
# The Effect of Addition Two Different Thickening Agent (Gelatine and Carrageenan) for Shelf Life and Physicochemical Properties In Grass Jelly

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Faculty of Agro Based Industry

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## DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

_____

Student

Name: Nurul Atikah Binti Ismail

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I certify that the report of this final year project entitled "The Effect of Addition Two Different Thickening Agent (Gelatine and Carrageenan) for Shelf Life and Physicochemical Properties In Grass Jelly" by Nurul Atikah Binti Ismail, matric number F15A0182 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro-Based Industry, University Malaysia Kelantan.

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| | |
|---|---|
| ° C | Degree celcius |
| ± | Plus minus sign |
| % | Percentage |
| g | Gram |
| kg | Kilogram |
| ml | Milli letter |
| nm | Nano meter |
| SPF | Sweet Potato Flour |
| CF | Corn Flour |
| ANOVA | Analysis of Variance |
| SD | Standard Deviation |
| CFU | Colony Forming Unit |
| L* | Lightness Colour |
| a* | Redness Colour |
| b* | Yellowness Colour |
| SPSS | Statistical Package for the Social Science |
| TPA | Texture Profile Analysis |

**THE EFFECT OF ADDITION TWO DIFFERENT THICKENING AGENT (GELATINE AND CARRAGEENAN) FOR SHELF LIFE AND PHYSICOCHEMICAL PROPERTIES IN GRASS JELLY**

## ABSTRACT

Grass jelly agar and drink had been popular because of their nutrition especially high in dietary fibre and carbohydrates. There are some problem that can reduces consumer preferences and perception. This research was conducted to study the physicochemical analysis and shelf life in grass jelly made with two different thickening agent which are gelatine and carrageenan. This research was to determine appropriate percentage of addition gelatine and carrageenan. This study also conducted to improve the texture of grass jelly by addition of gelatine and carrageenan. Physicochemical properties which are pH, brix, colour and texture was analyse by weekly until week 4. Furthermore, shelf life of grass jelly with addition gelatine and carrageenan also will be evaluated. Grass jelly agar will be prepared with different percentage which are addition of gelatine 1%, 2%, 3% and 4% while addition of carrageenan 6%, 7%, 8% and 9%. The samples will be kept at constant temperature which is 4 $^{0}$C for 3 months. In order to test the shelf life, the parameters including pH, texture, colour and concentration of brix at week 1 will be determined, to select the best percentage of gelatine and carrageenan for microbiological analysis. The shelf life will be tested within 3 months on weekly basis and all parameters will be test weekly. Based on the result, shelf life of grass jelly addition with gelatine is much longer than carrageenan. Physicochemical analysis showed that grass jelly with addition of gelatine at the percentage 3% and addition carrageenan at 7% is the most preferable sample. In future study, another thickening agent can be used to replace with gelatine and carrageenan for another analysis.

Keywords: shelf life, grass jelly, gelatine, carrageenan, parameter

**KESAN PENAMBAHAN DUA AGEN PENEBALAN (GELATINE DAN KARRAGEENAN) UNTUK JANGKA HAYAT DAN SIFAT FIZIKOKIMIA DALAM AGAR JELI**

## ABSTRAK

Agar jeli dan minuman jeli telah popular kerana nutrisi mereka terutama tinggi dalam serat makanan dan karbohidrat. Terdapat beberapa masalah yang boleh mengurangkan pilihan dan persepsi pengguna. Oleh itu, kajian ini dijalankan untuk mengkaji analisis fizikokimia dan jangka hayat cincau yang dibuat dengan dua agen penebalan yang berbeza iaitu gelatine dan karragenan. Kajian ini bertujuan untuk menentukan peratusan tambahan gelatin dan karagenan. Kajian ini juga dilakukan untuk meningkatkan tekstur cincau dengan penambahan gelatin dan karagenan. Selain itu, sifat fizikokimia iaitu pH, brix, warna dan tekstur dianalisis secara mingguan selama minggu ke-empat. Jangka hayat agar jeli dengan tambahan gelatine dan carrageenan juga akan dinilai. Agar jeli akan disediakan dengan peratusan yang berbeza iaitu penambahan gelatin 1%, 2%, 3% dan 4% manakala penambahan karragenan 6%, 7%, 8% dan 9%. Sampel akan disimpan pada suhu malar iaitu $4^0$C selama 3 bulan. Untuk menguji jangka hayat, parameter termasuk pH, tekstur, warna dan kepekatan brix pada minggu 1 akan ditentukan, untuk memilih peratusan terbaik gelatine dan karragenan untuk analisis mikrobiologi. Jangka hayat dalam masa 3 bulan setiap minggu dan semua parameter akan diuji setiap minggu. Berdasarkan hasilnya, jangka hayat cincau dibuat dengan gelatine jauh lebih panjang daripada karragenan. Analisis fizikokimia menunjukkan bahawa cincau dengan penambahan gelatin pada peratusan 3% dan tambahan karragenan pada peratusan 7% adalah sampel yang paling disukai. Dalam kajian masa depan, satu lagi agen penebalan boleh digunakan untuk menggantikan dengan gelatine dan karrageenan untuk analisis yang lain.

Kata kunci: jangka hayat, agar jeli, gelatin, karrageenan, parameter

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Grass jelly (*Mesona chinensis*) or known as cincau in Indonesia is jelly-like dessert mostly popular served as sweet dessert in Hong Kong, Mainland China and Southeast Asia. According to Sasmita and Ling (2017), extraction of grass jelly in traditional way has been used as herbal drinks to promote health and vitality. Phenolic compounds extracted from grass jelly significantly contributed to the antioxidant activity and free radical scavenging effects (Yen & Hung, 2000).

Physical preferences including texture is the most important character of grass jelly and gelling agent that is gelatine and carrageenan make the texture more desirable and meet consumer demand. In addition, Yuris, Goh, Hardacre, and Matia-Merino (2017) reported grass jelly categorized among the less common polysaccharide fraction and show an interesting interaction with starch. The polysaccharides usually act to improve food functionality.

Extraction of grass jelly is black in colour and just has a low viscosity and when has been heated and cooled, its cannot form own gel (Yuris et al., 2017). The grass jelly in market usually has a longer shelf life and maintain in shining texture. Unfortunately,

the quality and cannot be stored in longer period of time. Thus, the objective in this research study is to determine the shelf life of grass jelly made with gelatine and compared it with commercial grass jelly drink.

## 1.2 Problem Statement

Physical properties are the main subject that needs to be maintained for grass jelly products. The production of the grass jelly must be hygienic and in a good quality as preferred by consumers. The influence to choose the food product is based on the quality and condition of the food and beverage products. Contamination from microorganisms, enzyme and pathogen can be the main distributor of food spoilage thus, affecting the shelf life of grass jelly. Nowadays, people are more concerned to consume healthier and organic product that had higher demand to avoid synthetic preservative. Grass jelly produced locally by a small and medium-sized enterprises (SMEs) using sweet potato flour and corn flour demonstrated shorter shelf life compared to commercial grass jelly. The addition of gelatine and carrageenan may improve the texture of the grass jelly but no information regarding the shelf life. Therefore, this study was conducted to analyse the physicochemical analysis of grass jelly made with gelatine and carrageenan stored up to three months.

## 1.3 Hypothesis

Shelf life of the grass jelly made with gelatine and carrageenan is different from the commercial grass jelly drink. It is hypothesized that: -

$H_0$: Grass jelly made with gelatin and carrageenan have longer shelf life compared to commercial grass jelly drink

$H_1$: Grass jelly made with gelatin and carrageenan have shorter shelf life compared to commercial grass jelly drink.

## 1.4 Objectives

The objectives of this study are:

1) To determine the appropriate concentration of thickening agent, gelatine and carrageenan in making of grass jelly
2) To analyse physicochemical properties of grass jelly from two different thickening agent which are gelatine and carrageenan
3) To evaluate the effect of shelf life in grass jelly with gelatine and carrageenan during storage at 4 $^0$C

## 1.5 Scope of Study

The scope of this study is to develop a better quality grass jelly. This study also conducted to look for the shelf life of grass jelly made with gelatine and carrageenan as the thickening agent compared to commercial grass jelly drink. In this study, the parameter that had been analysed is the percentages of thickening agent, gelatine 0%, 1%, 2%, 3%, 4% and for carrageenan 6%, 7%, 8% and 9% respectively. The timeline for testing the shelf life was three months. The constant parameters are storage temperature and sugar syrup concentration. The shelf life was testing by using spread plate technique.

## 1.6 Significance of Study

This research was conducted to determine the shelf life of grass jelly made with gelatine and carrageenan. Throughout this study, the shelf life of grass jelly samples had been compared to a locally produced grass jelly, and commercial grass jelly drink available in the market. This research was identify whether the addition of gelatine and carrageenan may increase the grass jelly shelf life over time without the addition of preservatives compared to the locally produced grass jelly.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Grass Jelly and Its Application

Grass jelly is also known as cincau which is mean by green grass in Chinese. There are various plants used to make grass jelly including green grass jelly (*Cyclea barbata*), black cincau (*Mesona palustris*) and cincau perdu (*Melastoma polyanthum*). Alternative name of grass jelly is leaf jelly. According to Laurent (2013), grass jelly had it other names such as *chao kual* in Thailand and *xian cao* in Chinese. In manufacturing sector, black grass jelly is manufactured in the form of instant powder like other jellies and agar. The powder form is easier to use.

There are many types of grass jelly form in the market such as grass jelly powder, grass jelly drink and grass jelly desserts. Different place have different way to serve the grass jelly. In Asian country, grass jelly is available as a beverage or mixed with a juice. Differ from China, grass jelly commonly served with evaporated milk or sugar syrup. People love to put the grass jelly in beverages like soy drink or "bandung" drink as accessories to the drink. It is a healthy drink because of the benefits content inside the grass jelly. As it is known in recent years, black grass jelly become more popular within public because of its benefit and uses.

### 2.1.1 Morphology of Grass Jelly

*Mesona chinesis* is a type of plants used to make black grass jelly. It belongs to the genus *Platostoma* of the mint family. *Mesona chinesis* also known as *Plastoma palustre*. This species is cultivated on flat ground or areas with slight slope. *Mesona chinesis* grows extensively in East Asia such as south east China and Taiwan. The grown of this species are prefers ravines, grassy, dry and sandy area for growth. The plants are from 15-100 cm high with hairy stems and leaves. The leaves are tear-drop shaped and serrated. The *Mesona chinesis* plant is processed by harvesting all the aerial portions above the root. The suitable place to planting the grass jelly is at loose soil with acidity of 5.5 to 6.5.

### 2.1.2 Benefits of Grass Jelly

In Kreungngern and Chaikham (2016) study, it was illustrated that *Mesona chinesis* is a herbal plant with many health benefits including lowering blood pressure and diuretic effects. Yen and Huang (2001), reported extraction of phenolic compounds from grass jelly significantly contributed to free radical scavenging effects and antioxidant activity.

There is high soluble fiber content in the grass jelly and it also has been used as a folk medicine (Dewanti Widyaningsih, Wijayanti, Handayani, & Prasetyo, 2016). Grass jelly also effective against diabetes, hypertension and heat-shock. Grass jelly leaf gum is a potent polar antioxidant which interacts with wide range of species that are directly responsible for oxidative damage (Lai, Chou, & Chao, 2001).

### 2.1.3 Nutritional Value of Grass Jelly

Grass jelly beneficial due to the nutrient content inside the agar. Grass jelly is higher in dietary sugar and carbohydrates. The grass jelly is relatively low calorie count which is beneficial for diet. Nutritional facts of grass jelly drink consists of carbohydrate, protein, calories and calcium. Grass jelly is one of the best meal for diet behaviour to get ideal weight and posture.

## 2.2 Thickening Agent Added In Grass Jelly

Thickening agent is a substance that can increase viscosity of a liquid without changing its properties. It can improve the suspensions or emulsions of other ingredients which can increase the stability. Gelling agent commonly regulated as food additives that are used to thicken various foods. Gelatine, corn flour and sweet potato flour are the gelling agents had been used in production of grass jelly.

### 2.2.1 Gelatine

Gelatine is derived by hydrolytic degradation of collagen, the principal protein component of white fibrous connective tissue likes tendon, bone and skin (Ross-Murphy & Simon, 1992). Normally, gelatine is dissolved in warm water at temperature of 40 $^{0}$C. If the temperature is above 40 $^{0}$C, the polypeptide will form in flexible single form.

Term ‘gel’ is derived from the word of gelatine. In the past, the structure and mechanical behaviour of gel from the polypeptide gelatine was widely studied. There is

three modern technique in structural and molecular properties, which are categorized as molecular, macromolecular and supra-molecular. In this study, gelatine will be substituted in grass jelly agar as thickening agent. The percentages of gelatine used are 1%, 2%, 3% and 4%.

### 2.2.2 Carrageenan

Reported by Van De Velde, Lourenço, Pinheiro, and Bakker (2002)**,** carrageenan is a food-grade and biocompatible support material extracted from red seaweeds. Carrageenan acts as an immobilization support and major in stability improvement. Uses of carrageenan in food application is started in a long time ago almost 600 years. Carrageenan had been applied in various applications especially food industry for sauces, jellies and yogurt. Other than that, carrageenan also had been applied in cosmetic and pharmaceutical industry.

In Europe, carrageenan is in list of permitted gelling agent. The number and position of sulphate group in carrageenan is differ in several types. As carrageenan had feature of gel formation, then, it is suitable to be used in immobilisation techniques. Carrageenan consists of mechanical stability acts as the gel hardening. In this research, carrageenan is also the substitution in grass jelly agar as thickening agent. The percentage of carrageenan used is 6%, 7%, 8% and 9%.

### 2.2.3 Corn Flour

Flour is fine powder made from cereals or other starch based produce. It is most commonly made from wheat. The addition of corn flour as thickening agent will

affect physical properties such as thickness and rheological characterization, chemical which is elasticity and ruptured and also formaldehyde emission. Uses of corn flour can improve physical and mechanical properties. Corn flour is abundant, renewable, inexpensive and readily available (Moubarik et al., 2013). In this study, corn flour will be used as one of the thickening agent in constant weight which is 2.5 gram, 0.87%.

