OPTIMIZATION OF BROWN SEAWEED FLOUR (*KAPPAPHYCUS ALVAREZII*) TO REPLACE GELATIN USING RESPONSE SURFACE METHODOLOGY (RSM) FOR GUMMY CANDY PREPARATION

ALYA AZWANI BINTI ZUL YUSFAMI

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by

ALYA AZWANI BINTI ZUL YUSFAMI

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

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CERTIFICATE

This is to certify the dissertation entitled 'OPTIMIZATION OF BROWN SEAWEED FLOUR (KAPPAPHYCUS ALVAREZII) TO REPLACE GELATIN USING RESPONSE SURFACE METHODOLOGY (RSM) FOR GUMMY CANDY PREPARATION' is the record of research work done by ALYA AZWANI BINTI ZUL YUSFAMI, MATRIC NUMBER 158994 during period record of August 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 of Nutrition with Honours.

Main supervisor

PROFESSOR DR. WAN ROSLI WAN ISHAK
School of Health Sciences
Universiti Sains Malaysia
Health Campus
16150 Kubang Kerian
Kelantan, Malaysia
Date: 5 January 2025

DECLARATION

I hereby declare that this dissertation is the results of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as 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, and promotional purposes.

ALYA AZWANI BINTI ZUL YUSFAMI

Date: 5 January 2025

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LIST OF SYMBOLS

cm	Centimetre
>	Greater than
<	Lower than
L	Litre
mL	Mililitre
g	Gram
mg	Miligram
kg	Kilogram
%	Percentage
P	p-value
°C	Degree Celcius

LIST OF ABBREVIATIONS

Terms	**Operational definition**
CCD	Central Composite Design
DoE	Design of Experiment
KA	*Kappaphycus alvarezii*
κ -carrageenan	kappa carrageenan
RSM	Research Surface Methodology (RSM)
TPA	Texture Profile Analysis

PENGOPTIMUMAN TEPUNG RUMPAI LAUT MERAH (*KAPPAPHYCUS ALVAREZII*) UNTUK MENGGANTIKAN GELATIN MENGGUNAKAN KAEDAH TINDAKBALAS PERMUKAAN (RSM) UNTUK PENYEDIAAN GULA-GULA BERGETAH

ABSTRAK

Industri konfeksioneri bergetah kini menunjukkan minat yang semakin meningkat untuk menambah bahan baru memandangkan pengguna mencari produk yang lebih sihat dan berkhasiat. *Kappaphycus alvarezii* merupakan sumber penting hidrokoloid seperti karagenan yang berfungsi sebagai agen pengegelan. Oleh itu, kesan interaksi pembolehubah tak bersandar seperti serbuk rumpai laut (4%, 5% & 6%), gula (10%, 18% & 26%) dan air (62%, 81% & 100%) dari formulasi asal telah dikaji untuk menilai formulasi kandi bergetah yang optimum menggunakan Kaedah Respons Permukaan (RSM) berdasarkan tindak balas analisis profil tekstur. Dua formulasi optimum diperoleh daripada RSM dengan kekerasan minimum, kepejalan dan kelikatan yang lebih rendah serta atribut melantun yang tinggi: sampel yang dioptimumkan (rumpai laut 4%, air 100%, gula 26%) dan (rumpai laut 5%, air 81%, gula 18%). Analisis perbandingan telah dijalankan antara kandi bergetah kawalan dan dua sampel optimum ini. Keputusan menunjukkan bahawa penambahan rumpai laut meningkatkan kandungan lembapan (56.86%), abu (1.63%) dan serat (0.39%) secara ketara sambil mengurangkan kandungan lemak (0.04%) dan karbohidrat (39.06%). Penilaian sensori menunjukkan bahawa kandi bergetah dengan rumpai laut kurang digemari berbanding sampel kawalan, dengan perbezaa pada tekstur, rasa dan penerimaan keseluruhan. Pertumbuhan kulat dipantau selama tiga minggu menunjukkan bahawa semua sampel bebas kulat dalam peti sejuk, tetapi kandi bergetah dengan rumpai laut menunjukkan pertumbuhan kulat dalam masa tiga hari. Penemuan ini menunjukkan potensi kandi bergetah yang diperkaya dengan rumpai laut sebagai makanan berfungsi, menekankan

keperluan untuk pengoptimuman lanjut bagi mengimbangi pemakanan yang dipertingkatkan dengan daya tarikan sensori dan kestabilan simpanan.

OPTIMIZATION OF BROWN SEAWEED FLOUR (*KAPPAPHYCUS ALVAREZII*) TO REPLACE GELATIN USING RESPONSE SURFACE METHODOLOGY (RSM) FOR GUMMY CANDY PREPARATION

