetarianism.

Table 4.5: Sociodemographic profiles of the respondents

Demographic Characteristics	**Distribution**	**Frequency (n)**	**Percentage (%)**
Gender	Male	46	27.9
	Female	119	72.1
Course	Science	109	66.1
	Non-science	56	33.9
Education level	Year 1	57	34.5
	Year 2	45	27.3
	Year 3	52	31.5
	Year 4	9	5.5
	Above year 4	2	1.2
Religion	Buddhism	135	81.8
	Christian	16	9.7
	Muslims	3	1.8
	Others	11	6.7
Diet pattern	Non-vegetarian	159	96.4
	Vegetarian	6	3.6

4.4.2 Knowledge of PBM Items

Table 4.6 provides a summary of respondents' knowledge responses on plant-based meat (PBM) items. The majority of the respondents (72.1%) believed that PBM consumption could benefit health, the environment and animal welfare. Also, 81.8% of them agreed that isolated plant proteins, including soy, wheat gluten and peas were the principal sources of PBM production. PBM items were regarded as highly processed products by 72.7% of the respondents, and 76.4% were aware that PBM items are available in various forms on the market. Overall, the general knowledge of respondents on PBM items was good, as nearly three-quarters of them responded correctly to the first four questions (Part A).

Notably, half of the respondents (49.1%) disagreed that PBM items contain fewer calories than animal-based meat. In addition, 58.8% and 63.0% of respondents indicated that PBM items were higher in carbohydrates and dietary fibers compared to animal-based meat, respectively. Almost 70% of the respondents chose the correct statement that PBM items had lower fat content over animal-based meat. Remarkably, less than half of respondents believed that PBM items have lower amino acid profiles (43.0%), as well as better antioxidant activity and polyphenolic compounds (46.1%) in comparison to animal-based meat. In general, it can be concluded that consumers' knowledge in the aspect of nutrient compositions of PBM items was limited, as only around half of them were able to answer the questions in Part B correctly.

Table 4.6: Knowledge towards plant-based meat (PBM) items

Questions	n (%)		
	Yes	**Not sure**	**No**
1. Do you think plant-based meat consumption can bring health benefits, as well as environmental sustainability and animal welfare preservation?	119 (72.1)	19 (11.5)	27 (16.4)
2. Do you know that plant-based meat items are primarily made from isolated plant proteins such as soy, wheat gluten and peas?	135 (81.8)	15 (9.1)	15 (9.1)
3. Do you think plant-based meat items involve a high degree of processing method?	120 (72.7)	30 (18.2)	15 (9.1)
4. Do you know that plant-based meat on the market comes in various forms, including plant-based meat dumplings, plant-based meat sausages and plant-based meatballs etc.?	126 (76.4)	19 (11.5)	20 (12.1)
In comparison to animal-based meat,			
5. Does plant-based meat have lower calorie content?	84 (50.9)	46 (27.9)	35 (21.2)
6. Does plant-based meat have higher carbohydrates?	97 (58.8)	42 (25.5)	26 (15.8)
7. Does plant-based meat have higher dietary fiber?	104 (63.0)	40 (24.2)	21 (12.7)
8. Does plant-based meat have lower fat content?	117 (70.9)	26 (15.8)	22 (13.3)
9. Does plant-based meat have lower amino acid profile?	71 (43.0)	63 (38.2)	31 (18.8)
10. Does plant-based meat have higher antioxidant activity and polyphenolic compounds?	76 (46.1)	70 (42.4)	19 (11.5)

4.4.3 Level of Knowledge Towards PBM Items

The level of knowledge for PBM items is shown in Table 4.7. Among the respondents, the majority (37%) had a low knowledge level towards PBM items, while 36.4% and 26.7% had high and moderate knowledge levels of PBM items, respectively.

Table 4.7: Level of knowledge towards plant-based meat (PBM) items

Variable	Classification	Frequency (n)	Percentage (%)
Knowledge	Low level	61	37.0
	Moderate level	44	26.7
	High level	60	36.4

CHAPTER 5

DISCUSSION

5.1 Proximate Analysis of PBM Items

5.1.1 Moisture

As shown in Table 4.1, all food samples had a substantial difference in moisture content, with SC having the greatest level and DCB having the lowest. Moisture comprised the primary component of all food samples (Marshall, 2010). The considerably greater moisture content in SC could be related to the inclusion of watery ingredients, specifically the creamy sauce which is made from a combination of milk and water (Tarmizi, Daud and Rahman, 2020).

In addition, the procedures and temperatures used for the cooking process could influence the extent of moisture loss (Kassama and Ngadi, 2016; Tarmizi, Daud, and Rahman, 2020). Yet, the cooking techniques and temperatures were not specified by the collaborative vegan restaurant in this study. Based on Durazzo, et al. (2019), who have listed the preparation methods for Italian traditional dishes, it is noted that pasta requires a total of 13 minutes for boiling followed by pan-frying, while pizza requires a baking duration of 1 to 2 minutes in the oven. Additionally, burgers were typically grilled at restaurants (Cooper, 2022). Therefore, we hypothesized that the cooking techniques used in PBM items preparation were similar to those documented in prior research. KP and DCB were predominantly prepared by dry-heat cooking techniques, which require an

elevated temperature (above 150 ºC) without the addition of moisture. Whereas SC was predominantly prepared by moist-heat cooking techniques, which entail cooking in a liquid medium at lower temperatures due to the restricted boiling point of water (Alfaro, 2022).

