University of San Carlos
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Laboratory Report

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CHE 3110L
Laboratory Simulation of Industrial Product Manufacture

Carrageenan Production

A laboratory report submitted to

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by

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1. Introduction

Food additives are a common ingredient found in many processed foods. Typically, these additives come in the form of hydrocolloids which are a group of long-chain polymers (such as polysaccharides and proteins) that have the ability to modify food properties such as viscosity and texture. The term hydrocolloid originates from the term hydro meaning water and colloid meaning glue-like (Wüstenberg, 2015). Hence, hydrocolloids are used commonly in different industries as a thickener, stabilizer, emulsifier, and gelling agents like food gums, proteins, and starches (Saha & Bhattacharya, 2010).

Few of the most common food gums and gelling agents used in the industry are extracted from seaweeds. Due to the differences between the characteristics of these seaweeds, these gelling agents are categorized into three, namely, alginates, agar, and carrageenan. Out of the three, carrageenan is the most versatile due to the differing chemical structure and properties and is widely used not only in the food industry but also in cosmetics, pharmaceutical products, printing, and textiles (Das, 2016).

Carrageenan that is commercially used are classified into three categories: Iota, Lambda, and Kappa. These classifications represent the different abilities each variety of carrageenan can impart. Iota ι-carrageenan forms elastic gels with calcium salts that are stable even when frozen and thawed. This type of carrageenan exhibits a thixotropic behavior, which means that the gel can be stirred and can flow like a thick liquid but will gradually reform a gel once left to rest. Lambda λ-carrageenan forms no gels but are suitable in forming high-viscosity solutions. Kappa κ-carrageenan forms strong, rigid gels with potassium salts and brittle gels with calcium salts that are slightly opaque and become transparent with the addition of sugar. Chemically, the difference between the three carrageenan types is the number of sulfate groups per disaccharide. κ-carrageenan has one sulfate per disaccharide, ι-carrageenan has two, and λ-carrageenan has three sulfates per disaccharide (Husin, 2014).

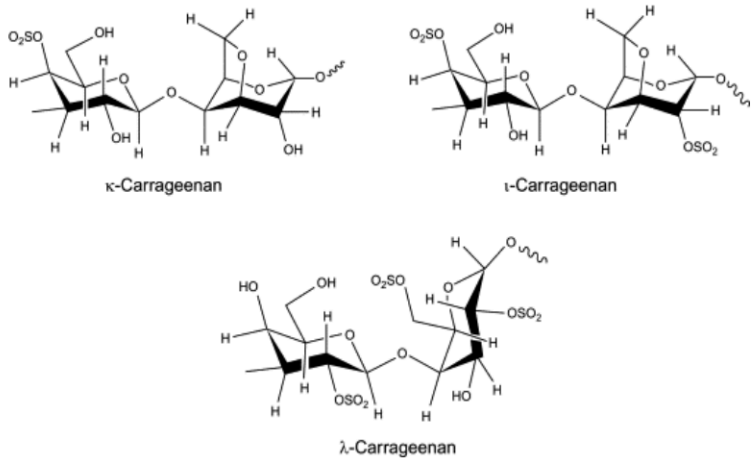


Figure 1. *Chemical Structures of the Different Carrageenan Types*

In the production of carrageenan, different types of seaweed produce different carrageenan varieties. For instance, Chondrus Crispus, also known as Irish Moss, contains a mixture of kappa and lambda carrageenan. In the Philippines, two most commonly cultivated species of seaweed namely, Kappaphycus alvarezii (Cotonii), the elkhorn sea moss and Eucheuma denticulatum (Spinosum), which are both known in the Philippines as "guso" contains mainly kappa-CGN and iota-CGN, respectively (McHugh, 2003).

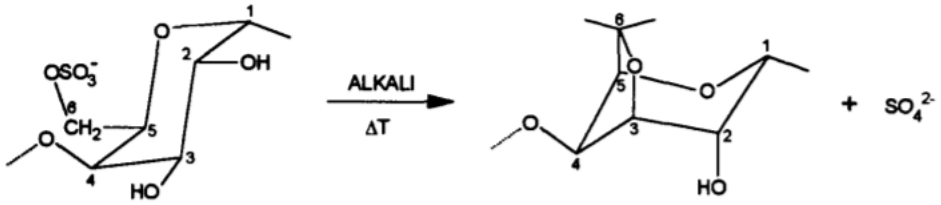
Figure 2*. Irish Moss (photo obtained from: healthy.net)*

Figure 3. *Cotonii (photo obtained from: thalgovietnam.vn)*

Figure 4. *Spinosum (photo obtained from:caraga.bfar.da.gov.ph)*

Prior to extraction, the raw material is dried to contain as little moisture as possible ideally, less than 15% water by mass. Fresh seaweeds typically have a 10 to 1 wet to dry ratio. This means that after complete drying, the original weight of the seaweed will be decreased ten times. However, most seaweed in the market is sold with a minimum moisture content of 35% (McHugh, 2003). The most common means of drying involve sun-drying or oven-drying.

To maximize the extraction of carrageenan from the raw materials, the seaweed is washed to remove sand, salts, and other foreign matter. The seaweed is then partially dried and treated with hot water containing an alkali such as sodium hydroxide (NaOH) or potassium hydroxide (KOH) for about two hours to obtain a solid residue containing mostly carrageenan and cellulose (Hoffmann, 1995). An alkali is used because it removes some of the sulphate groups and increases the formation of 3,6-anhydrogalactose, which leads to an increased gel strength in the final product (McHugh, 2003).



(1)

There is no one way to extract carrageenan from seaweeds. Depending on the quality and the costs, different methods may be applied (McHugh, 2003). One method involves producing semi-refined carrageenan which only involves cleaning, alkalization, drying, and milling which makes it the cheapest of all carrageenan production methods. Although inexpensive, the carrageenan produced by this method is colored, and generally impure because the carrageenan

is not "extracted" from the seaweed but components other than the carrageenan are dissolved in the alkali solution. Also, this method only works best in the production of kappa carrageenan, which is why the seaweed, *Kappaphycus alvarezii*, is most suitable for this method. Another method follows the SRC process but takes an extra step in refining the carrageenan. Once alkalization is done, the mixture is filtered, and alcohol is added to the filtrate to precipitate the carrageenan. Alcohol is recovered from the filtrate, and the precipitate is washed, dried, and milled, producing high-quality, colorless, carrageenan. Although this method is applicable for all carrageenan types, this method is deemed the most expensive. Another method also involves alkalization, but the resulting alkaline mixture is filtered, and KCl is added to the filtrate to precipitate the carrageenan. The mixture is then pressed, forcing water out of the gel, hence the name gel pressing method. The gel is dried and milled as usual, producing high purity kappa carrageenan. However, this gel method is only applicable in producing kappa carrageenan as it relies on the ability of kappa carrageenan to form gels with potassium salts. Finally, processed Eucheuma seaweed, also known as Philippine Natural Grade Carrageenan (PNG) is produced by modifying the SRC process by removing the color with the use of bleach. The bleach is removed using a closed dryer or by alcohol washing followed by alcohol recovery via vacuum evaporation.

2. Objectives of the Experiment

1. Prepare a process flowsheet for the manufacture of carrageenan, complete with details in process conditions and stream specification;

2. Monitor overall and component mass and energy flows during the lab-scale implementation of the product manufacture;

3. Calculate component yields in every process step and for the entire process; and

4. Identify critical steps in the process based on laboratory data and the entire experience of generating the product.

3. Methodology

3.1. Methodological Framework

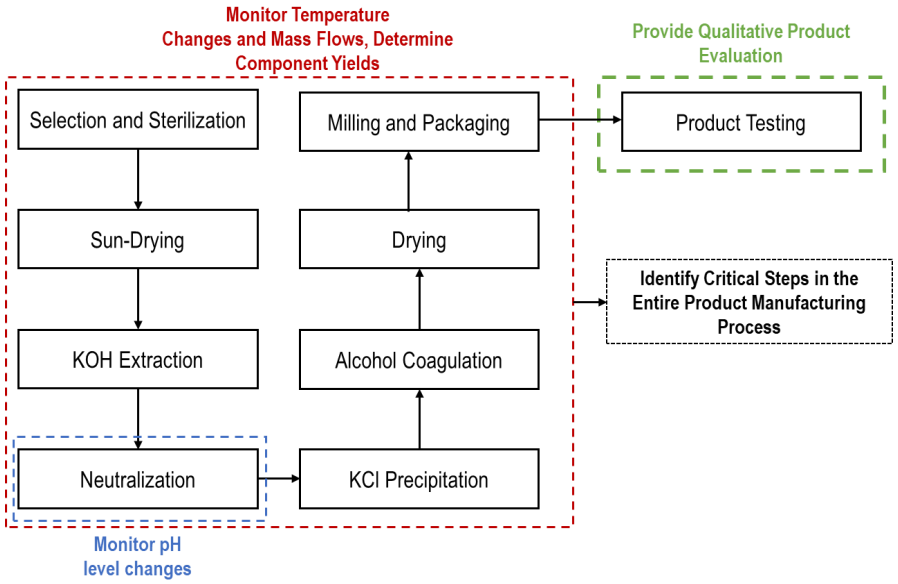


Figure 5. *Methodological Framework for the Production of Carrageenan*

As shown in figure 5, the process of carrageenan production is better visualized via a process flow diagram containing the key steps. Due to the unavailability of the laboratories, the energy flows were only monitored via temperature changes. The mass flows were constantly monitored to aid in the determination of the component yields in every inflow and outflow streams. Additionally, the pH level of the mixture was closely monitored during the neutralization step. Critical steps were also identified during the actual conduct of the experiment based on the generated data and observations. Finally, review and testing were done to evaluate the final product.

