NUTRITIONAL VALUES AND ANTIOXIDANT ACTIVITY OF NOODLES INCORPORATED WITH BLACK FACE GENERAL (*Strobilanthes crispus*)

By

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ABSTRACT

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Set Shu Hui

Strobilanthes crispus, known as Black Face General is one of the herbal plants readily found in Malaysia. It has been used in some traditional remedies due to its antioxidant potential and pharmaceutical properties. In this study, the noodle was made by incorporating different concentrations of fresh *S. crispus* leaves (3, 5, and 10%) to determine the nutritional values, antioxidant activities (TPC, DPPH, and FRAP), and physicochemical properties (texture and color). Analysis of variance (ANOVA) and Tukey test were carried out to examine the significant difference of results at p= 0.05 level. Based on the result, proximate analysis revealed that there was a significant difference ($p<0.05$) in the amount of moisture, ash, protein, fat, and fiber across samples except carbohydrates content ($p>0.05$). In all antioxidant assays, 10% *S. crispus* noodles showed the highest antioxidant capacity with significant difference between samples ($p<0.05$): TPC (1.75 ± 0.11 mg GAE/mL), DPPH (IC_{50} = 1958.4 ± 194.58 μM/mL) in references to ascorbic acid (IC_{50} = 27.64 ± 0.74 μM/mL), and FRAP (141.44 ± 8.22 μM Fe(II)/mL). The antioxidant activity of 0% control noodles were 1.38 ± 0.15 mg GAE/mL, IC_{50} = 2676.34 ± 362.95 μM/mL, and 60.03 ± 6.4 μM Fe(II)/mL for TPC, DPPH, and FRAP respectively. There was a significant decrease in hardness along the *S. crispus*

concentration where the 10% *S. crispus* noodles had the lowest value (42.66 ± 0.89 N). Besides, the tensile strength difference among samples was not significant ($p>0.05$). As the concentration of *S. crispus* increases, L* and a* values decreased, b* values increased, this indicated that the noodle became darker and stronger in a green and yellow hue due to the presence of chlorophyll pigment. Overall, 10% *S. crispus* noodles effectively enhance antioxidant activity compared to 3% *S. crispus* and control noodles.

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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

SET SHU HUI

APPROVAL SHEET

This final year project report entitled **"NUTRITIONAL VALUES AND ANTIOXIDANT ACTIVITY OF NOODLES INCORPORATED WITH BLACK FACE GENERAL (*Strobilanthes crispus*)"** was prepared by SET SHU HUI and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) Food Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **SET SHU HUI** (ID No:**17ADB02447**) has completed this final year project report entitled "NUTRITIONAL VALUES AND ANTIOXIDANT ACTIVITY OF NOODLES INCORPORATED WITH BLACK FACE GENERAL (*Strobilanthes crispus*)" under the supervision of Dr. Tan Yen Nee (supervisor) from the Department of Agricultural and Food Science, Faculty of Science from the Department of Agricultural and Food Science, Faculty of Science.

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,

(SET SHU HUI)

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
$CuSO_4$	Copper (II) sulfate
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EC_{50}	Effective concentration at which 50% of activity is observed
FC	Folin-Ciocalteau
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
H_2O_2	Hydrogen Peroxide
IC_{50}	Half maximal scavenging concentration
R^2	Coefficient of determination
ROS	Reactive oxygen species
Rpm	Revolutions per minute
SC	*Strobilanthes. crispus*
SPSS	Statistical package for the social science
TPC	Total phenolic content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine

CHAPTER 1

INTRODUCTION

Nowadays, many of studies revealed that oxidative stress degrades cells and tissues, impairs metabolic function, and plays a significant role in the onset and progression of various health diseases and conditions. For instance, cardiovascular risk diseases, certain kinds of cancer, inflammatory diseases, Alzheimer's disease, Parkinson's disease, aging, et cetera (Liguori, et al., 2018). Oxidative stress happened when the reactive oxygen species (ROS) or free radicals become excessive and the critical balance between ROS and antioxidant defenses is out of balance (Lobo, et al., 2010). In this case, much attention needs to be paid to the prevention of many free-radical-induced diseases by scavenging excessive free radicals through antioxidants. Due to concerns regarding the health effect of long-term consumption, natural antioxidants tend to replace synthetic antioxidants. Vegetables, fruits, and herbs are all-natural sources of the antioxidant that the body requires (Lourenco, Moldao-Martins & Alves, 2019).

Malaysia is endowed with rainforests, which habitat to approximately 2000 species of medicinal plants that have been linked to positive effects on human health (Fazleen Izzany, et al., 2018). Black Face General is one of the well-known medicinal plants in Malaysia, also referred as ‘Pecah beling’, ‘Jin batu’, ‘Pecah kaca’, ‘Karang jin’, ‘Bayam karang’, and ‘Hei mian jiang jun’ in Mandarin (Ghasemzadeh, Jaafar & Rahmat, 2015). Black Face General with

the scientific name, *Strobilanthes crispus*, is widely grown alongside the riverbank or wilderness fields (Koay, et al., 2013). Traditionally, the fresh leaves of Black Face General are chewed and ingested or boiled in water and consumed as herbal tea (Bakar, et al., 2006; Samuel, et al., 2010). The compress of fresh leaves is also applied externally to the wound or snake bites (Koay, et al., 2013). The local population has been using this herb as antidiabetic, antilytic, diuretic, anti-constipation, and wound healing treatment (Fadzelly, Asmah & Fauziah, 2006). In recent research, the Black Face General has scientifically proven to offer several beneficial properties, including, antioxidant, antibacterial, antiulcerogenic, anti-diabetic, wound healing, and anticancer due to various phytochemicals present (Nurraihana & Norfarizan-Hanoon., 2013; Ng, et al., 2021). On the other hand, the demand for the herbal-related market in Malaysia is predicted to develop at a rate of 15 to 20% annually and account for 40 to 100 billion USD (Tan, et al., 2020). Hence, in response to this market potential, incorporating medicinal plants into related food products that accompany beneficial effects can be developed.

Noodle is one of the traditional staple foods comprised primarily of wheat flour, water, and salt. It contributes to approximately 20-50% of the total wheat flour consumption across countries. Among these countries, 30% of Malaysia's wheat flour was used in the manufacture of noodles (Hou, 2011). Wheat noodles can be classified into two types which are white salted and yellow alkaline (Hou, 2020). Noodles are one of the fast-growing industries in Asian countries due to the affordable pricing, ease of preparation, unique taste, and texture (Cato & Li, 2000). Nevertheless, conventional noodles lack critical

nutritious components such as dietary fiber, vitamins (particularly vitamin B), and minerals since these nutrients were lost during the wheat flour refining process (Pakhare, Dagadkhair & Udachan, 2018). To date, there was limited study reviews on the *S. crispus* related product except for herbal tea and cookie (Nurraihana & Norfarizan-Hanoon., 2013; Wirawan & Yan, 2021). Consequently, there is a current interest in the inclusion of Black Face General into wheat noodles to improve nutritional values, notably dietary fiber, and health benefits such as antioxidants. The objectives of this project were:

i. To examine the nutritional values of noodles incorporated with different concentrations (3,5,10%) of Black Face General through proximate analysis.
ii. To determine the antioxidant activities of noodles incorporated with Black Face General at different concentrations.
iii. To investigate the physicochemical properties including texture and color of different concentrations Black Face General noodle.

CHAPTER 2

LITERATURE REVIEW

2.1 Wheat Noodles

Wheat noodles can be categorized into two types: white salted and yellow alkaline. Both types of wheat noodles are made up of wheat flour (100 parts), water (28-32 parts), salt (1-2 parts), and egg (optional) but yellow alkaline noodles with the addition of 1-part alkaline salts (Na_2CO_3 or K_2CO_3). The processing steps are similar for both noodles. The white salted noodle is also available in Chinese and Japanese styles. The distinctions between the Chinese style and the Japanese style are outlined in the following table (Hou, 2020):

Table 2.1: Distinctions between Chinese and Japanese style white salted noodles

	Chinese style	**Japanese style**
Types of wheat flour	Hard flour with medium to high protein	Semi-hard flour with low protein, high starch
Texture	Firmer	Softer
Differentiation of noodle type	Based on the processing methods	Based on the width of noodle strand

Table 2.1(Continued): Distinctions between Chinese and Japanese style white salted noodles

	Chinese style	**Japanese style**
Examples	Fresh raw, air dried, frozen, par-boiled (70-80% complete cooked), and instant (deep fried)	So-men (1.0-1.2 mm), Hiya-mugi (1.3-1.7 mm), Udon (2.0-3.9 mm), Hira-men (5.0-7.5 mm)

(Hou & Kruk, 1998; Hou, 2020; Fu, 2008)

2.1.1 Noodle Quality

Appearance and texture are the major criteria to assess for a high-quality noodle. The traditional wheat noodles are often white to cream-white or pale yellow to strong yellow in color with a bright appearance and smooth surface (Hou, 2010). Moreover, the texture should be firm, chewy, non-sticky, and have high tensile strength (Ahmed, Qazi & Jamal, 2015). According to Xu and co-researchers (2020), the physicochemical properties of white salted wheat noodles include 15.23 N of hardness, 9.02 N of chewiness, 0.24 N of tensile strength, and the L*, a*, and b* values were 83.78, −0.3, and 19.13 respectively.