Likewise, Abasi, et al. (2009) found that when temperature increased, the moisture content of the samples lowered. This revealed that higher cooking temperatures in KP and DCB induce a greater moisture loss from evaporation, whereas boiling preserves more moisture in SC (Tarmizi, Daud and Rahman, 2020). Nevertheless, it was observed that DCB showed a significantly lower moisture content in comparison to KP, suggesting a greater dehydration rate during grilling rather than baking, comparable to that described by Marimuthu, et al. (2012) in snakehead fish. This can be explained by the rationale that the baking process in KP produces more tightly bound and immobilized water (Oppong, et al., 2021).

5.1.2 Ash

The ash content can be utilized as an indicator for estimating the mineral concentrations (Marshall, 2010). According to Table 4.1, SC had the lowest ash concentration with significant differences, followed by DCB and KP, both of which did not differ substantially. Ash concentration in food samples might vary depending on the cooking method used. As highlighted by Cheong, Ahmad, and Tengku Rozaina (2022), the ash content increased when dry-heat cooking methods were used, but it dropped when moist-heat cooking methods were used.

In this study, the high temperatures used in the preparation of DCB and KP resulted in greater moisture loss and thus increment of dry matter and ash content. The findings agreed with research done in fish samples by Marimuthu, et al. (2012) and Karimian-Khosroshahi, et al. (2016). Moreover, the watery ingredients used in SC contributed to a rise in moisture content, subsequently leading to a reduction in ash level. The inclusion of salt, sugar, and spices in food samples could contribute to the ash content (Tarmizi, Daud and Rahman, 2020; Zaki, et al., 2020).

5.1.3 Fat

Table 4.1 shows a significant difference in fat content between SC with KP and DCB. The KP has the greatest fat content, followed by DCB and SC. As stated by Karimian-Khosroshahi, et al. (2016), the total fat content has been reported to have an indirect relationship to the moisture content. In comparison to SC, the greater moisture loss reported in KP and DCB allows more oil to infiltrate into the food items, and thereby contain a higher fat concentration. Furthermore, the result obtained concurred with the study done by Cheong, Ahmad and Tengku Rozaina (2022), who concluded that the application of dry-heat cooking techniques led to an elevation in crude fat content. Also, Zaki, et al. (2020) highlighted that the nutrient concentration plays a role in increasing crude fat content. Apart from that, the lower cooking temperature employed in SC resulted in smaller moisture loss, which has been accountable for its lowest fat content among all samples (Tarmizi, Daud and Rahman, 2020).

Besides, the overall fat composition of the food samples was influenced by the inclusion of fat-containing substances including dairy products such as cheese, dairy milk, as well as cooking oil. Based on Clegg, et al. (2021), the inclusion of dairy products in plant-based diets generally offers high levels of total fat and saturated fat. This, in turn, may elevate the consumption of dietary saturated fat and subsequently associated with an elevated risk of cardiovascular disease (CVD). Notably, cheese, as mentioned by Nolden and Forde (2023), stands out for its significant amount of saturated fat. However, a meta-analysis has determined that the consumption of dairy milk lacks significant association with an increased likelihood of CVD and death. The absence of an impact on CVD risk factors by dairy products, particularly cheese, may be related to the existence of micronutrients and food matrices (Clegg, et al., 2021). This observation indicates that there exists a multifaceted interaction of several factors that must be taken into account when evaluating the effects of dairy products in PBM items on health status.

5.1.4 Protein

With references to Table 4.1, DCB had the highest protein content, followed by KP, however the difference was not statistically significant. On the other hand, SC had the lowest protein content and was found to differ significantly between samples. The availability of dairy items such as mozzarella and cheddar cheeses (Durazzo et al., 2017), as well as plant-based meat patty (Pointke et al., 2022) may explain the greater protein content in DCB and KP. Moreover, the protein content was inversely related to moisture content (Karimian-Khosroshahi, et al.,

2016). Dry-heat cooking methods are associated with lower moisture levels and higher protein levels in DCB and KP (Marimuthu, et al., 2012; Karimian-Khosroshahi, et al., 2016), while the elevated moisture content of SC indicated that it had the lowest protein content among all food samples (Karimian-Khosroshahi, et al., 2016). Also, SC only contained a small portion of plant-based minced meat, lowering the protein value of the samples.

According to a study conducted by Nolden and Forde (2023), there is a significant disparity in protein content between plant-based and animal-based products. For instance, chicken burgers contained a greater protein content (7.57 ± 0.63%) when compared to DCB (0.66 ± 0.06%). Also, various studies have reported a lower protein content in PBM alternatives than animal-based products (Curtain and Grafenauer, 2019; Alessandrini, et al., 2021; Safefood, 2021; Harnack, et al., 2021; Cutroneo, et al., 2022). This is because animal-derived products are the primary source of protein (Durazzo, et al., 2017), and thus, the available plant-based diet options may not offer comparable protein quantities in replacement of animal-derived diets (Nolden and Forde, 2023).