3.2. Materials

The key materials for this experiment were seaweed, potassium hydroxide solution, hydrochloric acid, potassium chloride solution, ethyl alcohol and distilled water.

The primary material needed in the production of carrageenan was seaweed. In this experiment, one (1) kilogram of a particular type of seaweed, *Kappaphycus Alvarezii*, was used because of its abundance in the Philippine market. The seaweed used in this experiment was purchased from the local public market in Lapu-Lapu City.

***Figure 6**. Actual photo of seaweed used in the experiment*

To extract the carrageenan, an alkali was needed, and potassium hydroxide solution was prepared by dissolving KOH crystals in distilled water. This solution extracts the carrageenan out of the fresh seaweed by removing the sulfate groups and producing 3,6-anhydrogalactose, as shown in equation 1.

***Figure 7.** KOH crystals*
(photo obtained from: https://rayeneh.com/koh-potassium-hydroxide/)

The resulting mixture from the alkalization step was highly basic and must be neutralized prior to any further treatment. With this, a dilute hydrochloric acid was prepared by mixing 29% hydrochloric acid (muriatic acid) with distilled water.

***Figure 8.** Muriatic acid*
(photo obtained from: https://gmart.com.ph/shop/apollo-muriatic-acid-250ml/)

The carrageenan in the mixture was precipitated into gels via the addition of potassium chloride solution. This solution was prepared by dissolving a specific amount of KCl salt in distilled water.

***Figure 9**. KCl Salt*
(photo obtained from: https://cicig.co/product/yidfopu1a)

95% ethyl alcohol solution was used to coagulate the carrageenan gels previously precipitated by the KCl solution.

***Figure 10.** Ethyl alcohol*
(photo obtained from: https://www.nebraskascientific.com/chemicals-d-l/3938-ethyl-alcohol-95-denatured-lab-grade-1l.html)

Distilled water was used to prepare the aforementioned solutions, to sanitize equipment surfaces and to sterilize the seaweed prior to treatment.

***Figure 11.** Distilled water*
(photo obtained from: https://shopee.ph/Absolute-Distilled-Drinking-Water-6000-ml-i.238345847.5727760372)

3.3. Equipment

The equipment used in the actual conduct of experiment were a gas stove, an oven, a digital weighing scale, a mechanical weighing scale, pH paper strips, a blender, a cooking pot, wash basins, measuring cups, strainers, kitchen thermometer, soft-bristled brush, and plastic containers.

For the heating and drying processes, a triple burner gas stove and a 30x30 cm oven were used. The oven used had the capacity to reach temperatures up to 280°C.

Figure 12. *3-burner stove and oven*
(photo obtained from:
https://shopee.ph/Labelle-3-Burner-Gas-Range-with-Oven-(a-product-of-La-Germania)-i.10158735.3613798238)

The digital weighing scale was used to measure smaller masses like measuring the amounts of solutes in preparing solutions and measuring the final product. This weighing scale was calibrated to measure in grams (g) up to one decimal place.

Figure 13. *Digital weighing scale*
(photo obtained from:
https://shopee.ph/I-2000-3kg-digital-kitchen-scale-jewelry-mini-superior-i2000-3000g-i.15263198.5614799735?gclid=EAIaIQobChMIu8fMmozD7QIVUz5gCh0jhADlEAQYBiABEgKhuvD_BwE)

Because the digital scale available was small, the mechanical weighing scale was used to measure heavier materials and is calibrated to measure in kilograms (kg).

Figure 14. *Mechanical weighing scale*
(photo obtained from:
https://shopee.ph/Fuji-Mechanical-Table-Scale-%28Weighing-Scale%29-30kg-and-20kg-i.53329915.5239728236)

The pH of the mixture was monitored via a pH paper that came along with a color chart that indicate the pH level. This was utilized during the neutralization process.

Figure 15. *pH Paper with Color Indicators*
(Image Obtained From: https://www.aliexpress.com/item/4000523185088.html)

The type of thermometer used in the experiment was a kitchen thermometer, one that is enclosed in glass and can read temperatures both in Fahrenheit and in Celsius.

Figure 16. *Kitchen thermometer*
(photo obtained from: https://www.aliexpress.com/item/32284460901.html)

For the size reduction processes a 500-watt blender was used to grind the final dry product into powder form.

Figure 17. *500-watt blender*
(photo obtained from: https://www.abenson.com/kw-4724.html)

The rest of the equipment used were a cooking pot for the actual cooking process, wash basins, measuring cups used to measure volumes from 5mL up to 1000 mL, strainers, a kitchen knife for cutting, soft-bristled brush, and plastic containers for the final packaging.

3.4. Procedures (Manuhara, Praseptiangga, & Riyanto, 2016)

A process flow sheet was prepared beforehand to serve as a guide in monitoring the process conditions, including temperature and pH levels. The masses were monitored closely throughout the whole experiment.

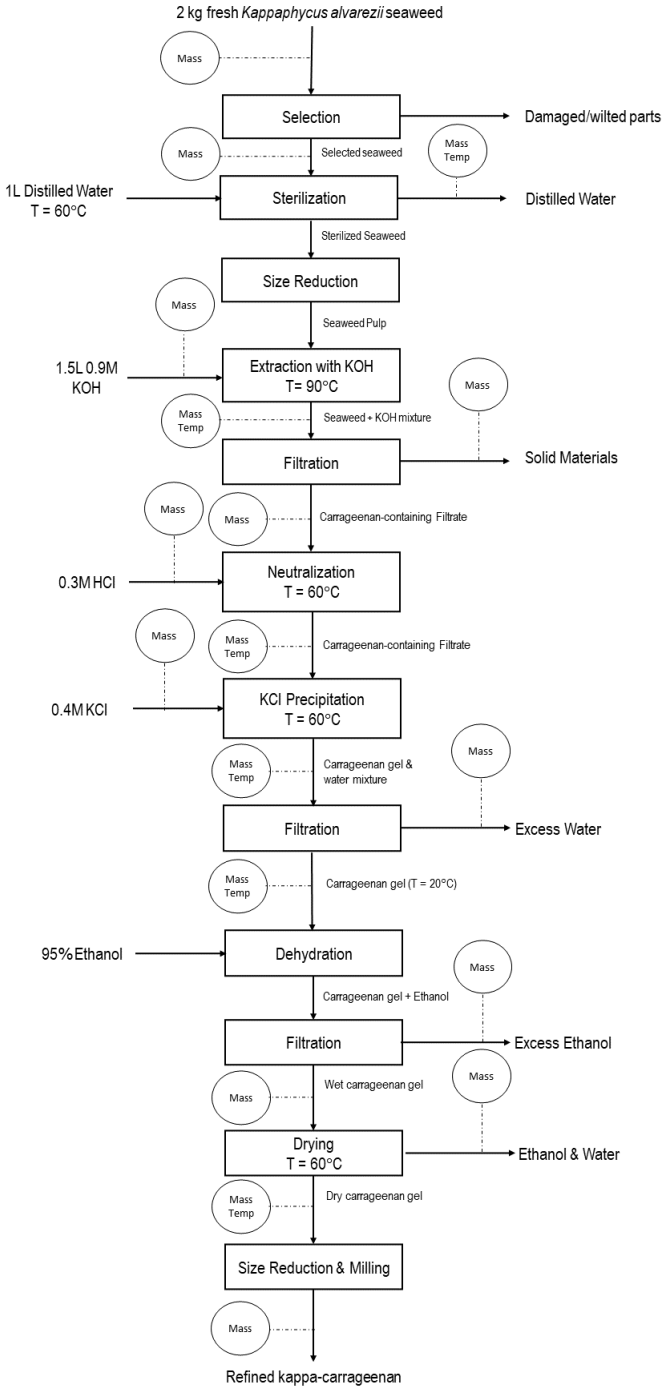


Figure 18. *Process flow for the production of carrageenan*

Prior to the actual conduct of the experiment, the materials used along with working surface and equipment were cleaned with soap and running water and sterilized with hot water, at about 70°C. One (1) kilogram of the fresh *Kappaphycus Alvarezii* seaweed was weighed and washed with about 20 liters of running tap water to get rid of unwanted materials such as sand and other plants tangled with the seaweed. Fortunately, the seaweed was pre-washed at the market and contained little to no sediments. The seaweed was weighed again, and the material still weighed one (1) kilogram on the mechanical scale. The seaweed was reduced to smaller pieces by cutting it with a kitchen knife. The freshly cut seaweed was placed in a stainless-steel basin and was sterilized by soaking it in 1000g of distilled water that was heated to 60°C for an hour. Then, the seaweed was transferred in a colander and excess water was allowed to drain off for 4 hours. The seaweed was weighed again, and the seaweed weighed 873 grams, indicating that the seaweed had lost 127 grams of water in the draining process.