### 2.2.4 Sweet Potato Flour

Sweet potato flour is low viscosity and very useful in the development of caloric-rich specialty food (Ramesh Yadav, Guha, Tharanathan, & Ramteke, 2006). Sweet potato flour contains of maltose which is used in wide range of food and pharmaceutical industry. The maltose behaviour is known as good thermal stability, mild sweetness, lack of colour formation and low viscosity in solution (Noda, Furuta, & Suda, 2001). In this study, sweet potato flour will be used as one of the thickening agent in constant weight which is 10 gram, 3.48%.

## 2.3 Shelf Life of Grass Jelly Using Spread Plate Method

Shelf life study will summarize the storage results by examining the microbial growth. Shelf life must meet the food hygiene regulations. This will ensure safety among consumer and contribute to maintain brand's reputation. Shelf life will assess the quality of the product. Shelf life was implied in food, cosmetic and pharmaceutical industry.

In this study, shelf life of grass jelly agar made with gelatine and carrageenan will be determined. Shelf life will be conducted using spread plate method. This is technique to plate a liquid sample. A successfully spread plate will have a countable number of

isolated colonies evenly distributed on the plate (Choi & Rogers, 2015). Countable range of colony is 20 to 250 colonies.

## 2.4 Analysis of Grass Jelly Made with Gelatine and Carrageenan

### 2.4.1 pH Value

According to Sigel, Zuberbuhler, and Yamauchi (1991), pH measurement is not just a simple method which is only need us to switch on the pH, dip into solution and taking the meter reading. Analysis of pH reading must be clarified to achieve the objective of analyse pH value. pH is to measure of the hydrogen ion concentration of the water. The lower pH meter of water, the more acidic it is. The higher pH meter of water, the more neutral or alkaline it is.

### 2.4.2 Texture

Texture analysis is comprehension test to determine textural properties of foods. It commercially used in pharmaceutical, personal care food and gel industry. There are attributes will be analysed by the texture analyser such as hardness and cohesiveness. Food companies usually applied texture analysis technique in new product development and as a part of quality control in finished process food.

### 2.4.3 Colour

Interaction between visible light and materials both extrinsically and intrinsically will form colour. The colour analysis will detect the lightness, redness and brightness. Colour is one of quality attribute in food and bioprocess industry. It will influence consumer attractiveness, choice and preferences. Colour measurement in food had been considered as indirect measurement in term of quality attributes.

### 2.4.4 Percentage of Brix

Brix is amount of light bends passes through a liquid. It is a measurement for concentration of sugar content. Percentage of brix is the sugar content in the aqueous solution. Traditionally, brix is usually used in wine, fruit juice, honey industries and carbonated beverage. Amount of one gram of sucrose is equal to one degree of brix. Measurement percentage of brix can be measured by using refractometer. The refractometer has advantages over other methods of estimating IgG concentration in that the Brix refractometer is inexpensive, readily available, less fragile, and less sensitive to variation in temperature, season of the year and other factors (Quigley, Lago, Chapman, Erickson, & Polo, 2013).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Raw Materials

The grass jelly leaves was bought from traditional Chinese shop in Kota Bharu Then, other basic ingredients of the grass jelly include corn flour, sweet potato flour, gelatine, carrageenan, sodium carbonate and sugar was purchased from local market. Nutrient agar was prepared by suspended 28 g in 1 litre of distilled water. Peptone water was prepared by suspended 15 g in 1 litre of distilled water. Then both nutrient agar and peptone water was dissolved completely before sterile by autoclaving at 121 $^{0}$C for 15 minutes.

### 3.1.2 Apparatus

Apparatus that will be used in this experiment are large pot, large bowl, beakers, strainer, knife, chopping board, spoon, thermometer, disposable petri dish, measuring cylinder, spatula, muslin cloth, distilled water, water, small container with caps, 250 ml, hockey stick and Bunsen burner.

### 3.1.3 Equipment

Equipment that will be involved in this study are Brookfield CT3 Texture analyser, stove, laboratory weighing balance, pH meter, Chroma meter, autoclave, incubator, chiller, refractometer and pipette.

## 3.2 Methods

### 3.2.1 Extraction of Grass Jelly Leaves

A 50 gram of the dried grass jelly leaves was weighed using laboratory weighing balance and washed using distilled water to clean from dirt and any impurities. Then, the grass jelly leaves was placed into a pot and added with 2.25 litter of water and 10 ml sodium carbonate. The solution was soaked around 15 minutes and boiled for one hour. The solution of grass jelly agar was stirred using a spatula to prevent evaporation. After boiling, the solution was filtered using muslin cloth. The extraction was kept for production of grass jelly agar.

### 3.2.2 Production of Grass Jelly Agar

Grass jelly extract, sweet potato flour, corn flour, carrageenan and gelatine was mixed together and added with 25 gram of water to dissolve. The mixture was heated until boiling and continuously stirred using spatula to ensure it completely dissolved. The mixture was transferred out and allowed to cool down. Then, the mixture was kept in the fridge for future used. The formulation of grass jelly agar is according to Table 3.1

Table 3.1: Ingredients for standard grass jelly agar production

| Ingredients | Weight (gram) | Percentage (%) |
|---|---|---|
| Grass jelly extract | 250 | 86.95 |
| Sweet potato flour | 10 | 3.48 |
| Corn flour | 2.5 | 0.87 |
| Gelatine/Carrageenan | 0 | 0 |
| Water | 25 | 8.70 |
| Total | 287.5 | 100 |

### 3.2.2.1 Addition of Gelatine

Gelatine as one of the thickening agent was substituted at 0% (control), 1%, 2%, 3% and 4% (Table 3.2). The weight of water, sweet potato flour and corn flour is 25 g, 10 g and 2.5 g respectively. It will be the constant variable. Amount of grass jelly extract was changed according to the changes percentage of gelatine put into it. Five concentrations of gelatine will be used.

Table 3.2: Gelatine addition in grass jelly agar

| Ingredients | Amount addition of gelatine (g) | | | | |
|---|---|---|---|---|---|
| | 0% | 1% | 2% | 3% | 4% |
| Grass jelly extract | 250 | 247.5 | 245 | 242.5 | 240 |
| Sweet potato flour | 10 | 10 | 10 | 10 | 10 |
| Corn flour | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Gelatine | 0 | 2.5 | 5 | 7.5 | 10 |
| Water | 25 | 25 | 25 | 25 | 25 |
| Total (g) | 287.5 | 287.5 | 287.5 | 287.5 | 287.5 |

### 3.2.2.2 Addition of Carrageenan

Carrageenan as one of the thickening agent was substituted at 0% (control), 6%, 7%, 8% and 9% (Table 3.3). The weight of water, sweet potato flour and corn flour is 25 g, 10 g and 2.5 g respectively. It will be the constant variable. Amount of grass jelly extract was changed according to the changes percentage of carrageenan put into it. Five concentrations of carrageenan will be used.

Table 3.3: Carrageenan addition in grass jelly agar

| Ingredients | Amount addition of carrageenan (g) | | | | |
|---|---|---|---|---|---|
| | 0% | 6% | 7% | 8% | 9% |
| Grass jelly extract | 250 | 235 | 232.5 | 230 | 227.5 |
| Sweet potato flour | 10 | 10 | 10 | 10 | 10 |
| Corn flour | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Carrageenan | 0 | 15 | 17.5 | 20 | 22.5 |
| Water | 25 | 25 | 25 | 25 | 25 |
| Total (g) | 287.5 | 287.5 | 287.5 | 287.5 | 287.5 |

### 3.3 Production of Grass Jelly Drink

In production of grass jelly drink, the grass jelly agar was carefully cut into small cube by sized 2 cm X 2 cm. The cube was weighted by using laboratory weighing balance around 20 g. The grass jelly had been cut was placed into sterile 250 ml bottle. Then, the prepared sugar syrup at 13 brix was added into the bottle.

### 3.4 Determining Shelf Life of Grass Jelly Agar

The colony forming units (CFU) were determined by spread plate methodologies. Serial dilution was prepared to get appropriate dilution. The dilution chosen was from factor $10^{-1}$ until $10^{-4}$. First, the grass jelly sample will be homogenised in peptone water by shake 90 times for each sample. The sample of grass jelly that had been diluted was spread with the hockey stick on the nutrient agar plate. After that, the plates were

incubated. Plate with CFUs between 25 and 250 were utilized to calculate the CFU/ gram. The sample was left for three months in the fridge at constant temperature which is 4 $^{0}$C for this test. The sample was checked frequently by weekly. All measurement were done in triplicate.

### 3.5 Determining Colour of Grass Jelly Agar

Colour of grass jelly agar was measured using Chroma meter. The data was shown on the Chroma meter screen. Data of colour differences using CIE L*a*b* coordinates. In this L*a*b* coordinates, L* indicates lightness, a* indicates redness and b* indicates yellowness. All measurement were done in triplicate.

### 3.6 Determining Texture of Grass Jelly Agar

Texture profile analysis (TPA) of grass jelly was conducted using a TA-XT plus Texture analyser (Stable Micro System, London, England). TPA was performed using 10 kg load cell and a 75 mm diameter compression platen (SMS P/75) which is forced to compress 75% of the sample height in two cycles of compression tests. The test conditions were; pre-test speed 2.0 mm/s, test speed 10.0 mm/s and post-test speed 10.0 mm/s; penetration distance 5 mm and a rest period of 5s between two cycles; trigger load 5 g. Three replicates for each samples was performed. Hardness, cohesiveness, and gumminess were calculated by the software instrument called Texture Exponent (Stable Micro System, London, England).

### 3.7 Determining pH Value of Grass Jelly Agar and Drink

Reading of pH value was measured by using a digital pH meter. The reading of pH meter were taken in triplicate of each sample.

### 3.8 Determining Brix Concentration of Grass Jelly Agar and Drink

Brix concentration was determined with a refractometer. The brix of grass jelly drink was measured in triplicate of each sample.

### 3.9 Determining colour of Grass Jelly Drink

By using UV spectrophotometer, the colour of the grass jelly drink in black colour that was leached out to the liquid after caped the bottle and storage was determined. The wavelength of UV Spectrophotometer was set at 309 nm. The pipetted solution of grass jelly drink in the cuvette was placed in UV Spectrophotometer and the reading of absorbance was recorded.

### 3.10 Statistical Analysis

Two way Analysis of Variance (ANOVA) using SPSS software will be using to evaluate all the data. Standard deviation and mean (means ± SD) will be determined with using of ANOVA calculation. Determination of significant differences among treatment means were done by Tukey's multiple range tests ($P \leq 0.05$).

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Screening Percentage of Gelatine and Carrageenan

From previous study, texture of grass jelly agar was not smooth like commercial grass jelly. So, this study was made to improve the physicochemical properties include test for the shelf life. Based on analysis, formulation of addition gelatine and carrageenan had been made. The percentage was 5%, 10%, 15% and 20%. But, the formulation was not matched correctly because of the observation made before run experiment. Below showed the negative observation of the first formulation before it changed to new percentage which are for gelatine (1%, 2%, 3%, 4%) while for carrageenan (6%, 7%, 8%, 9%).

| Samples | Observation |
|---|---|
| Gelatine 5% | Grass jelly agar made with gelatine 5 – 20% was not significant because the texture was hard. Grass jelly agar with gelatine 20% was the most hard among others percentage. So, the percentage of gelatine had been decreased to 1 until 4 percent. It is just nice for addition of gelatine. |
| Gelatine 10% | |
| Gelatine 15% | |
| Gelatine 20% | |
| Carrageenan 5% | Grass jelly agar made with carrageenan 5 – 20% also not significant because of its texture and appearance. Grass jelly agar made with addition carrageenan 5% was so soft and not in texture of grass jelly. But, grass jelly agar made with carrageenan 10 – 20% was harder. So, the percentage of carrageenan was change to 6 – 9% because it is in between 5% and 10%. |
| Carrageenan 10% | |
| Carrageenan 15% | |
| Carrageenan 20% | |

## 4.2 Physicochemical Analysis for Grass Jelly Agar

Physicochemical analysis for grass jelly agar was pH analysis, brix analysis, colour analysis indicates lightness, L*, redness a* and brightness b* and texture analysis including 5 attribute which are hardness, cohesiveness and gumminess.

### 4.2.1 pH Analysis for Grass Jelly Agar

Table 4.1: pH analysis data for grass jelly agar by week

| Sample | pH analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 8.757 ± 0.005774 | 8.743 ± 0.025166 | 8.717 ± 0.015275 | 8.653 ± 0.023094 |
| Gelatine 1% | 8.927 ± 0.005774 | 8.867 ± 0.015275 | 8.663 ± 0.005774 | 8.260 ± 0.017321 |
| Gelatine 2% | 8.860 ± 0.020000 | 8.670 ± 0.017321 | 8.427 ± 0.011547 | 8.257 ± 0.005774 |
| Gelatine 3% | 8.637 ± 0.015275 | 8.437 ± 0.035119 | 8.397 ± 0.005774 | 8.223 ± 0.015275 |
| Gelatine 4% | 8.217 ± 0.005774 | 8.127 ± 0.011547 | 8.070 ± 0.010000 | 8.027 ± 0.005774 |
| Carrageenan 6% | 8.940 ± 0.010000 | 8.920 ± 0.010000 | 8.867 ± 0.025166 | 8.833 ± 0.037859 |
| Carrageenan 7% | 9.333 ± 0.011547 | 9.287 ± 0.011547 | 9.250 ± 0.010000 | 9.193 ± 0.011547 |
| Carrageenan 8% | 9.007 ± 0.005774 | 8.963 ± 0.020817 | 8.933 ± 0.005774 | 8.890 ± 0.010000 |
| Carrageenan 9% | 9.087 ± 0.005774 | 9.020 ± 0.010000 | 8.933 ± 0.005774 | 8.897 ± 0.015275 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

