THE NUTRITIONAL COMPOSITION, TEXTURE, COLOUR, AND SENSORY EVALUATION OF CHOCOLATE BAR FORMULATED WITH BROWN SEAWEED (*KAPPAPHYCUS ALVAREZII*) USING RESPONSE SURFACE METHODOLOGY (RSM)

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By

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Dissertation submitted in partial fulfilment of the requirements for the degree of Bachelor in Nutrition with Honours

January 2025

CERTIFICATE

This is to certify that the dissertation entitled "THE NUTRITIONAL COMPOSITION, TEXTURE, COLOUR, AND SENSORY EVALUATION OF CHOCOLATE BAR FORMULATED WITH BROWN SEAWEED (*KAPPAPHYCUS ALVAREZII*) USING RESPONSE SURFACE METHODOLOGY (RSM)" is the record of the research work done by WAN NURFATIHAH BINTI WAN NASRI, MATRIC NUMBER 157343, during the period record of March 2024 until January 2025 under my supervision. I have read this dissertation, and, in my opinion, it confirms acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation to be submitted in partial fulfilment for the degree of Bachelor in Nutrition with Honours.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, or promotion purposes.

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Wan NurFatihah Binti Wan Nasri

Date: 4 February 2025

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LISTS OF SYMBOLS

cm	Centimeter
>	Greater than
<	Lower than
H_A	Alternative hypothesis
H_O	Null hypothesis
mL	Milliliter
g	Gram
mg	Milligram
kg	Kilogram
%	Percentage
P	p-value
°C	Degree Celsius
a*	Redness coordinate to Greenness coordinate
b*	Yellow coordinate to Blueness Coordinate
L*	Brightness Coordinate
R^2	Coefficient of determination

LIST OF ABBREVIATIONS

Terms	Operational definition
RSM	Response Surface Methodology
CCD	Central Composited design
K. alvarezii	*Kappaphycus Alvarezii*
KA	*Kappaphycus Alvarezii*
OBP	Overripe Banana Powder
AOAC	Association of Official Agricultural Chemists
WHO	World Health Organisation
FAO	Food and Agriculture Organisation
DF	Dietary Fiber
ISF	Insoluble dietary fiber
SDF	Soluble dietary fiber
T2DM	Type 2 Diabetes
T. cacao	*Theobroma cacao*
ORBS	Overripe Banana Sweetener
DM	Diabetes mellitus
CVD	Cardiovascular disease

KANDUNGAN NUTRISI, TEKSTUR, WARNA, DAN SENSORI COKLAT BAR DIFORMULASIKAN DENGAN RUMPAI LAUT (*KAPPAPHYCUS AVAREZII*) MENGGUNAKAN METODOLOGI PERMUKAAN TINDAKBALAS (RSM)

ABSTRAK

Dengan memasukkan serbuk rumpai laut (*Kappaphycus alvarezii*) ke dalam resipi coklat, kajian ini bertujuan untuk menghasilkan coklat yang berkhasiat dan tinggi serat. Di samping itu, garam telah ditambah kepada coklat untuk menambah baik rasa sambil juga menentukan komposisi pemakanannya. Pemanis pisang ranum, atau OBP, digunakan sebagai ganti gula yang pada asalnya digunakan untuk menghasilkan coklat. Sebanyak dua faktor telah ditetapkan, masing-masing dengan peratusan rumpai laut yang berbeza (1%, 2%, dan 3%) dan garam (0%, 0.5%, dan 1%). Nisbah/paras rumpai laut dan garam putih dalam formulasi coklat susu telah dioptimumkan menggunakan reka bentuk komposit Pusat (CCD) metodologi permukaan tindakbalas (RSM). Menurut CCD RSM, kekerasan coklat meningkat apabila lebih banyak rumpai laut dan garam ditambah. Begitu juga, rumpai laut meningkatkan jumlah serat mentah dalam coklat, manakala garam mempunyai sedikit kesan ke atasnya. Eksperimen 1 (rumpai laut 2%, garam 1%) dan 9 (rumpai laut 2%, garam 0.5%), masing-masing menghasilkan dua formulasi optimum berdasarkan CCD. Analisis pemakanan kedua-dua ujian ini dijalankan secara perbandingan dengan kumpulan kawalan, yang terdiri daripada 0% rumpai laut dan 0% garam. Penambahan 2% rumpai laut dan 0.5% garam ke dalam formulasi meningkatkan kelembapan (2.54%), protein (0.65%), lemak (0.65%), dan abu (2.30%), manakala komposisi lemak (46.37%). dan karbohidrat (46.64%) berkurangan sedikit. Dalam penilaian sensori, Eksperimen 9 menunjukkan keputusan dalam penampilan, warna, aroma dan rasa yang paling tinggi berbanding sampel kawalan. Kesimpulannya, gabungan 0.5% garam dan 2% rumpai laut mempunyai potensi berfungsi dengan baik untuk menghasilkan coklat yang berkhasiat. *Kappaphycus alvarezii* berupaya

menambah baik kandungan nutrisi coklat dan boleh digunakan sebagai elemen alternatif dalam penciptaan kuih-muih berserat tinggi dan padat dengan nutrien.