After the preparation of the raw material, the seaweed was placed on a tray to prepare for sun-drying. After 48 hours of sun-drying the seaweed weighed 435 grams. Since the seaweed was glossy and wet underneath, it was placed in a net bag and sun-dried for two more days. After a total of 96 hours of drying, the seaweed weighed 270 grams and its green color was completely gone.

For the alkalization step, about two (2) liters of 0.9M KOH solution was used and this was prepared by mixing 100.8 grams of KOH crystals with 2000 grams of distilled water (assuming the density is 1g/mL). The dried seaweed was placed in a pot and was allowed submerged in the solution. The pot was then placed over a gas stove and was heated for 2 hours. The temperature of the mixture was monitored using a kitchen thermometer and the temperature was maintained at 90°C. After heating, the mixture was allowed to cool to room temperature (20°C) so that it would be safe enough to handle during the next step.

With the aid of a round spoon, the mixture was scooped into the strainer and the viscous filtrate was allowed to drain off, leaving the solid materials on the strainer. The solid materials were left t in the strainer set-up for about an hour to let most of the filtrate drain off. The remaining solid materials weighed 650 grams and were discarded.

The viscous filtrate was very basic and was determined to have a pH level of 11 based on the pH color chart. To neutralize the filtrate, a 1.37% v/w was prepared by diluting 25 mL of 29% v/w HCl solution in 505 grams of distilled water. The solution was poured slowly into the mixture until the pH indicator paper turns into a light green color (about pH 7) which indicates a neutral pH.

Prior to the precipitation step, the neutralized filtrate was weighed to determine the amount of KCl solution needed. Since the filtrate weighed about 1903 grams, about 1000 grams of 0.4 M KCl solution was prepared to make roughly a 2:1 filtrate to solution ratio. The KCl solution was prepared by dissolving 29.8 grams of KCl salt in 1000 grams of distilled water. While the solution was being prepared, the mixture was reheated to 60°C and the heating time amounted to a total of 27 minutes. Once the mixture reached the desired temperature, the prepared KCl solution was poured into the mixture while stirring continuously for 15 minutes. After this step, the mixture was allowed to cool to room temperature for it to be safely handled in the next step.

Since the precipitated gels from the KCl precipitation step are small, a piece of a clean linen cloth was placed over a sieve to strain the mixture, leaving the gel precipitates. About 1 liter of the 95% v/v ethyl alcohol solution was mixed in the remaining mixture to coagulate the gels. The alcohol solution was poured gradually with continuous stirring. The coagulated gels were strained, and the remaining wet gel weighed to be about 308.2 grams.

For the drying process, the oven was preheated to 150°C (lowest setting available in the oven). The gel was spread out on a baking tray lined with a silicone mat to prevent from sticking. The tray was covered in aluminum foil that was poked with holes to allow the contents in the tray to heat evenly while allowing steam to escape. The drying was done in 10-minute intervals and after a total of 3 hours and 40 minutes of drying, the dried gel weighed 28.3 grams.

For the milling process, a 500-watt blender was used to grind the final product into smaller bits, preferably in powder form. However, even after 30 minutes of total operation time with the blender, the dried gels were only partially pulverized. The resulting product was then transferred to a container with a soft-bristled brush to get most of the product in the blender. However, due to the difficulty in getting all of the powder from the nooks and

crannies of the blender, the final weight of the product was only 25.5 grams and the remaining 2.8 grams were counted as a loss.

Finally, the product was tested by incorporating it in fruit jam, specifically mango jam. Mango was the chosen fruit since mangoes are low in pectin and do not readily form a gel unlike most fruit jams (Adamant, 2019). The testing was done by comparing two jam samples, one containing carrageenan and one without.

In calculating for the component yield per process step, some of the desired output material (e.g. amount of extracted carrageenan in the alkalization step) cannot be quantified with the equipment at hand. Hence, the component yield was calculated for the process steps where applicable (e.g. separation processes) and the yield for the entire process:

$$Percent\ Yield\ = \frac{Mass\ of\ Desired\ Material\ in\ the\ Output\ Stream}{Total\ Mass\ of\ Input\ Streams} x100\% \quad \textbf{(2)}$$

$$Percent\ Yield,\ \%\ = \frac{Mass\ of\ Final\ Product,\ \ g}{Mass\ of\ Seaweed\ Used,\ \ g} \times 100\% \quad \textbf{(3)}$$

4. Results and Discussions

4.1 Process Conditions, Stream Specifications, and Mass Flows

The production of carrageenan involved eight key processes, namely: sterilization, sun-drying, extraction, neutralization, precipitation, coagulation, drying, and milling. The conditions and stream specifications for each process are indicated in the process flow sheet in ANNEX I.

The first key process was the sterilization step. This was necessary to remove biological contaminants that might be present in the raw material. Prior to this, the raw seaweed weighed about 1 kg based on the reading from the mechanical weighing scale. After washing, cutting, and soaking the seaweed for 1 hour in 60°C hot distilled water, the sterilized seaweed was allowed to drain off excess water for 4 hours, hence leaving only about 873 grams of seaweed.

Following this process was the sun-drying. Since fresh seaweed typically have a 10:1 wet to dry ratio (McHugh, 2003), the seaweed was dried to minimize its water content so as to minimize the dilution of the carrageenan and increase the efficiency of the extraction. From 873 grams, the mass of the seaweed went down to 270 grams after a 96-hour drying period. The seaweed became stiff and the green pigmentation was gone. The water content of the seaweed was reduced from 90% w/w to 61% w/w.

Extraction with KOH was done immediately after drying. The mixture was heated to 90°C for 2 hours with occasional stirring. The mixture was allowed to cool to room temperature (~20°C) before it was handled in the next step. The total mass of the mixture amounted to 2370.8 grams and the mass of the discarded material was 650 grams, leaving 1720.8 grams of filtrate.

Since the filtrate was at pH level 11 based on the pH chart, neutralization was necessary. About 182.2 grams of the prepared 1.37% v/w HCl solution was used, reducing the pH level to a neutral pH, or about 7 in the pH chart. This step was done in room temperature (~20°C) and produced a total of 1903 grams of neutralized filtrate.

Prior to the addition of KCl solution, the mixture was heated to 60°C for 27 minutes. A total of 1029.8 grams of 3% w/w KCl solution was added to precipitate the carrageenan.

The entire mixture weighed 2932.8 grams after. The mixture was cooled to room temperature in preparation for the next step.

The gels were coagulated using 1 liter (~790 grams) of 95% ethyl alcohol solution and the resulting mixture weighed 3722.8 grams. The coagulated gels formed were white in color and formed long "threads" that tangled up during mixing. The gel was strained using a sieve and the filtrate from the process was a clear, yellow to brown liquid. 308.2 grams of the wet gel was obtained from this process.

For the drying process, the oven temperature was set at 150°C and the drying was done in 10-minute intervals. The total drying time accumulated to a total of 220 minutes, and the weight of the dried gel was measured to be at 28.3 grams, losing about 279.9 grams of moisture.

Finally, the milling process was done in room temperature in a 500-watt blender that was operated for about 30 minutes. The dry gel was ground up to the blender's capacity and was recovered from the equipment with the use of a soft brush. Unfortunately, some of the product was stuck in the equipment that only 25.5 grams of the carrageenan powder was recovered. The remaining 2.8 grams were recorded as a loss. Photo documentation of the actual conduct of experiment is also provided in ANNEX IV.