ABSTRACT

The gummy confectionery industry is showing a growing interest in adding novel ingredients to conventional formulations since consumers are looking for healthier and more nutritious products. *Kappaphycus alvarezii* is a well-known source of important food hydrocolloids such as carrageenan which acts as gelling agents. Thus, in this study, the interaction effects of independent variables such as seaweed powder (4%, 5% & 6%), sugar (10%, 18% & 26%) and water (62%, 81% & 100%) from the original formulation was investigated to evaluate the optimal gummy candy formulation using the Response Surface Methodology (RSM) based on the texture profile analysis response. Two optimal formulations were obtained from RSM with minimum hardness, lower firmness and stickiness and high springiness attributes. Those optimized samples are gummy candy with (seaweed 4%, water 100%, sugar 26%) and (seaweed 5%, water 81%, sugar 18%). Comparative analyses were conducted between control gummies and these two most optimized samples. Results revealed that seaweed addition significantly increased moisture (56.86%), ash (1.63%), and fibre (0.39%) content while reducing fat (0.04%), and carbohydrate (39.06%) levels. Sensory evaluation indicated that gummies with seaweed were less preferred compared to control samples, with differences noted in texture, taste, and overall acceptability. Mould growth was monitored over three weeks, revealing that all samples remained mould-free in refrigeration, though gummies with seaweed exhibited mould in 3 days. The findings highlight the potential of seaweed-enriched gummies as functional foods, emphasizing the need for further optimization to balance enhanced nutrition with sensory appeal and shelf stability.

Chapter 1: INTRODUCTION

1.1 Study Background

Seaweeds have a huge biodiversity in the coastal environment. Based on the colour of the algae, seaweed is classified as brown (phaeophycea), red (rhodophyta), or green (chlorophyta). The most prevalent kind of algae is red algae, which has 6000 species (Ahmad et al., 2018). The aquaculture of seaweed is a significant contributor to the Asian economy. In 2019, the Asian region accounted for 97% of the total 35.8 million tonnes of seaweed produced worldwide. Cultivation of the eucheumatoid seaweeds Kappaphycus and Eucheuma is a major contributor to the economy, food supply and rural livelihood in the ASEAN region (Lim et al., 2021). Malaysia, with its extensive coastline and suitable agroclimatic conditions, stands among the top global producers of seaweed. Sabah, particularly Kudat and Semporna, is the hub of seaweed cultivation, with species like *E. spinosum* and *Kappaphycus alvarezii* being farmed for their carrageenan content (FAO,2018).

Kappaphycus and *Eucheuma* are farmed for carrageenan as it is a hydrocolloid used in the food, beverage, and cosmetics industries. Hydrocolloids, a substances that yield a gel when mixed with water. Chemically, they are high molecular weight hydrophilic biopolymers. Together, they act as thickening, stabilizing, and gelling agents to raise quality and lengthen the shelf life of goods. In both the food and non-food industries (medicine, research, pharmaceutical, health, and livestock agriculture), these extracts find extensive use as an additive. Furthermore, those who follow vegetarian, vegan, or halal diets for health or religious reasons can utilize carrageenan as a substitute for bovine or porcine gelatin (FAO,2018).

Additionally, seaweeds also are gaining major attention due to the benefits they give to our health with high nutritional value. Seaweeds have been known to contain bioactive compounds that are derived from sulfated polysaccharides, polyphenols, carotenoids, proteins and lipids (Premarathna et al., 2022). Seaweed is also recognized as a powerhouse of vitamins and minerals that are highly beneficial to health. They have been used as edible commodities due to their ability to reduce the risks of many non-communicable diseases as seaweed demonstrates anti-inflammatory, wound healing, anti-cancer, anti-diabetic activities and anti-degenerative activities (Premarathna et al., 2022).

The confectionery industry, particularly the gummy candy sector, has seen increased demand for healthier, functional, and vegan-friendly products. Consumers now prefer healthier gummy candy with lower sugar content, and vegan-friendly components with the addition of vitamins, antioxidants, phenolic components. To meet such demands, some strategies are used to improve gummy candies formulations and create specific functional properties with incorporation of dietary fibre, bioactive compound and by using substitutes for gelatin (Šeremet et al., 2020). In this regard, some hydrocolloids and gelatin replacers such as carrageenan was successfully used in some studies for gummy candy products (Mandura et al., 2020).

To achieve this, response surface methodology (RSM) is employed to optimize gummy formulations. RSM is the most popular multivariate statistic technique, which has been used in the optimization of various processes. Optimization is the process of choosing the best element from a group of potential options or variables to satisfy a set of criteria (Mueller K., 2019). Response surface methodology (RSM) is one of the tools of optimization that examines the connections between some explanatory factors and one or more response variables through the proper Design of Experiment (DoE) (Karmoker et al., 2019). RSM is a collection of statistical and mathematical methods established on the fit of a polynomial model to the data that must depict the behaviour of a data set with the purpose of making statistical predictions. The approach is useful for optimizing, designing, developing, and improving processes where a response or responses are affected by several variables (Ghorbannezhad et al., 2016).

Thus, this study aims to find the optimized formulation of seaweed that will partially replace gelatin as a gelling agent while maintaining the texture of the gummy candy by using Response Surface Methodology (RSM) and determining the physicochemical characteristic and sensory of the optimized gummies.

1.2 Problem Statement

The increasing demand for healthier snacks has prompted the development of functional gummy candies enriched with natural ingredients like seaweed, known for its high fibre and mineral content. Moreover, texture plays a vital role in gummy candies because it influences their chewability and each consumer has different expectations regarding the chewability of gummies (Mahat et al., 2020). Therefore, optimizing well-designed gummy candies is important to meet consumer expectations, which include ingredient information about water, sugar, and gelling agent alternatives.

