The Effect of Semi-Refined Carrageenan and Potassium Chloride Added on Physico-Chemical Characteristics of Instant Grass Jelly

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Abstract. This study focused on the effect of the combination effect of semi-refined carrageenan and potassium chloride on the instant grass jelly formulation. As in the traditional method of grass jelly production, the cornstarch was added to the grass jelly extract to give the gelling texture of grass jelly. Semi-refined carrageenan was added to replace the starch and the physicochemical characteristics and nutrient composition of the product was evaluated. The cincau leaves was boiled in a certain volume of water and the extraction was then converted to powder form via spray dryer. A pre-study has been conducted in determining the best method to produce grass jelly extract which based on the result of production yield, as well as conducted the physic-chemical tests. The finding showed that the extraction of 125g dried leaves of cincau with 4.5 L of water which boiled for 2.5 hours was the best extraction recovery with 75.92%. As a conclusion, SRC can be used as a starch replacer in grass jelly production as an instant product and texture is retained as well as nutrient contents of the product is improved.

Keywords: Semi-refined carrageenan, potassium chloride, grass jelly, cincau

INTRODUCTION

Cincau (Mesona chinensis) is known as an herbaceous plant which belongs to the Lamiaceae family and most widely used of agricultural plants in China and Southeast Asia such as Indonesia, Vietnam, and Burma (Adisakwattana *et al.,* 2014). The herbaceous plant known as *Mesona palustris* BL in Indonesia but in Taiwan, it is known as the "hsian-tsao" (*Mesona procumbens* Hemsl) (Haryadi and Construction, 2002). At present, the herbaceous plants used as herbal drinks and dessert which known as grass jelly or black grass jelly by local people in Indonesia and "hsian-tsao" in Taiwan and China (Widyaningsih and Adilars, 2013). Grass jelly is also well-known as refreshments for the Asian community, which commercialized as grass jelly, can drink while the grass jelly is mixed with the ais kacang dessert which existed as indigenous cultural dessert in Malaysia. Besides that, Euromonitor (2014) showed that there is a steady demand for the beverages which including grass jelly drinks in Malaysia.

In Malaysia, kappa carrageenan is derived from red seaweed *Kapphaphycus alvarezii* which are widely cultured especially in Tawau, Sabah (Campo *et al.,* 2009). Kappa carrageenan is the most frequently used among the different classes of carrageenan in food industry as its gelling properties which able to form elastic gels with low content (Bono *et al.,* 2011). Interaction between carrageenan with food products provide several advantages such contributions and improvements in the water holding capacity (WHC), the reduction of fat, the possibility of replacing animal gelatin in dairy products such as yogurt. In addition, carrageenan also provides benefits such as antimicrobial activity and development of textured products (Venugopal, 2009). Kappa carrageenan formed random coil and irregular chain when exposed to high temperature and form a rigid double helix structure during cooling. Kappa carrageenan can form a three-dimensional network in the presence of monovalent and divalent cations such as K^+, Ca^+ and Na^+ where K^+ able to form a strong gel with kappa carrageenan with the range between 0.5 - 2.5% (Chen *et al.,* 2002). Furthermore, the gel formation of gel between carrageenan

with K^+ cation is stronger than Ca^+ cation in which the gelation with K^+ cation is shown to be a continuous and compact network structure (Mac Artain et al., 2003). Thus, the aim of this study is to focus on the effect of the combination effect of semi-refined carrageenan (SRC) and potassium chloride (KCL) on the instant grass jelly formulation in which SRC is expected to be a potential hydrocolloid in producing the instant grass jelly product with good quality of physicochemical characteristics and sensory aspects.

METHODS
Materials
The main materials which used in this study, including the dried leaves of grass jelly which derived from species of Mesona palustris BL, kappa semi-refined carrageenan powder samples are provided by Tacara Sdn. Bhd., Tawau, Malaysia (TA150) which produced from Kappaphycus alvarezii harvest in Tawau, Sabah. Other materials include potassium chloride (Kalisel, 1019GERI), maltodextrin with DE 4 by Euro Chemo-Pharma and refined white sugar (Prai) and alkaline water which supplied by the Spring Water Filter Mart of Penampang, Sabah, Malaysia.