2.1.2 Gluten Network

Gluten has played a crucial role in supporting the end-product quality of noodles by providing the desired firm and elastic characteristics. Gluten is a substance composed of monomeric gliadins and polymeric glutenin protein (Day, 2011). This substance is considered a source of protein in noodles because it accounts for 80-85% of total wheat flour protein (Ooms and Delcour,

2019). The 3D gluten network is produced after the addition of water, and the intermolecular disulphide bonds, iso-peptide bonds, dityrosine linkages, and hydrogen bonds are formed and extensively cross-linked across the polymers (Lucas, Becker & Jekle, 2018). During the kneading phase, the gluten network is further developed as the interaction between polymers increases and the linkages are strengthened (Cappelli, Bettaccini & Cini, 2020). Gliadin and glutenin are almost water-insoluble but soluble in alcohol. The tensile strength of gluten comes from the gliadin fraction, while glutenin is accountable for the elasticity (Slukova, et al., 2017). Hence, these viscoelastic properties of gluten are responsible for the unique mouthfeel of resistance breaking during the bite and partially retaining its original shape (Mioduszewski & Cieplak, 2021).

Moreover, the gluten network can be strengthened by the addition of a small amount of salt. The step can decrease the electrostatic repulsive force and thus encourage agglomeration between glutenin and gliadin by shielding the charges on them (Wellner, et al., 2003; Tuhumury, Small & Day, 2014). In addition, the varieties of wheat flour can influence the formation of gluten matrix. A hard wheat flour consisting 10-14% of protein can produce a stronger gluten matrix, whereas a soft wheat flour consisting of 8-10% protein generates a weaker gluten matrix (Issarny et al., 2017).

2.2 Black Face General (*S. crispus*)

Black Face General (*Strobilanthes crispus*) is a species belonging to the genus Strobilanthes, which is part of the family Acanthaceae, the order Lamiales, the class Magnoliopsida, the division Magnoliophyta, and the kingdom Plantae

(MyBIS, 2022). It is a woody shrub that can reach heights of up to one meter and thrives at elevations ranging from 50 to 1200 meters above sea level (Ramadhani, et al., 2021). The stem of Black Face General is greenish-grey in color; the leaf shape varies from oblong, elliptic to lanceolate and has a slightly crenate edge with both rough surfaces encased in short hairs; and it has a yellow flower with five funnel-shaped corolla petals. (Nurraihana & Norfarizan-Hanoon., 2013; Ramadhani, et al., 2021). The plant has a slightly bitter flavour because of the presence of a high amount of cystoliths composed of calcium carbonate and alkaline infusion (Ng, et al., 2021).

Figure 2.1: Fresh, elliptic-shaped *S. crispus* leaves.

Figure 2.2: *S. crispus* shrub beside pathway in Block D at Universiti Tunku Abdul Rahman (Kampar).

2.2.1 Health benefits

Black Face General is a traditional folk herb that compromises various therapeutic properties, notably antimicrobial, antioxidant, anticancer, and antiaging (Asmah & Fauziah, 2006). Black Face General as an anticancer medication is the most widespread among these. Table 2.2 summarizes studies that supported health benefits. Moreover, this herb is not recommended for pregnant women (Medicinal Herb Info, 2022),

Table 2.2: A summary of the findings supporting the health benefits of *S.crispus*.

Health benefits	**Type of study**	**Evaluated against**	**Findings**	**References**
Antioxidant	*In vitro*	DPPH and FRAP	Mature leaves exhibit greater antioxidant activity than young leaves due to bioactive compound accumulation.	Mohd Fadzelly, et al., 2006.
Antioxidant	*In vitro*	DPPH and FRAP	DPPH (73.8 %), and FRAP (267.5 μM of Fe (II)/g) with a half-maximal inhibitory concentration (IC50) of 44.1 μg/mL	Ghasemzadeh, Jaafar & Rahmat, 2015.
Antioxidant	*In vivo* animal study	Male Sprague Dawley rats	The treatment with *S. crispus* inhibited Fe-NTA- and H^2O^2-induced lipid peroxidation by 45–53%, and also shown an 18–30% dose-dependent reduction of DNA damage.	Mohammad Iqbal, et al., 2010.
Antihyperglycemic effect	*In vivo* animal study	Sprague dawley rats	The result revealed that *S. crispus* tea significantly decreased blood glucose levels compared to control rats.	Mohd Fadzelly, Asmah & Fauziah, 2006.

Table 2.2 (Continued): A summary of the findings supporting the health benefits of *S.crispus*.

Health benefits	**Type of study**	**Evaluated on**	**Results**	**References**
Antimicrobial	*In vitro*	*Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium, and Escherichia coli*	Strong inhibitory effect against Gram-positive bacteria (7.8-125.0 µg/mL), and moderate against the Gram-negative bacteria (31.0-250.0 µg/mL)	Koay, et al., 2013.
Antimicrobial	*In vitro*	*Staphylococcus aureus* and *Escherichia coli*	Maximum diameter inhibition zones of 5.75 mm and 5.25 mm for *S. aureus* and *E. coli*, respectively, indicating that a concentration of 100% extracts inhibits the development of these bacteria.	Adibi, et al., 2017.

Table 2.2 (Continued): A summary of the findings supporting the health benefits of *S.crispus*.

Health benefits	Type of study	Evaluated on	Results	References
Wound healing	*In vivo* animal study	Male Sprague Dawley rats	Using leaf extract accelerated dermal wound healing compared to a placebo-controlled treatment, resulting in a smaller scar width after wound closure and a wound that included less inflammatory cells and more collagen with angiogenesis.	Al-Henhena, et al., 2011.
Anticancer	*In vitro*	HeLa (cervical cancer)	With 50% inhibition at 182.5 μg/mL against the HeLa cancer cell line, the leaf extracts demonstrated promising anticancer activity.	Ghasemzadeh, Jaafar & Rahmat, 2015.
Anticancer	*In vitro*	breast and prostate cancer cell lines	The dichloromethane extract induced a killing effect on breast and prostate cancer cell lines with low EC_{50} via apoptosis.	Yaacob, et al., 2010.

Table 2.2 (Continued): A summary of the findings supporting the health benefits of *S.crispus*.

Health benefits	Type of study	Evaluated on	Results	References
Anti-ulcerogenic	*In vivo* animal study	Mucosal injured Sprague Dawley rats	The leaf extract significantly increases gastric mucus production and gastric fluid pH accompanied by a reduction in gastric lesion development. The stomach was protected more effectively at 1000 mg/kg.	Mahmood, et al., 2011.

2.2.2 Toxicity

To date, there were no toxic side effects found from the consumption of *S. crispus* products based on animal studies. However, there are lacking of the clinical research for the toxicity associated with long-term use of *S. crispus* (Nurraihana & Norfarizan-Hanoon., 2013). According to In vivo animal study of Al-Henhena, et al (2015), the oral intake of 2500 mg/kg of *S. crispus* extract to rats did not induce any toxicity. During the 14 days of observation, neither mortality nor obvious signs of hepatotoxic and nephrotoxic consequences were seen. There are no significant variations in blood parameters. In another study, the dose of *S. crispus* was maximized to 4900 mg/kg of rat body weight, and no substantial toxicity was found with unaltered levels of creatinine, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, and albumin (Norfarizan-Hanoon, et al., 2012). Hence, the *S. crispus* is considered safe to consumed at 4.9 g/kg.

2.2.3 Phytochemicals

Phytochemicals are non-nutritive and bioactive plant metabolites associated with pharmaceutical activities which are crucial to scavenge ROS or free radicals when oxidative stress has been exerted (Liu, 2013). Carotenoids, alkaloids, phytosterols, and polyphenols (including phenolic acid and flavonoids) are different types of phytochemicals (Huang, et al., 2016). Numerous studies have revealed that various phytochemicals have been identified in the leaves of *S. crispus* as indicated in Table 2.3.

Table 2.3: Phytochemicals identified from the leaves of *S. crispus*.

Phytochemicals	Examples	References
Phenolic	- caffeic acid, ferulic acid, cinnamic acid, gentisic acid, p-caumeric acid, p-hydroxybenzoic acid chlorogenic acid, vanilic acid, syringic and gallic acid - tannin	Ghasemzadeh, Jaafar & Rahmat, 2015.
Flavonoids	- quercetin, rutin, myricetin kaempferol, (+)-catechin, (−)-epicatechin, luteolin, naringenin, and apigenin	Ghasemzadeh, Jaafar & Rahmat, 2015; Liza, et al., 2010.
Phytosterols	- α-sitosterol, campesterol, and stigmasterol	Muslim, et al., 2010.
Vitamins	- ascorbic acid (C), riboflavin (B2), thiamin (B1)	Ismail, et al., 2000.
Essential oils	- main components: phytol, α-cadinol, taumurolol, ledol, and eugenol	Asmah, et al., 2006.

According to a study by Ghasemzadeh, Jaafar & Rahmat (2015), caffeic acid (2.95 mg/g DM), ferulic acid (1.76 mg/g DM), and gallic acid (1.45 mg/g DM) are the most abundant phenolic acids, whereas quercetin (1.95 mg/g DM), rutin (1.48 mg/g DM), and catechin (1.12 mg/g DM) are the most prevalent flavonoids in *S. crispus*.

2.2.4 Antioxidant Properties

Antioxidants, as the name implies, defend against oxidative stress on a physiological and biological level. It is able to prevent or detect the propagation of an oxidative chain by stabilizing the generated radical, thereby reducing oxidative damage. The most abundant bioactive phytochemicals that serve as key antioxidants are phenolic compounds. Typically, the phenolic compound act as a hydrogen donor to inhibit free radicals from its hydroxyl group or chelate metal ions (Santos-Sanchez, et al., 2019).