Additionally, the protein values in this study were generally low. Since ready-to-eat dishes contain a combination of heterogeneous components, including carbohydrates, lipids and a range of micronutrients, protein accessibility may be impacted by the interconnection of food components, matrices and various nutrients. Moreover, the hydrolysis process resulted in a decrease in the amount

of some amino acids during protein determination (Mæhre, et al., 2018). Thus, there is a possibility that these factors will result in lower protein content.

Nevertheless, the Kjeldahl method lacks specificity and only allows a general measurement of the overall nitrogen level present in food samples. The inability of the Kjeldahl method to distinguish between protein nitrogen and non-protein nitrogen (NPN) gives an underestimated measurement of actual protein content. NPN, which encompasses nitrate, ammonia, urea, nucleic acids and free amino acids, constitutes around 25% of the overall nitrogen content (Moreno-Villares and Marta Germán-Díaz, 2019). Particularly, vegetables tend to exhibit greater levels of NPN compared to animal-derived dietary sources **(**Mæhre, et al., 2017). According to Hayes (2020), the Kjeldahl method could result in an overestimation of protein content ranging from 40 to 71%, even when precise conversion factors are employed. Hence, overstatement of the possibility, financial viability, and marketability of these novel protein sources will probably occur since items with increased protein contents have a higher market value (Hayes, 2020). Additionally, there is the risk of food adulteration by the inclusion of NPN, which might endanger consumer food safety (Mæhre, et al., 2017). Therefore, further research is needed to better understand the ingredients utilized in PBM items and to determine their protein composition using more precise methods such as amino acid analysis methods (Hayes, 2020).

5.1.5 Carbohydrate

In Table 4.1, the carbohydrate content among the samples varied significantly. There was no significant difference in carbohydrate content between DCB and KP. On the contrary, SC had the lowest carbohydrate content, which differed from the other samples. The carbohydrate contents of this study ranged from 28.18 to 33.37 %, which is much higher than isolated PBM products, which ranged from 7.9 to 16.7% (Curtain and Grafenauer, 2019). The selected PBM items for this study were in the form of ready-to-eat meals, which are typically served together with cereal-based ingredients, including wheat, bread, and pasta (Tarmizi, Daud and Rahman, 2020), thereby contributing to their overall carbohydrate content.

Besides, the carbohydrate content of food samples can be greatly influenced by various cooking methods. According to Cheong, Ahmad and Tengku Rozaina (2022), the application of moist-heat cooking leads to a reduction in carbohydrate levels. The boiling process in SC may be potentially attributed to the loss of nutrients (Bahado-Singh et al., 2006) and sugar (China, et al., 2019) in the liquid medium, thus lowering the carbohydrate content. Conversely, it has been shown that dry-heat cooking methods tend to have a higher carbohydrate level (Cheong, Ahmad and Tengku Rozaina, 2022). Since higher temperature used in KP and DCB promotes moisture loss, causing an accumulation of free sugars within the food item (Bahado-Singh et al., 2006). Additionally, it is generally acknowledged that the heating process promotes starch breakdown, which in turn leads to a reduction in carbohydrate complexity and an elevation

in sugar content, and therefore contributes to a higher glycemic index (GI) value (Bahado-Singh et al., 2006).

5.1.6 Fiber

Crude fiber refers to the remaining plant material following a series of extractions using solvents. This residue contains various insoluble dietary fiber components, such as hemicelluloses, lignins and cellulose, which are quantified after the removal of soluble components (Trowell, 1976). Rehman, Islam and Shah (2002) stated that the process of cooking resulted in varying degrees of loss for cellulose and hemicellulose, whereas lignin exhibited little changes.

All the samples exhibited comparable crude fiber content and did not demonstrate any significant differences from one another, as indicated in Table 4.1. The primary sources of dietary fiber in the dishes are derived from cereals, legumes and vegetables (Durazzo, et al., 2017). This implies that the fiber-origin ingredients included in the samples for this study were all comparable in composition and quantity. Still, DCB had the highest fiber content among the samples. Joshua, Timothy, and Suleiman (2012) investigated the crude fiber content of raw and cooked vegetables grown locally in Nigeria and discovered that the amount of fiber declines with a longer cooking time (shown as 15 minutes). Moreover, the incorporation of plant ingredients and fiber-rich thickeners during PBM product formulation, makes them contain more fiber than animal-based products (Bakhsh, et al., 2021a). Hence, it is believed the usage of raw vegetables and PBM patty increases the fiber content of DCB.

In accordance with Barber et al. (2020), there is evidence suggesting that fiber can enhance metabolic health, cardiovascular and colonic health, intestinal movement, and minimize the incidence of colorectal cancer. Still, it is important to emphasize that the average daily intake of dietary fiber in Malaysia falls significantly below the recommended limit of 20 to 30 g/day (Lee and Muda, 2018). Therefore, PBM items consumption might be a viable approach for improving fiber intake and overall dietary quality in Malaysia.

5.2 Antioxidant Properties of PBM Items

5.2.1 Total Phenolics Content (TPC) and Total Flavonoids Content (TFC)

According to Table 4.2, the total phenolics content (TPC) and total flavonoids content (TFC) of all PBM items did not differ significantly. TPC values varied between 1.36 to 1.94 mg GAE/g sample, while TFC varied between 0.96 to 1.19 mg QE/g sample. Overall, the TPC values were all higher than TFC values. This is consistent with the statement proposed by Sulaiman and Balachandran (2012) that flavonoids are the dominant subgroup of phenolic compounds. Specifically, flavones and flavonols are identified as the most abundant phenolics within this category.