4.2 Component Yield/Breakdown

The component yields for individual process steps are calculated where applicable. In process steps that involve mixing, the component yields are 100% hence it is no longer indicated below. In the sterilization process, the feed composed of about 1000 grams of seaweed and 1000 grams of 60°C distilled water. Since the fresh seaweed contains a 10:1 wet to dry ratio, the components for this step were categorized as the dry solids and water as shown on the table below:

Table 1. *Component Breakdown for the Sterilization Process*

<table>
<tr><th colspan="2">Input Stream</th><th colspan="2">Output Stream</th></tr>
<tr><th>Component</th><th>Mass</th><th>Component</th><th>Mass</th></tr>
<tr><td>Seaweed
$x_{dry\text{-}solids} = 0.1$
$x_{water} = 0.9$</td><td>1000 g</td><td rowspan="2">Seaweed + water mixture
$x_{dry\text{-}solids} = 0.05$
$x_{water} = 0.95$</td><td rowspan="2">2000g</td></tr>
<tr><td>Water</td><td>1000g</td></tr>
</table>

x_{water} = 1.0			

The seaweed-water mixture was left to drain in a colander for 4 hours and significant water loss was observed and the water content of the seaweed itself reduced from 90% w/w to 88% w/w. The water in the colander was discarded (1127 g).

***Table 2**. Component Breakdown for the Draining Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Seaweed + water mixture $x_{dry\text{-}solids}$ = 0.05 x_{water} = 0.95	2000g	Seaweed $x_{dry\text{-}solids}$ = 0.12 x_{water} = 0.88	873 g
		Water x_{water} = 1.0	1127 g

To further reduce the water content of the seaweed, sun-drying was done for a total of 96 hours. More water was removed from the material, reducing the total water content of the seaweed down to 61%. The seaweed obtained in this process amounts to 43.65% of the entire input stream.

***Table 3.** Component Breakdown for the Drying Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Seaweed $x_{dry\text{-}solids}$ = 0.12 x_{water} = 0.88	873 g	Seaweed $x_{dry\text{-}solids}$ = 0.39 x_{water} = 0.61	270 g
		Water (moisture) x_{water} = 1.0	603 g

The next key process was the extraction with potassium hydroxide. A total of 100.8 grams of KOH was dissolved in 2000 grams of distilled water. The 270 grams of dried seaweed was immersed in the prepared solution, resulting to a total of 2370.8 grams of the entire seaweed-KOH solution mixture.

***Table 4**. Component Breakdown for the Alkalization Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Seaweed $x_{dry\text{-}solids}$ = 0.39 x_{water} = 0.61	270 g	Seaweed + KOH mixture $x_{dry\text{-}solids}$ = 0.05	2370.8 g

5% w/w KOH Solution $x_{KOH} = 0.05$ $x_{water} = 0.95$	2100.8 g	$x_{water} = 0.91$ $x_{KOH} = 0.04$	

For the separation process, the rest of the components other than the dry solid seaweed was regarded as the filtrate itself. The discarded material weighed 650 grams, leaving 1720.8 grams of the viscous filtrate.

Table 5. *Component Breakdown for the Separation Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Seaweed + KOH mixture $x_{dry\text{-}solids} = 0.05$ $x_{filtrate} = 0.95$	2370.8 g	Discarded material $x_{dry\text{-}solids} = 0.18$ $x_{filtrate} = 0.82$	650 g
		Filtrate $x_{filtrate} = 1.0$	1720.8 g

The proceeding step was the neutralization process. 182.2 grams of the 1.37% v/w HCl solution was added into the mixture. The neutralized filtrate then weighed 1903 grams which is 72.58% of the output stream.

Table 6. *Component Breakdown for the Neutralization Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Filtrate $x_{filtrate} = 1.0$	1720.8 g	Neutralized Filtrate	1903 grams
1.37% v/w HCl solution	182.2 g		

In the precipitation step, 1029.8 grams of the 3% w/w KCl solution was added to the neutralized filtrate, making 2932.8 grams of the precipitated gel mixture.

Table 7. *Component Breakdown for the Precipitation Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Neutralized Filtrate	1903 g	Precipitated gel mixture	2932.8 g
3% w/w KCl solution	1029.8 g		

$x_{KCl} = 0.03$ $x_{water} = 0.97$			

The coagulation process required the use of 1 liter (~790 g) of 95% ethyl alcohol solution. The resulting mixture containing the coagulated gels weighed about 3722.8 grams.

***Table 8**. Component Breakdown for the Coagulation Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Precipitated gel mixture	2932.8 g	Coagulated gel mixture	3722.8 g
95% v/v ethyl alcohol	790 g		

The coagulated gel was separated from the filtrate and the gel obtained weighed 308.2 grams which is 8.27% of the input material. This indicates that the discarded filtrate has a mass of 3414.6 grams.

***Table 9.** Component Breakdown for the Separation Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Coagulated gel mixture	3722.8 g	Discarded Filtrate	3414.6 g
		Wet gel	308.2 g

Following the coagulation and separation process was the drying step. 308.2 grams of the wet gel was dried in an oven for to a total of 220 minutes, and the weight of the dried gel was measured to be at 28.3 grams (9.18% of input material), losing about 279.9 grams of moisture.

***Table 10.** Component Breakdown for the Drying Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Wet gel	308.2 g	Dried carrageenan gel	28.3 g
		Water and other volatile materials	279.9 g

Lastly, the milling and the packaging process was done. 28.3 grams of the dried gel was ground into powder form and recovered using a soft brush. Unfortunately, only 25.5

grams (90.10% of initial dried gel mass) of the powder was obtained because of the difficulty in recovering some of the material that got stuck in the equipment.

Table 11. *Component Breakdown for the Milling Process*

Input Stream		Output Stream	
Component	Mass	Component	Mass
Dried carrageenan gel	28.3 g	Carrageenan Powder	25.5 g
		Loss	2.8 g

The percent yield for the entire process is calculated by dividing the mass of the product material over the mass of the initial material used. Hence, the overall percent yield for 1 kg (1000 g) of fresh seaweed is 2.55%.

Calculations on mass flows are also illustrated in ANNEX V.

4.3 Critical Steps in the Production of Carrageenan

The production of carrageenan was not done in a laboratory but instead, in a kitchen. It was necessary to observe proper sanitation of surfaces and sterilization of materials to avoid contamination since carrageenan is a food additive.

Prior to treatment, it was a necessary step to dry the raw seaweed material and lessen its water content. Not only does this enhance the efficiency of the extraction process but this also reduces the volume of the material, making it easier to operate in smaller vessels.

In the entire production process, the most critical step is the extraction process as this will determine the amount of the final product. The extraction must be done in optimal process conditions, which in this case was a temperature of 90°C, to ensure that the components react well with each other and the carrageenan is extracted well from the raw material to the filtrate. Heating must also be monitored closely as to not overcook the mixture and cause the seaweed to disintegrate into the mixture.

Another critical step is the precipitation and the coagulation processes. These processes allow the extracted carrageenan to solidify in the form of a gel and makes the separation process easier. It is noted that continuous stirring must be done to ensure that all of the carrageenan is precipitated and coagulated. Based on the experiences of doing the experiment, one can be sure that all of the gel is coagulated when the filtrate in the separation step is clear and no white gel strains appear.

Drying and milling of the coagulated gel was necessary to obtain a dry and powdered carrageenan product. It is important to note that the drying must be done in intervals to ascertain that the product loses moisture gradually and not abruptly. In the milling process, it is recommended to utilize a smaller grinding machine. It was observed in this experiment that a large grinding equipment was not able to totally reduce the product into powder form because of the excess space in the grinding container.

Finally, the final product must be stored in a sealed container, away from moisture and direct heat to prevent contamination.

5. Conclusions

The production of carrageenan was adopted from the methods of Manuhara, Prasenptiangga, and Riyanto (2016). One kilogram of fresh raw seaweed was dries and was treated with 5% w/w KOH solution to extract the carrageenan. The neutralization, precipitation, and coagulation process were done by adding 1.37% v/w HCl solution, 3% w/w KCl solution, and 95% ethyl alcohol solution, respectively. Drying and milling were the final downstream processes and the final product weighed 25.5 grams which was 2.55% of the fresh seaweed.