2.2.4.1 Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the abundant aglycone-type flavonoid glycosides found in *S. crispus*, which the human body is incapable of synthesizing the substance (Anand David, Arulmoli & Parasuraman, 2016). It possesses 5 hydroxy groups, a catechol group on its aromatic ring (B), and a 5-OH group on the aromatic ring (A) to form an intramolecular hydrogen linkage with the C-4 carbonyl group. Quercetin can prevent low-density lipoproteins oxidation through the scavenge free radicals and chelate transition metal ions abilities (Kato, et al., 2006).

2.2.4.2 Caffeic Acid

Caffeic acid (3,4-dihydroxycinnamic acid) is phenolic acid that synthesized via the secondary metabolism of plants. It has the structure of phenylpropanoid bearing a 3,4-dihydroxylated aromatic ring to a carboxylic acid through a trans-ethylene link (Espindola, et al., 2019). The antioxidant and prooxidant

activities of caffeic acid are attributed to a combination of radical scavenging and prevention of lipid peroxidation (Khan, Maalik & Murtaza, 2016).

2.2.4.3 Mechanisms Action of Antioxidants

Antioxidants are able to inhibit oxidation by neutralizing free radicals through different mechanisms including:

i. Hydrogen atom transfer (HAT)
ii. Single electron transfer mechanism (SET)

HAT mechanism is the coordinated movement of a proton and an electron in a single kinetic step, in which a free radical removes one hydrogen atom from the antioxidant, transforming the antioxidant into a radical (Liang & Kitts, 2014). For example, caffeic acid acts as both metal chelator and hydrogen donor to donate a hydrogen atom to hydroperoxide which hinders the lipid peroxidation chain reaction and results in a reduction of lipid-derived peroxyl and alkoxyl radicals (Genaro-Mattos, et al., 2015). The SET reaction is emulated by the transfer of a single electron from the nucleophile to the substrate, resulting in the formation of a radical intermediate whose fate can be determined by a variety of subsequent reactions (Liang & Kitts, 2014). For instance, quercetin contribute a proton and merge with the radical itself, the unpaired electron is delocalized by resonance, making the quercetin radical low in energy to prevent it from oxidizing other macromolecules (Bentz, 2017).

2.3 Proximate Analysis

Wheat noodles are belonging to high moisture food which made up approximately 28-38% of moisture content while increasing to 60-70% after cooking (Santiago, et al., 2016; Obadi, et al., 2022). Noodles have moderate carbohydrates and protein content, but however low in dietary fiber, vitamins, and minerals (Litaay, et al., 2022). Black Face General leaves are high in crude fiber and mineral content. For instance, a study by Isrianto, Kristianto & Wilujeng (2021) identified 15 types of macro and micro minerals, namely silicon, phosphorus, sulfur, potassium, calcium, titanium, manganese, iron, nickel, copper, zinc, strontium, molybdenum, barium and strontium. Thus, the fiber and ash content of noodle is expected to increase after incorporation of Black Face General. The proximate composition of Black Face General leaves is determined by Ismail and co-researchers (2000), as 69.3% of moisture, 21.6% of ash, 13.3% of protein, 4.3% of carbohydrate and 13.9% of crude fiber.

2.4 Antioxidant Assays

2.4.1 Total Phenolic Content (TPC) Assay

The total phenolic contents in foods were measured using the colorimetric Folin–Ciocalteu (FC) reagent, a mixture of phosphomolybdic acid and phosphotungstic acid (Cao, et al., 2020). The FC reagents will react and generate a blue chromophore complex when the phenolic compounds contribute electron in an alkaline solution (Blainski, Lopes & Mello, 2013). Using a UV-visible spectrophotometer, the intensity of the chromophore complex is determined at 760 nm based on the quantity of phenolic chemicals (Dai & Mumper, 2010).

2.4.2 2,2-Diphenyl-2-picrylhydrazyl (DPPH) Assay

2,2-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that persists steadily in organic solvents since it is unable to undergo dimerization (Gulcin, et al., 2008). The delocalization of spare electrons produces an intense violet color in ethanol solvent and has the maximum absorption at the wavelength of 517 nm using a spectrophotometer at room temperature (Kedare & singh, 2011). When the DPPH radical solution reacts with free radical scavengers that donate hydrogen, resulting discoloration of violet color to pale yellow due to the reduction of the DPPH solution (Gulcin, 2011). This assay is a popular method to evaluate the radical scavenging activity due to its simplicity and rapid process based on an electron transfer reaction, and hydrogen-atom abstraction (Liang & Kitts, 2014). The DPPH antioxidant activity is generally expressed as half maximal scavenging concentration (IC_{50}), which defined as the concentration of antioxidants required to obtain a 50% radical scavenging activity (Rivero-Cruz, et al., 2020).

2.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric Reducing Antioxidant Power (FRAP) assay is a non-specific, oxidation and reduction linked, colorimetric assay that analyzes the ability of antioxidants to reduce the ligand complex ferric ion (Fe^{3}-TPTZ) to a vivid blue color of ferrous ions (Fe^{2+}-TPTZ) (Liang & Kitts, 2014). In order to maintain the iron solubility, a low pH 3.6 buffer is required to reduce the ionization potential, and increase redox potential. The ferric reducing activity is quantified spectroscopically at the wavelength of 593 nm (Gulcin, 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The leaves of the plant *S. crispus* were collected beside a pathway in Block D at Universiti Tunku Abdul Rahman (UTAR), Kampar, with the assistance of Dr. Tee Chong Siang from the Department of Biological Science. The mature leaves from the middle part of the plant were desired. The wheat flour and salt were bought from Econsave at Kampar, Malaysia. Moreover, 2, 2-diphenyl1-picrylhydrazyl (DPPH) powder was purchased from Sisco Research Laboratories Pvt. Ltd., India. Other analytical-grade solvents and chemicals included methanol, Folin-Ciocalteau's phenol reagent, gallic acid, L-ascorbic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ferrous sulphate, ferric chloride hexahydrate, anhydrous sodium acetate, glacial acetic acid were provided by Department of Agricultural and Food Science, UTAR.

3.2 Overview of Methodology

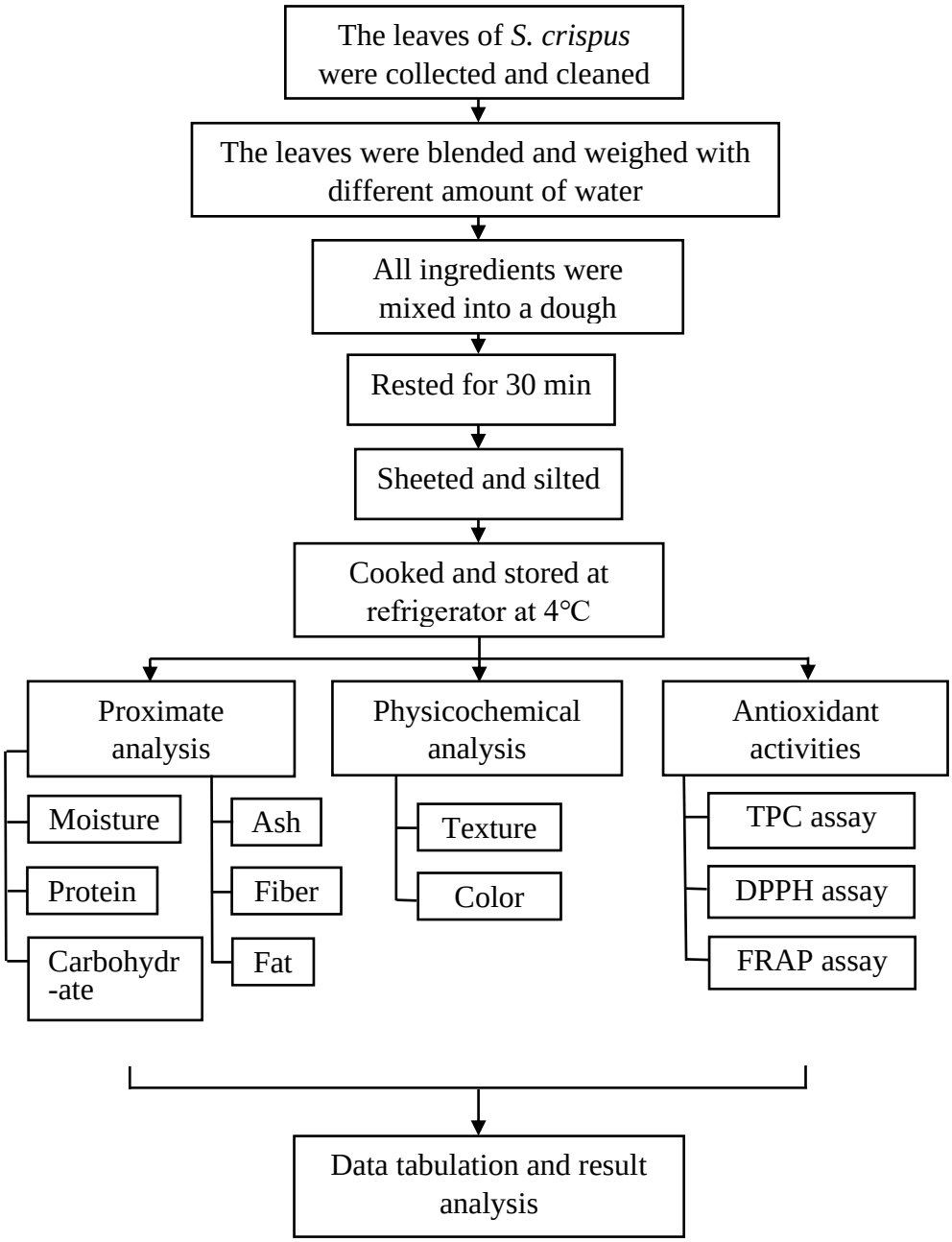

Figure 3.1: Overview of methodology in this project.