Phenolic compounds are abundantly present in plant-derived foods, such as grains, fruits and vegetables (Gutiérrez-Grijalva, et al., 2016). In this study, KP contained the highest TPC and TFC levels among all samples, as kale possesses a high content of bioactive phytochemicals, including total phenolics (Armesto, et al., 2016), total flavonoids and antioxidant capacity (Armesto, et al., 2019). Nevertheless, no significant differences were observed in TPC and TFC values between samples, suggesting that equivalent amounts of phenolic-rich materials were used in these PBM items preparation (Durazzo, et al., 2017).

Despite the absence of significant differences in TPC and TFC, each PBM item nevertheless contributes to the dietary intake of phenolic chemicals, which could have advantageous effects on health. Phenolic compounds have been reported to possess antioxidant, anti-inflammatory, and anti-carcinogenic properties, which help to prevent a wide range of chronic illnesses like cancer, diabetes, Alzheimer's disease and CVD (Gutiérrez-Grijalva, et al., 2016). This is because polyphenols contain hydroxyl groups that efficiently squelch damaging free radicals, thereby exhibiting potent antioxidant characteristics (Kupina, et al., 2018).

Furthermore, cooking was found to significantly reduce antioxidant compounds. As mentioned by Sikora and Bodziarczyk (2012), cooking decreased the dry mass of polyphenols and antioxidant activity in vegetables by 56% and 38%, respectively. The loss of polyphenol content might be due to heat-induced deterioration or leaking into the cooking water (Armesto, et al., 2016).

Additionally, flavonoids are soluble in water and susceptible to oxidation. Armesto, et al. (2019) claimed that the leaching rate of flavonoids may be associated with heightened temperatures and exposure to water and oxygen while cooking, all of which are believed to trigger cellular breakdown and facilitate oxidation processes. Therefore, the current study's findings of no significant difference in TPC and TFC content were supported.

5.2.2 Antioxidant Capacities

The antioxidant capacities of PBM items were determined using two methods, including DPPH and ABTS free radical scavenging assays. Rebaya, et al. (2015) argued that a universally applicable and precise approach for quantitatively evaluating antioxidant activity does not exist. Consequently, it is recommended to perform a minimum of two methodologies for assessing antioxidant activity. This may be because each assay has different strengths and drawbacks in terms of the mechanism of antioxidant function (Othman, et al., 2014). Besides, the antioxidant capacities were expressed as antioxidant activity percentage (AA%) and half-maximal inhibitory concentration (IC_{50}) values. The IC_{50} value is the concentration of a substance containing antioxidants that is necessary to eliminate 50% of the original concentration of free radicals. The ability to scavenge free radicals is directly proportional to AA%, but inversely proportional to IC_{50} value. Thus, a lower IC_{50} suggests more antioxidant activity (Olugbami, Gbadegesin and Odunola, 2014).

In Table 4.3, SC revealed the lowest AA% and the highest IC_{50} values in both assays, with a significant difference compared to the other samples. Meanwhile, KP had the highest AA% in DPPH assay and the lowest IC_{50} values in both assays. This might be due to fresh kale exhibits a strong antioxidant capacity, with IC_{50} DPPH values of 4.86 g/L and AA% of approximately 90% (Armesto, et al., 2016), as well as ABTS of 33.22 μM Trolox/g of fresh sample (Sikora and Bodziarczyk, 2012). Also, cooking parameters such as technique, temperature, duration, and portion served have a significant influence on antioxidant activity (Hwang, et al., 2012). Hwang, et al. (2012) also noted that greater losses were reported in moist-heat cooking methods compared to dry-heat cooking methods, due to the dissolution of water-soluble phytochemicals in liquid mediums (Akdaş and Bakkalbaşı, 2016). Similarly, Armesto et al. (2016) underlined the process of boiling had the most pronounced impact on the antioxidant capacity, resulting in a four-fold rise in IC_{50} compared to fresh samples. Overall, based on the above studies, it can be inferred that SC displayed the lowest antioxidant capacities among all samples, whereas KP seems to have the greatest antioxidant capacities, despite being comparable to DCB.

Generally, TPC and TFC were associated with antioxidant capacity (Aryal, et al., 2019). Nonetheless, in the current study, despite the statistical differences in antioxidant capacity, the amounts of TPC and TFC were comparable across the samples. This emphasized the necessity for further investigations to identify the potential antioxidant roles of other phytochemicals in PBM items. Apart

from that, the AA% determined by DPPH assay was generally lower than the ABTS assay. This could be because the limited solubility of DPPH radicals in organic solvents restricts their use in hydrophobic systems, while ABTS can be used for both hydrophilic and lipophilic antioxidant systems (Floegel, et al., 2011). Thus, DPPH is a key limiting factor in understanding the hydrophilic antioxidant functions (Othman, et al., 2014). Interestingly, KP showed the significantly highest AA% in DPPH assay, but in ABTS assay, DCB was found with the highest AA% despite being comparable to KP. This finding could postulate that DCB contains more hydrophilic antioxidants in nature than KP, which can only be detected using ABTS assay.