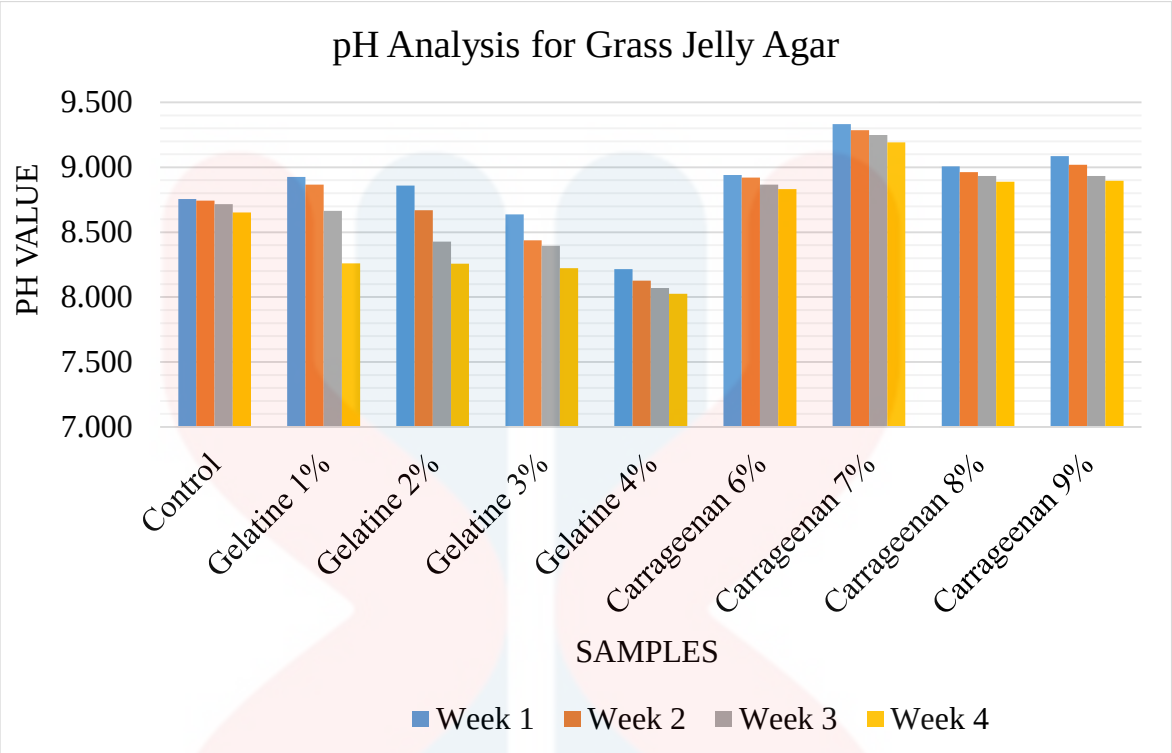


Figure 4.1: Graph of pH analysis data for grass jelly agar by week

Figure 4.1 shows the pH analysis data for grass jelly agar by week. Through figure 4.1, the pH value for all samples is decreasing by week. Carrageenan 7% have the higher pH value compared to others with average pH value 9.333, 9.287, 9.250 and 9.193 from week 1, 2, 3 and 4 respectively. The decreasing pH value for each sample is just slightly difference. The lowest pH value is gelatine 4% which are 8.217, 8.127, 8.070 and 8.027 from week 1, 2, 3 and 4 respectively.

Based on figure 4.1, the pH analysis for grass jelly agar with addition gelatine show decreasing from week 1 to week 4 for each percentage. The pH was in range 8.9 to 8.0. So, it is in alkaline behaviour and still safe to be eaten.

Based on figure 4.1, the pH analysis for grass jelly agar with addition carrageenan show decreasing from week 1 to week 4 for each percentage. The pH was in range 9.3 to 8.8. So, it is in alkaline behaviour and still safe to be eaten.

#### 4.2.1.1 Effect of Different Concentration of Thickening Agent on pH Value of Grass Jelly Agar

**a) Gelatine**

Gelatine with percentage 1 until 4 show decreasing pH value. When percentage addition of gelatine increases, the pH value will be decreases.

**b) Carrageenan**

Carrageenan with percentage 6 until 9 show increasing pH value. When percentage addition of carrageenan increases, the pH value will be increases.

### 4.2.2 Brix Analysis for Grass Jelly Agar

Table 4.2: Brix analysis data for grass jelly agar by week

| Sample | Brix analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 2.833 ± 0.057735 | 2.467 ± 0.152753 | 2.533 ± 0.115470 | 2.100 ± 0.100000 |
| Gelatine 1% | 5.167 ± 0.152753 | 4.733 ± 0.057735 | 4.467 ± 0.115470 | 3.400 ± 0.200000 |
| Gelatine 2% | 7.133 ± 0.115470 | 6.733 ± 0.115470 | 6.233 ± 0.057735 | 5.767 ± 0.057735 |
| Gelatine 3% | 8.300 ± 0.100000 | 7.733 ± 0.115470 | 7.333 ± 0.115470 | 6.233 ± 0.152753 |
| Gelatine 4% | 7.867 ± 0.115470 | 7.633 ± 0.057735 | 7.133 ± 0.115470 | 6.633 ± 0.057735 |
| Carrageenan 6% | 7.767 ± 0.057735 | 7.333 ± 0.115470 | 6.533 ± 0.115470 | 6.500 ± 0.100000 |
| Carrageenan 7% | 10.667 ± 0.057735 | 10.167 ± 0.057735 | 9.467 ± 0.115470 | 8.400 ± 0.100000 |
| Carrageenan 8% | 8.267 ± 0.057735 | 7.767 ± 0.057735 | 7.167 ± 0.057735 | 6.267 ± 0.115470 |
| Carrageenan 9% | 8.767 ± 0.057735 | 8.333 ± 0.115470 | 7.533 ± 0.057735 | 6.667 ± 0.115470 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

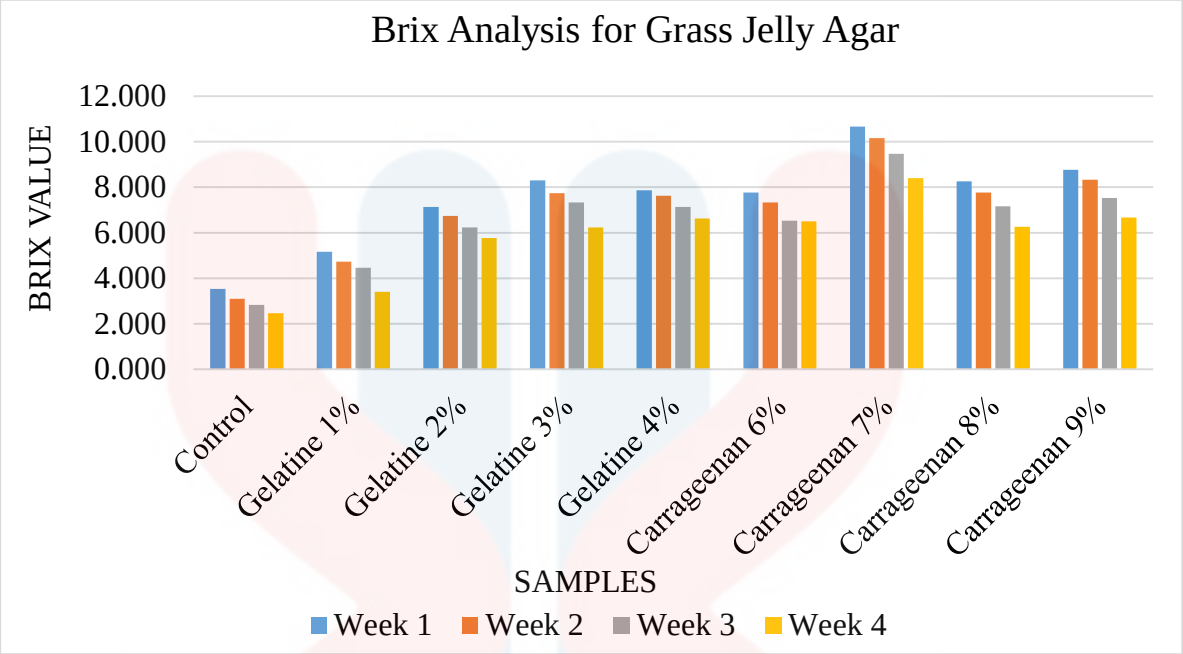


Figure 4.2: Graph of brix analysis data for grass jelly agar by week

According to the result in figure 4.2, the brix value was decreased by week for each samples. The brix value for sample carrageenan 7% resulted the highest value compared to other samples. The average brix value are 10.667, 10.167, 9.467 and 8.400. Control sample was the lowest brix reading which are 2.833, 2.467, 2.533 and 2.100. The decreased value of brix proved that the sugar syrup content in grass jelly agar was decreasing by week.

#### 4.2.2.1 Effect of Different Concentration of Thickening Agent on Brix Value of Grass Jelly Agar

**a) Gelatine**

Gelatine with percentage 1 until 4 show increasing brix value. When percentage addition of gelatine increases, the brix value will be increases.

**b) Carrageenan**

Carrageenan with percentage 6 until 7 show increasing brix value, but from percent 7 until 9, the brix value start to decline. This show that the

graph trend was unstable. This condition can happen because of the changes environment temperature and evaporation of oxygen.

### 4.2.3 Colour Analysis for Grass Jelly Agar

#### a) Lightness

Table 4.3: Colour analysis data of Lightness, L* value for grass jelly agar by week

| Sample | Colour Lightness value analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 20.480 ± 0.317962 | 19.433 ± 0.192180 | 23.673 ± 0.185023 | 17.693 ± 0.204042 |
| Gelatine 1% | 21.463 ± 0.159478 | 19.230 ± 0.269629 | 22.193 ± 0.113725 | 23.113 ± 0.205020 |
| Gelatine 2% | 23.200 ± 0.573760 | 20.090 ± 0.298664 | 25.650 ± 0.235160 | 20.640 ± 0.206640 |
| Gelatine 3% | 20.530 ± 0.070000 | 17.390 ± 0.240208 | 17.687 ± 0.327159 | 18.817 ± 0.085049 |
| Gelatine 4% | 22.970 ± 0.764918 | 18.700 ± 0.450333 | 20.917 ± 0.070238 | 22.490 ± 0.30315 |
| Carrageenan 6% | 25.147 ± 0.568624 | 20.840 ± 0.276225 | 24.520 ± 0.170880 | 23.307 ± 0.201329 |
| Carrageenan 7% | 22.093 ± 0.397031 | 17.483 ± 0.250067 | 21.403 ± 0.223010 | 24.710 ± 0.091652 |
| Carrageenan 8% | 27.980 ± 0.692820 | 25.620 ± 0.137477 | 29.093 ± 0.015275 | 26.510 ± 0.277849 |
| Carrageenan 9% | 29.817 ± 0.444110 | 24.447 ± 0.100167 | 28.473 ± 0.055076 | 28.467 ± 0.228108 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

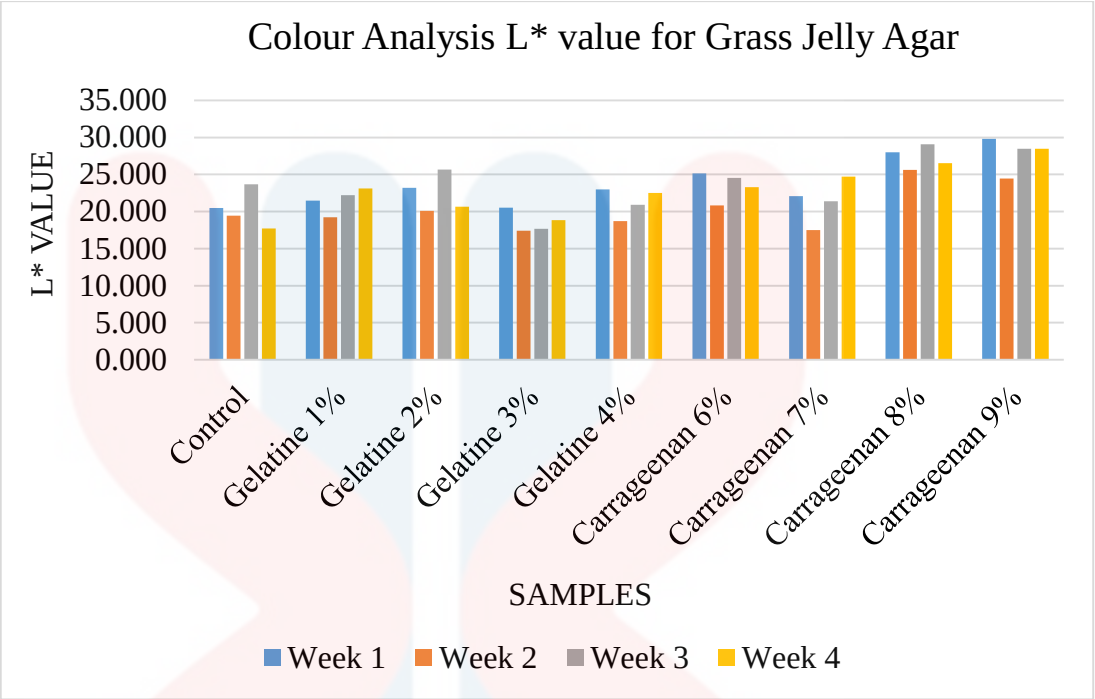


Figure 4.3: Graph of colour analysis data of Lightness, L* value for grass jelly agar by week

Figure 4.3 showed that colour analysis of lightness, L* value for grass jelly agar by week. The average of L* value highest at week 1 that indicated for carrageenan 9% and the lowest L* value was at week 2 that indicated for gelatine 3%. From the result, its show that gelatine 3% has the lowest L* value compared to other samples. There is differences of L* value among all samples.

**b) Redness**

Table 4.4: Colour analysis data of Redness, a* value for grass jelly agar by week

| Sample | Colour Redness value analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 3.767 ± 0.448813 | 2.497 ± 0.030551 | 4.267 ± 0.040415 | 3.593 ± 0.144684 |
| Gelatine 1% | 9.293 ± 1.128288 | 3.217 ± 0.037859 | 5.593 ± 0.065064 | 5.597 ± 0.289885 |
| Gelatine 2% | 6.147 ± 0.230290 | 3.580 ± 0.052915 | 5.730 ± 0.130000 | 4.587 ± 0.035119 |
| Gelatine 3% | 3.630 ± 0.130767 | 2.020 ± 0.085440 | 3.867 ± 0.158850 | 3.613 ± 0.092916 |
| Gelatine 4% | 5.400 ± 0.141774 | 3.817 ± 0.066583 | 5.870 ± 0.108167 | 5.137 ± 0.066583 |
| Carrageenan 6% | 7.630 ± 0.549454 | 4.850 ± 0.051962 | 6.680 ± 0.351568 | 6.087 ± 0.060277 |
| Carrageenan 7% | 6.570 ± 0.570351 | 3.490 ± 0.238118 | 6.067 ± 0.070946 | 6.057 ± 0.181475 |
| Carrageenan 8% | 10.043 ± 0.160728 | 6.190 ± 0.070000 | 8.797 ± 0.256970 | 8.330 ± 0.124900 |
| Carrageenan 9% | 8.763 ± 0.381095 | 8.057 ± 0.040415 | 8.647 ± 0.447363 | 9.157 ± 0.090185 |

Means in the different column followed the different letters are significantly different (P≤0.05). Each data point is the average of three replications (n=3).