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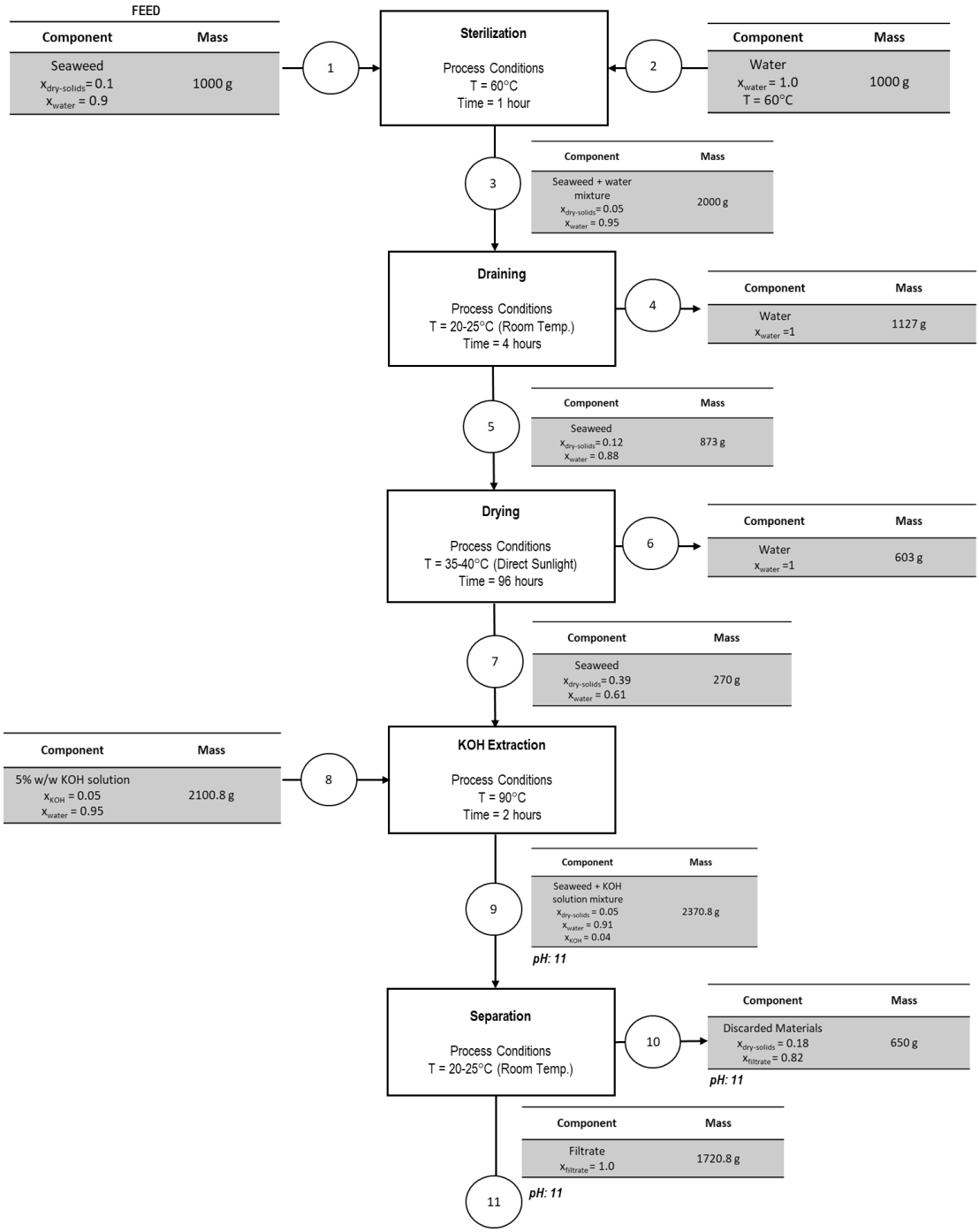
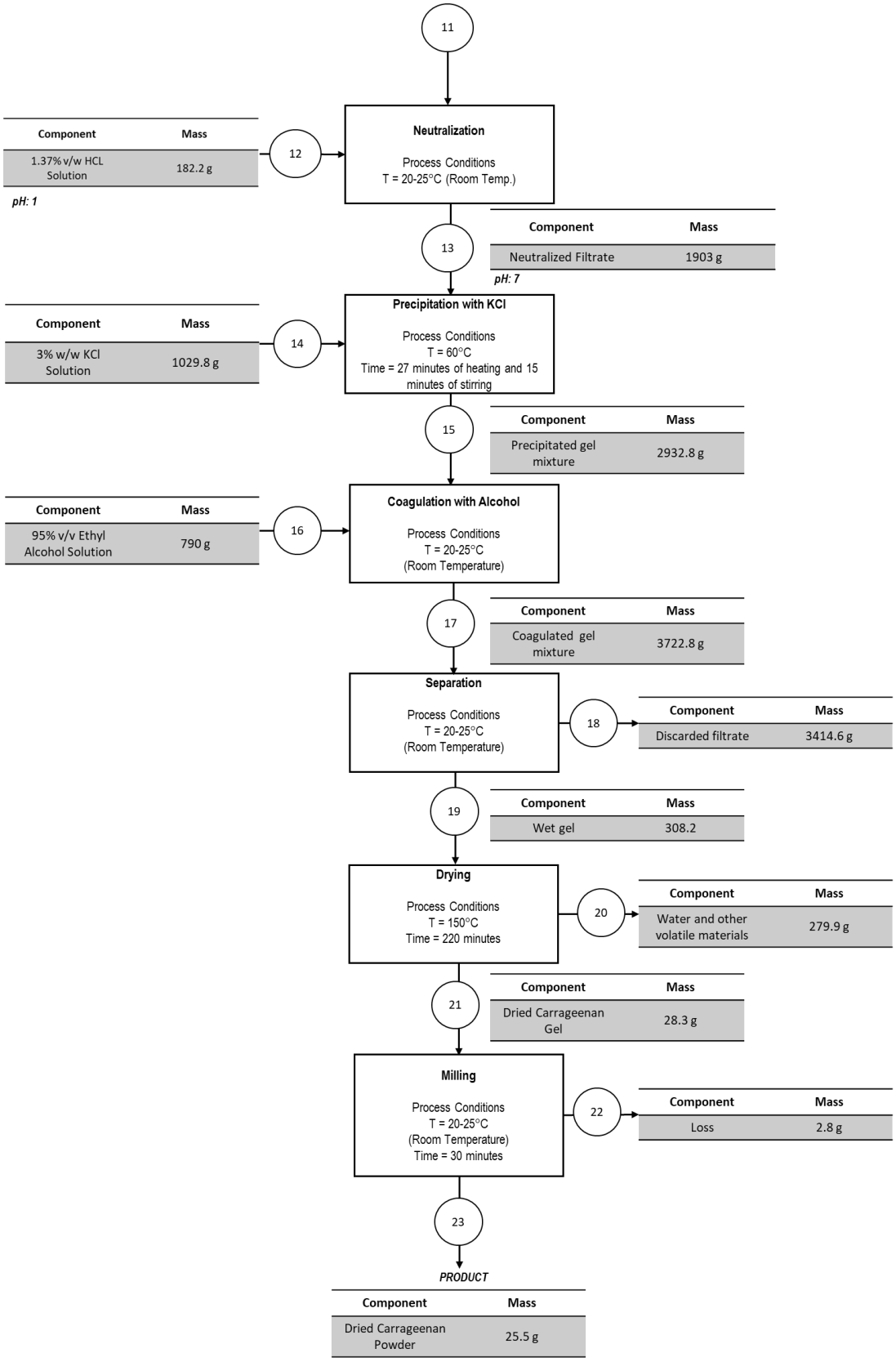
ANNEX I

Process Flowsheet




11
Component
Mass
1.37% v/w HCL Solution
182.2 g
pH: 1
12
Neutralization
Process Conditions
T = 20-25°C (Room Temp.)
13
Component
Mass
Neutralized Filtrate
1903 g
pH: 7
Component
Mass
3% w/w KCl Solution
1029.8 g
14
Precipitation with KCl
Process Conditions
T = 60°C
Time = 27 minutes of heating and 15 minutes of stirring
15
Component
Mass
Precipitated gel mixture
2932.8 g
Component
Mass
95% v/v Ethyl Alcohol Solution
790 g
16
Coagulation with Alcohol
Process Conditions
T = 20-25°C
(Room Temperature)
17
Component
Mass
Coagulated gel mixture
3722.8 g
Separation
Process Conditions
T = 20-25°C
(Room Temperature)
18
Component
Mass
Discarded filtrate
3414.6 g
19
Component
Mass
Wet gel
308.2
Drying
Process Conditions
T = 150°C
Time = 220 minutes
20
Component
Mass
Water and other volatile materials
279.9 g
21
Component
Mass
Dried Carrageenan Gel
28.3 g
Milling
Process Conditions
T = 20-25°C
(Room Temperature)
Time = 30 minutes
22
Component
Mass
Loss
2.8 g
23
PRODUCT
Component
Mass
Dried Carrageenan Powder
25.5 g

ANNEX II

Financial Report

Table 12. *Expense Summary for Materials*

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Fresh *Kappaphycus alvarezii* seaweed	2 kg	₱ 50/kg	₱ 100.00
[2] Distilled Water	4 L	₱ 12/L	₱ 48.00
[3] KOH Crystals	80 g	₱ 0.45/g	₱ 36.00
[4] 5M HCl solution	250 mL	₱ 0.1/mL	₱ 25.00
[5] KCl salt	50 g	₱ 0.4/g	₱ 20.00
[6]Ethanol (95 %)	1L	₱ 105.00/L	₱ 150.00
		Total	**₱ 379.00**