However, incorporating seaweed into gummy formulations poses challenges, including potential alterations in texture, nutrient composition, sensory acceptability, and shelf life. While seaweed enhances the nutritional value, it can affect the chewiness and firmness of the gummy, leading to consumer dissatisfaction. Thus, this study aims to optimize the formulation of seaweed as a viable alternative to gelatin in gummy candies and identify the properties of texture in particular, hardness, firmness, stickiness and springiness, produced with a selective amount of seaweed. From the previous study, it could be seen that the hardness attribute of gummy candy increased significantly with seaweed powder levels (6-8%) (Haziq, 2023). To reduce the hardness, the seaweed level used in this study was reduced to 4-6% to replace gelatin. Then, the impact of the seaweed ratio on gummy texture was assessed as the increasing amount of gelling agents more likely led to a gel-strengthening effect but did not excessively effect the texture of the gummy confectionary.

Additionally, the impact of seaweed on mould growth and overall product stability during storage remains underexplored. Addressing these issues is essential for creating a balanced gummy candy that meets consumer preferences for texture and taste while providing enhanced nutritional benefits.

1.3 Study Rationale

Response surface methodology (RSM) is used in this study to substitute gelatin in gummy candy with brown seaweed flour. By systematically optimizing the gummy candy formulation using RSM, it is possible to produce gummy candies with the desired texture characteristics while maintaining product quality when gelatin is substituted with brown seaweed flour. This kind of optimization is essential to preserve customer acceptability.

A proximate analysis of the gummy candies was conducted including the ash, protein, fibre and fat. The moisture, colour and texture profile analysis will be also conducted in this study. The texture profile analysis included the test of the firmness, springiness, hardness and stickiness of the gummy candies. This analysis provides valuable insights into the physical characteristics of the candies, highlighting their potential as a functional food product. Not only that, the sensory evaluation was also conducted to test the gummy candy's appearance, colour, aroma, softness, flavour, and general acceptability based on the preference of consumers. These tests are important to identify the quality of the gummy candies that is prepared by different formulation of seaweed, sugar and water. Thus, by doing this study, the preference of Malaysians regarding gummy candies will be seen through the test of gummies consumption.

1.4 Objective

1.4.1 General objective

To optimize the use of brown seaweed flour (*Kappaphycus alvarezii*) as a replacement for gelatin in gummy candy production using Response Surface Methodology (RSM).

1.4.2 Specific objective

1. To optimize the ratio of sugar, water and brown seaweed flour using Central Composite Design (CCD) of Response Surface Methodology (RSM)
2. To determine texture profiles and nutritional composition of gummy candy developed with brown seaweed flour to replace gelatin
3. To evaluate the sensory acceptability of brown seaweed flour as a replacement for gelatin in gummy candy
4. To observe the growth of visible mould on gummy candy samples stored under controlled refrigerated and room temperature conditions over a period of 3 week

1.5 Research questions

1. What is the optimal ratio of sugar, water and brown seaweed flour for gummy candy production?
2. What are the differences in the texture profiles and nutritional composition between gummy candy with brown seaweed flour and gelatin?
3. What is the acceptability of consumers of brown seaweed flour as a replacement for gelatin in gummy candy?
4. What is the effect of different ratio of seaweed, water and sugar on visible mold growth in gummy candies during 3 weeks of storage under different temperature conditions?

1.6 Hypothesis

Alternative hypothesis

There is a significant difference in the proximate, texture and sensory attributes of gummy candy produced using brown seaweed flour as a replacement for gelatin.

Null hypothesis

There is no significant difference in the proximate, texture and sensory attributes of gummy candy produced using brown seaweed flour as a replacement for gelatin.

CHAPTER 2: LITERATURE REVIEW

2.1 Seaweed (*Kappaphycus alvarezii*)

Marine macroalgae, widely known as seaweeds, can be classified into many classes and families. It covers 90% of plant species that grow in the sea and serves as a critical food source in the food chain. Seaweed grows luxuriantly in coastal waters, on rocky sea fronts or float freely, rather than on sand or gravel. In some areas, coastal seaweed colonies can extend for miles to the sea (Pereira, 2021). Seaweeds have blades, stipes, and holdfasts, but no actual stems, roots, or leaves. Despite their appearance as plants, seaweed belongs to Eukaryota domain, the most complex organism in the algae family. There are three main taxonomic groups of macroalgae, Chlorophyta (green algae), Rhodophyta (red algae) and phylum Ochrophyta, class Phaeophyceae (brown algae).

Marine macroalgal resources are gaining importance as one of the promising feedstocks to produce food, feed, chemicals and energy which are upwardly growing worldwide because of their easy cultivation (Baghel et al., 2020). Furthermore, it is worth mentioning that seaweed cultivation is well aligned with the principles of sustainable production since seaweed can mitigate around 20 tons of CO^2 per hectare per year, there is no need for freshwater, and no competition for land, concerning food production (Hargreaves et al., 2013). Besides, the cultivation of algae species promotes advantages such as absorbing carbon dioxide through the process of photosynthesis. This capture of carbon dioxide can help minimize the greenhouse effect (Nigam & Singh, 2011). Moreover, algae produce more oxygen than consume in contrast to terrestrial plants (Wei et al., 2013).