5.3 Association Between TPC, TFC and Antioxidant Capacities

A large portion of the antioxidant activity in plants or plant products is provided by phenolics, the biggest class of phytochemicals (Sulaiman and Balachandran, 2012). In general, foods with higher phenolic content have stronger antioxidant activity (Fadly, Purwayantie and Arundhana, 2020). This aligns with the study's result in Table 4.4, where a strong linear correlation between TPC and both the AA% of DPPH assay ($r = 0.94$) and ABTS assay ($r = 0.90$), indicating a higher concentration of TPC could potentially enhance the antioxidant activities. Similar findings were also reported by Rebaya, et al. (2015), Awang-Kanak, Bakar and Mohamed (2019) and Cheong, Ahmad and Tengku Rozaina (2022).

Nevertheless, TFC only displayed a moderate linear correlation with AA% of DPPH ($r = 0.46$) and weak inversely correlation with AA% of ABTS ($r = -0.31$), indicating that the antioxidant effects of flavonoids on PBM items were limited. The outcome was in line with Othman, et al. (2014), who hypothesized that only specific flavonoid structures, especially specific locations of hydroxyl groups within the molecules, are likely to possess antioxidant characteristics. Additionally, the presence of other bioactive substances within the food samples was also a factor in determining the antioxidant capacities. In addition to flavonoids, several subcategories under phenolic compounds may contribute to antioxidant roles, including simple phenols, phenolic acids, coumarins, stilbenes, hydrolyzable and condensed tannins, lignans and lignins (Blainski, Lopes and Mello, 2013). Future in-depth studies are necessary to identify this. Apart from that, studies conducted by Awang-Kanak, Bakar, and Mohamed (2019) and Armesto, et al. (2019) found opposite findings, showing a positive association between TFC and DPPH and ABTS.

5.4 Level of Knowledge Towards PBM Items

In Malaysia, there is a growing market for plant-based protein products due to a modern dietary trend (Austrade, 2021). Many studies have attempted to identify facilitators and barriers in the consumption of plant-based food or PBM items. In general, younger generations and well-educated individuals demonstrated a greater willingness to replace (Carlsson, Kataria, and Lampi, 2022) and preferences (Van Loo, Caputo, and Lusk, 2020) for PBM alternatives,

which well represents the target populations of university students in the current study.

Nonetheless, this study found that UTAR undergraduate students have a low level of knowledge towards PBM items (Table 4.7). This might potentially be attributed to most participants declaring themselves as non-vegetarian (96.4%), as seen in Table 4.5. Based on a survey conducted among German consumers, it was found that 66.7% of participants who identified as omnivores claimed to consume PBM products either monthly or not at all (Pointke, et al., 2022). It could be hypothesized that non-vegetarians have limited exposure to PBM items and hence little knowledge of them. Moreover, despite the long duration since the introduction of PBM products, there has been no apparent reduction in meat consumption (Safdar, et al., 2022). In 2022, the per capita consumption of poultry in Malaysia reached 50 kg. This statistic positions Malaysia as a prominent global consumer of chicken meat (Statista Research Department, 2023). Also, previous research has highlighted the tendency of consumers to exhibit reluctance in accepting innovative food technologies (Siegrist and Hartmann, 2020).

According to Table 4.6, respondents had a good general knowledge of PBM items, but their nutrition-related knowledge of PBM items was limited. Similar to the findings of Pointke, et al. (2022), majority of the participants were able to recognize the popular ingredients of PBMAs, classifying PBMAs as ultra-processed foods, and claimed the reasons for their consumption. Despite this,

they lacked information regarding the health-promoting components of PBMAs. Bucher, Müller and Siegrist (2015) observed that consumers often overlook the content of individual nutrients when evaluating the healthfulness of foods, presuming that consumers tended to rely on more general evaluations instead of considering particular nutritional information.

Likewise, a study conducted in Beijing found that consumers' knowledge of PBMs is still limited, and they have an adverse preference for them. Interestingly, their likelihood to purchase PBM improved significantly after nutritional information was provided, instead of information regarding food safety and environmental concerns (Wang, et al., 2022). This implies that consumers' acceptance of PBM would change whenever external knowledge related to individual advantages and interests was provided. Hence, it is recommended that detailed information regarding the nutrition aspects of PBM items be provided in order to improve knowledge and familiarity of PBM items among UTAR undergraduate students.

5.5 Strengths and Limitations

Most of the prior research done abroad has focused on investigating the nutritional compositions of PBM products in isolated forms. Also, most studies relied on product labels and recipes to obtain nutrient information, but these sources could only provide limited data on macronutrients. Therefore, the current study is in its efforts to include PBM items in ready-to-eat forms that could reflect the actual dietary choices of consumers. Also, validated chemical

analytical methods were employed to provide more comprehensive assessments of the nutritional and antioxidant contents of PBM items, comprising the analysis of nutrients not listed on the label. Furthermore, this study can be regarded as a preliminary study to identify the present knowledge levels on PBM items among Malaysian undergraduate students, which will serve as a reference for future researchers when working on this topic. The survey also revealed that undergraduates had limited nutritional knowledge of PBM items, underlining the necessity of educational efforts to raise their understanding in this aspect.