Table 13. *Expense Summary for Equipment/Apparatus*

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Weighing Scale	1 unit	₱ 630 / unit	₱ 630.00
[2] Ph Paper	10 strips	₱ 1.46/strip	₱ 14.6.00
[3] Colander	1 pc	₱ 115/pc	₱ 115.00
[4] Wooden Spatula	1 pc	₱ 59/pc	₱ 59.00
[5] Wooden Ladle	1 pc	₱ 59/pc	₱ 59.00
[6] Spoon	1 pc	₱ 59/pc	₱ 9.00
[7] Thermometer	1 pc	₱ 250/pc	₱ 250.00
[8] Pot	1 pc	₱ 800/pc	₱ 800.00
[9] Plastic Containers	1 pc	₱ 10/pc	₱ 20.00
[10] Gas Stove	2 kg	₱ 63.63/kg	₱ 127.26
[11] Measuring Cup	1 pc	₱ 85/pc	₱ 85.00
[12] Cheese Cloth	1 meter	₱ 32/ meter	₱ 32.00
[13] Plastic Plate	1 pc	₱ 10/pc	₱ 10.00
[14] Pitcher	1 pc	₱ 80/pc	₱ 80.00
[15] bowl	1 pc	₱ 67/pc	₱ 67.00
		Total	**₱2,357.86**

Table 14. *Room Rental Costs*

Room	*No. of days*	*Cost/day*	*Subtotal*
[1] ChE Unit Operation Laboratory	1	₱ 500/day	₱ 500.00
		Total	**₱ 500.00**

Table 15. *Total Costs*

Grand total	**₱ 3,531.75**

Grand total (with 20% contingency)	**₱ 4,238.10**

ANNEX III

Raw Data Sheets

Table 16. *Raw data from Preparation and Drying Process*

	Quantity	Unit
Mass of fresh seaweed	1	kg
Mass of cleaned and trimmed seaweed	1	kg
Mass of 1L distilled water	1000	g
Temperature of heated distilled water	60	^{O}C
Mass of seaweed after draining	873	g
Mass of seaweed after 48 hours of drying	435	g
Mass of seaweed after 96 hours of drying	270	g
Final mass of dried seaweed	270	g

Table 17. *Raw data from Alkali Treatment Process*

	Quantity	Unit
Mass of KOH crystals	100.8	g
Mass of distilled water	2000	g
Concentration of KOH solution	0.9	M
Temperature of mixture while cooking	90	^{O}C
Total time of heating	2	hrs.
Mass of discarded solids	650	g

Table 18. *Raw Data from Neutralization Process*

	Quantity	Unit
pH of filtrate before neutralization	~11	
Concentration of HCl prior to dilution	29	%v/w
Amount of water added	505	g
Volume of HCl added	25	mL
New concentration of HCl solution	1.37	%v/w
pH of filtrate after neutralization	~7	

Table 19. *Raw data from KCl Precipitation Process*

	Quantity	Unit
Mass of container	255	g
Mass of container + filtrate	2158	g
Mass of filtrate	1903	g
Temperature of filrate upon reheating	60	°C
Time of heating	27	mins.
Mass of KCl salt	29.8	g

Mass of distilled water	1000	g
Concentration of KCl solution	0.4	M
Stirring time	15	mins.

Table 20. *Raw data from Coagulation and Drying Process*

	Quantity	Unit
Volume of 95% EtOH added	~1	L
Mass of wet gel	308.2	g
Temperature of pre-heated oven	150	^{O}C
Mass of gel after 2 hours and 10 minutes of baking	64.7	g
Mass of gel after 3 hours and 40 minutes	28.3	g
Total time of oven-drying process	3.67	hrs.

Table 21. *Raw data from Milling and Packaging Process*

	Quantity	Unit	Remarks
Mass of dried carrageenan gel before pulverizing	28.3	g	
Mass of pulverized carrageenan powder	25.5	g	2.8 g counted as loss

Table 22. *Raw data from Post-Production Analytics Process*

	Quantity	Unit
Mass of carrageenan powder before use	25.5	g
Amount of carrageenan powder used	0.5	tsp
Mass of carrageenan powder left in container	24.8	g
Mass of carrageenan powder used in testing	0.7	g

Homemade mango jam product – Observations	Without carrageenan	With carrageenan
Consistency	Dripping easily; spreads better	More viscous, settles like a gel as it cools; thicker, hard to spread
Color	Light yellow	Still yellow, but slightly darker
Scent	Mango scent	Mango scent, no significant peculiar scent
Taste	Typical mango jam taste; sweet.	Still has a mango taste; a little aftertaste but not too overpowering.

ANNEX IV

Actual Conduct of Experiment

Documentation:

I. Preparation

a. Selection

b. Weighing of selected seaweed (1kg)

c. Cleaning of seaweed

d. Size reduction

e. Sterilization with distilled water heated to 60°C. Soaked for 1 hour.

f. Straining for 6 hours

g. Weighing of cleaned and sterilized seaweed

II. Drying

a. Placing of seaweed on a baking tray

b. Drying of seaweed under direct sunlight

c. Weighing of seaweed after day 2

d. Drying of seaweed under direct sunlight using a net bag

e. Weighing of seaweed after day 4

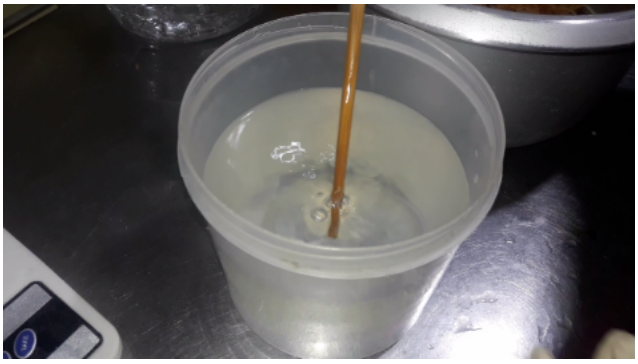
III. Alkalization

a. Preparation of 0.9M KOH solution (done twice to produce about 2L of KOH solution)

i. Weighing of 1000 grams distilled water

ii. Weighing of 50.4 grams of KOH crystals

iii. Mixing the KOH crystals with water



b. Pouring the solution into the pot with the seaweed

c. Heating the mixture for 2 hours until the temperature reaches 90°C

d. Straining

IV. Neutralization

a. Preparation of 1% HCl solution

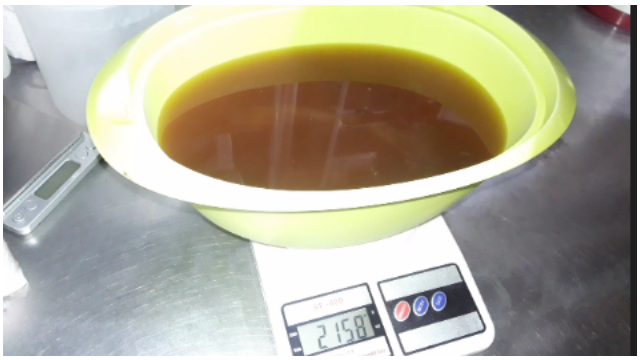
b. Initial pH testing

c. 5 mL additions of 1% HCl solution

d. After 600 mL total addition of 1% HCl solution

V. KCl Precipitation

a. Weigh filtrate to get an estimate of the volume



b. Reheat filtrate until it reaches 60°C

c. Prepare ~1L KCl solution

d. Add KCl solution to mixture

VI. Coagulation with Alcohol

a. Pour ~1L of 95% ethyl alcohol

b. Straining the gel

c. Weighing the wet gel

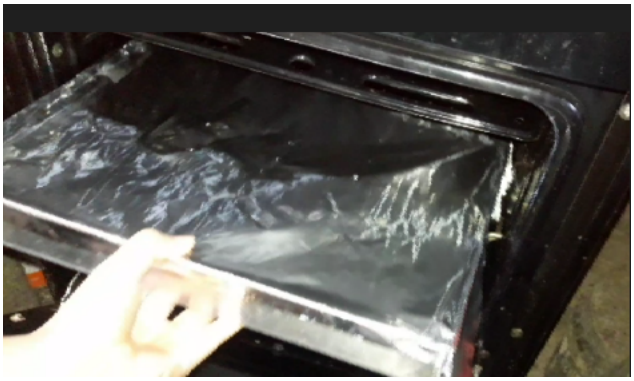
VII. Drying

a. Spreading over a baking tray lined with silicone mat

b. Covering with aluminum foil

c. Poking the foil with holes

d. Baking at 150°C at 10-minute intervals



e. After a total of 1 hour

f. After a total of 2 hours and 10 minutes

g. After a total of 3 hours baking

VIII. Grinding

a. Pulsing in the blender at high speed

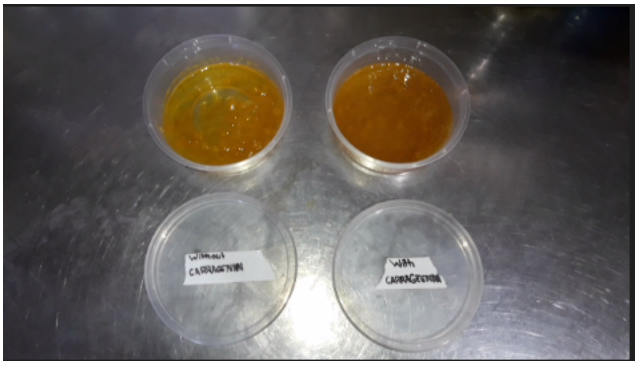
b. Transferring the powder using a clean brush