3.3 Collection and Preparation of *S. crispus* Leaves

The fresh leaves of *S. crispus* were collected and cleaned thoroughly with tap water to remove the debris. The water on the leaves was wiped away before usage. Different concentration of herb incorporation was prepared by blending different amount of leaves with different amount of water as shown in Table 3.1 using a blender.

3.4 Wheat Noodle Making

One hundred grams of all-purpose wheat flour, 48g of water, and 2 g of salt were weighed and mixed together to produce a consistent control sample dough. The dough was covered with plastic wrap and rested for 30 minutes at room temperature. After that, the dough was sheeted multiple times with a noodle-making machine until a sheet thickness of 1 mm was attained. The dough sheet was then silted into 6 mm width noodle strands using the cutting roller attached to the machine. The noodles were cooked in boiling water (1500 mL) for 7 minutes and rinsed with cold water for 30 seconds (Sui, Lucas & Corke., 2006). The cooked noodles were allowed to drain excess water prior to being placed in a separate plastic bag. Finally, the packed noodles were stored in refrigerator at 4 ℃. Different concentrations of *S. crispus* (3, 5, and 10 %) were incorporated into the dough by adjusting the weight of ingredients as shown in Table 3.1 and then using the same technique for *S. crispus* (SC) noodle manufacture as described previously.

Table 3.1: The ingredients and formulations of control and noodle samples incorporated with 3, 5, and 10% of *S. crispus.*

Ingredients	**Concentrations of *S. crispus***			
	Control	**3%**	**5%**	**10%**
All-purpose flour (g)	100	97	95	90
Water (g)	48	46.5	45.5	43
Salt (g)	2	2	2	2
***S. crispus* leaves (g)**	0	4.5	7.5	15
Total weight of dough (g)	150	150	150	150

3.5 Proximate Analysis

The Association of Official Analytical Chemists (AOAC, 1990) methods were adopted to obtain the nutritional values of cooked *S. crispus* noodles including protein, fat, moisture, ash, crude fiber and carbohydrates. There were four cooked noodle samples (0, 5, 10 and 15%) subjected to this proximate analysis.

3.5.1 Moisture Content

The moisture content was determined by the loss on drying method, which utilizes a drying oven (Binder GmbH, Germany). The empty crucibles with lids were pre-oven dried at 105 ℃ for 3 hours, then transferred to a desiccator to allow cold. The empty crucibles with lids were weighed as W_1. Five grams (W_i) of each sample were weighed and distributed evenly over each crucible in a triplicate manner. The crucibles with samples were dried in the oven at 105 ℃ for at least 7 hours. The sample-filled crucibles were then transferred to a desiccator to allow cooling. After cooling, the crucibles with dried samples

were weighed (W_2). The following equation was used to compute the percentage of moisture content.

$$M\ (\%) = \frac{W_i - (W_2 - W_1)}{W_i} \times 100$$

Where,

M (%) = Percentage of the moisture content

W_i = Initial weight of sample, g

W_1 = The weight of empty crucible with lid, g

W_2 = The weight of crucible with lid and dried sample, g

3.5.2 Ash Content

The crucibles with lids were placed in a muffle furnace (Naberthem) at 550 ℃ for at least 8 hours to remove the impurities on the crucible surface. After 30 minutes of cooling in a desiccator, the crucibles with lids were then weighed (W_1). About 2 g (W_i) of each sample was weighed inside the crucible. Hot plates were used to slowly heat crucibles containing samples without lids to liberate organic matter. Once the smoke stop producing, the crucibles and lids were placed aside in the furnace at 550 ℃ for 8 hours. After incineration, the crucibles were covered with lids to avoid loss of ash. The crucibles were cool down in a desiccator. The weight of the crucible together with ash and lid was recorded as W_2. The sample was required to re-incinerate in the furnace for further ashing if the sample failed to turn grey. The percentage of ash content was calculated using the equation below.

$$A(\%) = \frac{(W_2 - W_1)}{W_i} \times 100$$

Where,

A (%) = Percentage of ash content

W_i = Initial weight of sample, g

W_1 = The weight of empty crucible with lid, g

W_2 = The weight of crucible with lid and ash, g

3.5.3 Fat Content

The fat determination was conducted by using the Soxhlet method through a Fat Analyzer (Gerhardt). The extraction beakers with 3 pieces of boiling stones were pre-heated in the drying oven at 105 ℃ for 1 hour. The extraction beakers were then cooled down in a desiccator to ensure all readings are consistent. The weight of extraction beakers with boiling stones was measured and recorded (M_1). Five grams of each dried powdered sample (M_0) were wrapped with filter paper and inserted into the bottom of the extraction thimble. A piece of cotton wool was placed on the upper part of sample. The thimbles together with thimble holders were inserted into each beaker respectively. In the fume hood, roughly 90 mL of petroleum ether was introduced to the extraction beakers and then connected to the Fat Analyzer. SOXTHERM Manager control software was operate to perform fat extraction. After extraction, the extraction beakers with the removal of the thimble were heated at 105 ℃ for 1 hour in the drying oven. The extraction beakers were then cooled for 1 hour in a desiccator. The

weight of the extraction beakers was recorded as M_2. The percentage of fat content was calculated using following equation.

$$F(\%) = \frac{M_2 - M_1}{M_0} \times 100$$

Where,

F (%) = Percentage of fat content

M_0 = Weight of dried sample, g

M_1 = Weight of extraction beaker with boiling stone before extraction, g

M_2 = Weight of extraction beaker with boiling stone after extraction, g

3.5.4 Protein Content

The protein determination was carried out by using the Kjeldahl method through a Kjeldahl system including speed digester K-436 and distillation unit K-350 (Buchi). A Kjeldahl catalyst was pre-prepared by mixing 7 g of potassium sulphate (K_2SO_4) with 0.8 g of copper sulphate ($CuSO_4$). Two grams of dried powdered sample were weighed and inserted into a digestion tube. Five grams of Kjeldahl catalyst and 20 mL of concentrated sulfuric acid (98% H_2SO_4) were added to the digestion tube subsequently in the fume hood. The tubes were inserted back into the rack of the digester unit to perform the digestion process until the solution became clear. After digestion ended, the digested solution was set for 1 h to cool down prior to distillation process. The tubes were installed within the distillation unit. A few drops of colour indicator (20 mg of Methyl red and 100 mg of Bromocresol green in 100 mL of 95%

ethanol) and 30 mL of 4% boric acid were added to a conical flask to form a pink solution. The conical flask was attached to the receiving vessel compartment of distillation unit. An appropriate amount of distilled water (in the ratio of 2:1 to acid) and sodium hydroxide (NaOH) (in the ratio of 3:1 to acid) was added. The process of distillation was done when the pink color solution turned green. The conical flask containing boric acid mixture was titrated with 0.25 M Sulfuric acid (H_2SO_4) until reached the end point where the green color mixture turned pink. The amount of H_2SO_4 used was recorded. Using the following equation, the percentage of protein was calculated.

$$\mathrm{P}(\%) = \frac{(V_s - V_b) \times N \times 14.007 \times 5.70 \times 100}{W \times 1000}$$

Where,

P (%) = Percentage of protein content

V_S = Volume of H_2SO_4 used in sample titration, mL

V_b = Volume of H_2SO_4 used in blank titration, mL

N = Normality of acid

W = Weight of sample, g

14.007 = Molecular mass of nitrogen, g/mol

5.70 = Nitrogen-to-protein conversion factor of wheat product

3.5.5 Crude Fiber Content

Gravimetric method was employed to determine the crude fiber content using a crude fiber analyzer (Gerhardt). The empty fiber bags were pre-dried in a drying oven at 105℃ for 2 h. The fiber bags were transferred to a desiccator to allow cooling and the fiber bag for sample was weighed as M_1 while another

fiber bag for blank was weighed as B_1. Around 1g of dried powdered sample (M_2) was added into the fiber bag except for the blank. After that, the fiber bag was fitted with a glass spacer and then loaded onto carousel in a beaker. De-fatting preliminary step was unnecessary since the fat content was less than 10%. The hotplate of the instrument was preheated for 5 minutes. To digest fiber, about 360 mL of 0.13 M H_2SO_4 was added into the beaker and thoroughly mixed with the samples by rotating the carousel for one minute. The samples were heated to a gentle simmer after being brought to a boil for 3-5 minutes. The carousel with samples was removed after 30 minutes and rinsed three times in hot distilled water to remove excess acid. Likewise, the samples were boiled slowly in 360 mL of 0.31 M NaOH for 30 minutes. The alkali residue was removed by rinsing three times of the carousel together with samples in hot distilled water. After that, the fiber bags were inserted into the pre-incinerate crucibles and oven dried at 105℃ for 4 hours. The crucibles with sample fiber bags were then placed in a desiccator until cooled and weighed as M_3, while the blank fiber bag with crucible was weighed as B2. The crucibles were incinerated in a muffle furnace at 550℃ overnight. The crucibles containing ash-filled fiber bags were allowed cool in a desiccator before weighing and recorded as M_4. The crucible with blank fiber bag ash was weighed as B_3. Percentage of crude fiber was calculated using the equation below (Madhu, et al., 2017).

$$\mathrm{CF}(\%) = \frac{(\mathrm{M_3} - \mathrm{M_1} - \mathrm{M_4}) - (\mathrm{B_2} - \mathrm{B_1} - \mathrm{B_3})}{\mathrm{M_2}} \times 100$$

Where,

CF (%) = Percentage of crude fiber

M_1 = Weight of empty fiber bag, g

M_2 = Weight of dried sample, g

M_3 = Weight of crucible with fiber bag (dried), g

M_4 = Weight of crucible with fiber bag (ash), g

B_1 = Weight of blank fiber bag, g

B_2 = Weight of blank fiber bag with crucible (dried), g

B_3 = Weight of blank fiber bag with crucible (ash), g

3.5.6 Carbohydrate Content

The carbohydrate content (C%) was calculated by difference method using the equation below (FAO, 2003).

$$C(\%) = 100\% - (\%\ \text{of moisture} + \text{ash} + \text{fat} + \text{protein})$$

3.6 Noodles Extraction

The noodle was extracted according to the method described by Syahirah and Rabeta (2019) with slight modifications. The fresh noodles were oven dried at 60℃ for 2 days and ground into powder form. One gram of dried noodle sample with 100 mL of 80% methanol in a conical flask was sealed with parafilm. The mixture was agitated overnight (300 rpm, 29℃) using a shaking incubator (Infors, Switzerland). The sample was centrifuged (2500 rpm/min, 4℃) for 20 minutes using Hettich Universal 320R refrigerated centrifuge. The filtered supernatant with Whatman filter paper was collected and subjected to a

rotary evaporator (40℃) to remove methanol. The crude extract was stored in the dark at 4℃ until future analysis.