Determination of the best extraction method for grass jelly extracts
A pre-study was carried out to determine the best extraction method for grass jelly extract with higher recovery and shorter preparation time, which based on the production yield of the spray dried grass jelly extract powder. Two variables of water added (4500 ml and 3500 ml) and cooking time (2.5 hours and 3 hours) is designed to determine the best extraction method for grass jelly extract and 125 g of cincau dried leaves with 30 ml of alkaline water were used. The extract produced is then spray dried at temperature at 170-175˚C inlet and outlet temperature at 85 to 95 ° C with a flow rate of 15 ± 2 g / min (Adisakwattana et al., 2014). Maltodextrin of 40 DE is used with the percentage of 10 % as the encapsulating agent in the spray drying process of grass jelly extract.

$$\text{Yield recovery (\%)} = \frac{Total\ mass\ of\ powder\ produced}{Total\ mass\ of\ sample\ solid\ content} x100\% \qquad (1)$$

Physicochemical analysis
There are nine formulations of instant grass jelly powder produced which premix with different percentage of SRC and KCL and subjected to various physic-chemical test which including the solubility test, gel strength, turbidity, color, DSC and syneresis.

Table 1: Formulation of instant grass jelly with different concentration of semi-refined carrageenan (SRC) and potassium chloride (KCL).

Formulation	Grass Jelly Extract (%)	Refined White Sugar (%)	SRC (%)	KCL (%)
F1				0.15
F2	5	5		0.25
F3			0.5	0.35
F4				0.15
F5	5	5		0.25
F6			1.5	0.35
F7				0.15
F8	5	5		0.25
F9			2.5	0.35

Sample preparation
The instant grass jelly with semi-refined carrageenan was produced by dissolving the premix powder of grass jelly extract, SRC, KCL and refined sugar with required amount of distilled water (80-

90 ˚C) until it reaches 100 % with continuous manually stirring (see Table 1). The complete dissolved solution is then poured into a container and warms it at room temperature for the gel formation.

Solubility test

Determination of solubility for instant grass jelly powder is determined and modified from Lai *et al.* (2009). Amount of 10g of premixed sample was put into a beaker and water added up to 100 % and the solution is stirred with the vortex stirrer. Dissolving time is calculated by stopwatch and recorded once the instant grass jelly powder was dissolved completely. The solubility rate calculated by the mass of the sample divided by the time of its completely dissolved.

Gel strength measurement

The samples were prepared by dissolving the required amount of the raw materials in distilled water until it reaches 100 % with manually stirring at (80-90˚C) until it's completely dissolved. The samples were then poured into a standardized size of plastic container and left to mold and samples were stored in the refrigerator ambient temperature of 10°C overnight and then deducted into a cylindrical shape with a diameter of 20mm and a length of 30 for guest gel rupture strength of sample grass jelly. TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) was used to evaluate the strength of the gel for instant grass jelly samples. A load cell of 5 kg was applied where the maximum force is ± 5 kg. Fixtures and probe analysis (P 0.5 / R = 12.7mm diameter) is compressed against instant grass jelly samples to a depth of 15 mm at a crosshead speed of 2 mm s-1. Each measurement was repeated 3 times to obtain an average value of elasticity (Bono *et al.,* 2014).

Turbidity test

Instant grass jelly samples were prepared according to the required amount and stored for overnight prior the analysis. The turbidity of instant grass jelly is measured by Pye Unicam PU 8600 UV / Visible Spectrophotometer (Pye Unicam LTD., Cambridge, UK) at 550nm (Lau *et al,* 2000). Each measurement was repeated three times to obtain an average value.

Color

The color of instant grass jelly samples was measured using the calorimeter Hunter Lab Color flex. The parameters L *, a * and b * were recorded from the screen where L * represents whiteness or brightness / darkness, a * represented redness / green and b * represents the yellow / blue. Each measurement was repeated three times to obtain an average value.

DSC measurements

A Perkin Elmer Diamond DSC Instrument was used to measure the Tg for the instant grass jelly samples. The instrument was calibrated with pure indium and an empty pan of the same type as the sample pan was used as the reference pan. Samples with 10.00 ± 0.02 mg were measured in an aluminum pan prior the analysis. The measurement was carried out starting from temperature range of 20 - 100˚C with the scan rate of 5°C per min. After the first heating cycle, the sample is maintained at a temperature of 100°C for 15 minutes. Later, the same grass jelly sample is cooled from 100°C to 20°C at 5°C per min. Each transition temperature measurement for melting and gelling were repeated three times to obtain an average value (Medina-Torres et al., 2006).