Kappaphycus alvarezii, also known by its commercial name *Eucheuma cottonii*, is a class of red seaweed that can grow up to two meters long and is one of the biggest tropical red macroalgae, 6000 diverse species which is the highest among all three types of seaweeds. It has the highest growth rate among other Kappaphycus seaweeds

(Mohammad et al., 2019). Red seaweed is a eukaryotic alga and the brown-red colour comes from a pigment called phycoerythrin, pycocyanin and chlorophyll A. They have vast benefits to humans, for instance, as a food source because they are rich in vitamins, minerals, calcium, magnesium and many more (Shafie et al., 2022). *Kappaphycus alvarezii* is one of the species abundantly found in East Malaysia as it is highly demanded for its cell wall polysaccharide (kappa carrageenan) (Chang et al., 2017).

2.1.1 Seaweed as a Replacer of Gelatin

The major part of harvested seaweed is extracted to produce tones of polysaccharide isolates (hydrocolloids). *Kappaphycus alvarezii* is the major industrial source of unique polysaccharides, in particular carrageenan and agar, accounting for up to 40-50% of the dry weight (Torres et al., 2019). These are the most common polysaccharide isolates of seaweed as they are commonly used in the food industry for various purposes such as stabilizing and gelling agents to maintain the structure of food, the same functions of gelatin with gelling abilities.

Carrageenan is a mixture of water-soluble, linear, sulfated galactans. It is formed by alternate units of d-galactose and 3,6-anhydro-galactose (3,6-AG) linked by α-1,3- and β-1,4-glycosidic linkages. (Bagal-Kestwal et al., 2019). Carrageenan can be classified as lambda (λ), kappa (κ), or iota (i) according to the gel-forming ability. *Kappaphycus alvarezii* is a kappa carrageenan source. K-carrageenan is a gel-forming carrageenan which in ionic solutions and in the presence of salts, usually potassium ions, k-carrageenan will form a strong, rigid gel (Husin, 2014). The structure of kappa carrageenan is a right-handed double helix of parallel chains. This structure allows kappa carrageenan to form its durable thermos-reversible gels by itself.

The production of carrageenan gums involves a few processes like cleaning the half-dried seaweed by washing, alkaline extraction then filtrations, freeze-thaw cycles and lastly, drying grinding as well as blending. One of the properties is that it can form different kinds of gels at room temperature due to the structure (Shafie et al., 2022). Due to its biocompatibility, mechanical strength and high-water retention, kappa carrageenan can act as a gelling, thickener and stabilizing agent that is vital for its use in the food industry. Therefore, k-carrageenan is used for the production of confectionery, ice cream, cheese, jam and bread as a gelling agent (Rudke et al., 2020). The chewy texture of candy can be produced by adding ingredients that contain gelling agents such as carrageenan. Carrageenan from seaweed also can be an alternative to gelatin as it is a plant-based gelatin. Due to gelatin being animal-derived (porcine and bovine) is not suitable for some individuals following vegetarian or certain religious dietary restrictions.

2.1.2 Nutrition Composition of Seaweed

Seaweed, consumed mainly by Asian people, is one of the largest producers of biomass in the marine environment and is rich in bioactive compounds. It has now been discovered as a nutrient-dense food as they are good source of carbohydrates, vitamins, essential minerals and dietary fibre (Shannon & Abu-Ghannam, 2019). Seaweed contains a complete source of nutrition in varying amounts. The content of can vary due to differences in species, location and seasonal temperature, condition of harvest, and age of harvest.

Seaweeds also possess a low lipid content, nonetheless enriched in polyunsaturated fatty acids (Lomartire et al., 2021). Fat content can vary from 1 - 6%, while the fibre and protein content range from 33 - 50% and 5.6 - 24%, respectively. The essential amino acid content of seaweed is considered high (45 - 49%) compared to the total amino acid content. Seaweeds are a natural source of macro (Na, Ca, K, Mg, S, Cl and P) and microelements (I, Zn, Cu, Se, Ni, Co, B and Mn) and contain plenty of iodine which has an important role in preventing goitre disease in humans (Pati et al., 2016).

Seaweed contains polyphenols and sulfated polysaccharides which are regarded as preventive agents against lifestyle-related diseases as this compound offers a wide range of physiological functions such as antioxidant, anti-allergenic, anti-cancer, anti-atherogenic, anti-thrombin, anti-coagulant, anti-inflammatory properties, and cardioprotective (Liu et al., 2019). The incorporation of seaweed in the food matrix has been found to improve the physicochemical and nutritional properties, and technological aspects of foods (Mohammad et al., 2019)

2.2 Gelatin

Gelatin is a high molecular weight and water-soluble polypeptide which is made of hydrolytic degradation of protein from collagen. It is a collagen-hydrolyzed substance that is the main protein in certain mammalian species hides, smooth connective tissues and bones. Gel strength and viscosity are the most important physical properties of gelatin. The quality criteria of the finished products are influenced by factors such as water content, temperature, pH, glass transition temperature, molecular weight and natural gelling capabilities of gelling agents. Gelatin's solubility increases with increasing temperature. When gelatin is dissolved in water at around 35–45°C and cooled slowly it will form a gel. The gel obtained from gelatin is thermoreversible.