3.7 Antioxidant Assay

3.6.1 Preparation of Samples for Antioxidant Test

One milliliter of each crude extract was dispensed into a pre-dried crucible respectively and the weight of the empty crucible was recorded beforehand. The crucibles were proceeded to oven drying at 105℃ overnight. The concentration of crude extract in terms of mg/mL was then measured and calculated.

3.6.2 Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of each noodle extract was assessed using a spectrophotometric assay based on the Folin-Ciocalteu reagent described by Chai and Wong (2012).

3.6.2.1 Preparation of Sodium Carbonate Solution (20%, w/v)

Sodium Carbonate (Na_2CO_3) weighing 20 g was dissolved with 100 mL of distilled water and the mixture was kept at room temperature.

3.6.2.2 Preparation of Gallic Acid Standard

In this analysis, gallic acid was chosen and served as the standard reference. A stock solution with a concentration of 100 mg/L was prepared by dissolving 10 mg of gallic acid in 100 mL of distilled water. The gallic acid solution was stored in a dark Scott bottle to prevent light exposure. Using distilled water,

serial dilutions from the stock were carried out until different concentrations (10-90 mg/L) were obtained.

3.6.2.3 Protocol

For TPC assay, each sample extract was diluted down to 1.0 mg/mL with distilled water. Next, 200 μL of extract was mixed with 800 μL of distilled water and 100 μL of Folin-Ciocalteu reagent. The mixture was initially incubated for 3 minutes at dark and room temperature. Then, 300 μL of 20% sodium carbonate was added. The mixture was vortexed and incubate in dark for 2 hours. The mixture absorbance was read at a wavelength of 765 nm using a UV-vis spectrophotometer (Thermo Scientific, US). A blank control was proceeded by substituting 200 μL of the extract with distilled water. For gallic acid reference, same procedures were applied to the gallic acid ranging from 0 to 100 mg/mL. A standard curve was plotted using absorbance against the concentration of gallic acid and the result was expressed as mg of gallic acid equivalent per 1 mL extract (mg GAE/mL extract).

3.6.3 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH radical scavenging activity (RSA) and IC_{50} of each noodle extract was determined according to the protocol outline by Ng, et al (2022).

3.6.3.1 Preparation of 0.15 mM DPPH solution

The 0.15 mM DPPH solution was freshly prepared by dissolving 5.91 mg of DPPH powder in 100 mL of 80% methanol. A dark Scott bottle was used to store the solution.

3.6.3.2 Preparation of Ascorbic Acid Standard

Ascorbic acid was selected as the standard reference. A stock solution with a concentration of 500 μM was prepared by dissolving 8.8 mg of ascorbic acid in 100 mL distilled water and further diluted to 100 μM. Using distilled water, serial dilutions from the stock were carried out until different concentrations (50, 40, 30, 20, 10 μM) were obtained.

3.6.3.3 Protocol

Different concentrations of sample extract ranged 250, 500, and 1000 μg/mL were prepared through the serial dilution technique. Five hundred μL different concentrations of extract was treated with 500 μL of 0.15 mM DPPH using a vortex. The mixture was incubated in dark conditions for 30 minutes at room temperature. After that, the absorbance at 517 nm was measured. The sample was substituted with methanol as control while 1000 μL methanol was used as blank. Ascorbic acid reference also followed the same procedures to the concentration ranging from 0 to 50 μM. The radical scavenging activity (%) was calculated using the following equation. To determine the IC_{50} value, a graph of radical scavenging activity against sample or reference standard concentrations was plotted. The IC_{50} value was obtained from the regression line respectively by substituting y equal to 50 into the regression equation (Xiao, et al., 2020).

$$\text{RSA (\%)} = \frac{A_b - A_s}{A_b} \times 100$$

Where,

RSA (%) = Percentage of radical scavenging activity

A_b = Absorbance of blank

A_s = Absorbance of sample or reference

3.6.4 Determination of Ferric Reducing Antioxidant Power (FRAP)

According to the procedure described by Ng, et al (2022), the ferric reducing antioxidant power (FRAP) assay was conducted on each noodle extract.

3.6.4.1 Preparation of sodium acetate buffer (300 mM)

A 182.12 mg of anhydrous sodium acetate was dissolved in 20 mL of distilled water, followed by 1.58 mL of glacial acetic acid. The buffer was done by topping up to a final volume of 100 mL using distilled water.

3.6.4.2 Preparation of $FeCl_3$ solution (20 mM)

Ferric chloride hexahydrate ($FeCl_3$. 6 H_2O) with the amount of 108.12 mg was dissolved in 20 mL of distilled water.

3.6.4.3 Preparation of TPTZ solution (10 mM)

In 20 mL of 40 mM hydrochloride acid, 62.46 mg of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) powder was dissolved.

3.6.4.4 Preparation of FRAP Working Reagent

In a ratio of 10:1:1, 100 mL of sodium acetate buffer was mixed with 10 mL of $FeCl_3$ solution and 10 mL of TPTZ solution. Before the test, the FRAP solution was freshly produced and incubated at 37℃ in water bath.

3.6.4.5 Preparation of Fe_2SO_4 standard

The standard reference for the FRAP assay was ferric sulfate heptahydrate (Fe_2SO_4. 7 H_2O). In order to make a 500 μM stock solution, 13.9 mg of Fe_2SO_4 in 10 mL of distilled water and then diluted to 100 μM. Serial dilution was performed to reach 50, 40, 30, 20, and 10 μM concentrations.

3.6.4.6 Protocol

Each noodle extract was diluted with distilled water to a concentration of 1.0 mg/mL. Next, 200 μL of each extract was mixed with 1.8 mL of FRAP working reagent (37℃). After vortexing, the mixture was incubated for 5 minutes in dark and at room temperature. The absorbance was measured at 593 nm. The extract substituted with distilled water served as blank. Identical procedures were applied to the ferric sulphate standard (0-50 μM). A standard curve was plotted using absorbance against the concentration of ferric sulphate and the result was expressed as mg of ferric sulphate equivalent per 1 mL extract (μM Fe(II)/mL extract).

3.8 Texture Analysis

3.7.1 Hardness Measurement

The TA-XT Plus Texture Analyzer (Stable Micro System, UK) equipped with a cylinder probe (P/35) was calibrated with a 5 kg load cell. Two 6 cm cooked noodle strands were selected and placed side by side on the instrument platform to perform analysis through Exponent software (Nouri, Nafchi & Karim., 2015).

Table 3.2: Instrument setting for hardness measurement

Parameters	**Setting**
Test mode	Compression
Pre-test speed	2.0 mm/s
Test speed	2.0 mm/s
Post-test speed	2.0 mm/s
Strain	75%

3.7.2 Tensile Strength

The tensile strength was assessed using a TA-XT Plus Texture Analyzer (Stable Micro System, UK) outfitted with spaghetti tensile grips (A/SPR) according to Hong, et al. (2021) with minor modification. Calibration of height and weight was done using a 5 kg load cell. One long cooked noodle strand was self-locking tied to the grips before being analyzed by Exponent software.

Table 3.3: Instrument setting for tensile strength measurement**.**

Parameters	Setting
Test mode	Tension
Pre-test speed	1.0 mm/s
Test speed	3.0 mm/s
Post-test speed	10.0 mm/s
Distance	100 mm

3.9 Color Measurement

In this assessment, color was measured by using a CM-600d chromameter (Konica Minolta, Japan) as described previously by Nouri, Nafchi & Karim (2015) with slight modifications. The cooked samples of SC noodles and control noodles were placed onto a small Petri dish. Before carrying out the measurement, zero calibration was completed based on the reflectance of the floor, and white calibration was accomplished with the aid of a calibration cap. The CIE-Lab parameters of each sample, L*, a*, and b* values, were recorded as follows: L* denoted brightness (0 = black, 100 = white), -a* indicated greenness, +a* represented redness, -b* represented blueness, and +b* represented yellowness.

3.10 Statistical Analysis

All results from this project were presented in triplicate manner as mean ± standard deviation (SD). The IBM SPSS statistics software (version 26) was applied in performing one-way analysis of variance (ANOVA) to analyze the data. The means of significant differences between groups of samples at $p < 0.05$ were differentiated using the Tukey's HSD comparison test.