However, there are few limitations in the study. Firstly, the study only included limited food samples from a single restaurant, and all data was merely measured in duplicate. This implies that these data failed to accurately represent the range of PBM items available in the market and the statistical power is weak to identify significant differences across variables. Second, this study was unable to obtain a complete list of ingredient composition, cooking techniques, and preparation times for PBM items, all of which could potentially impact the evaluation of the nutritional and antioxidant content of PBM items. Thirdly, the questionnaire was self-developed and the respondents were recruited based on convenient sampling. Hence, it might introduce unreliability and selection bias, making it inappropriate to make a conclusion on the knowledge levels of PBM items among all UTAR undergraduate students. Also, the questionnaire did not provide a clear definition of PBM items, respondents might have answered the questions with varying ideas of PBM products in mind. Moreover, the data was

only limited to a specific group of respondents and was not representative of the entire Malaysian population or any other nation.

5.6 Recommendations for Future Studies

There are several recommendations can be suggested for future studies. Firstly, future studies could increase the sample size of PBM items. Also, sample processing, storage and extraction protocols should be optimized to avoid prolonged defrosting durations, thus improving results consistency. In addition to proximate analysis and antioxidant profiles, research can focus on other nutritional components of PBM items, such as micronutrient content analyses including vitamins and minerals, particularly sodium content as PBM items are regarded as ultra-processed foods. This may assist in generating more thorough nutritional profiles of PBM items available in the market, making it simpler for consumers to make informed food choices. Moreover, sensory evaluation can be performed to assess customer preferences and acceptability of PBM items. Data from sensory analysis might be used for estimating product marketability and development, as well as recognizing factors that influence consumer choices.

In terms of survey study, researchers may explore the knowledge, attitudes and practices toward PBM items on undergraduate students or other populations. The expansion of the sample size of respondents is recommended to yield more precise and representative outcomes. Finally, research can be planned by incorporating survey data and laboratory analyses. For example, laboratory

analyses can be carried out on several PBM items that survey participants most frequently picked. This would help in determining consumers' current dietary patterns and guaranteeing that research efforts are utilized on food items that have the biggest influence on consumers' diets.

CHAPTER 6

CONCLUSION

In this study, the nutritional compositions, total phenolics and antioxidant capacities of PBM items, as well as consumer knowledge of PBM items among undergraduates have been investigated. In general, moisture comprised the largest proportion of all samples, followed by carbohydrates, fat, fiber and ash, while protein made up the smallest proportion. KP and DCB were found to have lower moisture content, higher ash, protein, fat and carbohydrate contents in comparison to SC. This variation in nutritional contents was attributed to the diversity of ingredients and cooking methods used in PBM item preparation. Nonetheless, there were no significant differences in fiber content, TPC and TFC across all samples. Furthermore, SC was shown to have the lowest antioxidant capacities with significant differences from other samples in both antioxidant capacities tests (DPPH and ABTS). TPC exhibited a strong association with antioxidant capacity, while TFC had a weak association. Other than that, UTAR undergraduate students reported a low level of knowledge of PBM items, particularly regarding nutritional knowledge of PBM items. Additional studies are recommended to investigate the nutritional and antioxidant profiles of other PBM items, as well as to explore the knowledge, attitudes and practices toward PBM items in undergraduate students.

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APPENDICES

Appendix A

Ethical Approval Letter from UTAR

UNIVERSITI TUNKU ABDUL RAHMAN DU012(A)
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Re: U/SERC/62/2023

20 March 2023

Dr Teh Lai Kuan
Head, Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti, Bandar Baru Barat
31900 Kampar, Perak.

Dear Dr Teh,

Ethical Approval For Research Project/Protocol

We refer to the application for ethical approval for your students' research projects from Bachelor of Science (Honours) Dietetics programme enrolled in course UDDN3108. We are pleased to inform you that the application has been approved under Expedited Review.

The details of the research projects are as follows:

No	Research Title	Student's Name	Supervisor's Name	Approval Validity
1.	Nutritional Compositions, Total Phenolics, and Antioxidant Capacities of Plant-Based Meat Items	1. Chen Yu Wei 2. Elisa Bong Tsyr Yin 3. Oo Xing Joe	Dr Chang Sui Kiat Dr Ee Kah Yaw	20 March 2023 – 19 March 2024
2.	Microbiological Analyses and Undergraduate Student's Attitude and Perceptions About Microbiological Risk of Plant-based Meat Items	1. Careen Chong Kai Lyn 2. Siew Fei Kie 3. Wong Siew Ching	Dr Chang Sui Kiat Dr Lam Ming Quan	

The conduct of this research is subject to the following:

(1) The participants' informed consent be obtained prior to the commencement of the research;

(2) Confidentiality of participants' personal data must be maintained; and

(3) Compliance with procedures set out in related policies of UTAR such as the UTAR Research Ethics and Code of Conduct, Code of Practice for Research Involving Humans and other related policies/guidelines.

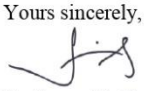
(4) Written consent be obtained from the institution(s)/company(ies) in which the physical or/and online survey will be carried out, prior to the commencement of the research.

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

Should the students collect personal data of participants in their studies, please have the participants sign the attached Personal Data Protection Statement for records.