Gelatin is classified into 2 types based on the extraction process. Type A gelatin is produced via acid extraction, while type B gelatin is produced via alkaline extraction (Luo et al., 2022). Besides, it also can be obtained from partial hydrolysis of collagen. Gelatin is nearly tasteless, colourless, translucent, dense and dried (Tukiran et al., 2023). As a source of protein-matrix, it has a high protein content in certain nutritionally balanced foods. Chemically, gelatin is made up of 18 varieties of complex amino acids, 57% of glycine, proline and hydroxyproline are the major compounds, while the remaining, 43% are other distinguished amino acid families such as glutamic acid, alanine, arginine, and aspartic acid (Sultana et al., 2018).

2.2.1 Application of Gelatin in Food Products

Gelatin is a key component of the food industry in modern cuisine owing to its gelling capabilities. Manufactures gourmet desserts using gelatin for texture, foaming and clarity as well as stabilizing the food structure. For canned meat products such as sausages, frankfurters, loaves and hams, gelatin has also been used to retain juices lost and to provide a good heat transfer medium during cooking (Alipal et al., 2021). Gelatin is also used in dairy products. Gelatin could enhance the water-holding capacity without increasing the firmness of acid milk and make it an ideal stabilizer in acid milk products, as its allows for better moisture retention without compromising the texture of the milk. The gelatin also acted as a stabilizer in preventing crystal formation and improving the overall texture of the ice cream (Ahmad et al., 2023).

Since gelatin exhibits good water binding, gel-stabilizing, and even ice crystal inhibition capabilities, it may be applied in bakery products to provide improved structure and texture (Ahmad et al., 2023). Pigskin gelatin formed a highly resistant gluten structure against the distortion of ice crystals, embodying a larger bread volume and more uniform bread crumb (Yu et al., 2020). This mechanism plays a crucial role in retarding the deterioration of bread quality over time. The gelatin acts as a barrier, preventing the migration of water within the bread and inhibiting the formation of new molecular structures that contribute to the hardening of the breadcrumb (Ahmad et al., 2023).

2.3 Gummy Candies

Gummy candies are confectionery products with a gel-like structure, which are commonly composed of fruit extract, sugar, acids, aroma, food colourants, flavouring and gelling agents such as gelatin (Teixeira-Lemos et al., 2021). Due to the chewy texture and as a carrier to incorporate essential vitamins into daily diets, gummy candies were well-liked by children under seventeen (Ng et al., 2023). Gummy candy recipes are typically developed by experienced food technologists and chemists. By blending different ingredients, they can control the various characteristics of gummy candy, such as texture, taste, and appearance. The primary ingredients include water, gelatin, sweeteners, flavours, and colours (Charoen et al., 2015).

The production of gummy candy generally involves several steps: mixing, cooking, cooling, shaping (or starch molding), conditioning, molding, coating, and packaging. Processes can vary from plant to plant in the manufacturing of gummy candies. The first step is the mixing of the main ingredients which are sucrose, glucose syrup, water, and polymer (or gelling agent) solution (Tireki et al., 2023). Polymer solution is prepared by the hydration of polymers in the water for a few hours. After the mixing step, cooking of the slurry takes place and after the slurry is cooled after cooking, acids, flavourings, and colours are added. Then, the slurry is deposited into starch molds and conditioned until the correct texture is achieved. Gummy candies are molded after conditioning, they are coated with glazing agents, such as carnauba wax, and as the final step candies are packaged (Tireki et al., 2023).

2.3.1 Gummy Candies as Healthy Food

In recent decades, consumer demand in the field of food production has changed significantly, as they have become increasingly aware of food quality and the health benefits associated with different food products. Meanwhile, in general perception, gummy candies are types of soft confectionery products with high amounts of sucrose and glucose syrup along with artificial additives that are not good for general health. However, the development of some new formulations can provide desirable health benefits and pleasant sensory (Tarahi et al., 2023).

Reducing or replacing sugar with alternative sweeteners might represent healthier alternatives for gummies. One of the most popular alternative sweeteners is honey. It is a natural sweetener with a unique flavour profile and utilizing honey in gummy candies may create confectionery products with natural, nutrient-rich and potentially beneficial options (Tarahi et al., 2023). One study examined the effect of honey and sucrose on the glycemic response to gummy candies. The results indicated that gummy candies sweetened with honey had a lower glycemic index, which suggests a potentially more favorable effect on blood sugar levels, making them a suitable option for individuals monitoring their glucose levels (Rivero et al., 2019).

The use of natural flavours and colourants like oranges, strawberries and other red fruits has been considered for the manufacturing of gummies. These can not only improve the organoleptic properties (colour, flavour and texture) of gummies but also produce healthier formulations with antioxidant properties (De Moura et al., 2019). The berry fruits used in the formulations, which included strawberries, raspberries, and blueberries, contain high amounts of anthocyanins and other phenolic compounds with antioxidant activity. Dietary antioxidants can also help in the maintenance of oral health and might influence periodontal disease management, potentially improving clinical outcomes (Teixeira-Lemos et al., 2021). Gummy candies also can act as a carrier to incorporate essential vitamins and minerals into daily diets, which are gaining much more attention these days due to their favorable, taste and structure, especially for children (Tarahi et al., 2023).