CHAPTER 4

RESULTS

Figure 4.1: The appearance of cooked noodles incorporated with *S.crispus.* (A) control noodle, (B) 3% SC noodle, (C) 5% SC noodle, (D) 10% SC noodle.

4.1 Proximate Analysis

The nutritional values of each noodle sample were summarized in Table 4.1. Only carbohydrate content did not differ significantly ($p>0.05$) among the samples while did differ significantly ($p<0.05$) in moisture, ash, protein, fat, and fiber content. Overall, moisture comprised the highest percentage content of all noodle samples, followed in descending order by protein, carbohydrates, ash, and finally fat.

Table 4.1: Proximate (nutritional) constituents of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%).

	Nutrition composition (%)					
	Moisture	**Ash**	**Fat**	**Protein**	**Crude fiber**	**Carbohy -drate**
Control noodle	71.46 ± 0.29^{D}	0.57 ± 0.01^{C}	0.02 ± 0.00^{C}	17.10 ± 0.64^{A}	0.15 ± 0.05^{C}	10.85 ± 0.71^{A}
3% SC noodle	72.66 ± 0.41^{C}	0.78 ± 0.01^{B}	0.09 ± 0.02^{B}	16.23 ± 0.92^{AB}	0.18 ± 0.06^{BC}	10.23 ± 1.35^{A}
5% SC noodle	73.65 ± 0.25^{B}	0.82 ± 0.01^{B}	0.13 ± 0.01^{AB}	15.24 ± 1.00^{AB}	0.29 ± 0.04^{B}	10.17 ± 1.21^{A}
10% SC noodle	74.76 ± 0.40^{A}	1.17 ± 0.03^{A}	0.17 ± 0.03^{A}	14.17 ± 0.72^{B}	0.48 ± 0.03^{A}	9.73 ± 0.32^{A}

$^{A-D}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

4.1.1 Moisture Content

The moisture content was slightly increased from control (71.46 ± 0.29%) to 15% SC noodles (74.76 ± 0.40%) with significant differences ($p=0.00$) across all samples.

4.1.2 Ash Content

The control and SC noodles were incinerated until greyish ash was formed for each sample as shown in Table 4.2. The ash content was significantly ($p=0.00$) increased from control (0.57 ± 0.01%) to 15% SC noodles (1.17 ± 0.03%) but 3% (0.78 ± 0.01%) and 5% (0.82 ± 0.01%) of SC noodles did not differ significantly from each other.

Table 4.2: Images of ash for control and various SC% noodles.

Control	3% SC noodles	5% SC noodles	10% SC noodles
			
Figure 4.2: Ash of control	**Figure 4.3**: Ash of 3% SC noodle	**Figure 4.4**: Ash of 5% SC noodle	**Figure 4.5**: Ash of 10% SC noodle

4.1.3 Fat Content

There was a statistically significant difference (p=0.00) in fat content between samples, the lowest fat content was in control noodles (0.02 ± 0.00%). The fat content increased when a higher percentage of *S. crispus* was introduced, with the highest fat content in 10% SC noodles (0.17 ± 0.03%). The control noodles, 3%, 10% of SC noodles were significantly different from each other, however, 5% of SC noodles cannot differ from 3 and 10% of SC noodles.

4.1.4 Protein Content

The protein content was the highest for control noodles (17.1 ± 0.64%). There was a slight significant (p=0.013) decreased from control to 15% SC noodles (14.17 ± 0.72%). There was a significantly differ between control and 15% SC noodle but the noodles with 3% (16.23 ± 0.92%) and 5% (15.24 ± 1.00%) of *S. crispus* incorporated did not differ from control or 15% SC noodles.

4.1.5 Crude Fiber Content

The control samples had the lowest crude fiber content (0.15 ± 0.05%) while 15% of SC noodles had the highest crude fiber content (0.48 ± 0.03%). The fiber content increased significantly (p=0.00) with the increased concentrations of *S. crispus* incorporated in noodles. All samples were different except for 3% SC noodles (0.18 ± 0.06%) which cannot differ from the control and 5% SC noodles (0.29 ± 0.04%).

4.1.6 Carbohydrate Content

There were non-significant differences found in carbohydrate content across all samples (p=0.597). The carbohydrate content was decreased as the concentration of SC noodles increased. The control noodles contained the highest (10.85 ± 0.71%) carbohydrates while 15% of SC noodles contained the least (9.73 ± 0.32%)

4.2 Antioxidant Activity

4.2.1 Total Phenolic Content

The total phenolic contents of control and SC noodles samples extracts were determined using a standard curve of gallic acid (Figure 4.5). A satisfactory linear standard curve was obtained with a regression correlation coefficient of R^2 = 0.9991. The equation derived from the standard curve is y= 0.0136x + 0.0152. The TPC was calculated and shown in Table 4.3. TPC was ranged from 1.38 to 1.75 mg GAE per mL extract with significantly different (p= 0.012) among samples. There was no significant difference between the control and 3% SC noodles. The 5% SC noodle did not differ significantly from other noodles.

Table 4.3: Total phenolic content of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%).

Samples extract	**Total Phenolic Content (mg Gallic Acid Equivalent/mL extract)**
Control noodle	1.38 ± 0.15^{B}
3% SC noodle	1.42 ± 0.03^{B}
5% SC noodle	1.58 ± 0.11^{AB}
10% SC noodle	1.75 ± 0.11^{A}

$^{A-B}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

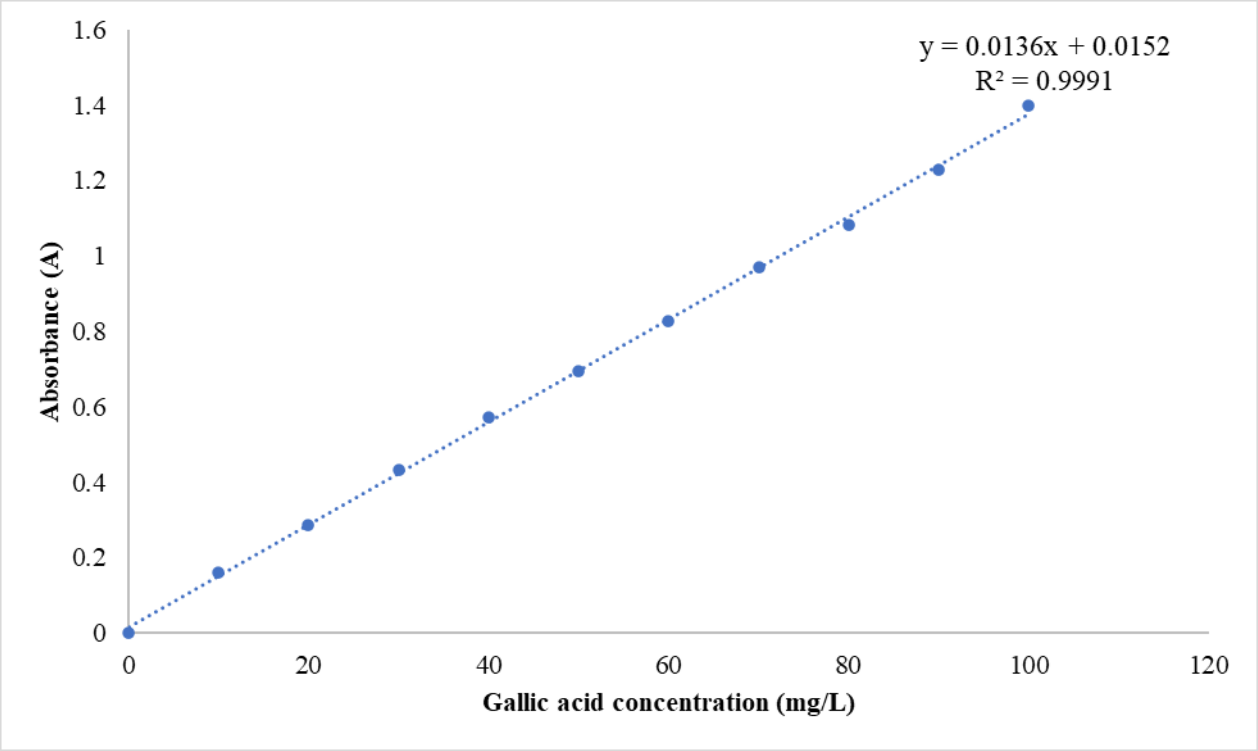

Figure 4.6: Standard curve of absorbance against gallic acid concentration for TPC.

4.2.2 DPPH

The DPPH activities of all samples are shown in terms of radical scavenging activity (RSA) and IC_{50} in Table 4.4. DPPH radical scavenging activity of the control, 3%, and 10% of SC noodles were significantly different (p=0.00) from each other and increased in response to the higher concentrations of *S. crispus*. The concentration of sample required to reach 50% of RSA is estimated as the IC_{50} value. The IC_{50} value was significantly different (p=0.041) between the control noodle and 10% of the SC noodle. In comparison to ascorbic acid standard, the antioxidant activity based on IC_{50} value in descending order was as follow: control noodle > 5% SC noodle > 3% SC noodle > 10% SC noodle > ascorbic acid.

Table 4.4: Radical scavenging activity and IC_{50} of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%) based on DPPH assay.