Thank you.

Yours sincerely,



Professor Ts Dr Faidz bin Abd Rahman
Chairman
UTAR Scientific and Ethical Review Committee

c.c Dean, Faculty of Science
Director, Institute of Postgraduate Studies and Research

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
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Appendix B

Questionnaire of Study

PERSONAL DATA PROTECTION NOTICE

Please be informed that in accordance with Personal Data Protection Act 2010 ("PDPA") which came into force on 15 November 2013, Universiti Tunku Abdul Rahman ("UTAR") is hereby bound to make notice and require consent in relation to collection, recording, storage, usage and retention of personal information.

1. Personal data refers to any information which may directly or indirectly identify a person which could include sensitive personal data and expression of opinion. Among others it includes:

   a) Name
   b) Identity card
   c) Place of Birth
   d) Address
   e) Education History
   f) Employment History
   g) Medical History
   h) Blood type
   i) Race
   j) Religion
   k) Photo
   l) Personal Information and Associated Research Data

2. The purposes for which your personal data may be used are inclusive but not limited to:

   a) For assessment of any application to UTAR
   b) For processing any benefits and services
   c) For communication purposes
   d) For advertorial and news
   e) For general administration and record purposes
   f) For enhancing the value of education
   g) For educational and related purposes consequential to UTAR
   h) For replying any responds to complaints and enquiries
   i) For the purpose of our corporate governance
   j) For the purposes of conducting research/ collaboration

3. Your personal data may be transferred and/or disclosed to third party and/or UTAR collaborative partners including but not limited to the respective and appointed outsourcing agents for purpose of fulfilling our obligations to you in respect of the purposes and all such other purposes that are related to the purposes and also in providing integrated services, maintaining and storing records. Your data may be shared when required by laws and when disclosure is necessary to comply with applicable laws.

4. Any personal information retained by UTAR shall be destroyed and/or deleted in accordance with our retention policy applicable for us in the event such information is no longer required.

5. UTAR is committed in ensuring the confidentiality, protection, security and accuracy of your personal information made available to us and it has been our ongoing strict policy to ensure that your personal information is accurate, complete, not misleading and updated. UTAR would also ensure that your personal data shall not be used for political and commercial purposes.

Consent:

6. By submitting or providing your personal data to UTAR, you had consented and agreed for your personal data to be used in accordance to the terms and conditions in the Notice and our relevant policy.

7. If you do not consent or subsequently withdraw your consent to the processing and disclosure of your personal data, UTAR will not be able to fulfill our obligations or to contact you or to assist you in respect of the purposes and/or for any other purposes related to the purpose.

8. You may access and update your personal data by writing to us at_____.

Acknowledgment of Notice

[ ] I have been notified and that I hereby understood, consented and agreed per UTAR above notice.

[ ] I disagree, my personal data will not be processed.

.....
Name:
Date:

Section A: Sociodemographic profiles

This section consists of 5 questions.
Please fill in your answer to each question accordingly.

4. Gender *

Mark only one oval.

- ◯ Male
- ◯ Female

5. Course *

Mark only one oval.

- ◯ Science
- ◯ Non-science

6. Education level *

Mark only one oval.

- ◯ Year 1
- ◯ Year 2
- ◯ Year 3
- ◯ Year 4
- ◯ > Year 4

7. Religion *

Mark only one oval.

- Christian
- Buddhism
- Muslims
- Others

8. Diet pattern *

Mark only one oval.

- Vegetarian
- Non-vegetarian

Section B: Consumer's Knowledge on Plant-Based Meat Items

This section will be separated into 2 parts:
Part 1 - General knowledge of PBM items
Part 2 - Knowledge on nutritional composition and antioxidant properties of PBM items in comparison to animal-based meat

Part 1: General knowledge of plant-based meat (PBM) items

This section consists of 4 questions.
Please read each of the question carefully.

Please choose "Not sure" if you do not know the answer.

Based on your opinion, please select the answer (Yes/ No/ Not sure) for the following questions:

9. Do you think plant-based meat consumption can bring health benefits, as well as environmental sustainability and animal welfare preservation? *

Mark only one oval.

- Yes
- No
- Not Sure

10. Do you know that plant-based meat items are primarily made from isolated plant proteins such as soy, wheat gluten and peas? *

Mark only one oval.

- Yes
- No
- Not Sure

11. Do you think plant-based meat items involve a high degree of processing method? *

Mark only one oval.

- Yes
- No
- Not Sure

12. Do you know that plant-based meat on the market comes in various forms, including plant-based meat dumplings, plant-based meat sausages and plant-based meatballs etc.? *

Mark only one oval.

- Yes
- No
- Not Sure

Part 2: Knowledge on nutritional composition and antioxidant properties of PBM items in comparison to animal-based meat

This section consists of 6 questions.
Please read each of the question carefully.

Please choose "Not sure" if you do not know the answer.

In comparison to animal-based meat,

13. Does plant-based meat have lower calorie content? *

Mark only one oval.

- Yes
- No
- Not Sure

14. Does plant-based meat have higher carbohydrates? *

Mark only one oval.

- Yes
- No
- Not Sure

15. Does plant-based meat have higher dietary fiber? *

Mark only one oval.

- Yes
- No
- Not Sure

16. Does plant-based meat have lower fat content? *

Mark only one oval.