c. Weighing of the final output

IX. Packaging

a. Sealing in a plastic container

X. Product Testing with Mango Jam

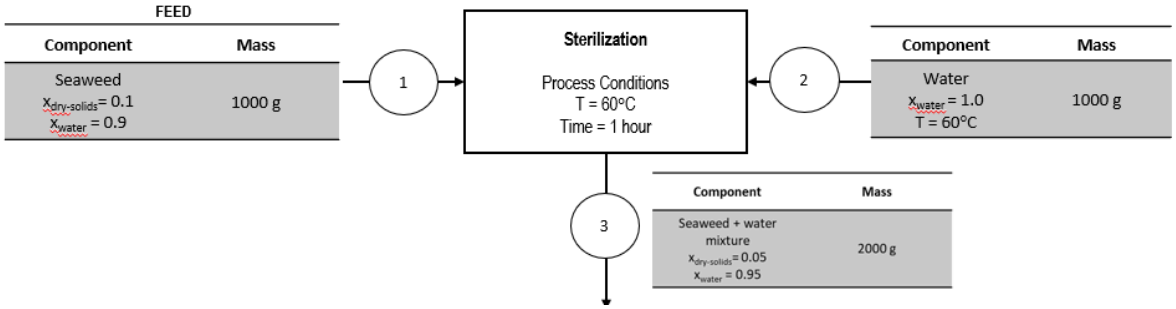


Video Documentation:
https://drive.google.com/file/d/1-2lZlTl3UkM5K-sqrLMsqMfgDysdJ364/view?usp=sharing

ANNEX V

Sample Calculations

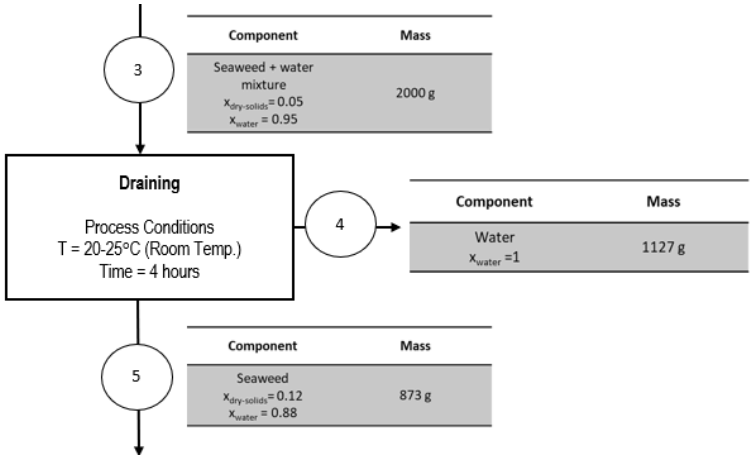
Preparation (Sterilization) Process



Since seaweed has a 10:1 wet to dry ratio, the mass fraction for the dry solids were taken to be 10% of the feed material.

$$m_1 = 1000gm_2 = 1000gm_3 = m_1 + m_2 = 2000\ g$$

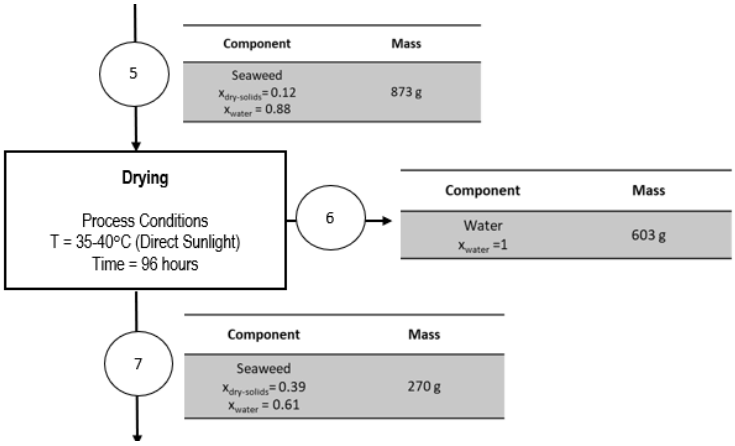
Draining Process



$$m_3 = 2000\ gm_5 = 873\ g\ (measured)m_4 = m_3 - m_5 = 1127\ g$$

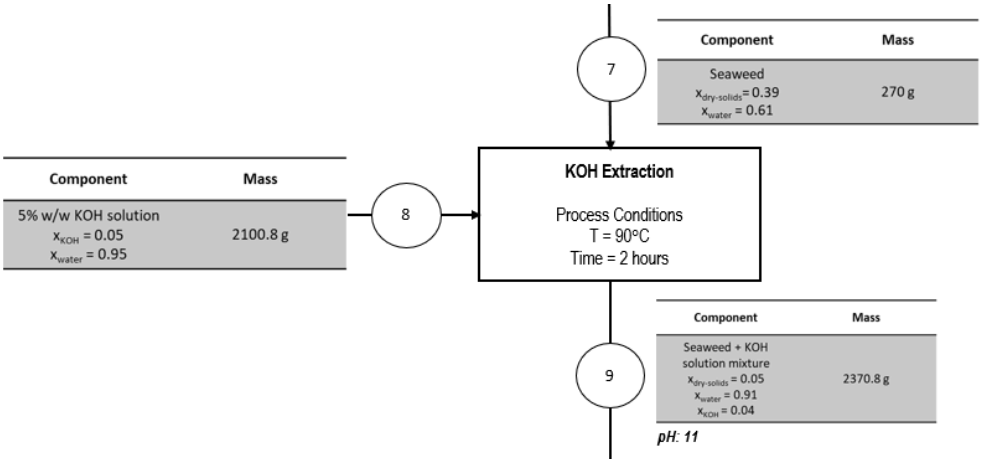
$$Percent\ Yield = \frac{873\ g}{2000\ g} x100\% = 43.65\%$$

Sun-Drying Process



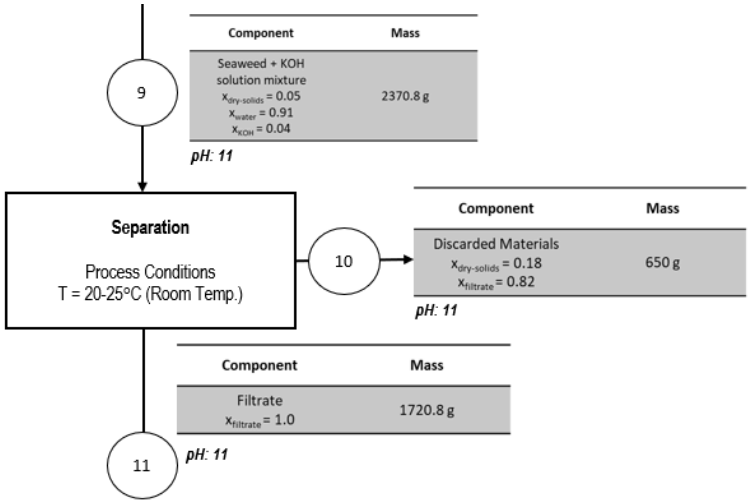
$$m_5 = 873\ g\ (measured) m_7 = 270\ g\ (measured) m_6 = m_5 - m_7 = 603\ g$$

Extraction Process



$$m_7 = 270\ g\ (measured) m_8 = 2100.8\ g\ (measured) m_9 = m_7 + m_8 = 2370.8\ g$$