Samples extracts	**DPPH**	
	RSA (%)	**IC_{50} (µM /mL)**
Control noodle	21.86 ± 2.16^{C}	2676.34 ± 362.95^{A}
3% SC noodle	26.28 ± 0.99^{B}	2258.97 ± 277.11^{AB}
5% SC noodle	29.12 ± 1.68^{AB}	2272.89 ± 121.21^{AB}
10% SC noodle	32.67 ± 1.59^{A}	1958.37 ± 194.58^{B}
Ascorbic acid	-	27.64 ± 0.74

$^{A-C}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

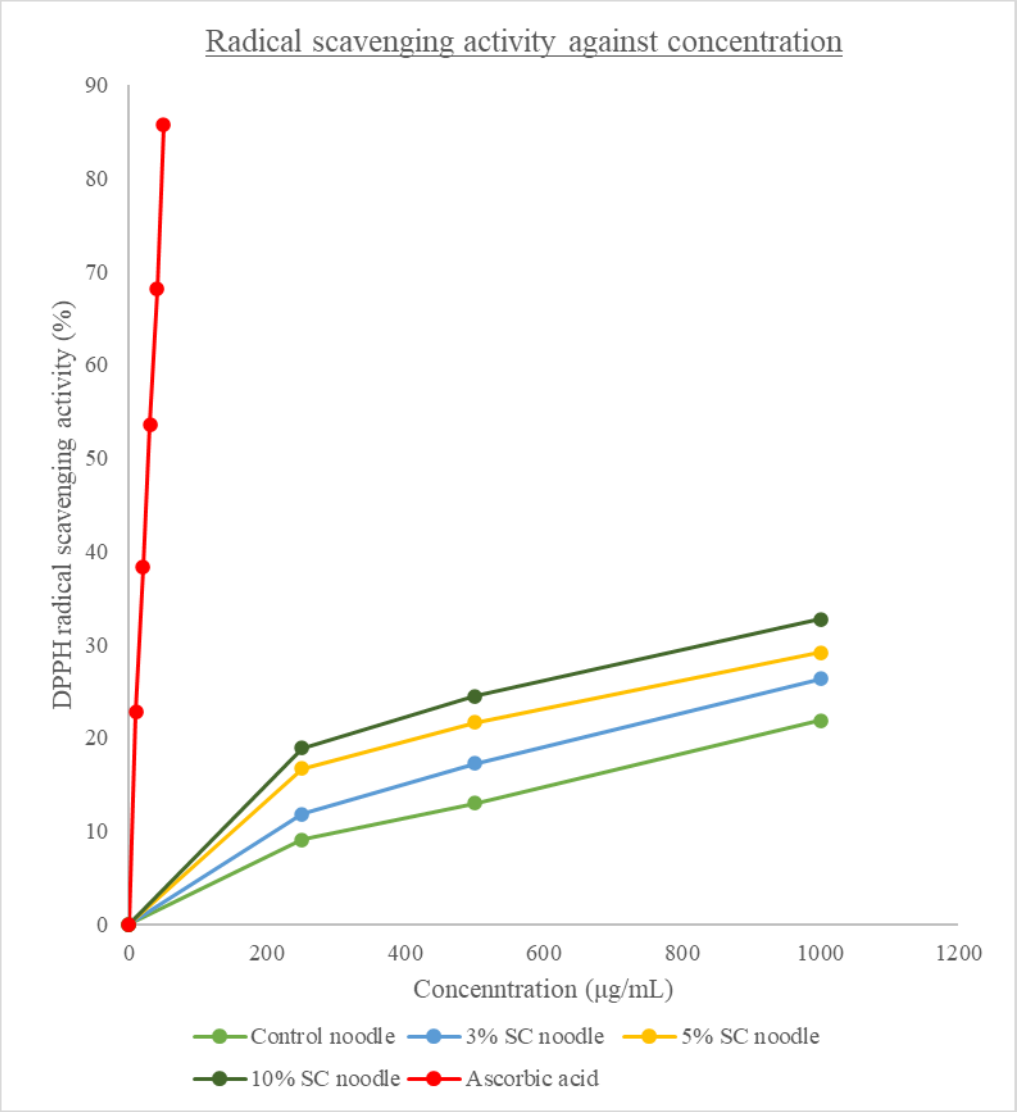

Figure 4.7: DPPH radical scavenging activity of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%) with ascorbic acid as comparison.

4.2.3 FRAP

The FRAP activity of control and SC noodles samples extracts were determined using a standard curve of ferric sulphate (Figure 4.7). A satisfactory linear standard curve was obtained with a regression correlation coefficient value of $R^2 = 0.9994$. The FRAP activity for each sample was calculated based on the equation derived from the standard curve (y= 0.0208x + 0.0089) and presented in Table 4.5. The FRAP activity was significantly increased (p=0.00) with the increase in *S. crispus* concentrations. The control noodle and 3% SC noodles were no significant differences from each other but differ significantly from 5 and 10 % SC noodles.

Table 4.5: The antioxidant activity of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%) based on FRAP assay.

Samples extract	**FRAP (µM Fe(II)/mL)**
Control noodle	60.03 ± 6.4^{C}
3% SC noodle	73.49 ± 8.72^{C}
5% SC noodle	104.58 ± 9.49^{B}
10% SC noodle	141.44 ± 8.22^{A}

$^{A-C}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

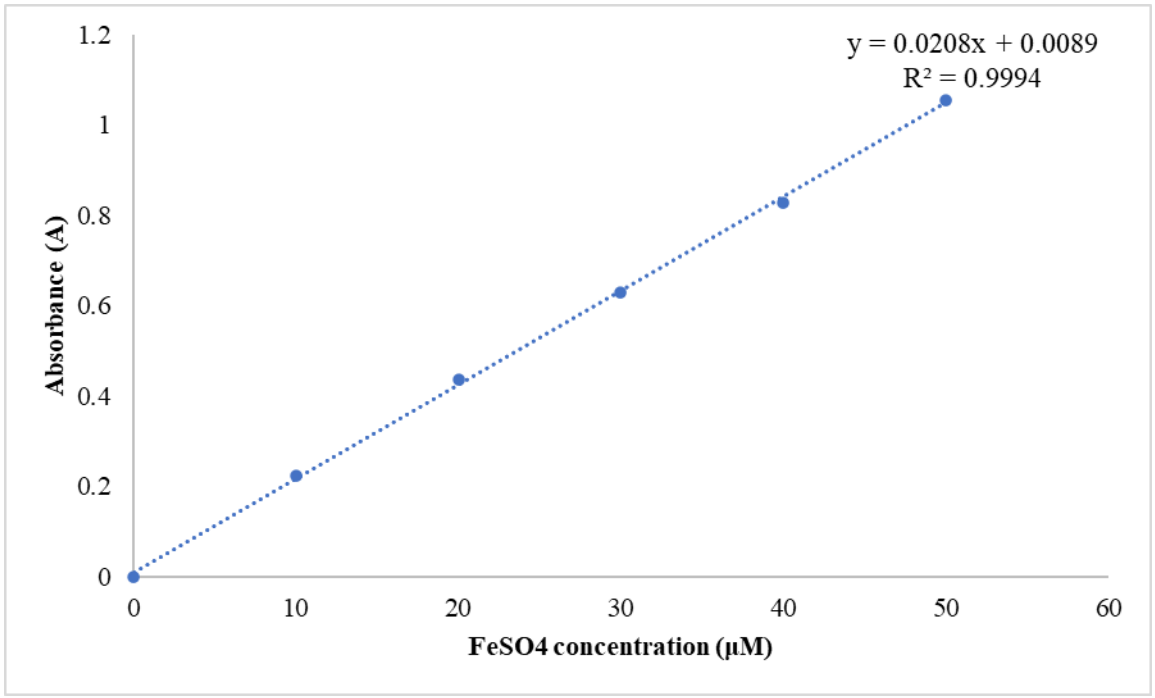

Figure 4.8: Standard curve of absorbance against ferric sulphate concentration for FRAP.

4.3 Texture Measurement

Table 4.6: The texture measurement of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%).

Samples	**Texture**	
	Hardness (N)	**Tensile strength (N)**
Control noodle	78.89 ± 8.25^{A}	0.18 ± 2.93^{A}
3% SC noodle	63.83 ± 4.73^{B}	0.15 ± 0.02^{A}
5% SC noodle	57.77 ± 2.43^{B}	0.14 ± 0.03^{A}
10% SC noodle	42.66 ± 0.89^{C}	0.13 ± 0.04^{A}

$^{A-C}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

4.3.1 Hardness

The hardness or firmness measurement revealed a significant difference ($p=0.00$) found across samples, except for 3 and 5% of SC noodles. The hardness was decreased when the concentration of *S. crispus* increased. The control noodle had the hardest texture (78.89 ± 8.25N) while the 10% SC noodle has the softest texture (42.66 ± 0.89).

4.3.2 Tensile Strength

There was no significant difference ($p=0.289$) found in tensile strength among the samples. A decreasing trend of tensile strength along the higher concentration of *S. crispus* incorporated was observed. The control noodle had the maximum tensile strength (0.18 ± 2.93), whereas the 10% SC noodle had the lowest (0.13 ± 0.04).

4.4 Color Measurement

The CIE-Lab parameters of each sample were recorded in Table 4.7. There were significant differences (p=0.00) found in L*, a*, and b* values. Decrease trends were observed in L* and a* values along the higher concentration of SC noodles. In a* value, 5 and 10 % of SC noodles cannot differ from each other. An increasing trend was observed in the b* value along with the higher concentration of SC noodles. The 5% SC noodle was not significantly different from the 3 and 10% of SC noodles. Overall, the noodle after incorporated with *S. crispus* were tend to decrease in L* and a* values while increasing in b*.

Table 4.7: The color measurement of noodles incorporated with different concentration of *S. crispus* (control, 3, 5, and 10%).