2.4 Optimization of Formulation using Response Surface Methodology (RSM)

Response surface methodology (RSM) is a statistical method that is used to optimize the level of components and process conditions (Sravani et al., 2023). The objective is to optimize a response that is influenced by several independent variables (Chelladurai et al., 2021). RSM was used to optimize the process parameters and develop a regression equation to predict a response. It can assess the effect of various parameters and their inter-relations on dependent variables and can be used to fit a quadratic polynomial model equation by selecting various design experiments (Sravani et al., 2023).

The experiment design consists of several levels, the two levels, 2^k and three-level, 3^k design evaluates each factor at low, -1 and high +1 setting, while the three-level, 3^k design also considers the factor at the center, 0. The experiment design uses coded variables, which $x_1, x_2, \ldots, x_n$ will be centered on 0 and extend at +1 and -1 from the center region of the experiment. The natural and actual unit is then centered and rescaled to the range from +1 to -1. There are several types of design including full, fractional factorial, Central Composite Design (CCD), Box-Behnken, Etc.

It is always been a tedious task to choose a suitable experimental design, which can easily explain many response variables. Such variables often end as a quadratic surface model. For such kind of interpretation, CCD or known as The Box and Wilson design can be an excellent choice (Bhattacharya, 2021). CCD can fit a full quadratic model. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points, also called star points that can estimate curvature. CCD can be used to efficiently estimate first- and second-order terms and model a response variable with curvature by adding center and axial points to a previously-done factorial design.

Three independent variables will be selected for evaluation, namely: seaweed ratio (%), water ratio (%), and sugar ratio (%). Four responses of textural profile variables will be considered for optimization: hardness, firmness, springiness, and stickiness. These parameters are employed to establish the response variables and the independent variables for optimizing the formulation using the RSM approach.

2.5 Development and Optimization of Seaweed-Based Gummy Candies: Nutritional Properties, Microbial Stability, and Sensory Acceptability

In the study on seaweed gummy candy using Response Surface Methodology (RSM), the optimal formulation was found to enhance the textural properties of the candy while increasing its fibre and mineral content (Haziq et al., 2023). Similarly, the incorporation of k-carrageenan in gummy guyabano candies enriched the product with higher carbohydrate, crude fibre, and ash content, while reducing fat content (Minguito, 2023). These nutritional enhancements make seaweed-based candies a healthier snack alternative.

A critical aspect of gummy candy development is ensuring microbial stability, particularly against mould growth. Seaweed-based gummies, with their high moisture content and nutrient composition, can be susceptible to microbial contamination if not processed and stored properly. Research on thin candy made from green seaweed (*Caulerpa* sp.) highlighted the risks of mould growth due to the production of hydrolytic enzymes by moulds, which focus on carbohydrate and protein-rich food (Fransiska et al., 2020). Mitigation strategies include reducing water activity, incorporating preservatives such as potassium sorbate, and optimizing storage conditions to prevent mould proliferation. These findings underscore the importance of balancing nutritional benefits with microbial safety.

The sensory attributes of seaweed-based gummy candies play a crucial role in consumer acceptability. Studies indicate that incorporating seaweed enhances the texture, elasticity, and chewiness of gummy candies. For instance, the addition of κ-car in guyabano gummy candies at an optimal concentration (3%) resulted in the most favourable sensory attributes, including taste, texture, and aroma (Minguito, 2023). Similarly, RSM optimization in seaweed gummy candy formulation achieved a desirable balance of firmness, springiness, and low stickiness (Haziq et al., 2023). However, excessive seaweed content may introduce undesirable sensory qualities, such as cloudiness or a seaweed-like taste, which can impact consumer preferences.

CHAPTER 3: MATERIAL AND METHOD

3.1 Study Design

The type of study that applied was an experimental study. This study involved a control sample and 20 experimental samples according to Central Composite Design (CCD) Response Surface Methodology (RSM) using Design-Expert software (v.7.0.0, State-Ease, Inc., Minneapolis, USA.) Three independent variables had been chosen, which are, brown seaweed flour ratio (X1), water ratio (X2) and sugar ratio (X3) for the response: hardness, firmness, stickiness and springiness to produce gummy candy with the best texture. The control sample is the gummy candies without the incorporation of seaweed flour (0%). In comparison, the experimental samples were gummy candies incorporated with seaweed flour ranging from 4 to 6% that replaced the gelatin from the original formulation. In contrast to previous research (Haziq, 2023), the seaweed range used in this study was smaller to assess the impact of the seaweed ratio on gummy hardness. The coded levels and actual values of the independent variables are presented in Table 1.

TABLE 3.1 CCD response surface set for low, centre and high limit for all factors for optimization of seaweed gummies

Independent variables (factors)	**Symbols**	**Coded levels**		
		-1	0	1
Seaweed	X1	4	5	6
Water	X2	62	81	100
Sugar	X3	10	18	26

There are 20 experiments involved in this Central Composite design for the formulation of gummy candy samples containing different ratios of seaweed powder, water and sugar. All experiments with different combinations of all factors are shown below (Table 3.2).