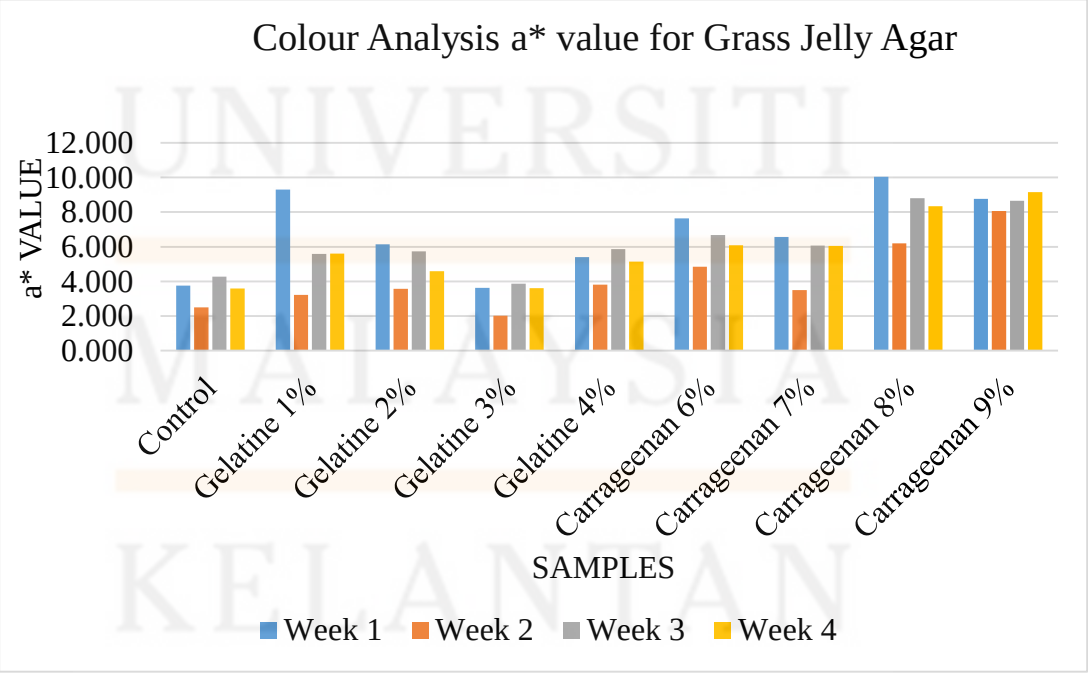


Figure 4.4: Graph of colour analysis data of Redness, a* value for grass jelly agar by week

Figure 4.4 show that colour analysis of redness, a* value for grass jelly agar by week. The highest average of a* value was at week 1 that indicated for carrageenan 8% and the lowest a* value was at week 2 that indicated for gelatine 3%. Each sample show differences a* value by week.

**c) Yellowness**

Table 4.5: Colour analysis data of Yellowness, b* value for grass jelly agar by week

| Sample | Colour Yellowness value analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 9.197 ± 0.342685 | 5.340 ± 0.045826 | 10.870 ± 0.103923 | 10.940 ± 0.281603 |
| Gelatine 1% | 18.247 ± 1.489340 | 5.393 ± 0.047258 | 12.313 ± 0.080829 | 14.153 ± 0.330807 |
| Gelatine 2% | 14.377 ± 0.738738 | 6.683 ± 0.096090 | 14.657 ± 0.391067 | 13.330 ± 0.202978 |
| Gelatine 3% | 9.570 ± 0.281603 | 4.883 ± 0.130128 | 11.857 ± 0.075056 | 11.733 ± 0.115902 |
| Gelatine 4% | 12.997 ± 0.170978 | 7.007 ± 0.066583 | 15.737 ± 0.060277 | 14.817 ± 0.240069 |
| Carrageenan 6% | 17.763 ± 1.422053 | 7.967 ± 0.020817 | 14.543 ± 0.240069 | 13.837 ± 0.198578 |
| Carrageenan 7% | 14.377 ± 0.883761 | 6.383 ± 0.487579 | 14.400 ± 0.121655 | 12.487 ± 0.597188 |
| Carrageenan 8% | 20.223 ± 0.115036 | 9.417 ± 0.104083 | 18.447 ± 0.321299 | 16.623 ± 0.197569 |
| Carrageenan 9% | 19.453 ± 0.477214 | 17.583 ± 0.035119 | 18.620 ± 0.948736 | 19.610 ± 0.310000 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

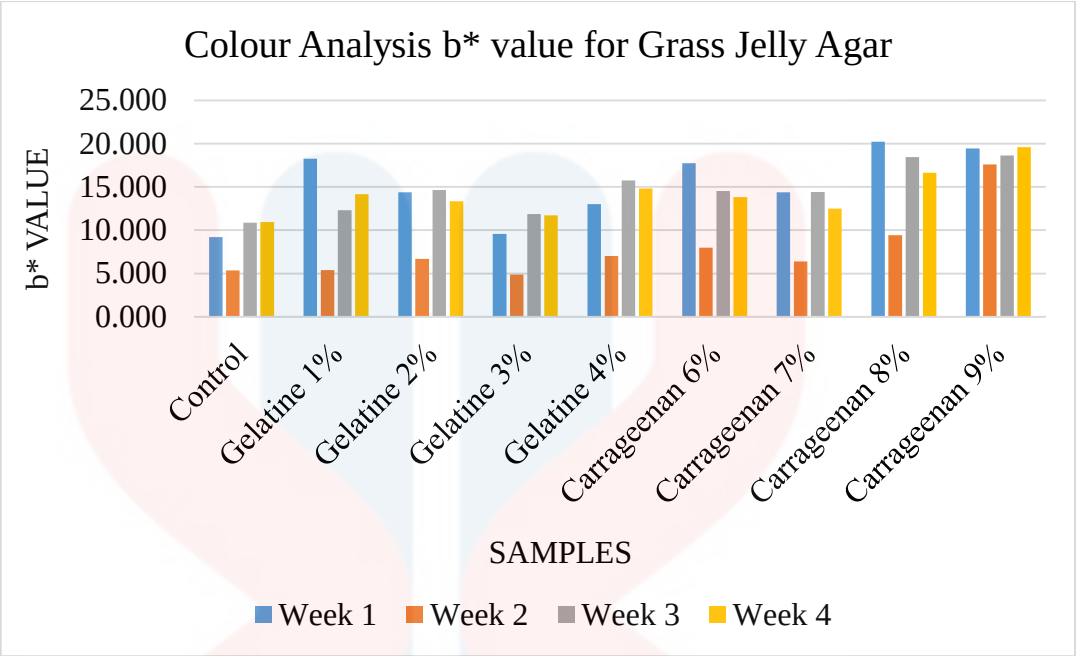


Figure 4.5: Graph of colour analysis data of Yellowness, b* value for grass jelly agar by week

Figure 4.5 show that colour analysis of yellowness, b* value for grass jelly agar by week. The highest average of b* value was at week 1 that indicated for carrageenan 8% and the lowest b* value was at week 2 that indicated for gelatine 3%. Each sample show differences b* value by week.

### 4.2.4 Texture Profile Analysis for Grass Jelly Agar

Texture is the most important characteristic of grass jelly agar and the addition of several gelling agents including potato flour, agar, carrageenan and other hydrocolloids has been displayed to provide desirable texture, relating to the consumer acceptance (Kreungngern & Chaikham, 2016).

**a) Hardness**

Hardness is defined as the force necessary to attain a given deformation (Kreungngern & Chaikham, 2016). Table 4.6 show the texture analysis data of hardness for grass jelly agar by week.

Table 4.6: Texture profile analysis data of hardness for grass jelly agar by week

| Sample | Texture analysis (Hardness) | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 5356.000 ± 2.000000 | 5174.333 ± 1.527525 | 6198.000 ± 1.000000 | 5925.000 ± 2.000000 |
| Gelatine 1% | 5388.000 ± 1.000000 | 5383.667 ± 1.527525 | 5267.333 ± 1.527525 | 5225.667 ± 2.516611 |
| Gelatine 2% | 5051.333 ± 0.577350 | 5203.000 ± 1.000000 | 5116.000 ± 2.000000 | 5026.000 ± 2.645751 |
| Gelatine 3% | 5012.667 ± 1.154701 | 5102.333 ± 1.527525 | 5163.333 ± 1.527525 | 5087.333 ± 2.081666 |
| Gelatine 4% | 5076.667 ± 2.081666 | 5134.667 ± 1.154701 | 5147.000 ± 1.732051 | 5164.667 ± 3.055050 |
| Carrageenan 6% | 5223.000 ± 1.000000 | 5236.667 ± 1.527525 | 5176.667 ± 2.081666 | 5337.000 ± 2.000000 |
| Carrageenan 7% | 5121.000 ± 1.000000 | 5175.333 ± 3.511885 | 5142.667 ± 1.527525 | 5133.000 ± 3.605551 |
| Carrageenan 8% | 5183.333 ± 1.527525 | 5275.667 ± 2.081666 | 5244.333 ± 2.516611 | 5263.000 ± 2.645751 |

| | | | | |
|---|---|---|---|---|
| Carrageenan 9% | 5184.333 ± 1.154701 | 5212.000 ± 3.000000 | 5113.667 ± 3.785939 | 5135.000 ± 1.000000 |

Means in the different column followed the different letters are significantly different (P≤0.05). Each data point is the average of three replications (n=3).

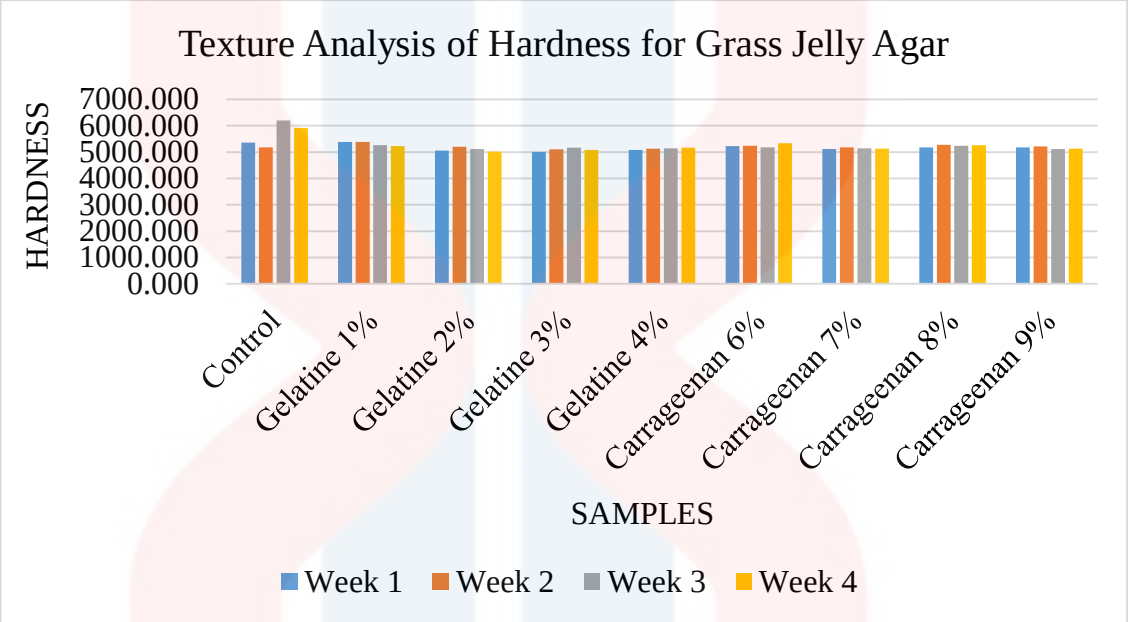


Figure 4.6: Graph of texture profile analysis data of hardness for grass jelly agar by week

From table 4.6, the hardness for each samples was just show slightly difference. The grass jelly agar with gelatine 1% show higher hardness value compared to other gelatine percentage. The grass jelly agar with carrageenan 7% show the higher hardness value compared to other carrageenan percentage. Sample control for week 3 show the highest hardness value.

**b) Cohesiveness**

Cohesiveness represents the strength of the internal bonds making up the body of the product. The lower the cohesiveness of a material, the more brittle it will be (Kreungngern & Chaikham, 2016). Table 4.7 show the texture analysis data of cohesiveness for grass jelly agar by week.

Table 4.7: Texture profile analysis data of cohesiveness for grass jelly agar by week

| Sample | Texture analysis (Cohesiveness) | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 1.373 ± 0.050332 | 1.550 ± 0.010000 | 1.140 ± 0.020000 | 2.070 ± 0.020000 |
| Gelatine 1% | 1.160 ± 0.010000 | 0.767 ± 0.057735 | 0.467 ± 0.152753 | 1.130 ± 0.020000 |
| Gelatine 2% | 0.767 ± 0.152753 | 0.847 ± 0.025166 | 0.763 ± 0.025166 | 0.967 ± 0.015275 |
| Gelatine 3% | 0.767 ± 0.011547 | 0.763 ± 0.020817 | 0.767 ± 0.015275 | 0.740 ± 0.010000 |
| Gelatine 4% | 1.057 ± 0.015275 | 0.947 ± 0.020817 | 1.030 ± 0.020000 | 1.457 ± 0.035119 |
| Carrageenan 6% | 0.980 ± 0.010000 | 0.937 ± 0.015275 | 0.837 ± 0.020817 | 0.933 ± 0.015275 |
| Carrageenan 7% | 1.050 ± 0.030000 | 1.227 ± 0.015275 | 1.260 ± 0.026458 | 0.963 ± 0.005774 |
| Carrageenan 8% | 0.957 ± 0.020817 | 1.063 ± 0.025166 | 1.023 ± 0.011547 | 0.947 ± 0.020817 |
| Carrageenan 9% | 1.363 ± 0.015275 | 1.017 ± 0.011547 | 1.030 ± 0.020000 | 1.033 ± 0.025166 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

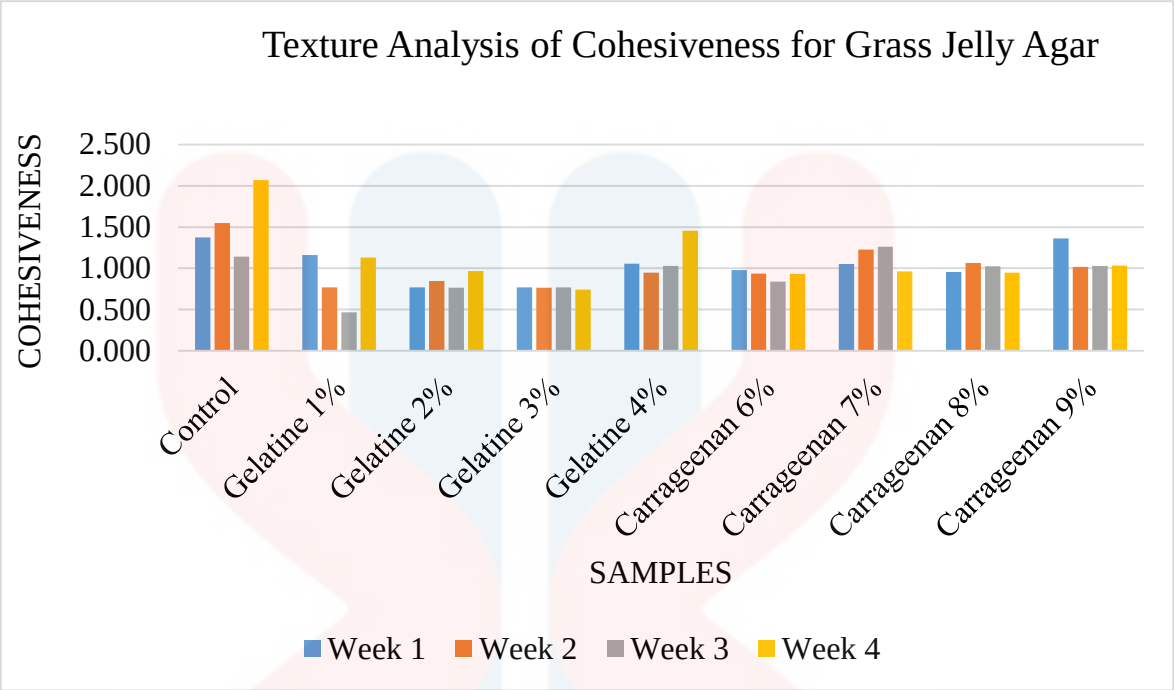


Figure 4.7: Graph of texture profile analysis data of cohesiveness for grass jelly agar by week

From table 4.7, the cohesiveness value of grass jelly agar with addition of carrageenan was higher than addition with gelatine. The highest value of cohesiveness is 2.070 which is indicated to control sample in week 4. Figure 4.7 also show that cohesiveness value of control sample more higher compared to grass jelly agar with addition gelatine and carrageenan. Sample with gelatine 3% show the lowest cohesiveness value.

**c) Gumminess**

Gumminess represents the energy required to disintegrate a semi-solid food product to a state ready for swallowing (Kreungngern & Chaikham, 2016). Table 4.8 show texture analysis data of gumminess for grass jelly agar by week.

Table 4.8: Texture profile analysis data of gumminess for grass jelly agar by week

| Sample | Texture analysis (Gumminess) | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 6539.000 ± 5.567764 | 7796.667 ± 3.214550 | 6935.000 ± 2.000000 | 6235.000 ± 2.645751 |
| Gelatine 1% | 6180.667 ± 3.055050 | 4327.333 ± 2.516611 | 5646.000 ± 2.000000 | 5824.667 ± 2.516611 |
| Gelatine 2% | 4055.667 ± 3.511885 | 4265.667 ± 3.214550 | 4043.000 ± 2.000000 | 4859.667 ± 1.527525 |
| Gelatine 3% | 3793.667 ± 1.154701 | 3945.333 ± 3.511885 | 4035.667 ± 2.516611 | 4233.667 ± 1.527525 |
| Gelatine 4% | 5266.000 ± 1.000000 | 4972.667 ± 3.055050 | 5194.667 ± 2.081666 | 5035.667 ± 3.214550 |
| Carrageenan 6% | 5194.000 ± 2.000000 | 4805.000 ± 3.000000 | 4376.333 ± 2.516611 | 4942.333 ± 2.516611 |
| Carrageenan 7% | 5173.333 ± 1.527525 | 5062.333 ± 1.527525 | 6434.667 ± 2.516611 | 4937.000 ± 2.000000 |
| Carrageenan 8% | 5058.333 ± 3.511885 | 5612.667 ± 2.081666 | 5395.000 ± 2.000000 | 5103.667 ± 1.527525 |
| Carrageenan 9% | 7074.667 ± 1.527525 | 6075.000 ± 2.000000 | 5210.333 ± 2.516611 | 5014.000 ± 2.000000 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

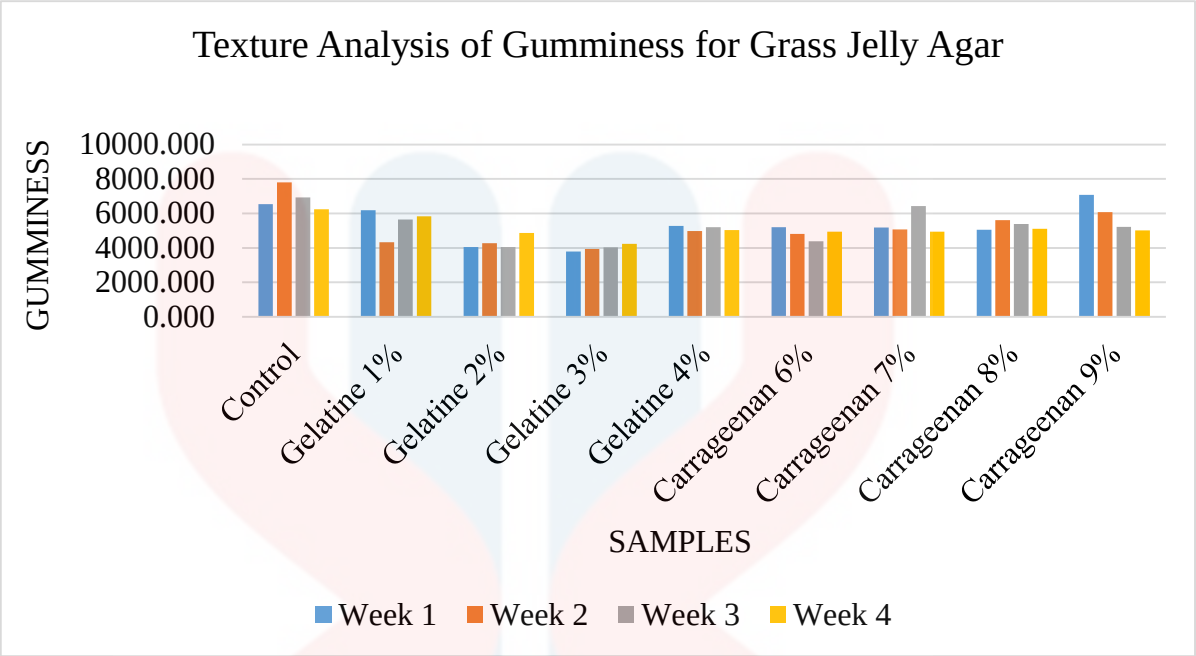


Figure 4.8: Graph of texture profile analysis data of gumminess for grass jelly agar by week

From table 4.8, gumminess value of control sample was higher and only degrade a slightly value from week 1 to week 4. The highest value of gumminess was sample carrageenan 9% which is 7074.667. The sample of grass jelly agar with addition of gelatine3% show the lowest value of gumminess which is in range 3000 to 4000. Overall sample showed slightly degradation of gumminess value from week 1 to week 4.

## 4.3 Physicochemical Analysis for Grass Jelly Drink

Physicochemical analysis for grass jelly drink was pH analysis, brix analysis, colour analysis indicates the absorbance value with 309 nm.

### 4.3.1 pH Analysis for Grass Jelly Drink

Table 4.9: pH analysis data for grass jelly drink by week

| Sample | pH analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 7.997 ± 0.015275 | 7.263 ± 0.015275 | 7.250 ± 0.017321 | 6.373 ± 0.015275 |
| Gelatine 1% | 6.357 ± 0.020817 | 5.537 ± 0.011547 | 4.877 ± 0.015275 | 4.737 ± 0.015275 |
| Gelatine 2% | 6.220 ± ± 0.01 | 4.957 ± 0.011547 | 4.677 ± 0.015275 | 5.430 ± 0.01 |
| Gelatine 3% | 6.707 ± 0.005774 | 4.837 ± 0.011547 | 4.543 ± 0.015275 | 4.237 ± 0.015275 |
| Gelatine 4% | 5.740 ± 0.01 | 5.283 ± 0.005774 | 5.183 ± 0.005774 | 4.800 ± 0.01 |
| Carrageenan 6% | 8.360 ± 0.01 | 7.227 ± 0.025166 | 5.647 ± 0.023094 | 2.170 ± 0.052915 |
| Carrageenan 7% | 8.260 ± 0.026458 | 7.147 ± 0.025166 | 6.050 ± 0.01 | 2.147 ± 0.015275 |
| Carrageenan 8% | 7.820 ± 0.02 | 5.980 ± 0.01 | 4.950 ± 0.01 | 2.043 ± 0.015275 |
| Carrageenan 9% | 6.640 ± 0.02 | 6.030 ± 0.01 | 5.020 ± 0.01 | 2.343 ± 0.015275 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

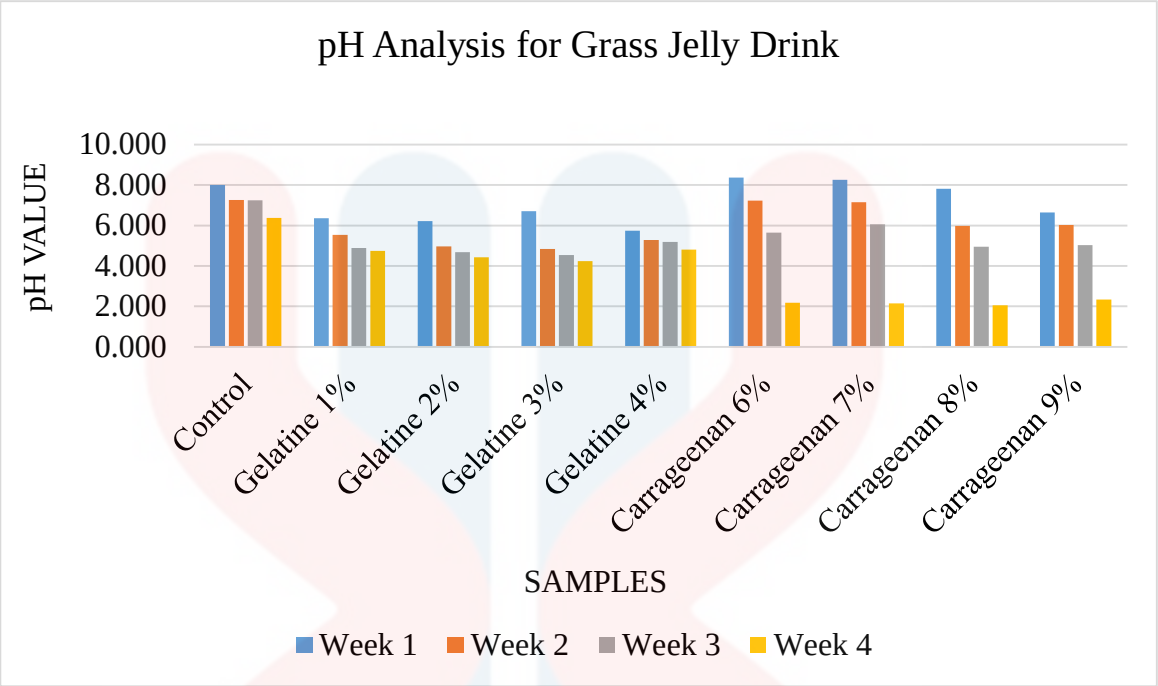


Figure 4.9: Graph of pH analysis data for grass jelly drink by week

Table 4.9 shows the pH analysis data for grass jelly drink by week. Through table 4.9, the pH value for all samples is decreasing by week. Carrageenan 6% for week 1 have the higher pH value compared to others with average pH value 8.360. The lowest pH value is carrageenan 8% in week 4 which is average value 2.043.

Based on figure 4.9, the pH analysis for grass jelly drink with addition gelatine show decreasing from week 6 to 7. But, the trend increasing from week 7 to 8 and decreasing back from week 8 to 9. It is may be because of internal factor that affected the pH value.

Based on figure 4.9, the pH analysis for grass jelly drink with addition carrageenan show decreasing from week 1 to week 4 for each percentage. The pH was in range 8.360 to 2.043. It means that the pH value is from alkaline to acidic and cannot be consume anymore.

#### 4.3.1.1 Effect of Different Concentration of Thickening Agent on pH Value of Grass Jelly Drink

**a) Gelatine**

Gelatine with percentage 1 until 4 show unstable reading of pH value.

**b) Carrageenan**

Carrageenan with percentage 6 until 9 show decreasing pH value. When percentage addition of carrageenan increases, the pH value will be decreases.

### 4.3.2 Brix Analysis for Grass Jelly Drink

Table 4.10: Brix analysis data for grass jelly drink by week

| Sample | Brix analysis | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 10.533 ± 0.057735 | 9.467 ± 0.057735 | 8.533 ± 0.057735 | 7.367 ± 0.057735 |
| Gelatine 1% | 9.633 ± ± 0.057735 | 9.267 ± 0.057735 | 8.200 ± 0.173205 | 7.533 ± 0.11547 |
| Gelatine 2% | 9.867 ± 0.057735 | 9.267 ± 0.057735 | 8.233 ± 0.057735 | 7.867 ± 0.057735 |
| Gelatine 3% | 9.967 ± 0.057735 | 9.267 ± 0.057735 | 8.200 ± 0.1 | 7.800 ± 0.1 |
| Gelatine 4% | 9.867 ± 0.057735 | 9.267 ± 0.11547 | 8.433 ± 0.11547 | 7.067 ± 0.11547 |
| Carrageenan 6% | 10.567 ± 0.152753 | 9.467 ± 0.152753 | 9.000 ± 0.173205 | 7.500 ± 0.1 |
| Carrageenan 7% | 11.433 ± 0.152753 | 10.867 ± 0.057735 | 9.600 ± 0.1 | 8.367 ± 0.152753 |
| Carrageenan 8% | 11.100 ± 0.1 | 10.700 ± 0.1 | 9.033 ± 0.057735 | 8.833 ± 0.057735 |
| Carrageenan 9% | 11.233 ± 0.057735 | 10.400 ± 0.2 | 9.200 ± 0.1 | 8.033 ± 0.057735 |

Means in the different column followed the different letters are significantly different ($P \leq 0.05$). Each data point is the average of three replications (n=3).

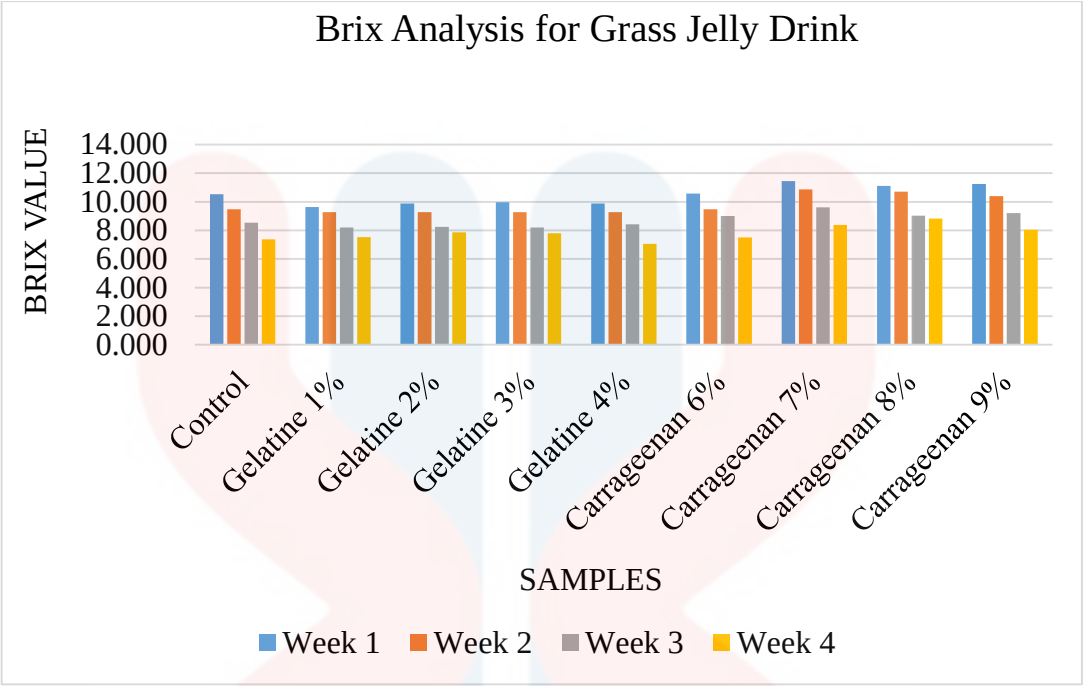


Figure 4.10: Graph of brix analysis data for grass jelly drink by week

Table 4.10 show the brix analysis for grass jelly drink by week. Through table 4.10, the brix analysis for each sample is decreasing from week 1 to week 4. Sample grass jelly drink with carrageenan 7% show the highest brix value which are 11.433, 10.867, 9.600 and 8.367 from week 1, 2, 3 and 4 respectively.

### 4.3.3 Colour Analysis for Grass Jelly Drink

Table 4.11: Colour analysis data of absorbance value at 309 nm for grass jelly drink by week

| Sample | Colour analysis (UV-Spectrophotometer) | | | |
|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 4.413 ± 0.047885 | 4.037 ± 0.007024 | 4.420 ± 0.143615 | 4.371 ± 0.020551 |
| Gelatine 1% | 4.432 ± 0.02203 | 4.314 ± 0.001528 | 4.422 ± 0.030089 | 4.081 ± 0.017039 |
| Gelatine 2% | 4.146 ± 0.045004 | 3.875 ± 0.057292 | 2.918 ± 0.005292 | 4.431 ± ± 0.006807 |
| Gelatine 3% | 4.346 ± 0.034356 | 3.598 ± 1.15248 | 2.958 ± 0.009452 | 2.847 ± 0.008386 |
| Gelatine 4% | 4.469 ± 0.028042 | 4.203 ± 0.058011 | 4.319 ± 0.07697 | 4.346 ± 0.011136 |
| Carrageenan 6% | 3.849 ± 0.033724 | 4.360 ± 0.023714 | 4.492 ± 0.042595 | 4.393 ± 0.006245 |
| Carrageenan 7% | 2.496 ± 0.004359 | 3.065 ± 0.011015 | 3.717 ± 0.080829 | 3.581 ± 0.013 |
| Carrageenan 8% | 2.203 ± 0.006506 | 2.472 ± 0.032868 | 3.056 ± 0.011719 | 4.320 ± 0.006807 |
| Carrageenan 9% | 2.395 ± 0.001 | 2.862 ± 0.011 | 2.565 ± 0.00611 | 3.652 ± 0.014189 |

Means in the different column followed the different letters are significantly different (P≤0.05). Each data point is the average of three replications (n=3).

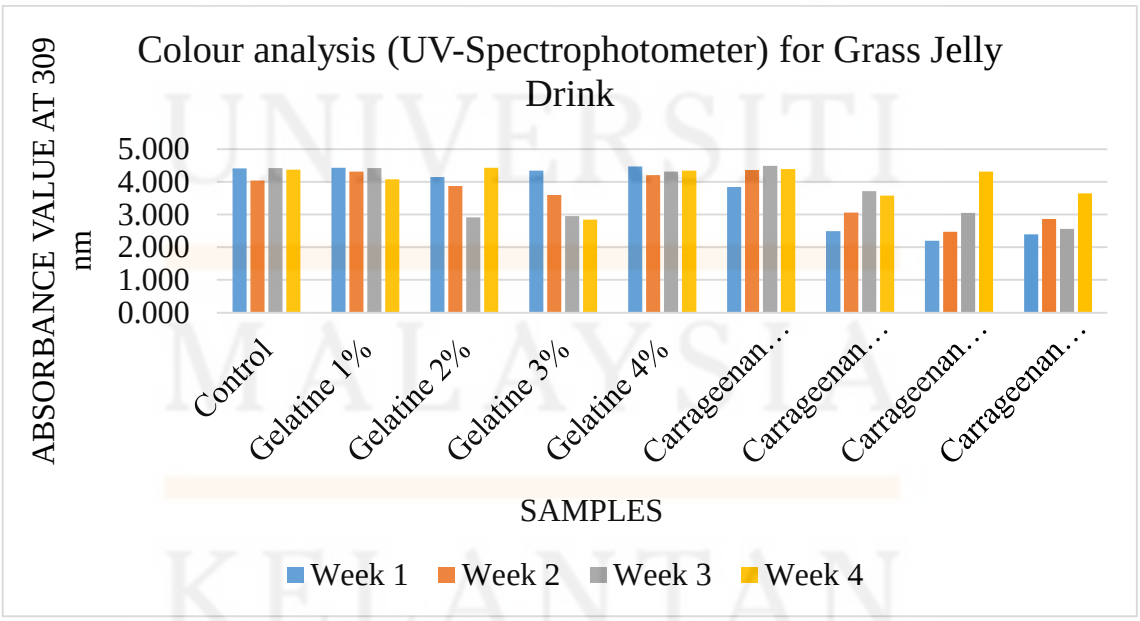


Figure 4.11: Graph of colour analysis data of absorbance value at 309 nm for grass jelly drink by week

Table 4.11 show the colour analysis data of absorbance value at 309 nm for grass jelly drink by week. Grass jelly drink with gelatine 3% show that the absorbance value is decreasing from week 1 to week 4 with value 4.346, 3.598, 2.958 and 2.847. grass jelly drink with carrageenan 8% show that the absorbance value is increasing from week 1 to week 4 with value 2.203, 2.472, 3.056 and 4.320.

## 4.4 Shelf Life of Grass Jelly

Shelf life of grass jelly was conducted by using spared plate count. The sample of control and one from each type of thickening agent was taken to be evaluate the shelf life. Grass jelly agar from addition thickening agent of gelatine 3% and carrageenan 7% was chosen to proceed with shelf life analysis. The sample was chosen based on the observation of its result from physicochemical analysis in week 1.

### 4.4.1 Plate Incubate at Temperature 37 $^0$C

Table 4.12: Number of colony count for storage in 37 $^0$C

| **Week** | **Samples** | **Number of Colony Count (cfu/ml)** | | | |
|---|---|---|---|---|---|
| | | **$10^{-1}$** | **$10^{-2}$** | **$10^{-3}$** | **$10^{-4}$** |
| 1 | Control | **57** | **42** | **25** | **11** |
| | Gelatine 3% | **NG** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **NG** | **NG** | **NG** | **NG** |
| 2 | Control | **103** | **67** | **38** | **39** |
| | Gelatine 3% | **3** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **27** | **NG** | **NG** | **NG** |
| 3 | Control | **TMTC** | **158** | **97** | **74** |
| | Gelatine 3% | **46** | **17** | **NG** | **NG** |

| | | | | | |
|---|---|---|---|---|---|
| | Carrageenan 7% | **91** | **41** | **23** | **NG** |
| 4 | Control | **TMTC** | **TMTC** | **153** | **110** |
| | Gelatine 3% | **73** | **58** | **44** | **NG** |
| | Carrageenan 7% | **145** | **86** | **59** | **NG** |
| 5 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **154** | **103** | **86** | **NG** |
| | Carrageenan 7% | **TMTC** | **128** | **92** | **NG** |
| 6 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **157** | **121** | **12** |
| | Carrageenan 7% | **TMTC** | **198** | **165** | **31** |
| 7 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **212** | **187** | **56** |
| | Carrageenan 7% | **TMTC** | **TMTC** | **204** | **69** |
| 8 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **247** | **205** | **103** |
| | Carrageenan 7% | **TMTC** | **TMTC** | **TMTC** | **119** |
| 9 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **TMTC** | **TMTC** | **157** |
| | Carrageenan 7% | **TMTC** | **TMTC** | **TMTC** | **192** |
| 10 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Carrageenan 7% | **TMTC** | **TMTC** | **TMTC** | **TMTC** |

- **NG No Growth**
- **TMTC To Many To Count (>250)**

Table 4.12 show number of colony count for storage in 37 $^{0}$C. The sample control start growing of colony in week 1 while for sample gelatine and carrageenan, the colony start to growth both in week 2. Colony count was TMTC for control sample in week 3 that means the sample cannot be acceptable anymore. Sample grass jelly agar with

addition carrageenan 7% start to TMTC in week 5 while for addition of gelatine 3% in week 6. In conclusion, the shelf life of control sample was the shortest followed by carrageenan and gelatine.

### 4.4.2 Plate Incubate at Temperature 4 $^0$C

Table 4.13: Number of colony count for storage in 4 $^0$C

| Week | Samples | Number of Colony Count (cfu/ml) | | | |
|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ |
| 1 | Control | **NG** | **NG** | **NG** | **NG** |
| | Gelatine 3% | **NG** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **NG** | **NG** | **NG** | **NG** |
| 2 | Control | **NG** | **NG** | **NG** | **NG** |
| | Gelatine 3% | **NG** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **NG** | **NG** | **NG** | **NG** |
| 3 | Control | **7** | **NG** | **NG** | **NG** |
| | Gelatine 3% | **NG** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **NG** | **NG** | **NG** | **NG** |
| 4 | Control | **67** | **24** | **NG** | **NG** |
| | Gelatine 3% | **NG** | **NG** | **NG** | **NG** |
| | Carrageenan 7% | **14** | **NG** | **NG** | **NG** |
| 5 | Control | **92** | **53** | **8** | **NG** |
| | Gelatine 3% | **21** | **7** | **NG** | **NG** |
| | Carrageenan 7% | **48** | **11** | **NG** | **NG** |
| 6 | Control | **129** | **76** | **21** | **6** |
| | Gelatine 3% | **57** | **28** | **NG** | **NG** |
| | Carrageenan 7% | **91** | **43** | **9** | **NG** |
| 7 | Control | **203** | **125** | **63** | **29** |

| | | | | | |
|---|---|---|---|---|---|
| | Gelatine 3% | **106** | **73** | **29** | **NG** |
| | Carrageenan 7% | **183** | **89** | **42** | **16** |
| 8 | Control | **TMTC** | **237** | **115** | **62** |
| | Gelatine 3% | **183** | **118** | **79** | **38** |
| | Carrageenan 7% | **TMTC** | **123** | **67** | **39** |
| 9 | Control | **TMTC** | **TMTC** | **TMTC** | **204** |
| | Gelatine 3% | **TMTC** | **175** | **132** | **71** |
| | Carrageenan 7% | **TMTC** | **227** | **105** | **74** |
| 10 | Control | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Gelatine 3% | **TMTC** | **TMTC** | **TMTC** | **TMTC** |
| | Carrageenan 7% | **TMTC** | **TMTC** | **TMTC** | **TMTC** |

- **NG No Growth**
- **TMTC To Many To Count (>250)**

Table 4.13 show number of colony count for storage in 4 $^0$C. The sample control start growing of colony in week 3 while for sample carrageenan 7% the colony start growth in week 4 and sample gelatine 3% colony start growth in week 5. Colony count was TMTC for control sample in week 8 that means the sample cannot be acceptable anymore. Sample grass jelly agar with addition carrageenan 7% start to TMTC in week 8 while for addition of gelatine 3% in week 9. In conclusion, the shelf life of control sample was the shortest followed by carrageenan and gelatine. Addition of thickening agent contributed to longer shelf life and adorable appearance of grass jelly (Kreungngern & Chaikham, 2016).

In conclusion, shelf life for grass jelly agar in storage 4$^0$C was longer than storage in 37$^0$C. The grass jelly agar made with gelatine had longer shelf life compared to grass jelly agar made with carrageenan. Number of countable colony count is in range 25 – 250 cfu/ml.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

For conclusion, the concentration of thickening agent, gelatine and carrageenan in making grass jelly had been determined which are gelatine (1%, 2%, 3%, 4%) and carrageenan (6%, 7%, 8%, 9%). The percentage chosen based on the observation of texture and appearance. The result show physicochemical properties for grass jelly agar and drink from gelatine and carrageenan. Samples grass jelly agar made with gelatine 3% and carrageenan 7% resulted the best result for physicochemical analysis. Both samples also chosen in evaluating the shelf life. In shelf life determination, the all sample will be longer shelf life when stored in 4C compared to 37C. The control sample had the shortest shelf life followed by carrageenan and gelatine.

## 5.2 Recommendation

In order to improve the texture and appearance of grass jelly agar, the future study can continue do a new research with using different thickening agent such as xanthan gum. The process throughout preparation of grass jelly also need to be focused more and implement safety and hygienic environment. For recommendations, I suggest that research could be conducted more specific about the ingredients used and processing step in order to produce grass jelly that comply high quality value and longer shelf life. Besides that, food industry also can grab opportunity to produce new formulation of high quality grass jelly with no preservative.

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## APPENDIX A

Table A.1 Two Way ANOVA for pH and brix analysis of grass jelly agar

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) Week | (J) Week | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| pH | WEEK 1 | WEEK 2 | .0811* | .00423 | .000 | .0700 | .0922 |
| | | WEEK 3 | .1674* | .00423 | .000 | .1563 | .1785 |
| | | WEEK 4 | .2811* | .00423 | .000 | .2700 | .2922 |
| | WEEK 2 | WEEK 1 | -.0811* | .00423 | .000 | -.0922 | -.0700 |
| | | WEEK 3 | .0863* | .00423 | .000 | .0752 | .0974 |
| | | WEEK 4 | .2000* | .00423 | .000 | .1889 | .2111 |
| | WEEK 3 | WEEK 1 | -.1674* | .00423 | .000 | -.1785 | -.1563 |
| | | WEEK 2 | -.0863* | .00423 | .000 | -.0974 | -.0752 |
| | | WEEK 4 | .1137* | .00423 | .000 | .1026 | .1248 |
| | WEEK 4 | WEEK 1 | -.2811* | .00423 | .000 | -.2922 | -.2700 |
| | | WEEK 2 | -.2000* | .00423 | .000 | -.2111 | -.1889 |
| | | WEEK 3 | -.1137* | .00423 | .000 | -.1248 | -.1026 |
| Brix | WEEK 1 | WEEK 2 | .4296* | .02808 | .000 | .3558 | .5035 |
| | | WEEK 3 | .8185* | .02808 | .000 | .7447 | .8924 |
| | | WEEK 4 | 1.5333* | .02808 | .000 | 1.4595 | 1.6072 |
| | WEEK 2 | WEEK 1 | -.4296* | .02808 | .000 | -.5035 | -.3558 |
| | | WEEK 3 | .3889* | .02808 | .000 | .3150 | .4628 |
| | | WEEK 4 | 1.1037* | .02808 | .000 | 1.0298 | 1.1776 |
| | WEEK 3 | WEEK 1 | -.8185* | .02808 | .000 | -.8924 | -.7447 |
| | | WEEK 2 | -.3889* | .02808 | .000 | -.4628 | -.3150 |
| | | WEEK 4 | .7148* | .02808 | .000 | .6410 | .7887 |
| | WEEK 4 | WEEK 1 | -1.5333* | .02808 | .000 | -1.6072 | -1.4595 |
| | | WEEK 2 | -1.1037* | .02808 | .000 | -1.1776 | -1.0298 |
| | | WEEK 3 | -.7148* | .02808 | .000 | -.7887 | -.6410 |

Based on observed means.
The error term is Mean Square(Error) = .011.

*. The mean difference is significant at the .05 level.

Table A.2 Post Hoc Tests (Tukey) for pH of grass jelly agar

**pH**

Tukey HSD[a,b,c]

| Sample | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| GELATIN 4% | 12 | 8.1100 | | | | | | | | |
| GELATINE 3% | 12 | | 8.4233 | | | | | | | |
| GELATINE 2% | 12 | | | 8.5533 | | | | | | |
| GELATINE 1% | 12 | | | | 8.6792 | | | | | |
| CONTROL | 12 | | | | | 8.7175 | | | | |
| CARRAGEENAN 6% | 12 | | | | | | 8.8900 | | | |
| CARRAGEENAN 8% | 12 | | | | | | | 8.9483 | | |
| CARRAGEENAN 9% | 12 | | | | | | | | 8.9842 | |
| CARRAGEENAN 7% | 12 | | | | | | | | | 9.2658 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .000.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.3 Post Hoc Tests (Tukey) for brix of grass jelly agar

**Brix**

Tukey HSD[a,b,c]

| Sample | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| CONTROL | 12 | 2.9833 | | | | | | |
| GELATINE 1% | 12 | | 4.4417 | | | | | |
| GELATINE 2% | 12 | | | 6.4667 | | | | |
| CARRAGEENAN 6% | 12 | | | | 7.0333 | | | |
| GELATIN 4% | 12 | | | | | 7.3167 | | |
| CARRAGEENAN 8% | 12 | | | | | 7.3667 | | |
| GELATINE 3% | 12 | | | | | 7.4000 | | |
| CARRAGEENAN 9% | 12 | | | | | | 7.8250 | |
| CARRAGEENAN 7% | 12 | | | | | | | 9.6750 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | .563 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .011.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.4 Two Way ANOVA for colour analysis of grass jelly agar

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) WEEK | (J) WEEK | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LIGHTNESS | WEEK 1 | WEEK 2 | 3.3830* | .08524 | .000 | 3.1588 | 3.6072 |
| | | WEEK 3 | .0078 | .08524 | 1.000 | -.2164 | .2320 |
| | | WEEK 4 | .8815* | .08524 | .000 | .6573 | 1.1057 |
| | WEEK 2 | WEEK 1 | -3.3830* | .08524 | .000 | -3.6072 | -3.1588 |
| | | WEEK 3 | -3.3752* | .08524 | .000 | -3.5994 | -3.1510 |
| | | WEEK 4 | -2.5015* | .08524 | .000 | -2.7257 | -2.2773 |
| | WEEK 3 | WEEK 1 | -.0078 | .08524 | 1.000 | -.2320 | .2164 |
| | | WEEK 2 | 3.3752* | .08524 | .000 | 3.1510 | 3.5994 |
| | | WEEK 4 | .8737* | .08524 | .000 | .6495 | 1.0979 |
| | WEEK 4 | WEEK 1 | -.8815* | .08524 | .000 | -1.1057 | -.6573 |
| | | WEEK 2 | 2.5015* | .08524 | .000 | 2.2773 | 2.7257 |
| | | WEEK 3 | -.8737* | .08524 | .000 | -1.0979 | -.6495 |
| REDNESS | WEEK 1 | WEEK 2 | 2.6141* | .07961 | .000 | 2.4047 | 2.8234 |
| | | WEEK 3 | .6363* | .07961 | .000 | .4269 | .8457 |
| | | WEEK 4 | 1.0096* | .07961 | .000 | .8003 | 1.2190 |
| | WEEK 2 | WEEK 1 | -2.6141* | .07961 | .000 | -2.8234 | -2.4047 |
| | | WEEK 3 | -1.9778* | .07961 | .000 | -2.1871 | -1.7684 |
| | | WEEK 4 | -1.6044* | .07961 | .000 | -1.8138 | -1.3951 |
| | WEEK 3 | WEEK 1 | -.6363* | .07961 | .000 | -.8457 | -.4269 |
| | | WEEK 2 | 1.9778* | .07961 | .000 | 1.7684 | 2.1871 |
| | | WEEK 4 | .3733* | .07961 | .000 | .1640 | .5827 |
| | WEEK 4 | WEEK 1 | -1.0096* | .07961 | .000 | -1.2190 | -.8003 |
| | | WEEK 2 | 1.6044* | .07961 | .000 | 1.3951 | 1.8138 |
| | | WEEK 3 | -.3733* | .07961 | .000 | -.5827 | -.1640 |
| YELLOWNEES | WEEK 1 | WEEK 2 | 7.2830* | .13152 | .000 | 6.9371 | 7.6289 |
| | | WEEK 3 | .5289* | .13152 | .001 | .1830 | .8748 |
| | | WEEK 4 | .9637* | .13152 | .000 | .6178 | 1.3096 |
| | WEEK 2 | WEEK 1 | -7.2830* | .13152 | .000 | -7.6289 | -6.9371 |
| | | WEEK 3 | -6.7541* | .13152 | .000 | -7.1000 | -6.4082 |
| | | WEEK 4 | -6.3193* | .13152 | .000 | -6.6652 | -5.9734 |
| | WEEK 3 | WEEK 1 | -.5289* | .13152 | .001 | -.8748 | -.1830 |
| | | WEEK 2 | 6.7541* | .13152 | .000 | 6.4082 | 7.1000 |
| | | WEEK 4 | .4348* | .13152 | .008 | .0889 | .7807 |
| | WEEK 4 | WEEK 1 | -.9637* | .13152 | .000 | -1.3096 | -.6178 |
| | | WEEK 2 | 6.3193* | .13152 | .000 | 5.9734 | 6.6652 |
| | | WEEK 3 | -.4348* | .13152 | .008 | -.7807 | -.0889 |

Based on observed means.
The error term is Mean Square(Error) = .234.

*. The mean difference is significant at the .05 level.

Table A.5 Post Hoc Tests (Tukey) for L* value of grass jelly agar

**LIGHTNESS**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| GELATINE 3% | 12 | 18.6058 | | | | | | |
| CONTROL | 12 | | 20.3200 | | | | | |
| GELATINE 4% | 12 | | | 21.2692 | | | | |
| CARRAGEENAN 7% | 12 | | | 21.4225 | | | | |
| GELATINE 1% | 12 | | | 21.5000 | | | | |
| GELATINE 2% | 12 | | | | 22.3950 | | | |
| CARRAGEENAN 6% | 12 | | | | | 23.4533 | | |
| CARRAGEENAN 8% | 12 | | | | | | 27.3008 | |
| CARRAGEENAN 9% | 12 | | | | | | | 27.8008 |
| Sig. | | 1.000 | 1.000 | .679 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .098.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.6 Post Hoc Tests (Tukey) for a* value of grass jelly agar

**REDNESS**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| GELATINE 3% | 12 | 3.2825 | | | | |
| CONTROL | 12 | 3.5308 | | | | |
| GELATINE 2% | 12 | | 5.0108 | | | |
| GELATINE 4% | 12 | | 5.0558 | | | |
| CARRAGEENAN 7% | 12 | | | 5.5458 | | |
| GELATINE 1% | 12 | | | 5.9250 | | |
| CARRAGEENAN 6% | 12 | | | | 6.3117 | |
| CARRAGEENAN 8% | 12 | | | | | 8.3400 |
| CARRAGEENAN 9% | 12 | | | | | 8.6558 |
| Sig. | | .495 | 1.000 | .053 | 1.000 | .187 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .086.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.7 Post Hoc Tests (Tukey) for b* value of grass jelly agar

**YELLOWNEES**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| CONTROL | 12 | 9.0867 | | | | | |
| GELATINE 3% | 12 | 9.5108 | | | | | |
| CARRAGEENAN 7% | 12 | | 11.9117 | | | | |
| GELATINE 2% | 12 | | 12.2617 | 12.2617 | | | |
| GELATINE 1% | 12 | | 12.5267 | 12.5267 | | | |
| GELATINE 4% | 12 | | | 12.6392 | | | |
| CARRAGEENAN 6% | 12 | | | | 13.5275 | | |
| CARRAGEENAN 8% | 12 | | | | | 16.1775 | |
| CARRAGEENAN 9% | 12 | | | | | | 18.8167 |
| Sig. | | .449 | .062 | .607 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .234.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.8 Two Way ANOVA for texture analysis of grass jelly agar

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) WEEK | (J) WEEK | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| HARDNESS | WEEK 1 | WEEK 2 | -33.4815* | .55556 | .000 | -34.9426 | -32.0203 |
| | | WEEK 3 | -108.0741* | .55556 | .000 | -109.5352 | -106.6129 |
| | | WEEK 4 | -77.8148* | .55556 | .000 | -79.2760 | -76.3537 |
| | WEEK 2 | WEEK 1 | 33.4815* | .55556 | .000 | 32.0203 | 34.9426 |
| | | WEEK 3 | -74.5926* | .55556 | .000 | -76.0537 | -73.1314 |
| | | WEEK 4 | -44.3333* | .55556 | .000 | -45.7945 | -42.8722 |
| | WEEK 3 | WEEK 1 | 108.0741* | .55556 | .000 | 106.6129 | 109.5352 |
| | | WEEK 2 | 74.5926* | .55556 | .000 | 73.1314 | 76.0537 |
| | | WEEK 4 | 30.2593* | .55556 | .000 | 28.7981 | 31.7204 |
| | WEEK 4 | WEEK 1 | 77.8148* | .55556 | .000 | 76.3537 | 79.2760 |
| | | WEEK 2 | 44.3333* | .55556 | .000 | 42.8722 | 45.7945 |
| | | WEEK 3 | -30.2593* | .55556 | .000 | -31.7204 | -28.7981 |
| COHESIVENESS | WEEK 1 | WEEK 2 | .0396* | .01152 | .005 | .0093 | .0699 |
| | | WEEK 3 | .1285* | .01152 | .000 | .0982 | .1588 |
| | | WEEK 4 | -.0852* | .01152 | .000 | -.1155 | -.0549 |
| | WEEK 2 | WEEK 1 | -.0396* | .01152 | .005 | -.0699 | -.0093 |
| | | WEEK 3 | .0889* | .01152 | .000 | .0586 | .1192 |
| | | WEEK 4 | -.1248* | .01152 | .000 | -.1551 | -.0945 |
| | WEEK 3 | WEEK 1 | -.1285* | .01152 | .000 | -.1588 | -.0982 |
| | | WEEK 2 | -.0889* | .01152 | .000 | -.1192 | -.0586 |
| | | WEEK 4 | -.2137* | .01152 | .000 | -.2440 | -.1834 |
| | WEEK 4 | WEEK 1 | .0852* | .01152 | .000 | .0549 | .1155 |
| | | WEEK 2 | .1248* | .01152 | .000 | .0945 | .1551 |
| | | WEEK 3 | .2137* | .01152 | .000 | .1834 | .2440 |
| GUMMINES | WEEK 1 | WEEK 2 | 163.6296* | .69537 | .000 | 161.8008 | 165.4585 |
| | | WEEK 3 | 118.2963* | .69537 | .000 | 116.4674 | 120.1252 |
| | | WEEK 4 | 238.8519* | .69537 | .000 | 237.0230 | 240.6807 |
| | WEEK 2 | WEEK 1 | -163.6296* | .69537 | .000 | -165.4585 | -161.8008 |
| | | WEEK 3 | -45.3333* | .69537 | .000 | -47.1622 | -43.5045 |
| | | WEEK 4 | 75.2222* | .69537 | .000 | 73.3934 | 77.0511 |
| | WEEK 3 | WEEK 1 | -118.2963* | .69537 | .000 | -120.1252 | -116.4674 |
| | | WEEK 2 | 45.3333* | .69537 | .000 | 43.5045 | 47.1622 |
| | | WEEK 4 | 120.5556* | .69537 | .000 | 118.7267 | 122.3844 |
| | WEEK 4 | WEEK 1 | -238.8519* | .69537 | .000 | -240.6807 | -237.0230 |
| | | WEEK 2 | -75.2222* | .69537 | .000 | -77.0511 | -73.3934 |
| | | WEEK 3 | -120.5556* | .69537 | .000 | -122.3844 | -118.7267 |

Based on observed means.
The error term is Mean Square(Error) = 6.528.

*. The mean difference is significant at the .05 level.

Table A.9 Post Hoc Tests (Tukey) for hardness value of grass jelly agar

**HARDNESS**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| GELATINE 3% | 12 | 5091.4167 | | | | | | | |
| GELATINE 2% | 12 | | 5099.0833 | | | | | | |
| GELATINE 4% | 12 | | | 5130.7500 | | | | | |
| CARRAGEENAN 7% | 12 | | | | 5143.0000 | | | | |
| CARRAGEENAN 9% | 12 | | | | | 5161.2500 | | | |
| CARRAGEENAN 8% | 12 | | | | | | 5241.5833 | | |
| CARRAGEENAN 6% | 12 | | | | | | 5243.3333 | | |
| GELATINE 1% | 12 | | | | | | | 5316.1667 | |
| CONTROL | 12 | | | | | | | | 5663.3333 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | .482 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4.167.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.10 Post Hoc Tests (Tukey) for cohesiveness value of grass jelly agar

**COHESIVENESS**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| GELATINE 3% | 12 | .7592 | | | | | |
| GELATINE 2% | 12 | | .8358 | | | | |
| GELATINE 1% | 12 | | .8808 | .8808 | | | |
| CARRAGEENAN 6% | 12 | | | .9217 | | | |
| CARRAGEENAN 8% | 12 | | | | .9975 | | |
| CARRAGEENAN 9% | 12 | | | | | 1.1108 | |
| GELATINE 4% | 12 | | | | | 1.1225 | |
| CARRAGEENAN 7% | 12 | | | | | 1.1250 | |
| CONTROL | 12 | | | | | | 1.5333 |
| Sig. | | 1.000 | .204 | .320 | 1.000 | .996 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .002.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.11 Post Hoc Tests (Tukey) for gumminess value of grass jelly agar

**GUMMINES**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| GELATINE 3% | 12 | 4002.0833 | | | | | | | | |
| GELATINE 2% | 12 | | 4306.0000 | | | | | | | |
| CARRAGEENAN 6% | 12 | | | 4829.4167 | | | | | | |
| GELATINE 4% | 12 | | | | 5117.2500 | | | | | |
| CARRAGEENAN 8% | 12 | | | | | 5292.4167 | | | | |
| CARRAGEENAN 7% | 12 | | | | | | 5401.8333 | | | |
| GELATINE 1% | 12 | | | | | | | 5494.6667 | | |
| CARRAGEENAN 9% | 12 | | | | | | | | 5843.5000 | |
| CONTROL | 12 | | | | | | | | | 6876.4167 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 6.528.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.12 Two Way ANOVA for pH, brix and colour analysis of grass jelly drink

**Multiple Comparisons**

Tukey HSD

| Dependent Variable | (I) WEEK | (J) WEEK | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| pH | WEEK 1 | WEEK 2 | 1.0933* | .00474 | .000 | 1.0809 | 1.1058 |
| | | WEEK 3 | 1.7670* | .00474 | .000 | 1.7546 | 1.7795 |
| | | WEEK 4 | 3.4244* | .00474 | .000 | 3.4120 | 3.4369 |
| | WEEK 2 | WEEK 1 | -1.0933* | .00474 | .000 | -1.1058 | -1.0809 |
| | | WEEK 3 | .6737* | .00474 | .000 | .6612 | .6862 |
| | | WEEK 4 | 2.3311* | .00474 | .000 | 2.3187 | 2.3436 |
| | WEEK 3 | WEEK 1 | -1.7670* | .00474 | .000 | -1.7795 | -1.7546 |
| | | WEEK 2 | -.6737* | .00474 | .000 | -.6862 | -.6612 |
| | | WEEK 4 | 1.6574* | .00474 | .000 | 1.6450 | 1.6699 |
| | WEEK 4 | WEEK 1 | -3.4244* | .00474 | .000 | -3.4369 | -3.4120 |
| | | WEEK 2 | -2.3311* | .00474 | .000 | -2.3436 | -2.3187 |
| | | WEEK 3 | -1.6574* | .00474 | .000 | -1.6699 | -1.6450 |
| BRIX | WEEK 1 | WEEK 2 | .6926* | .02784 | .000 | .6194 | .7658 |
| | | WEEK 3 | 1.7519* | .02784 | .000 | 1.6786 | 1.8251 |
| | | WEEK 4 | 2.6481* | .02784 | .000 | 2.5749 | 2.7214 |
| | WEEK 2 | WEEK 1 | -.6926* | .02784 | .000 | -.7658 | -.6194 |
| | | WEEK 3 | 1.0593* | .02784 | .000 | .9860 | 1.1325 |
| | | WEEK 4 | 1.9556* | .02784 | .000 | 1.8823 | 2.0288 |
| | WEEK 3 | WEEK 1 | -1.7519* | .02784 | .000 | -1.8251 | -1.6786 |
| | | WEEK 2 | -1.0593* | .02784 | .000 | -1.1325 | -.9860 |
| | | WEEK 4 | .8963* | .02784 | .000 | .8231 | .9695 |
| | WEEK 4 | WEEK 1 | -2.6481* | .02784 | .000 | -2.7214 | -2.5749 |
| | | WEEK 2 | -1.9556* | .02784 | .000 | -2.0288 | -1.8823 |
| | | WEEK 3 | -.8963* | .02784 | .000 | -.9695 | -.8231 |
| COLOUR | WEEK 1 | WEEK 2 | -.0041 | .05387 | 1.000 | -.1458 | .1376 |
| | | WEEK 3 | -.0228 | .05387 | .974 | -.1645 | .1189 |
| | | WEEK 4 | -.3634* | .05387 | .000 | -.5051 | -.2217 |
| | WEEK 2 | WEEK 1 | .0041 | .05387 | 1.000 | -.1376 | .1458 |
| | | WEEK 3 | -.0187 | .05387 | .986 | -.1604 | .1230 |
| | | WEEK 4 | -.3593* | .05387 | .000 | -.5010 | -.2176 |
| | WEEK 3 | WEEK 1 | .0228 | .05387 | .974 | -.1189 | .1645 |
| | | WEEK 2 | .0187 | .05387 | .986 | -.1230 | .1604 |
| | | WEEK 4 | -.3406* | .05387 | .000 | -.4823 | -.1989 |
| | WEEK 4 | WEEK 1 | .3634* | .05387 | .000 | .2217 | .5051 |
| | | WEEK 2 | .3593* | .05387 | .000 | .2176 | .5010 |
| | | WEEK 3 | .3406* | .05387 | .000 | .1989 | .4823 |

Based on observed means.
The error term is Mean Square(Error) = .039.

*. The mean difference is significant at the .05 level.

Table A.13 Post Hoc Tests (Tukey) for pH value of grass jelly drink

**pH**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| CARRAGEENAN 9% | 12 | 5.0083 | | | | | | | |
| GELATINE 2% | 12 | | 5.0708 | | | | | | |
| GELATINE 3% | 12 | | 5.0808 | | | | | | |
| CARRAGEENAN 8% | 12 | | | 5.1983 | | | | | |
| GELATINE 4% | 12 | | | | 5.2517 | | | | |
| GELATINE 1% | 12 | | | | | 5.3767 | | | |
| CARRAGEENAN 6% | 12 | | | | | | 5.8508 | | |
| CARRAGEENAN 7% | 12 | | | | | | | 5.9008 | |
| CONTROL | 12 | | | | | | | | 7.2208 |
| Sig. | | 1.000 | .891 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .000.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.14 Post Hoc Tests (Tukey) for brix value of grass jelly drink

**BRIX**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| GELATINE 1% | 12 | 8.6583 | | | | | | |
| GELATINE 4% | 12 | 8.6583 | | | | | | |
| GELATINE 3% | 12 | | 8.8083 | | | | | |
| GELATINE 2% | 12 | | 8.8083 | | | | | |
| CONTROL | 12 | | | 8.9750 | | | | |
| CARRAGEENAN 6% | 12 | | | | 9.1333 | | | |
| CARRAGEENAN 9% | 12 | | | | | 9.7167 | | |
| CARRAGEENAN 8% | 12 | | | | | | 9.9167 | |
| CARRAGEENAN 7% | 12 | | | | | | | 10.0667 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .010.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A.15 Post Hoc Tests (Tukey) for colour value of grass jelly drink

**COLOUR**

Tukey HSD[a,b,c]

| SAMPLE | N | Subset 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| CARRAGEENAN 9% | 12 | 2.8683 | | | | |
| CARRAGEENAN 8% | 12 | 3.0128 | 3.0128 | | | |
| CARRAGEENAN 7% | 12 | | 3.2373 | 3.2373 | | |
| GELATINE 3% | 12 | | | 3.4376 | | |
| GELATINE 2% | 12 | | | | 3.8426 | |
| CARRAGEENAN 6% | 12 | | | | | 4.2736 |
| CONTROL | 12 | | | | | 4.3099 |
| GELATINE 1% | 12 | | | | | 4.3123 |
| GELATINE 4% | 12 | | | | | 4.3343 |
| Sig. | | .690 | .140 | .259 | 1.000 | .998 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .039.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

## APPENDIX B

To count the colony forming units (CFU)

**By using this formula:**

$$CFU = \frac{number\ of\ colony}{volume\ plated} \times dilution\ factor$$

CFU for no dilution

Colonies per plate: 200

Volume dilution added to plate: 1 Ml

CFU $= \frac{200}{1\ Ml}$

=2000 cells/ 1 Ml

=2000 CFUs/ Ml

=2× $10^3$ CFUs/ Ml

CFU for dilution factor $10^{-2}$

Colonies per plate: 80

Dilution factor: 1:1× $10^3$

Volume dilution added to plate: 1 Ml

CFU= $\frac{80}{1\ Ml} \times 1000$

=80000 cells/ 1Ml

=80000 CFUs/ Ml

=8× $10^4\ CFUs/\ Ml$

## APPENDIX C

Images of grass jelly agar

FIGURE C.1 CONTROL

FIGURE C.2 GELATINE 1%

FIGURE C.3 GELATINE 2%

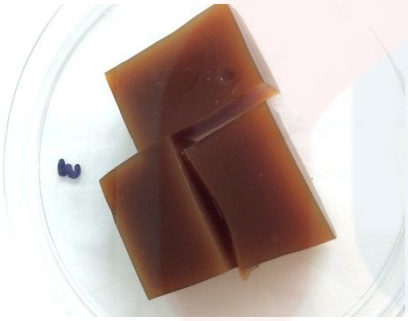

FIGURE C.4 GELATINE 3%

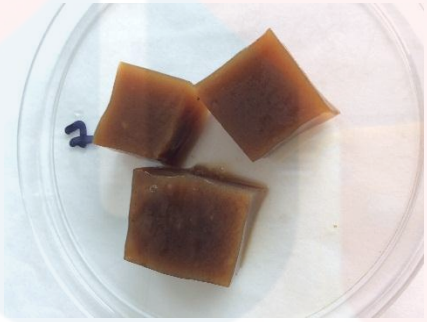

FIGURE C.5 GELATINE 4%

FIGURE C.6 CARRAGEENAN 6%

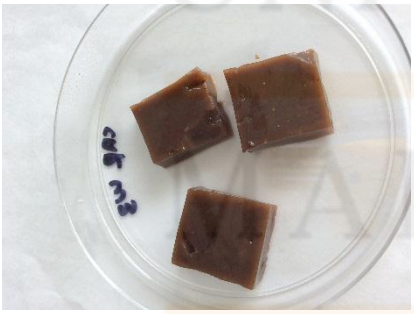

FIGURE C.7 CARRAGEENAN 7%

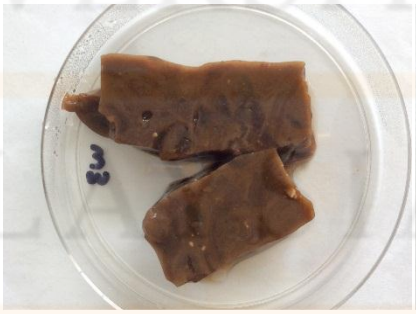

FIGURE C.8 CARRAGEENAN 8%

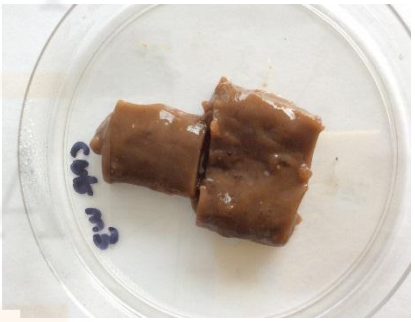

FIGURE C.9 CARRAGEENAN 9%