Samples	**Color**		
	L*	**a***	**b***
Control noodle	71.97 ± 0.96^{A}	0.06 ± 0.02^{A}	9.35 ± 0.13^{C}
3% SC noodle	61.94 ± 0.69^{B}	-0.44 ± 0.12^{B}	11.36 ± 0.26^{B}
5% SC noodle	54.76 ± 1.03^{C}	-0.59 ± 0.07^{C}	12.21 ± 0.37^{AB}
10% SC noodle	46.21 ± 1.15^{D}	-0.76 ± 0.06^{C}	12.83 ± 0.61^{A}

$^{A-D}$. Different superscripts in the same column indicate significant differences ($p<0.05$) whereas the data with the same superscript indicate no significant differences ($p>0.05$) using the Tukey test ($\alpha=0.05$).

CHAPTER 5

DISCUSSION

5.1 Proximate Analysis

As referred to the result of proximate composition in Table 4.1, there are significant differences ($p<0.05$) between all of the samples with regard to the contents of moisture, ash, fat, and protein as well as crude fiber. There was a slight increase in moisture content from control to 15% SC noodles from (71.46 ± 0.29%) to (74.76 ± 0.40%). This indicates that when the concentration of *S. crispus* increase, the noodle matrix absorbed or retained more moisture. This may be owing to the additional water given by *S. crispus* leaves, with a higher moisture content in noodles corresponding to a greater amount of leaves used. Moreover, the leaves puree can interrupt the formation of gluten network, weak and incomplete development of gluten network which did not cover the surface of noodle, causing more starch to expose to water during cooking. Hence, the starch granules tend to absorb more water (Moss, Gore & Murray, 1987).

A greater quantity of ash is proportional to a higher concentration of *S. crispus* incorporation in noodles. This is because the *S. crispus* leaves is a source of minerals. Based on Figures 4.1, 4.2, 4.3, and 4.4, a darker green-greyish color is observed along with the concentration of SC noodles. This may be due to the residue of green chlorophyll pigment. For crude fiber determination, 15% Sc

noodle obtained the highest percentage of fiber (0.48 ± 0.03%) since it has the highest leaves contain.

In terms of fat analysis, the fat content was rise significantly from control (0.02 ± 0.00%) to 15% SC noodles (0.17 ± 0.03%). The fat is contributed by the *S. crispus* leaves, including essential oils such as phytol (a phytyl ester in chlorophyll), cardinol, and eugenol (Asmah, et al., 2006).

Along with the concentration of SC noodles, a declining trend was noticed in the protein content. The control noodle found to have the highest protein content (17.1 ± 0.64%), whereas 15% SC noodle had the least (14.17 ± 0.72%). This is because, according to the formulation, the amount of wheat flour used was reduced along with the concentration of SC noodles, and it was determined that the *S. crispus* leaves did not significantly influence the protein level compared to wheat flour. Lastly, there was no significant differences ($p>0.05$) on the carbohydrate content but with slightly decreased down the samples. In this case, the carbohydrate content is also affected by the reduction of wheat flour as the main source.

5.2 Antioxidant Activity

5.2.1 Total Phenolic Content

According to Table 4.3, there are significant differences ($p<0.05$) between the total phenolic content for all samples. The total phenolic content increased progressively from the control (1.38 ± 0.15 mg GAE/mL) to 15% SC noodles as recorded as (1.75 ± 0.11 mg GAE/ mL). Black Face General is rich in phenolic components, including caffeic acid, ferulic acid, quercetin, rutin, catechin and quercetin (Ghasemzadeh, Jaafar & Rahmat, 2015). Therefore, this

has revealed a higher concentration of *S. crispus* incorporation, a higher phenolic content.

However, in comparison to research conducted by Ghasemzadeh, Jaafar & Rahmat (2015), the total phenolic content of fresh *S. crispus* extract (is 12.62 mg (GAE)/g) without undergoing food processing is much higher than the samples in this project. This happens because the phenolic compounds are thermally degradable during the cooking process. Besides, it might due to the functional components from noodles leaking into to the water during cooking, and oxidation happens throughout the noodle-making processes (Yu, et al., 2020).

5.2.2 DPPH Radical Scavenging Activity

Based on table 4.4, the radical scavenging activity (RSA) and IC_{50} value showed significantly different among all samples. The RSA value of the control noodle is 21.86 ± 2.16%, whereas the SC noodles are ranging from 26.28 ± 0.99 to 32.67 ± 1.59%. This indicated that the antioxidant was improved by the addition of *S. crispus* and exhibited more antioxidant activity when a larger concentration of *S. crispus* was added. Besides, the estimation of half maximal scavenging concentration (IC_{50}) value based on RSA decreases from control noodle (2676.34 ± 362.95 μM/mL) to 5% (2272.89 ± 121.21 μM/mL), 3% (2258.97 ± 277.11 μM/mL), and 10% SC noodle (1958.37 ± 194.58 μM/mL) to ascorbic acid (27.64 ± 0.74 μM/mL). A lower IC_{50} value A lower IC_{50} value indicates more antioxidant activity because it required a lower sample concentration to achieve 50% radical scavenging activity (Rivero-Cruz,

et al., 2020). The antioxidant activity of 3% and 5% IC_{50} value was not tally with RSA, as 5% SC noodle has a higher IC_{50} value. This may be due to the RSA percentages of 3 and 5% of SC noodle do not differ considerably, and the assessment of IC_{50} value is affected by linearity from each sample graph. According to the research, *S. crispus* leaves extract exhibited 73.8% of RSA and IC_{50} value of 5.44 ± 1.76 μmol/L, which close to vitamin C references (3.882 ± 0.628 μmol/L) (Al-Henhena, et al., 2015; Ghasemzadeh, Jaafar & Rahmat, 2015). This project's inclusion of *S. crispus* in noodles (maximum 15%) has substantially lower antioxidant activity than pure *S. crispus* extract found in the research. This may be a result of lesser *S. crispus* amount used in the food matrix and certain bioactive components are oxidized during food processing.

5.2.3 FRAP Activity

The FRAP activity is increase significantly ($p<0.05$) with the increase in *S. crispus* concentrations. The FRAP activity of control noodles is 60.03 ± 6.4 μM Fe(II)/mL, while SC noodles ranged from 73.49 ± 8.72 to 141.44 ± 8.22 μM Fe(II)/mL. This indicates that antioxidant activity is positively correlated with *S. crispus* concentration. However, the antioxidant activity is still lower than the research finding of 267.5 μM (Fe) II/g for pure *S. crispus* extract due to reasons stated above.

Overall, across all antioxidant assays, noodles infused with Black Face General were always higher than the control noodle. In addition, the DPPH radical scavenging activity and FRAP activity are linked positively with the total

phenolic content, as the phenolic component is accountable for the antioxidant activity (Aryal, et al., 2019).

5.3 Texture Measurement

Based on the result, a significant decrease in hardness and no significant decrease in tensile strength were reported. The control noodle has the highest hardness (78.89 ± 8.25 N) and the highest tensile strength (0.18 ± 2.93 N) than other noodles. The hardness test evaluates the structure's firmness, whereas the tensile strength analysis evaluates the structure's ability to withstand the greatest force before breaking. The noodles become softer and easy to break or have less elastic mouthfeel after being incorporated with a higher amount of *S. crispus*. The texture of SC noodles changed mainly depending on the formation of the gluten network. In this case, *S. crispus* puree with cellulose and fiber can cause steric hindrance to the gluten interaction, affecting the secondary structure of the gluten network (Nawrocka, et al., 2017). The greater the amount of *S. crispus* incorporated, the weaker the gluten network development, resulting in a softer texture that is easily deformed. Moreover, low protein content is associated by low levels of glutenin and gliadin, which lesser gluten synthesis (Liu, et al., 2018). It is less preferable to have a soft texture because a high-quality noodle should have a firm, chewy and elastic mouthfeel (Ahmed, Qazi & Jamal, 2015).

5.4 Color Measurement

According to Table 4.7, a decreasing trend in L* and a* with increasing trend of b* is observed. Since L* denoted brightness (0 = black, 100 = white), -a*

indicated greenness, +a* represented redness, -b* represented blueness, and +b* represented yellowness, the noodle after incorporated with *S. crispus* was become darker, yellower and greener. This is because the presence of green natural chlorophyll and yellow carotenoid pigment (Othman, et al, 2017).

5.5 Future Recommendations

In this *in vitro* study, the antioxidant properties of *S. crispus* noodles were only evaluated utilizing TPC, DPPH, and FRAP assays. Thus, an *in vivo* antioxidant and toxicology investigation can be conducted to provide more information about the antioxidant capacity associated with the human body. Next, the antimicrobial and shelf life study can be carried out to assess the potential pharmacological benefits of functional phytochemicals after incorporation with Black Face General. Besides, further texture and taste improvements of SC noodles can be done by investigating an optimum formulation that does not disrupt the gluten network and investigating a suitable preservative to lessen the bitter taste when a high dose of Black Face General is introduced.

CHAPTER 6

CONCLUSION

In conclusion, the incorporation of Black Face General (*Strobilanthes crispus*) into wheat noodles was capable of significantly improving some nutritional values including the moisture, ash, fat and crude fiber contents as well as higher mineral content. This project also demonstrated that the Black Face General is effective in enhancing the antioxidant activity of noodles, whereby 10% of SC noodles exhibited the strongest antioxidant activity across all assays and being significantly more potent than control and 3% of SC noodles. However, the outcome of texture analysis suggested that incorporation of Black Face General is less desirable because it resulted in a softer and more easily breakable texture. Lastly, SC noodle has a darker, greener and yellower appearance.

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Programme / Course	BACHELOR OF SCIENCE (HONOURS) FOOD SCIENCE-(FD)
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