- Yes
- No
- Not Sure

17. Does plant-based meat have lower amino acid profile? *

Mark only one oval.

- Yes
- No
- Not Sure

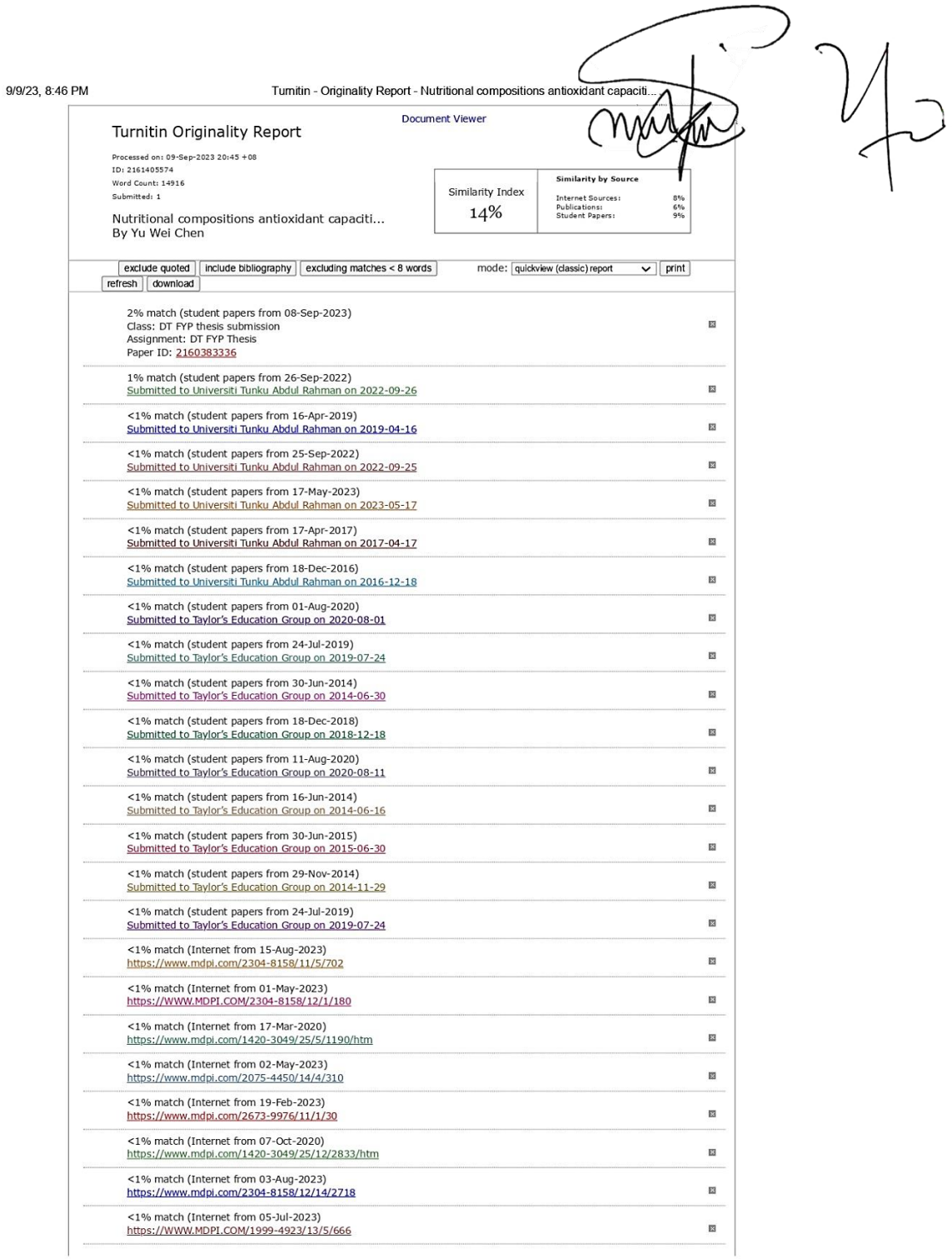
18. Does plant-based meat have higher antioxidant activity and polyphenolic compounds? *

Mark only one oval.

- Yes
- No
- Not Sure

Appendix C

Turnitin Report



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By Yu Wei Chen

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Appendix D

Turnitin Originality Report

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

UTAR
UNIVERSITI TUNKU ABDUL RAHMAN
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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	Chen Yu Wei
ID Number(s)	20ADB05681
Programme / Course	Bachelor of Science (Hons) Dietetics
Title of Final Year Project	Nutritional Compositions, Total Phenolics, Antioxidant Capacities, and Students' Knowledge Level About Plant-Based Meat Items

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 14 % **Similarity by source** Internet Sources: 8 % Publications: 6 % Student Papers: 9 %	
Number of individual sources listed of more than 3% similarity: 0	

Parameters of originality required and limits approved by UTAR are as follows:
(i) Overall similarity index is 20% and below, and
(ii) Matching of individual sources listed must be less than 3% each, and
(iii) Matching texts in continuous block must not exceed 8 words
Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor
Name: Dr. Chang Sui Kiat
Date: 13/9/2023

Signature of Co-Supervisor
Name: Dr. Ee Kah Yaw
Date: 13/9/2023