Syneresis

Syneresis measurement is carried out at room temperature by measuring the amount of moisture that evaporates from the grass jelly samples at a certain time interval. Grass jelly samples is prepared

and cut into the size of 30mm x 8mm and kept for 24 hours at a temperature of 10˚C for 2, 4, 6, 8 and 10 days. Original weight of the sample (W_0) together with its container is weighted and recorded. During the period specified interval, the water evaporates on the walls of the former absorbed by tissue or filter paper (Chen *et al.,* 2002). Then, the weights of the gels were measured (Wt). The syneresis of the gels was calculated as the cumulative weight of the water collected divided by the weight of the original sample and multiplied by 100

Syneresis (%) = $[(W_0 - W_t)/W_0]$ x 100 ……………………………………………………………………… (2)

Sensory evaluation

Sensory evaluation of the instant grass jelly with semi-refined carrageenan was conducted through the ranking test (Balanced Incomplete Block) and hedonic test to determine the acceptance level and choose the most favorable formulation of the instant grass jelly added with semi-refined carrageenan among the trained panelists. Ranking test (Balanced Incomplete Block) is used to select the formulations which have the highest level of liking among the panelists by ranking the order of the samples presented which according to Aminah (2000). Hedonic test was conducted to determine the level of likings of a few samples which selected from the ranking test among the panelists and the best will be chosen based on the mean score for the attributes tested, which given by the panelists, in which the hardness scale of 7 is used in this sensory test.

Chemical Composition Analysis

The analysis is carried out to determine the chemical composition and nutrient contents of the selected formulation for instant grass jelly with semi-refined carrageenan. The moisture content analysis was determined using air drying oven method (AOAC 943.01), whereas the protein content was determined by the micro Kjeldahl distillation method (AOAC 981.10). The ash content determination was carried out in at muffle furnace at 550 °C according to AOAC 930.05, while fat content was determined by a Soxhlet extraction method which using petroleum ether (AOAC 991.36). The total dietary fiber determination is carried out by using the enzymatic method as according to AOAC 985.29.

RESULTS AND DISCUSSION
Production Yield of Grass Jelly Powder Extract

The determination of the production yield for the grass jelly powder indicates the best extraction methods where there are four different liquid extract of grass jelly produced with different variables of water added and cooking time. Results showed that Extract 1 and Extract 2 showed higher recovery with 77.60 % and 75.92 % as compared to extract 3 and extract 4 with lower recovery of 55.26 % and 50.53 % respectively. It showed that the extract 3 and 4 which produced with lower amounts of water with 3500 is seen to be having lower production yield as compared with extract 1 and 2. It might be due to that extract 3 and 4 were produced with lower amount of water would contain lower amounts of solid content that it could be extracted during the boiling throughout the preparation process. Besides that, the more concentrated solution will indirectly affect the feeds of the extracts hard to be pumped and being sprayed during the spray drying process, which resulting the lower process efficiency and production yield of the grass jelly extract (Senka *et al.,* 2014). Extract 2 which produced with 4500 ml water added and boiled for 2.5 hours is selected as the best extraction recovery for the grass jelly powder as it compared with Newman method which used 3500 ml of water with cooking time of 3 hours. In addition, the maximum production capacity and cost saving factors are desired on an industrial processing level.

Table 2: Production yield of grass jelly extracts powder which based on different variables of water added and cooking time.

Grass Jelly Extract	Yield (%)
Extract 1	77.60
Extract 2	75.92
Extract 3	55.26
Extract 4	50.53

[1]Extract 1: Cooking time with 3 hours and 4500 ml water added.
[2]Ekstrak 2: Cooking time with 2.5 hours and 4500 ml water added.
[3]Ekstrak 3: Cooking time with 3 hours and 3500 ml water added.
[4]Ekstrak 4: Cooking time with 2.5 hours and 3500 ml water added.

Solubility

The solubility test is conducted to measure the reconstitution speed of spray-dried powder of grass jelly premix with SRC into the water. It is expressed as the time taken by the powder to fully reconstitute into the water by vortexing method (Larsson, 2010).
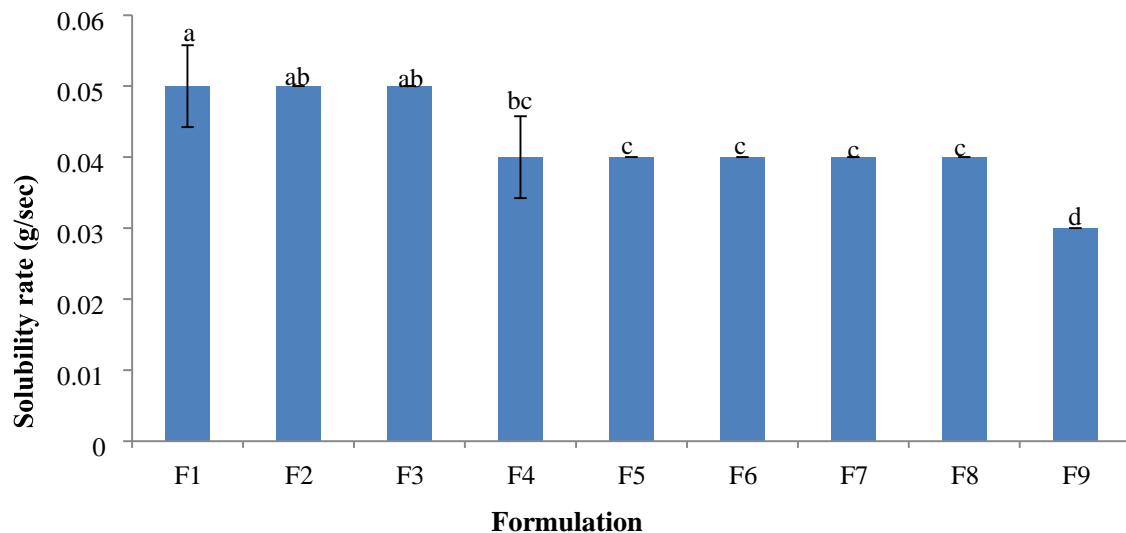

Figure 1: The mean value for the solubility rate of instant grass jelly with semi-refined carrageenan. The mean value which has the same alphabet bar shows no significant difference at $p < 0.05$.

Based on Figure 1, the solubility rate of the instant grass jelly powder with semi-refined carrageenan is decreased significantly ($p < 0.05$) as the concentration of semi-refined carrageenan used is increasing among the samples. The solubility rate of the samples is in the range of 0.03 – 0.53 g/sec. It is noted that the mean value for the F4 - F8 are not significantly different ($p > 0.05$) in which the mean value is in the range of 0.04. However, F9 showed significant differences with other formulations ($p < 0.05$) with the mean value recorded as 0.03 ± 0.00 g/sec which known as the lowest rate among the formulations. This is because F9 contains the highest solute content as compared with other formulations which needed a longer time to dissolve completely. Furthermore, the solubility of the carrageenan is reflected by the hydrophilic colloid in which the carrageenan is classified based on the number and position of the sulfate ester groups and the content of 3,6-anhydro-α-D-galaktopyranosyl. According to Necas and Bartosikova (2013), a high concentration of sulfate ester in carrageenan showed a low solubility in water. By this, kappa carrageenan, which contains sulfate ester content in the range of 25-30% indirectly affect its solubility properties in water.

Besides that, the solubility rate of the instant grass jelly powder is also affected by the other factors such as the dextrose equivalent value of the maltodextrin used and the inlet temperature (Chegini

and Ghobadian, 2005). Inlet temperatures used in the spray drying technique for grass jelly powder in this study are about 170 ° C and inlet temperature used in spray powder for food component is at 150-220 ° C (Phisut, 2012). When inlet temperature used is low, the rate of water evaporation is slower during the spray drying technique caused the powder in high moisture content. The high moisture content of the spray dried powder can increase the capacity of the solution solubility and tendency toward the agglomeration process (Chegeni and Ghobadian, 2005). Besides that, the maltodextrin value that is too high will directly affect the ability of the powder to dissolve as the high DE will cause the grass jelly powders to become sticky and thus affect the solubility of the powder (Tonon et al., 2009).

Gel Strength

The gel strength measurement of the instant grass jelly is measured with TA-XT2i Texture Analyzer by using an analytical probe (P 0.5/R=12. 7mm diameter) where the samples were compressed with a depth of 15mm at a crosshead speed of 2 mms s^{-1}.
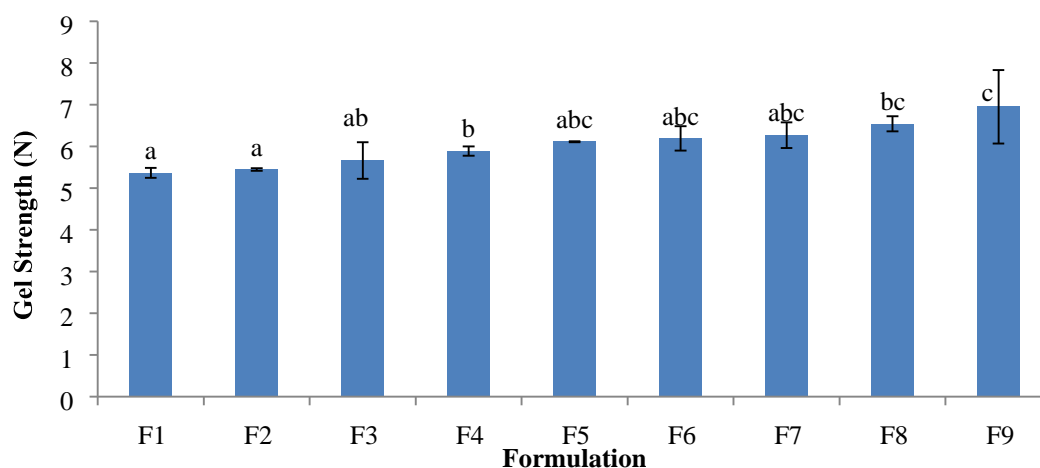

Figure 2: The mean value for the gel strength of instant grass jelly with semi-refined carrageenan. The mean value which has the same alphabet bar shows no significant difference at p<0.05.

Based on Figure 2, when the concentration of semi-refined carrageenan is increased among the samples, the gel strength of the grass jelly is also increased significantly (p<0.05). F9 exhibit higher gel strength among the formulations with 6.95 ± 0.88, whereas grass jelly of F1 recorded with lower gel strength as compared with the other formulations of 5.37 ± 0.12. This is due to the increase of the kappa semi-refined carrageenan concentration would induce the stronger and more compact of helix dimer and three-dimensional network with the presence of potassium cations. In addition, the stronger the gel structure is also associated with the microstructure system of the carrageenan gel which like the sectional tubular structure in gel with the characteristics of a diameter between 10 and 20 m (Mac Artain et al., 2003). At the same time, the ionic strength of the cation also plays an important role in enhancing the gel strength where the cations such as potassium cations are known to be specified with the semi-refined carrageenan for more compact and robust of gel formation (Haug et al., 2004).

Turbidity

Turbidity level of instant grass jelly is measured at 550 nm absorbance spectrum where turbidity on grass jelly is said to be progressively increased (p<0.05) in concentrations of semi-refined carrageenan used in the formulations.

Table 3: The mean value for the turbidly level of instant grass jelly with semi-refined carrageenan. The mean value which has the same alphabet in the same column shows no significant difference at p<0.05.

Formulation	Turbidity (nm)
F1	0.20 ± 0.00^a
F2	0.28 ± 0.00^b
F3	0.29 ± 0.01^b
F4	0.66 ± 0.00^c
F5	0.70 ± 0.00^d
F6	0.71 ± 0.00^d
F7	1.07 ± 0.01^e
F8	1.13 ± 0.00^f
F9	1.14 ± 0.00^f

Table 3 showed that F1 recorded the lowest turbidity level, which is at 0.20 ± 0.00 whereas F9 showed the highest level of turbidity among the formulations with the mean score of 1.14 ± 0.00. The semi-refined carrageenan powder (SRC) is said to have exhibited turbidity properties which due to the extraction process that used alkali treatment with 8.5% potassium hydroxide at a temperature of 60 ˚C (Prajapati *et al.,* 2014). Soaking and washing process with water after the alkali treatment will be able to get rid of waste minerals, protein and fat from carrageenan seaweed, yet a small portion of the cell walls of cellulosic waste of seaweed are still remained in partially processed of semi-refined carrageenan. Thus, semi-refined carrageenan typically contains cellulose residues, which contributed to the turbidity level of the semi-refined carrageenan powder used in this study (Prajapati *et al.,* 2014).

Color

As mentioned earlier, the L* value shown by the Hunter Lab calorimeter represents the brightness level where 100 represents white and 0 representing black.

Table 4: The mean value for the different parameter color of instant grass jelly with semi-refined carrageenan. The mean value which has the same alphabet in the same column shows no significant difference at p<0.05.

Formulation	Parameter		
	L*	a*	b*
F1	1.34 ± 0.11^a	0.60 ± 0.36^a	1.50 ± 0.34^a
F2	1.66 ± 0.12^b	0.63 ± 0.14^a	1.02 ± 0.02^{ab}
F3	1.94 ± 0.11^b	0.75 ± 0.16^a	1.39 ± 0.15^b
F4	2.42 ± 0.09^c	1.19 ± 0.09^a	2.35 ± 0.21^c
F5	2.68 ± 0.05^c	0.91 ± 0.23^a	2.74 ± 0.06^{cd}
F6	3.13 ± 0.07^d	0.89 ± 0.30^a	2.86 ± 0.07^d
F7	4.40 ± 0.15^e	1.04 ± 0.29^a	3.34 ± 0.03^e
F8	4.90 ± 0.07^f	1.16 ± 0.10^a	3.48 ± 0.04^e
F9	5.10 ± 0.11^f	0.61 ± 0.02^a	3.68 ± 0.04^e

The range for the mean score of L* was in between 1.34 to 5.10. The mean value of L* is the highest at F9 with the mean score of 5.10 ± 0.11 as compared to F1 with the mean value of L* which is the lowest one among the samples 1.34 ± 0.11. This reflects the degree of brightness is increased significantly (p<0.05) due to the concentration of semi-refined carrageenan used also increased among the samples. The semi-refined carrageenan is appeared to be pure white and slightly yellow, which resulted from the process of extraction of the seaweed *E. Cottonii* and thus contributed the degree of brightness in overall instant grass jelly appearance (Dunstan et al., 2001).

At the same time, the mean score for the b* also shows a sharp increase (p<0.05) when the concentration of semi-refined carrageenan used is increasing among the formulations. The positive

value of b* represents the color of yellow while a negative b* represents the color of blue. In conjunction with this, the L* and b* values are more emphasized in determining the color of carrageenan powder. On the other hand, the positive a* value which reflects the color of red showed there is no significant different (p>0.05) where the range for the a* is from 0.06 until 1.19. The low mean score of a* also showed that the instant grass jelly exhibit very low intensity of red color.

Differential Scanning Calorimeter (DSC)

The melting and gelling point for instant grass jelly with semi-refined carrageenan is measured by using the Differential Scanning Calorimeter instrument (DSC). Temperature shift occurs in a specified temperature range and the peak temperature taken as the melting point (Tm) and gelling point (T gel) for the samples.

Table 5: The mean value for the melting point (T_m) and gelling point (T_{gel}) for the instant grass jelly with semi-refined carrageenan. The mean value which has the same alphabet in the same column shows no significant different at p<0.05.

Formulation	Melting Point (T_m)	Gelling Point (T_{gel})
F1	33.69 ± 0.04^a	37.92 ± 0.03^a
F2	33.74 ± 0.04^a	40.73 ± 0.31^b
F3	33.66 ± 0.04^a	45.45 ± 1.72^d
F4	51.47 ± 0.11^b	38.87 ± 0.10^a
F5	62.32 ± 0.04^d	43.65 ± 0.05^c
F6	62.51 ± 0.02^d	43.99 ± 0.07^c
F7	61.61 ± 0.07^c	43.36 ± 0.09^c
F8	65.15 ± 0.08^e	47.58 ± 0.10^e
F9	70.06 ± 0.64^f	51.15 ± 0.18^f

Table 5 showed that F9 has the highest melting point (70.06 ± 0.64) and gelling point (51.15 ± 0.18) than the other formulations of the instant grass jelly. On the other hand, F1 shows the lowest melting point (33.69 ± 0.04) and gelling point (37.92 ± 0.31) among the samples. In overall, the transition temperature as well as the melting point and the gelling point were increased significantly (p<0.05) as the concentration of semi-pure carrageenan and potassium chloride used also increased. This is due to kappa carrageenan is sensitive to the monovalent cations K^+ in the gel network formation which form a strong and resilient gel, and thus further raised up the melting point during gelling phase and solution-gel transition (Lai *et al.,* 2000).

During the heating phase, the hydrogen bond is broken, and the double helix has undergone a series of changes in the aggregation network and subsequently separated. In the cooling process, the random nature coils formed helical shape during the endothermic transition temperature and subsequent gel formation occurs at a certain melting temperature (Nunez-Santiago and Tecante, 2007). The difference between melting and gelling is known as thermal hysteresis in which the occurrence of aggregation of the double helix that leads to the formation of gel (Mangione *et al.,* 2003).

Syneresis

The percentage of syneresis shows a significant increase in accordance with the jelly storage period, which lasted for 10 days (p<0.05). The slope of the graph showed that it significantly increased (p<0.05) when the concentration of semi-refined carrageenan used were decreasing among the instant grass jelly samples. Slope of F1 to F3 with a lower concentration of semirefined carrageenan showed a higher percentage of syneresis (p<0.05) which contrast with slop of F7 to F9 which have a higher content of semi-refined carrageenan showed lower slopes which reflect lower occurrence of the syneresis phenomenon (p <0.05).
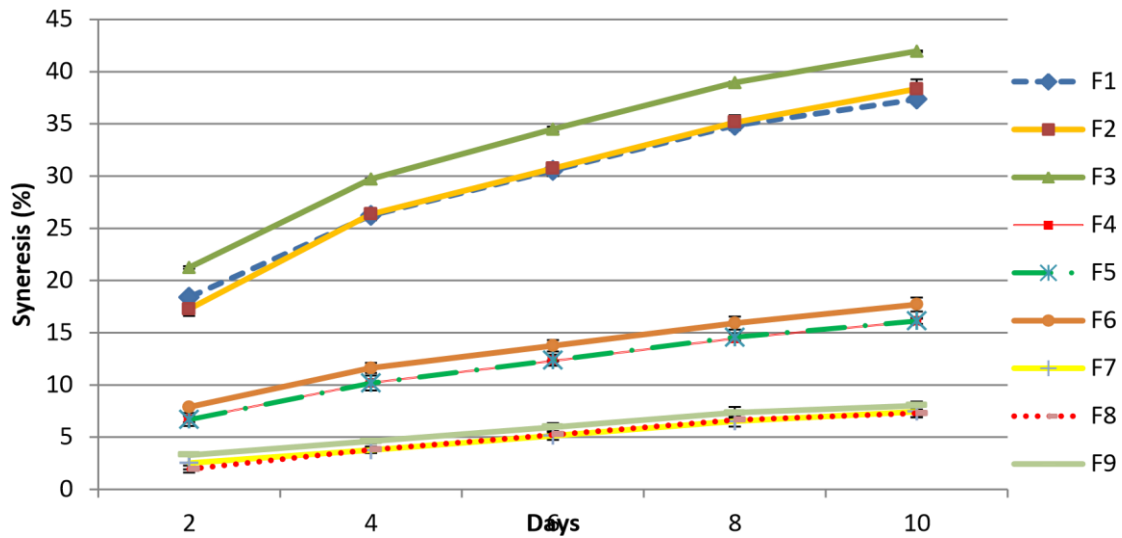
Figure 3: The mean percentage of syneresis of instant grass jelly with semi-refined carrageenan within 10 days of storage.

According to Munoz and Freile-Pelegerin (2004), an increase in the concentration of carrageenan in gel system can reduce the rate and the percentage of syneresis. This is due to shrinkage can be reduced in carrageenan gel when concentration of carrageenan used increases, at the same time it also helps to enhance gel elasticity which caused by the withdrawal capacity in the food gel system. Furthermore, syneresis can be reduced by increasing the concentration of carrageenan had also supported by Banerjee and Bhattacharya (2012). In addition, it can be observed that the increase of potassium chloride with the same concentration of semi-refined carrageenan among the samples as shown by F1 up to F3, where F3 has a greater mean of syneresis percentage which are higher than F1 and F2 ($p < 0.05$. This can be attributed to the fact that the use of high concentrations of cations will encourage carrageenan gel to suffer syneresis due to helical polymers in the gelling system which aggregate strictly and thus extrude water out from the gelling system of carrageenan (Thrimawithana et al., 2010).

Sensory Evaluation

There are three samples chosen from the ranking tests which are F4, F6 and F7 which have the lower total score value among the samples, whereby lower the score value higher the liking by the panelists. Besides that, the chosen samples are also compared with their physicochemical characteristics which based on the physical analyses that carried out prior to sensory tests. The chosen samples through the BIB ranking test will then be evaluated with hedonic test to select the formulation with the most acceptance and likings among the panelists. Hedonic test showed that F7 (2.5 % SRC, 0.02M KCL) is the most favorable formulation among the panelists.

The result in Table 6 showed that the attribute of texture and overall acceptance by F7 exhibit significant different ($p<0.05$) from F4 and F6. For the texture attribute, F7 recorded the highest mean score of 5.30 ± 1.20, followed by F6, which recorded a mean score of 3.95 ± 1.32 and F4 with the mean score of 3.93 ± 1.21. It can be associated that F7 exhibit higher gel strength than both other formulations. Besides that, it is also consistent with the fact that the increase in the concentration of SRC and KCL such as in F7 will provide elasticity properties and higher gel strength (Haug et al., 2004). This is because kappa carrageenan capable to form a three-dimensional network with potassium cations and establish a compact microstructure food gel system (MacArtain *et al.,* 2003).

Table 6: Result of hedonic test which based on the attributes evaluated. The mean value which has the same alphabet in the same row shows no significant difference at p<0.05.