Separation Process

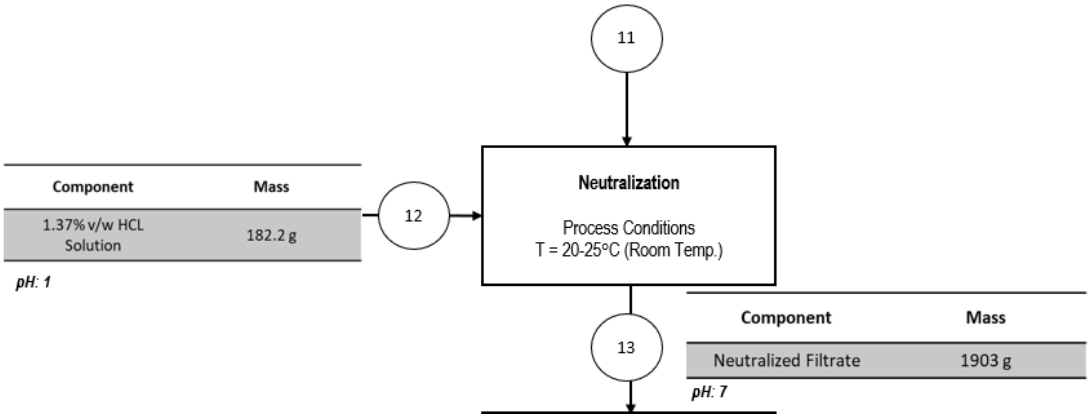


Since the amount of carrageenan extracted in the mixture is unknown, the liquid part of the mixture was considered as filtrate.

$$m_9 = 2730.8\ g m_{10} = 650\ g\ (measured) m_{11} = m_9 - m_{10} = 1720.8\ g$$

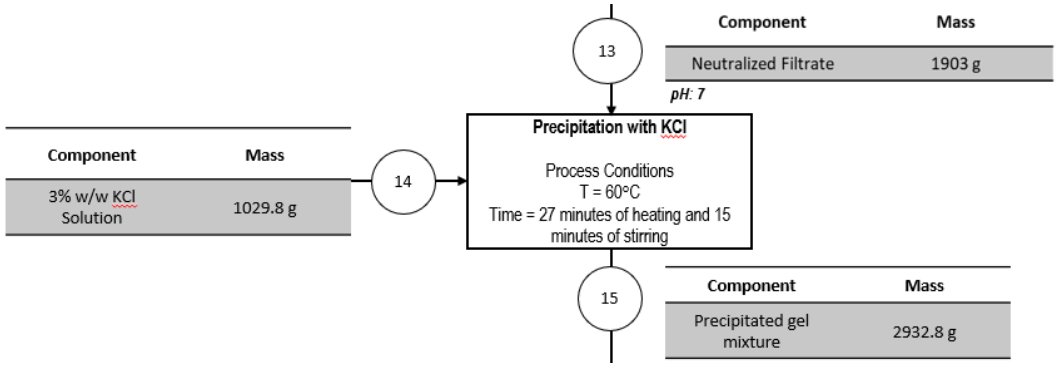
$$Percent\ Yield = \frac{1720.8\ g}{2370.8\ g} x100\% = 72.58\%$$

Neutralization Process



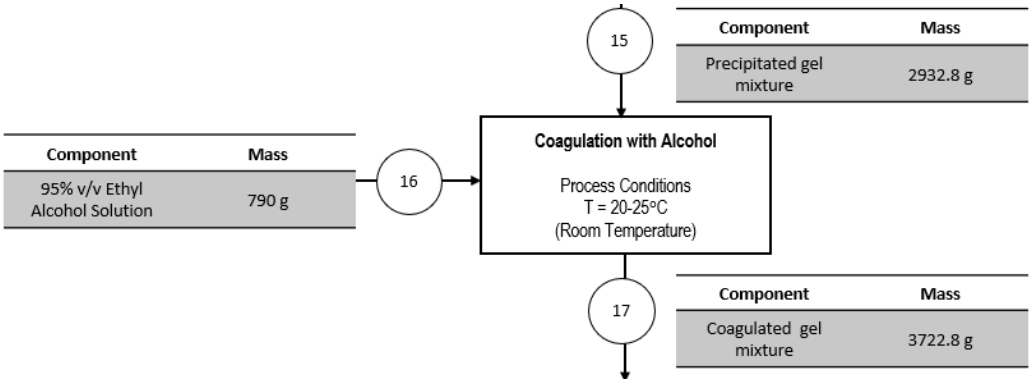
$$m_{11} = 1720.8\ g m_{13} = 1903\ g\ (measured) m_{12} = m_{13} - m_{11} = 182.2\ g$$

KCl Precipitation Process



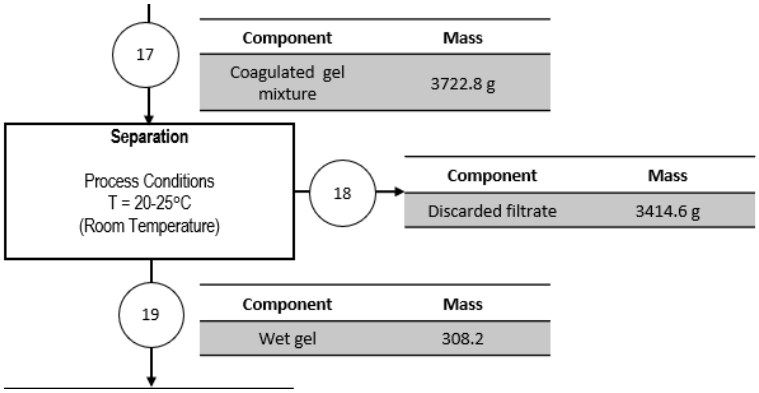
$$m_{13} = 1903\ g\ (measured) m_{14} = 1029.8\ g\ (measured) m_{15} = m_{13} + m_{14} = 2932.8\ g$$

Alcohol Coagulation Process



$$m_{15} = 2932.8\ g m_{16} = 790\ g m_{17} = m_{15} + m_{16} = 3722.8\ g$$

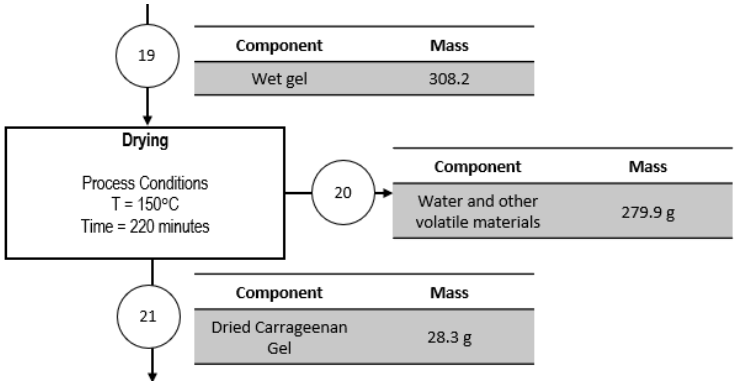
Separation Process



$$m_{17} = 3722.8\ g m_{19} = 308.2\ g\ (measured) m_{18} = m_{17} - m_{19} = 3414.6\ g$$

$$Percent\ Yield = \frac{308.2\ g}{3722.8\ g} x100\% = 8.27\%$$

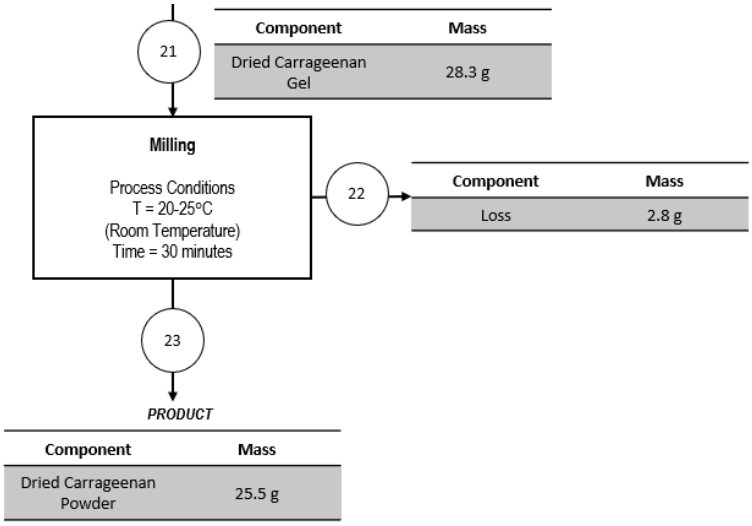
Drying Process



$$m_{19} = 308.2\ g m_{21} = 28.3\ g\ (measured) m_{20} = m_{19} - m_{21} = 279.9\ g$$

$$Percent\ Yield = \frac{28.3\ g}{308.2\ g} x100\% = 9.18\%$$

Milling Process



$$m_{21} = 28.3\ g m_{23} = 25.5\ g\ (measured) m_{22} = m_{21} - m_{23} = 2.8\ g$$

$$Percent\ Yield = \frac{25.5\ g}{28.3\ g} x100\% = 90.10\%$$

Entire Process Yield:

$$Percent\ Yield = \frac{25.5\ grams\ carrageenan\ powder}{1000\ grams\ raw\ seaweed} x100\% = 2.55\%$$