Standard order	Run order	Seaweed Ratio, %	Water Ratio, %	Sugar Ratio, %
1	17	5	81	18
2	9	4	81	18
3	16	5	81	18
4	10	6	81	18
5	18	5	81	18
6	5	4	62	26
7	15	5	81	18
8	20	5	81	18
9	6	6	62	26
10	7	4	100	26
11	2	6	62	10
12	1	4	62	10
13	13	5	81	10
14	11	5	62	18
15	3	4	100	10
16	12	5	100	18
17	4	6	100	10
18	14	5	81	26
19	8	6	100	26
20	19	5	81	18

TABLE 3.2. Treatments involved with different combinations of factors and responses according to CCD of experiment

The effect of texture profile of different treatments with different percentages of seaweed ratio (where gelatin being replaced partially with seaweed powder) was also compared with control gummy candy (100% gelatin).

3.2 Subject Criteria For Sensory Evaluation Test

Inclusion criteria

- Consume gummy candies
- Student and staff of School of Health Sciences, Health Campus, Universiti Sains Malaysia
- Age 18 years old and above

Exclusion criteria

- Unhealthy
- Allergic or sensitive to seaweed or gelatin

3.3 Sample Size Estimation

The estimated sample size for sensory evaluation is 30 (Aminah et al., 2000).

3.4 Sampling Method

The panels were recruited by using the convenience method. The advertisement for the sensory evaluation was disseminated on a platform such as social media, WhatsApp and e-mail for recruitment purposes. The first 30 volunteers of undergraduate students and staff from the School of Health Sciences who are interested in participating in the sensory evaluation and meet all the inclusion criteria, and absence of exclusion criteria, were selected or recruited.

3.5 Data Collection Method

20 formulations of gummy candy samples containing different ratios of seaweed powder, water and sugar of gummy candies were prepared in the food preparation laboratory and stored in the nutrition laboratory, at the School of Health Science. Each of the samples needs to be examined for its texture profile to find the two most optimized samples.

The two most optimized and control samples were transferred into a zip-lock plastic and stored at room temperature and in the refrigerator. The mould growth was observed with the naked eye within 3 weeks. Then, these three samples were examined for their nutritional composition. Moreover, each of the samples were also undergo a sensory evaluation assessment.

3.6 Preparation of Raw Materials

In this study, seaweed is used. Approximately, 1 kg of brown seaweed (*Kappaphycus alvarezii*) flour is directly obtained from Imtanomic Sdn Bhd (ISB). The flour was directly weighed and used to replace gelatin in gummy formulation according to the experiments set of CCDs as tabulated in Table 3.2. Other dry ingredients also be bought at the nearby supermarket in Kubang Kerian.

3.6.1 Preparation of gummy candies

List of ingredients of control gummy candies (gelatin) is shown in Table 3.6 (Haziq, 2023).

TABLE 3.6 List of ingredients of gummy candy

No	Ingredients	Quantity
1	Gelatin	15g
2	Water	120ml
3	Sugar	50g
4	Golden syrup	2.5ml
5	Lemon juice	5ml
6	Colouring (maroon)	1ml
7	Flavouring (strawberry)	1ml

Procedure

For the control candy, 15g of gelatin was added together with 60 ml of water into a cooking pan and placed on a cooking stove and set to low heat. The solution was stirred for about 45 seconds until it turned porridge-like. Next, 60ml of water, 50g of sugar, 2.5ml of golden syrup, 5.0ml of lemon juice and 5.0ml of colour and flavours were added altogether into the pan. Then, the mixture was stirred on low heat until it boiled. The solution was transferred into a mould and let cool inside the refrigerator for 1 hour. Exactly, 20 formulations were prepared with different combination ratios of seaweed, water and sugar as shown in Table 3.2.

3.7 Proximate Analysis

3.7.1 Determination of moisture content

The air-oven method was used to detect moisture, the empty dish will be dried overnight in an oven at 105^0C. After overnight, it was placed in a desiccator to chill before being weighed. 5g of the homogenized sample was weighed and dried overnight at 105^0C in an oven. After drying, the dish containing the sample was placed in the desiccator to cool until a consistent weight was achieved. The moisture content of the sample was calculated as the difference between the constant end weight and the beginning weight after drying. The following formula was used to calculate moisture content.

$$\text{Moisture } (\%) = \frac{w1 - w2}{w1} \times 100$$

$$\mathcal{W}1 = weight\ (g)\ of\ sample\ before\ drying$$

$$\mathcal{W}2 = weight\ (g)\ of\ sample\ after\ drying$$

3.7.2 Determination of ash

In determining the ash content, the crucible was oven-dried at 105ºC for three hours to ensure that impurities on the crucible were burned off and cooled in the desiccator immediately after drying until it reached room temperature. The crucible was weighed (W1), and 0.5 g of gummy candies samples were placed into the crucible (W2). The dried sample was charred until it ceased smoking by using an electric coil heating rack. The crucible was placed in the cold muffle furnace and heated at 550°C until whitish or greyish ash was obtained. The crucible was removed and cooled down in the desiccator until it reached room temperature. After cooling down, the crucible with the ash was weighed (W3). Then, the total ash content was calculated by using the formula:

$$\text{Ash (\%)} = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

3.7.3 Determination of protein

Kjeldahl method was used in determining the protein content.