Attributes	F4	F6	F7
Color	5.28±0.91[a]	5.03±0.86[a]	5.08±1.07[a]
Taste	4.85±1.15[a]	4.75±1.13[a]	4.88±1.04[a]
Aroma	4.68±1.00[a]	4.68±0.97[a]	4.63±0.95[a]
Transparency	4.80±1.04[a]	4.70±1.22[a]	5.13±0.91[a]
Texture	3.93±1.21[a]	3.95±1.32[a]	5.30±1.20[b]
Overall Acceptance	4.73±1.18[a]	4.55±1.13[a]	5.35±0.98[b]

Chemical Composition

The chemical composition of the instant grass jelly with the best formulation (F7) consists of five major categories, which are moisture, ash, fat, protein, and dietary fiber.

Table 7: Chemical composition of instant grass jelly powder with semi-refined carrageenan.

Chemical Composition	F7 (%)
Moisture	5.96 ± 0.12
Ash	6.04 ± 0.41
Fat	0.14 ± 0.06
Protein	0.29 ± 0.00
Dietary Fibre	18.54 ± 0.00

Based on Table 7, the moisture content of the grass jelly powder was 5.96 ± 0.12% and is said to be safe from microbiological aspects (Santhalakshmy *et al*., 2015). According Aziah and Komathi (2009), the quality of storage of powder-based products will be affected when moisture content is exceeded 14%, which will then contribute to the growth of fungi, insects, and clumping to be happened. Based on the Food Chemicals Codex (FCC) (1981), the maximum moisture content which allowed for carrageenan powder is below 12% as the tendency of carrageenan in moisture absorption during certain storage period.

Protein content of instant grass jelly powder which evaluated by Kjeldah recorded a value of 0.29 ± 0. This is because most of the powder which consisted of semi-pure carrageenan contain the composition of the protein is very low and can hardly be detectable (<1%), besides that the crude fiber also being broken through the alkali extraction process of seaweed (Chen et al., 2002). As shown in Table 4.7, the ash content in the instant grass jelly powder contains 6.04 of ± 0.41%, which reflected the various mineral contents that found in instant grass jelly powder. The International standards for ash level in carrageenan is not more than 40% and the sample is to be said fulfilled the standards (FCC, 1981).

The fat content in instant grass jelly powder is very low with a value of 0.14 ± 0.06%. This is partially true as carrageenan used in the formulation of instant grass jelly is derived from seaweed E. cottonii is a source of low-fat organism (Chen *et al.,* 2002). Besides that, the fat contained in the black grass jelly leaves merely 1% of the total content of nutrients which is relatively low (Food Directory Department of Health of the Republic of Indonesia, 1992). The content of dietary fiber in grass jelly powder in this study is recorded as 18.54% and based on the act of 18 (c) in the Food Act 1983, Schedule II, food products which contain high in dietary fiber must reach the 6g in 100g for solids. By this, instant grass jelly powder in this study is claimed to be a high dietary fiber product. This is due to the dietary fiber in this product are mainly derived from K. alvarezii which contain high composition of total dietary fiber with 49.7% dietary fiber soluble (Raman and Doble, 2015).

CONCLUSION

In this study, the best extraction method for grass jelly extract is determined which is the boiling time for 2.5 hours with water added as much as 4500ml to achieve the demanded recovery. Besides that, the effects of a combination of SRC and KCL on the physicochemical properties of instant grass jelly were investigated by several tests, which showed a significant difference in overall ($p<0.05$). The results showed that the high concentration of SRC gives high turbidity ($p<0.05$) and low rate of solubility, whereas higher concentrations of SRC would be able to increase the gel strength ($p<0.05$) with the range of 5.37 – 6.95 N. In addition, the colorimeter result showed that the more the SRC added, the brighter the product, yet still well accepted by the panelist in sensory tests. F7 (2.5 % SRC 0.02M KCl) are chosen as the best formulation which based on the combination results of physicochemical tests and hedonic test where it showed significant difference between the samples ($p<0.05$) for the attribute of texture and overall acceptance during hedonic test. Chemical composition analysis of F7 also showed that it exhibits 5.96 ± 0.12 of moisture, 6.04 ± 0.41 of ash, 0.14 ± 0.06 of fat, 0.29 ± 0.00 of protein and 18.54 ± 0.00 of dietary fiber. It reflects that the instant grass jelly powder added with semi-refined carrageenan is shown to be a good source of mineral and dietary fiber instant food product. As a conclusion, SRC can be used as a starch replacer in grass jelly production as an instant product and texture is retained as well as nutrient contents of the product is improved.

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