NUTRITIONAL COMPOSITION, TEXTURE, COLOUR, AND SENSORY EVALUATION OF CHOCOLATE BAR FORMULATED WITH BROWN SEAWEED (K*APPAPHYCUS ALVAREZII*) USING RESPONSE SURFACE METHODOLOGY (RSM)

ABSTRACT

By incorporating seaweed powder (*Kappaphycus alvarezii*) into the chocolate recipe, this study aimed to create a nutritious and high-dietary-fiber chocolate. In addition, salt has been added to the chocolate to improve its flavour while also determining its nutritional composition. Overripe banana powder, or OBP, was used in place of the sugar that originally was used to produce the chocolate. A total of two factors were set, each with different percentages of seaweed (1%, 2%, and 3%) and salt (0%, 0.5%, and 1%). The ratios/levels of seaweed and white salt in the milk chocolates' formulation were optimised using a Central composite design (CCD) of response surface methodology (RSM). According to CCD of RSM, chocolate's hardness increased as more seaweed and salt were added. Similarly, seaweed considerably raised the amount of crude fiber in chocolate, whereas salt had little effect on it. Experiments 1 (seaweed 2%, salt 1%) and 9 (seaweed 2%, salt 0.5%), respectively, produced the two optimal formulations based on CCD. The nutritional analysis of these two tests was conducted in comparison to the control group, which consisted of 0% seaweed and 0% salt. The addition of 2% seaweed and 0.5% of salt to the formulation increased the moisture (2.54%), protein (0.65%), fat (0.65%), and ash (2.30%), while the composition of fat (46.37%) and carbohydrate (46.64%) was slightly reduced. In the sensory evaluation, Experiment 9 showed results in the highest appearance, colour, aroma, and aftertaste compared to control samples. In conclusion, a combination of 0.5% salt and 2% seaweed potentially work well to create a nutritious chocolate. *Kappaphycus alvarezii* able to improves the nutritional content of chocolate and may be utilised as an alternative ingredient in the creation of a high-fiber, nutrient-dense confectionery.

CHAPTER 1

INTRODUCTION

1.1 Study Background

People all over the world enjoy chocolate, one of the most commonly consumed confections. The cocoa itself gives the chocolate a deep, sweet flavour with a touch of bitterness. *Theobroma cacao* is a plant that provides the source of cocoa. Although there aren't many varieties of chocolate bar available today, dark, milk, and white chocolate are the most widely available varieties. Ingredients, like the quantity of cocoa used in the chocolate's preparation, are what cause the variations in each variety of chocolate.

Due to the nutritious value of the cocoa, eating chocolate bar generally offers health benefits. For instance, chocolate contains flavonoids, and polyphenols. These substances have several advantages. For example, polyphenols are antioxidants that reduce the risk of cardiovascular disease (CVD) (Fernández-Murga et al., 2011). Minerals like iron, potassium, and magnesium are contributed by flavonoids, which have several advantages (Montagna et al., 2019). However, because chocolate bar is typically high in sugar and fat, which might raise the risk of certain diseases like diabetes mellitus (DM), cardiovascular disease (CVD), and obesity, its consumption should be moderate. As a result, it's essential to create chocolate bar that is high in specific nutrients yet low in sugar and salt without affecting the product's quality or flavour.

One of the functional ingredients that can be added to chocolate bar is seaweed, for example *Kappapyhcus alvarezii* which is known as brown seaweed or “cottoni” (Adharini et al., 2020). *K. alvarezii* considered a fiber-rich food and it also has a lot of benefits when been consumed. In formulated food, *K. alvarezii* considerably enhanced the level of dietary fiber.

Seaweed has 10–20 times the minerals of plants on land and concentrates minerals from seawater (Makkar et al., 2016). Apart of that, seaweed also provide variety of physiological functions, including anti-inflammatory and anti-microbial properties, are also provided by *K. alvarezii* (Mohammad et al., 2019). Researchers are convinced that some seaweed with potent anti-cancer properties will one day be useful in treating leukaemia and malignant tumours in humans (Pati et al., 2016). Thus, when there is additional seaweed in food product, it will directly increase the dietary fiber content.

Dietary fiber (DF) describes the parts of plant foods, such as polysaccharides and lignin, including oligosaccharides like inulin and resistant starch that are indigestible to human digestive enzymes. DF is naturally found in soybeans, fruits, wheat bran, rice bran, and others. DF has lately been suggested as a dietary modification that may aid in enhancing health outcomes. For instance, soluble fiber lowers blood cholesterol, whereas insoluble fiber promotes intestinal transit time, quickens stomach emptiness, and increases fecal volume, all of which contribute to healthy digestion (Soliman, 2019).

However, adding seaweed might alter the chocolate's texture, flavour, and acceptability overall, therefore, adding salt helps to balance out the chocolate's flavour. Apart from flavour, salt also plays roles in sensory, texture, and preservation of food development. The texture of the chocolate formulation could be impacted when there is the addition of white salt because salt has hygroscopic properties (Hao et al., 2024). As a result, it will help create balance in the chocolate's flavour, enhance its quality, and cover up the taste of the seaweed.

Overripe banana powder (OBP) was developed by using bananas (*Musa sapientum*) (Kumar et al., 2019). Bananas are highly available in tropical regions such as Malaysia. Bananas are easy to damage, which leads to the darkening colour of their skin and often being abandoned, which contributes to waste. To reduce the waste, OBP was developed, which has been used as a natural sweetener replacer to refined sugar since OBP is natural and healthier compared to refined sugar (Nie et al., 2024).

To develop optimal conditions of product development, a central composite design (CCD) of response surface methodology (RSM) helps determine the optimum condition to achieve an optimal outcome. It is a powerful mathematical technique that helps in the development, production, and enhancement of processes in the development of new products (Myers et al., 2016). RSM will provide an optimum ratio of responses based on our desirable outcome. Thus, by using this method, the creation of product will produce based on what we want to emphasise as an example, chocolate that is high in fiber but low in hardness.

1.2 Problem Statement

The prevalence of chronic diseases has been rising at an alarming rate in the past decade. Chronic conditions that cause early death include cancer, cardiovascular disease (CVD), and type 2 diabetes (T2DM) (Tagde et al., 2021). Diabetes mellitus (DM) is one of those chronic diseases that is currently a global public health concern because of its rising prevalence. Based on World Health Organization (WHO), in 2022, 14% of adults aged 18 and over had diabetes mellitus (DM), a 7% rise from 1990. Moreover, 59% of adults aged 35 and over had DM but were not taking medication to manage their disease. Malaysia is one of the countries in the Western Pacific region that had the highest rate of DM. Based on a national survey report, 3.6 million adults in Malaysia who were 18 years of age or older had diabetes in 2019; meanwhile, 49% of these cases went undiagnosed. In 2025, it was expected that 7 million adults in Malaysia who were 18 years of age or older would have DM (Akhtar et al., 2022).

The increasing trend of DM is due to age, depression, and food intake. This is because this inflammatory state will cause dysregulation of food intake, which impacts insulin resistance (Selman et al., 2022). The intake of food that is high in sugar will majorly impact insulin resistance, which will lead to an increase in the risk of DM. For example, chocolate bar. Several studies suggested that eating food that is high in sugar and fat helps to improve their mood by releasing dopamine (happy mood). As an example, a study showed that consuming 40g of dark and milk chocolate every day for 2 weeks will help women feel good mood because it contains cocoa polyphenols that help reduce stress (Al Sunni & Latif, 2014). People often perceive eating chocolate as unhealthy because most of the chocolate on the market is high in sugar and fat, so it is important to develop chocolate that is healthier by reducing the sugar and increasing its nutritional value, such as fiber.

Dietary fiber can be found in various sources, and one of the examples is seaweed. *Kappaphycus alvarezii* high in dietary fiber and can be found in Malaysia, especially in Sabah. The humid environment of Sabah is the optimum condition for growing seaweed, such as *Caulerpa lentillifere* and *K. alvarezii*. This is due to Sabah's location within the typhoon and monsoon belt (Hussin, 2017). However, overgrowing of the seaweed will destroy the marine ecosystem. So, by emphasising the use of *K. alvarezii*, we help to protect the ecosystem and also to get the nutritive value, especially the fiber content.

Adding salt to chocolate helps reduce the bitterness (Liu et al., 2024), which creates a balanced flavour between the sweetness and bitterness of the chocolate. Incorporating seaweed and salt into chocolate development improves the product's appeal and provides a rich flavour of chocolate. In addition, using overripe banana powder (OBP) instead of table sugar results in chocolate that is healthier because it is more natural and less sweet (Nie et al., 2024). Therefore, by incorporating seaweed together with salt in developing chocolate bar, will produce chocolate that has better nutritive value, taste, and is healthier compared to the chocolate in the market.

This study helps to increase the DF in chocolate bar by using brown seaweed (*K. alvarezii*) since seaweed is one of the fiber-rich foods. In this study, we also compared the chocolate that has been formulated with *K. alvarezii* and salt in terms of its nutritional composition, texture, colour, and sensory evaluation using response surface methodology (RSM). By using RSM, it will help to know the optimum ratio of seaweed and salt that affect the nutritional, physicochemical, and sensory properties.

1.3 Significance of Study

The purpose of this study is to compare the chocolate bar formulation with brown seaweed (*K. alvarezii*) and salt in terms of its nutritional composition, texture, colour, and sensory evaluation using RSM. The seaweed is also known by another name, “cottoni” (Adharini et al., 2020). *K. alvarezii* is a primary source of carrageenan and dietary fiber, which is used extensively in food production to improve texture, particularly gelling and viscosity of the food (Hotchkiss et al., 2016). People enjoy chocolate but are concerned about their health, so it is important to find out from this study whether incorporating *K. alvarezii* in the chocolate will enhance the overall quality, such as nutritional values focusing on dietary fiber, while maintaining the taste of the chocolate bar, so the consumers can accept it.

The researcher examined the nutritional composition, which includes moisture, ash, protein, fat, carbohydrate, and dietary fiber, which is crude fiber. The hardness of the chocolate bar is also being examined through texture profile analysis (TPA) to ensure that the chocolate bar can meet the quality standard of chocolate. The use of the response surface method (RSM) helped find the most appropriate ratio of seaweed and salt in developing the chocolate bar with the goal being to discover whether adding seaweed and salt to chocolate can considerably increase the amount of dietary fiber in the chocolate's composition.

A sensory evaluation also aims to determine the chocolate bar’s general acceptability, which is crucial in determining whether or not it satisfies panelist preferences. The panelists will evaluate the chocolate according to several criteria, including its appearance, colour, flavour, and other qualities. The results may reveal the panelists' chocolate preferences, and these sensory factors are essential in assessing whether the chocolate produced with *K. alvarezii* is acceptable in terms of quality.

1.4 Research Question

1. What is the optimized ratio of seaweed (*K. alvarezii*) and salt in chocolate bar formulated based on the central composite design (CCD) of response surface methodology (RSM)?
2. What are the differences in the nutritional composition, texture, and colour analysis between chocolate bar formulated with and without brown seaweed (*K. alvarezii*)?
3. What differences in the sensory acceptability of chocolate bar with and without formulated brown seaweed (*K. alvarezii*)?

1.5 Objective

1.5.1 General Objective

The general objective of this study is to produce optimized chocolate bar formulated with brown seaweed (*Kappaphycus alvarezii*) using response surface methodology.

1.5.2 Specific Objective

1. To identify the optimum condition (ratio of seaweed and salt) for the chocolate bar development using central composite design (CCD) of RSM.
2. To determine the nutritional composition, texture, and colour profile of optimized chocolate bar formulated with brown seaweed (*K. alvarezii*).
3. To assess the sensory acceptability of optimized chocolate bar formulated with brown seaweed (*K. alvarezii*).

1.6 Research Hypothesis

1.6.1 Null hypothesis, H_O

There is no significant difference in nutritional composition, texture, colour, and sensory evaluation of chocolate bar formulated with and without brown seaweed (*Kappaphycus alvarezii*) using response surface methodology (RSM).

1.6.2 Alternative hypothesis, H_A

There is a significant difference in nutritional composition, texture, colour, and sensory evaluation of chocolate bar formulated with and without brown seaweed (*Kappaphycus alvarezii*) using response surface methodology (RSM).

CHAPTER 2

LITERATURE REVIEW

2.1 Chocolate

Theobroma cacao is the plant source of the production of chocolate. The headwaters of the Amazon River, specifically in eastern Ecuador and Peru, was the place where the *T. cacao* was originated. *T. cacao* is also known by various names such as Cupui (Portuguese), Coklat (Indonesia), and Bacau (Colombia). There are specific conditions to plant the *T. cacao* such as the temperature, soil pH, and temperature (Jean-Marie et al., 2022).

White, milk, and dark chocolate are the three types of chocolate that are typically found. The ingredients and nutrients that set such chocolates apart from one another. Due to the high concentration of polyphenols and flavonoids in cocoa powder, as well as the fact that dark chocolate does not contain milk or milk powder like milk and white chocolate, dark chocolate has been shown to have positive health effects (Adriana & Ana, 2012). Apart from carbohydrates, proteins, and fat, cocoa flavonoid contains a wealth of beneficial nutrients, including bioactive compounds like polyphenols and tocopherols that act as antioxidants (Oracz & Zyzelewicz, 2020). Flavonoids are also rich in minerals, including potassium, iron, magnesium, and other nutrients (Montagna et al., 2019).

In addition to flavonoids, cocoa contain dietary fiber and other components of exceptional nutritional value. With reported levels ranging from 38% to 44% of total dietary fiber as non-starch polysaccharides plus Klason lignin (isolated lignin), cocoa husk has been recommended to be a good source of dietary fiber. The husks of cocoa beans are used to make chocolate. They also have features that contribute to the nutritional value of chocolate, such as their polyphenolic concentration, antioxidant capacity, and physicochemical properties (Lecumberri et al., 2007).

2.1.1 Benefits of chocolate

There are numerous advantages to eating chocolate for us. As evidence, the Kuna Indian population on the island of Panama had low rates of atherosclerosis, hypertension, and type 2 diabetes (T2DM) among them. This was attributed to the Kuna Indians' daily consumption of a homemade cocoa drink; this trait is not due to genetics, it started to disappear as the Kuna Indians migrated to the cities of mainland Panama, where their diets changed, and they consumed less cocoa (Latif, 2013).

Oxidative stress can lead to atherosclerosis and other cardiovascular dysfunctions together with hypertension and heart failure. The power of cocoa powder and cocoa extracts is shown as a greater antioxidant capability than other foods. According to a study, consuming dark chocolate, which has more polyphenols than white chocolate, increased young smokers' plasma antioxidant capacity while improving their flow-mediated vasodilation (Fernández-Murga et al., 2011).

In addition, cocoa has anti-diabetic properties that are particularly effective in treating T2DM. This happens by slowing down the digestion and absorption of carbohydrates in the gut, cocoa and flavonols essentially help to maintain glucose homeostasis. Alternatively, in insulin-sensitive tissues like the liver, adipose tissues, and skeletal muscle, cocoa, and flavonols help to improve insulin sensitivity by regulating the glucose transport and insulin signalling protein to prevent those tissues from suffering from the oxidative and inflammatory damage linked to the disease (Montagna et al., 2019).

Besides cocoa or dark chocolate, with their antioxidant, anti-inflammatory, and anti-obesity qualities, polyphenols have been tested in various kinds of in vitro and in vivo studies to enhance thermogenesis, which is a process of maintaining the body temperature by balancing the heat loss with generation (Osilla et al., 2022) and energy expenditure, reduce oxidative stress and inflammation, and aid in the management of weight loss. A subgroup analysis revealed lower weight and body mass index (BMI) after consuming more than 30g of chocolate per day in trials lasting between 4 to 8 weeks. Additionally, a group of normal obese women who consumed 100g of dark chocolate per day (70% cocoa) for one week showed an increase in high-density lipoprotein (HDL) cholesterol level, a decrease in LDL ratio, and a decrease in abdomen circumference (Montagna et al., 2019).

2.2 Seaweed

Seaweed can be referred to as a marine macroalga that is widely available and used not only in Malaysia but also in other countries. Seaweed consists of blades, stripes, and holdfasts, but seaweed does not consist of actual stems, roots, and leaves. Seaweed from the Eukaryto family and the seaweed was classified based on its colour, which is brown, green, and red (Ratni et al., 2023). Since 2008, the seaweed production in Malaysia has been about 118298 tonnes of seaweed, and it increased the market value to 97.3% from 2007 to 2008 (Mohammad et al., 2019).

Brown seaweed which is *Kappaphycus alvarezii* is one of the types of edible seaweed and widely being cultivated in food production. *K. alvarezii* is also known as “cottoni” and is the most famous seaweed that widely being commercially and cultivated in Indonesia (Adharini et al.,2020). *K. alvarezii* is an edible species that can be found in Malaysia, especially in Sabah. Asia, Africa, as well as the Pacific, has potential the farming of *K. alvarezii*. *K. alvarezii* consists of 29.4% of fiber and 27.4% carbohydrates (Mohammad et al., 2019). *K. alvarezii* is easy to cultivate, with a short production cycle as well as low production costs. *K. alvarezii* can be found in dark, red, brown, yellow, and greenish colours (Adharini et al., 2020). Dried *kappaphycus*, contains carrageenan (around 34.6% soluble fiber) and salt (20% potassium) (Wanyonyi et al., 2017). Consuming *K. alvarezii* has antimicrobial and antioxidant properties that aid with type 2 diabetes and cardiovascular problems. Additionally, the seaweed's carrageenan content aids in slowing down digestion, which raises metabolism.

Based on the Food and Agriculture Organisation of The United Nations (FAO), the total output of the world's seaweed industry amounts to around US$6 billion, with more than 8 million tons of wet seaweed used annually. FAO 2018 stated that, in 2016, the global production of aquatic plants, mostly seaweeds, reached 30.2 million tonnes. 40.7% of it came from *Eucheuma* and *Kappaphycus* (Alemañ et al., 2019).

2.2.1 Benefits of seaweed

Seaweed contains numerous nutrients that are good for our health, such as dietary fiber, protein, vitamins, as well as mineral. Seaweed has a range of total dietary fiber of 25-70%, of which 50–80% is soluble dietary fiber (Praveen et al., 2019). Aside from that nutrient, seaweed is also rich in other phytochemicals such as alkaloids, flavonoids, tannins, phenolic compounds, steroids, and terpenoids that help inhibit 1-diphenyl-2-picrylhydrazyl (DPPH) (Ratni et al., 2023). DPPH is a free radical that has the capability of being a hydrogen acceptor to the antioxidants.

The natural antioxidant that is present inside the seaweed (Abirami & Kowsalya, 2011) will act as a direct or indirect mechanism that inhibits the growth of free radicals. The free radicals lead to induced oxidative stress that can cause the development of certain diseases such as diabetes mellitus, cardiovascular disease, respiratory problems, cancer, and other types of diseases (Phaniendra et al., 2014). Sharan and Vennila (2021) stated that *K. alvarezii* has the highest phytochemical compared to other species, besides, it also consists of polyphenols, vitamins such as A, B1, B2, and C, as well as ascorbic acid that helps to inhibit the growth of the oxidation when being exposed to the light and oxygen when kept in storage. A high level of antioxidant activity in *K. alvarezii* demonstrates its capacity as a strong free radical eliminator, which gives an advantage for maintaining human health and preventing disease (Ratni et al., 2023).

Besides that, seaweed also acts as an antimicrobial. Antimicrobials inhibit and kill infectious organisms. For example, it helps in oral infections which are gingivitis and periodontitis, as well as help in oral cancer. This development happens when the bioactive component of the *K. alvarezii* is extracted with different polar solvents being used, such as ethanol, chloroform, and ethyl acetate (Sharan & Vennila, 2021). This will produce the extraction of *K. alvarezii,* which will indicate the potential efficacy against oral pathogens with potent strong antioxidant properties. The mixing between *K. alvarezii* with cinnamon essential oil help in prevent the growth of *Escherichia coli*, *Bacillus cereus*, *Aspergillus flavus*, and other few types of bacteria species in the antimicrobial film packaging's development (Ratni et al., 2023).

Seaweed is a strong source of dietary fiber and a tribute to nature's richness. Carrageenan is a powerful dietary hydrocolloid found in seaweed that serves several health benefits. The effects of carrageenan on blood pressure, serum lipid, and blood glucose level highlight how valuable seaweed itself (Mohammad et al., 2019). The carrageenan has the fourth-largest proportion of the worldwide food texture market after starch, gelatin, and pectin (Hotchkiss et al., 2016). Carrageenan not only provide health benefits but it also provides a good application in food production such as milk chocolate, yogurt, jellies, sauces and frozen dessert (Ranganayaki et al., 2014).

2.2.2 Development of seaweed in food

Seaweed, *K. alvarezii*, was added to the noodles (flat rice noodles “koay teow” and yellow alkaline noodles “mi kuning”) and fish sausage (‘keropok”). This is because noodles and fish sausage are the foods that are commonly consumed by Southeast Asians, especially in Malaysia. The formulation of seaweed into the noodles, as well as the fish sausage, shows that an increase in fiber and ash contents occurs when the use of seaweed puree increases (SP) (Mohammad et al., 2019). A similar experiment was done but using different types of seaweed puree, which are *Eucheuma cottoni, Gracilaria verrucosa*, and mixed between them, dried noodles with substitutes with the seaweed increased fiber and ash content compared to the dried noodles without seaweed puree . According to the survey that was done, the consumers were able to accept the fish sausage formulated with seaweed but, not in noodles (Mohammad et al., 2019).

The development of Choco’ Rula. Choco’ Rula is a mixture between chocolate and seaweed, which is *K. alvarezii* (Abas et al., 2024). The main idea of using the seaweed is because of the presence of carrageenan in the seaweed. This is because the use of carrageenan helps in thickening and also improves in mouthfeel (Hotchkiss et al., 2016). Apart from that, some studies about the use of seaweed *Ulva reticulata* in seaweed show consumption of seaweed chocolate increases the hemoglobin, which helps improve the iron status in the anemic adolescent girls because of the high content of iron present in *U. reticulata* (56 mg/100g) (Thahira Banu & Uma Mageswari, 2015). Based on the sensory taste that has been done by Siti et al (2024), 82.31% of consumers prefer chocolate formulated with the seaweed compared to the chocolate in the market. This shows that the potential of acceptance of chocolate formulated with seaweed may be accepted not only locally but also internationally.

2.3 Dietary Fiber

Dietary fiber is a word that has commonly been talked about but not known truth worthy of it. The phrase "dietary fiber" was used by Hipsley in the middle of the 20th century to describe plant materials that are resistant to being broken down by endoenzymes released by cells of mammals. Trowell used the term "dietary fiber" in the 1970s to refer to indigestible carbohydrates. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are the types of DF. Fiber classified as SDF is partially soluble in water but cannot be digested or absorbed by the human system, IDF is a kind of fiber that is insoluble in water and cannot be absorbed or digested by human systems (Yang et al., 2017). Whereas cellulose, lignin, and hemicellulose are examples of IDF, pectin, mucus, and gums are examples of SDF (Guan et al., 2021). Crude fiber is a subset of dietary fiber, which belongs to IDF. Apart from its water solubility, the DF can be categorised based on a few attributes, such as origin, the structure of the polymer, the amount of fiber contained, it is capacity to retain water, its ion-exchange capabilities, and its physiological qualities (Merenkova et al., 2020).

To provide optimal nutrition, dietary fibers are prioritised over other nutrients. An inadequate intake of fiber is linked to gastrointestinal disorders such as diarrhoea, colon cancer, and haemorrhoids, cardiovascular disorders such as hypercholesterolaemia, stroke, and ischemic heart, and metabolic disorders such as diabetes and obesity. Dietary fiber has been demonstrated in numerous studies to improve human health and aid in the prevention of certain chronic diseases that shorten life expectancy and raise mortality. Dietary fibers are thought to be extremely important for human health because of their many physiological impacts (Merenkova et al., 2019). Apart from that, DF also serves as a potential prebiotic for human health (Praveen et al., 2019).

Increased DF consumption has been shown to lower the chance of developing some diseases, including irritable bowel syndrome, constipation, and some cancers (Guan et al., 2021). In 2003, fast food restaurants had an 11% decline in French fry sales while seeing a 12% increase in salad orders. Moreover, DF increases intestinal flora, aids in gastrointestinal peristalsis to relieve constipation, absorbs toxic substances in the gut and facilitates their elimination, and supplies energy and nourishment to support the growth of probiotics (Yang et al., 2017).

2.3.1 Utilization of DF in food

Apart from seaweed as DF used in the development of the food, other dietary fiber such as banana, coffee grounds, and bran have also been used as DF in making better food, especially in terms of nutrition. This is because consumers start to take food that helps in improving their health to gain better well-being. This leads to more innovation to add other DF instead of seaweed in daily food items to encourage consumers to consume food or food ingredients that provide more benefits to them compared to the food that does not nourish by the DF.

Nowadays, food companies have started to develop flour that is enriched with DF. The usual DF is added to the flour products, such as noodles, biscuits, and whole-grain bread. We are aware that, in Asian countries, noodles are one of the staple foods that are used as part of their life (Mohammad et al., 2019). Usually, in producing the noodles, refined wheat flour will be used. Refined wheat flour is low in vitamins and DF as well as minerals compared to whole wheat flour (Zhang et al., 2019). This leads to the innovation of fiber-rich flour that adds bran start sold in our market to help in improving nutritional properties and improve the value of the product (Yang et al., 2017).

Moreover, the innovation of using bananas in cookies was also one of the utilisations that is currently being recognized. Banana is a fruit that is rich in DF and provides good nutritional health benefits. The incorporation of overripe banana pulp powder in chocolate cookies increases the DF and ach content of the cookies. However, due to its appearance and low quality, the overripe banana might not be seen as a good ingredient to be used even if it provides rich DF (Ng et al., 2020). Moreover, coffee ground in cookies also helps increasing the DF, not only increasing the nutritional value but also making the cookies more flavorful. This innovation of adding coffee grounds in cookies has potential value to prevent diabetes (Yang et al., 2017).

Besides, the utilisation of DF in meat products such as sausage. Meat products provide protein, essential fatty acids, minerals, and other micronutrients; however, meat products are usually low in DF. The DF as a fat replacer added into the sausage helps improve the quality, the process characteristics, and the increase in shelf life (Henning et al., 2016). As an example, the addition of pineapple improves the physical, textural, and chemical properties of the sausage. The use of DF not only improved in terms of nutritional value but also helped in improving the properties of the product itself.

2.4 Salt

Salt, also known as sodium chloride (NaCl), can naturally be found in rock salt deposits and seawater if an area of seawater is enclosed and the water evaporates due to solar heat. Essentially, salt consists of a 1:1 ratio of sodium and chloride ions. Salt was referred to as "white gold," although the same word was also used for other products, including sugar, water, and cotton (Elias et al., 2020).

Since prehistoric times, salt has been used to prepare food. Since ancient times, salt has been utilised in fermentation and dehydration processes to extend the shelf life of products. To preserve food using salt, dehydration was the first curing procedure and is still in use today (Elias et al., 2020). Saline solution, or salt water, is used in Chinese culture, for instance, to preserve meat and fish. This is done by soaking them in the solution, which lowers the water activity level below what various bacterial species need to thrive.

Salt is used in the food industry for processing, sensory, and preservation purposes. In processing, salt is typically utilised in meat, bread, biscuits, and other foods. For example, in bread production, adding salt helps develop the dough, and kneading process, and makes the bread softer. In the kneading process, salt helps promote the structure of the gluten of the bread (Rysová, J., & Šmídová, Z, 2021). In terms of the senses, salt not only contributes its distinct flavour but is also utilised to improve and alter the flavour of other components while also minimizing the experience of a particular product. However, no set amount of salt should be added to food rather, it depends on the consumer's preferences.

In general, salt enhances the flavour of foods like pasta, chocolate, gravy, and other items. Salt also plays important roles in food products, for example, in meat products, by increasing the shelf life by lowering the water activity of the product, which helps to increase the microbial safety of the food. Salt also helps in increasing the sensory properties of food products by creating the saltiness of the product and increasing the juiciness of the meat product. Cheese, seafood products, and chilled foods all employ salt as a preservation agent.

For instance, the salt in cheese will aid in slowing down the growth of the majority of bacteria (Kim et al., 2021).

2.5 Banana and Overripe Banana Powder

People worldwide, regardless of age, love the tasty and incredibly nutritious banana (*Musa sapientum*, genus Musa) (Kumar et al., 2019). In the world, bananas rank second after rice, wheat, corn, and potatoes as major food crops. The banana is recognised for its abundance of many health-promoting bioactive phytochemicals, as well as carbohydrates, dietary fiber, certain vitamins, and minerals. Bananas are used as food medications to treat conditions like infections, cancer, diabetes, and hypertension (Kumar et al., 2019). According to previous studies, consumers' intentions to purchase overripe bananas were considerably reduced because of their low quality, the appearance of brown spots, and decreased pulp firmness (Ng et al., 2020).

In terms of sugar, bananas contain sucrose, which is produced when starch is broken down into glucose and maltose. Sucrose synthase then turns these sugars into sucrose, which is further broken down by invertase into glucose and fructose as the fruit ripens. The findings indicate that the two main sugars in bananas at any stage of ripening were fructose and glucose, which contributed 12 to 13 g/100 g total in slightly ripe to overripe bananas and 3.2 g/100 g in unripe bananas. In unripe bananas, sucrose made up just 25% of the sugar, whereas in somewhat ripe or ripe bananas, it made up 22-27%, and in overripe bananas, 11% (Philips et al., 2021). The pulp of overripe bananas is used to make overripe banana powder (OBP). The overripe banana pulp will be processed to turn it into a powder to make OBP. OBP is described as a healthy substitute for refined sugars because OBP has a low glycemic index compared to refined sugar which will help to produce food that low in glycemic index (Ng et al., 2020), for example, making chocolate bars.

Natural sweeteners derived from high-sugar tropical fruits, including bananas, pineapples, mangoes, and pomegranates, are considered better alternatives. Overripe bananas are still underutilised, though, and not much research has been done to determine the optimal way to use them for food. Using overripe bananas will therefore contribute to both raising the value of food products and indirectly lowering food waste (Ng et al., 2020).

2.6 Optimizing Formulated using Response Surface Methodology (RSM)

Enhancing a system's, process’s or product's performance to reap the greatest possible benefits is referred to as optimizing. In analytical chemistry, the term "optimization" refers to the process of determining the ideal conditions for implementing a technique that yields the best possible result. The technique used for optimization is known as one variable at a time. The goal is to optimize the response which influenced by several factor (Chelladurai et al., 2021).

A set of statistical and mathematical techniques known as response surface methodology (RSM) is helpful in the development, enhancement, and optimization of processes. It is also very applicable to the conception, creation, and formulation of new products as well as to the enhancement of the designs of already existing ones (Myers et al, 2016). RSM also fits a polynomial equation to experimental data. The graphical picture that was produced once the mathematical model had been fitted gave rise to the name Response Surface Methodology.

RSM has been widely utilised in product formulation and food processing operations modelling and optimization. Modelling and optimization of significant food processing procedures, such as extrusion, fermentation, baking, and cooking, have been done with

RSM. RSM has been applied to several experimental designs such as factorial designs, D-optimal designs, central composite designs and their variations, and mixture designs (Bhattacharya, 2021). Box-Behnken designs and Central composite designs (CCD) are the two most popular types of RSM. The Central Composite design is the most popular fractional factorial design employed in the response surface model, which can fit a whole quadratic model.

CHAPTER 3

METHODOLOGY

3.1 Research Design

The type of study that was applied in this study was experimental design. This study involved a control sample and 13 experimental samples according to the Central Composite Design (CCD) of response surface methodology (RSM) using Design-Expert software (v. 7.0.0, State-Ease, Inc., Minneapolis, USA). Two independent variables were chosen: seaweed ratio (X1) and salt ratio (X2). Table 3.1 presents the coded levels and actual values of the independent variables. A set of 13 experiments was employed. The control sample was the chocolate bar without incorporating seaweed and salt, while the experimental samples were the chocolate bar incorporated with brown seaweed ranging from 1 to 3% and white salt from 0 to 1%.

Table 3.1 CCD response surface for the low, center, and high limit for all factors for seaweed chocolate bar

Independent variables (factor)	Symbols	Coded level		
		-1	0	1
Seaweed	X1	1	2	3
White Salt	X2	0	0.5	1

The CCDs of experiments provide modelling to response surfaces to optimize the independent variables for the response of physical characteristics, which are hardness and nutritional composition, which is crude fiber. There were 13 treatments involved in this CCD design for the formulation of a chocolate sample that contained different ratios of seaweed and salt (Table 3.2). The weight of salt and seaweed is a percentage of the total gram of 50g of chocolate.

Table 3.2 Treatment involves different combinations of factors and responses according to the CCD of the experiment

Standard order	Run order	Percent of seaweed ratio (Actual weight, g)	Percent of white salt Ratio (Actual weight, g)
1	8	2 (1.0)	1 (0.5)
2	4	3 (1.5)	1 (0.5)
3	2	3 (1.5)	0 (0)
4	6	3 (1.5)	0.5 (0.25)
5	13	2 (1.0)	0.5 (0.25)
6	3	1 (0.5)	1 (0.5)
7	1	1 (0.5)	0 (0)
8	9	2 (1.0)	0.5 (0.25)
9	11	2 (1.0)	0.5 (0.25)
10	10	2 (1.0)	0.5 (0.25)
11	7	2 (1.0)	0 (0)
12	5	1 (0.5)	0.5 (0.25)

13	12	2 (1.0)	0.5 (0.25)

3.2 Sample size calculation

The estimated sample size was 30 untrained panellists (Aminah, 2000).

3.3 Research Tool

The research tool for this study is a sensory evaluation form that is attached as Appendix 3. This sensory evaluation form was used during the sensory evaluation of chocolate. The panellists were staff and students of Universiti Sains Malaysia (Health Campus), Kubang Kerian, Kelantan, who had tested the chocolate through the sensory evaluation.

3.4 Data Collection Method

The chocolate bar was prepared in the food preparation laboratory and stored in the Nutrition Laboratory, at School of Health Sciences, USMKK. Each chocolate bar sample was analyzed in terms of its nutritional composition, texture, and colour which was recorded in Microsoft Excel. After that, the samples proceeded with sensory evaluation.

3.4.1 List of Ingredients

The main ingredient of this study is brown seaweed; 1 kg of brown seaweed (*K. alvarezii*) in powder form was supplied by IMTANOMIC Sdn Bhd (ISB) in powder form. The other ingredients are brought from a supermarket located near Kubang Kerian, Kelantan. Table 3.3 shows the list of ingredients used in this experiment. Overripe banana powder (OBP) was supplied by Prof. Dr Wan Rosli.