Reagents preparation

Sodium hydroxide, NaOH (40% w/v)

400g of NaOH were dissolved in 700 ml of distilled water in a volumetric flask with 1000 ml volume. The content was diluted to 1000 ml with distilled water.

Boric acid solution (4% w/v)

40g of boric acid was dissolved in a 1000ml volumetric flask that was halfway filled with distilled water. The content was diluted to 1000ml by using distilled water.

Hydrochloric acid, HCI (0.1N)

8.4ml of hydrochloric acid (HCI) was added into a 1000ml volumetric flask that was halfway filled with distilled water. The hydrochloric acid was diluted by volume up the solution until 1000ml mark by using distilled water.

Procedure

There are three steps involved in protein content determination which are digestion, distillation, and titration. For digestion, 1.000 ± 0.001 g of the sample was weighed and put into a Kjeldahl digestion flask. Two tablets of selenium catalyst and 20ml concentrated sulphuric acid were added into the digestion flask including four blank flasks without sample. Then, the mixture was heated in an inclined position on an electric coil heating rack in a fume cupboard. The Turbosog scrubber unit was turned on to remove and neutralize the acid fumes. Then, the mixture was heated gradually until the temperature achieved 400°C and a clear solution was observed. The flasks were cooled to room temperature after digestion was completed. The procedure was continued with the distillation process by using the Vadopest distillation unit. After setting all the information, the digestion flask was put into the unit one by one together with the receiver flask. The steam was passed through the distillation unit and ceased after three to four minutes. The content in the receiver flask was titrated until it became the original purplish colour by using 0.1N hydrochloric acid which will be delivered from the burette. All of the steps were included for blank as the blank is used for comparing the colour of the end product. The protein content was measured by using the nitrogen conversion factor of 6.25. The percentage of protein was determined by using the formula:

$$\% \text{ Protein } = \frac{(ml\ HCL - ml\ HCL\ blank) \times 14.008 \times 0.1 NHCL \times protein\ factor}{Weight\ in\ mg\ of\ the\ sample} \times 100$$

3.7.4 Determination of fat

The fat content of the gummy candies was measured by using the Soxhlet method. 3.000 ± 0.01g of sample were weighed and put into the cellulose thimble (W1). The thimbles are then connected to the thimble adapters of the Soxhlet extractor unit. The extraction vessels were dried in an air oven at 105°C for 3 hours and cooled in a desiccator. The vessels were weighed after they had attained room temperature (W2). 80 ml of petroleum ether was added to each of the extraction vessels and placed into position on the heating plate. There are three positions for the process of determining fat content. Firstly, the knob was placed at “immersion” position to immerse the thimble in the petroleum ether. After 30 minutes, the knob was changed to a “washing” position to remove the thimble from the solvent. After 30 minutes, the knob was switched to the “recovery” position and the stopcock was switched off. The step lasted for 45 minutes. Then, the collecting vessel was dried in the oven for 30 minutes to remove any excess petroleum ether. After drying, the collecting vessel was cooled in a desiccator and the weight was measured. The fat content was calculated by using the formula:

$$\text{Fat } (\%) = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3.7.5 Determination of crude fibre

The crude fibre content in gummy candies was measured by using the Weende method. The pre-dried crucible was placed on a balance and tare and 1.000g of Celite 545 was weighed. Then, 1.000g of sample in a crucible (W1) was weighed and placed in the crucible stand. The crucibles were positioned in the Fibertec Cold Extraction Unit and the valves were closed. 25 ml acetone was added to each crucible. Soak for 5 minutes and the solvent was filtered out by placing the valve in a “vacuum” position. The step was repeated three times. The “MAINS” button and button for R1 to heat reagents were pressed. Next, the crucibles were transferred using the holder and locked into position in front of the radiator in the Fibertec Hot Extraction Unit ensuring that the safety latch was engaged. 150 ml of pre-heated 1.25% H2SO4 was added into each column (R1) and 4 drops of n-Octanol were added to prevent foaming and the “HEATER” control was turned on clockwise. When the reagents started to boil, the moderate boiling was adjusted using the “HEATER” control and ‘START’ button was pressed and the boiling time was 30 minutes. At the end of the extraction, the heater was turned off. Then, the “vacuum” button was pressed to start the filtration and reversed pressure was used to wash the sample. Next, 150 ml of pre-heated 1.25% NaOH (R2) was added. The crucibles were released with the safety hook and the crucibles holder was used to transfer the crucibles to the Fibertec Cold Extraction Unit. The crucibles the positioned in the Fibertec Cold Extraction Unit and the valves are closed. 25 ml acetone was added to each crucible. Soak for 5 minutes and filter the solvent out by placing the valve in a “vacuum” position and repeating it three times. The crucible was removed and transferred to a crucible stand. Then leave at room temperature until the acetone has evaporated and the crucibles were dried at 130°C for 2 hours. The crucibles were cooled to room temperature in a desiccator and weighed (W2). The sample was ash in the crucibles for at least 3 hours at 525°C. The crucibles were cooled slowly to room temperature in a desiccator and weighed (W3) The crude fiber percentage was calculated by using this formula: