The Alkali Concentration Effect on Quality of Semi Refined Carrageenan Production : A Meta-Analysis

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Abstract

Semi-refined carrageenan (SRC) is a carrageenan product with a lower purity that it still contains a small amount of cellulose. Carrageenan which is produced from the extraction of red seaweed is part of the gelling compound and polysaccharide. Carrageenan has no nutritional value. In the food industry, carrageenan is used as a food additive and so its quality must be maintained properly especially during the process. The effect of processing on quality of carrageenan has been proven by several previous studies. This meta-analysis research was conducted to determine the effect of alkali concentration on carrageenan quality. Required data taken from previous studies was used to analyze the effect of alkali concentration on carrageenan quality. The variables of carrageenan quality analyzed were yield, water content, ash content, and viscosity. The results show the influence of the alkali concentration on carrageenan quality except water content. This can be seen from Z score and the effect size of each variable. Z score of yield, water content, ash content and viscosity are 6.3743, 1.3937, 2.1200, and 2.5694. The effect level of those variables are 1.8676, 0.2874, 1.1376 and 16.0021. All the p-value are below 0.05 (confidence level 95%).

Keywords
carrageenan, quality, food additive, meta-analysis, effect level.

1. Introduction

Food additives are ingredients that are not used as food and not a typical food ingredient; have or do not have nutritional value and intentionally added to produce a compound that affects the typical characteristics of food. One of the food additives that is widely used in the world is carrageenan. Carrageenan which is produced from the extraction of red seaweed is part of the gelling compound and polysaccharide. Although carrageenan has no nutritional value in the food industry. It is used as a food additive. There are two types of carrageenan: (1) semi refined carrageenan (SRC) and (2) refined carrageenan (RC). Semi refined carrageenan is a carrageenan product with a lower purity because it still contains a small amount of cellulose. The steps taken to processing red seaweed are as follows:

- Sorting dried seaweed to remove impurities such as gravel, coral, ropes and more.
- Washing dried seaweed with clean water.
- Extracting process using chemicals and water.
- Neutralization of pH using clean water.
- Drying
- Flour making (SRC product)
- Packaging

The process condition can affect the quality of SRC. Some previous studies have proven the relationship between processing and the quality of carrageenan. Table 1 shows the results of previous studies related to the process optimization of making carrageenan from red seaweed. In this study the red seaweed that will be discussed is *Eucheuma cottonii*.

Table 1. The Previous Studies Related to The Processing of Carrageenan

No.	Researcher	Year	Variable	Output (Dependent Variable)	Sample
1	Widiastuti	2002	Fixed variable; NaOH, water Isopropanol alcohol, extraction temp. is 90 °C extraction time is 18 hour, drying temperature is 60 °C. Independent variable; alkali concentration (0% - 5%).	• Yield; 24 - 47.9% • Viscosity; 45.4 – 110.7 cP • Gel strength; 211- 463.7gr/cm^2 • Gelling point; 20 – 30 °C	24
2	Yasita and Rachmawati	2009	Fixed variable; NaOH, water Extraction temp. 90 – 95 °C, Extraction time is 2 hour, solvent ratio 20:1 Independent variable; alkali concentration (0.1 – 0.3%), sedimentary type (etanol and isopropyl alcohol), type of bleach (H_2O_2).	• Yield; 11.4 - 39.71% • Water content; 12.5 - 68% • Ash content; 9.02 - 15.1% • Melting point; 46 - 49.5 °C • Gelling point; 27 - 32 °C	10
3	Mappiratu	2009	Fixed variable; KCl, HCl, aquades, extraction temperature 90 °C, solvent ratio 10:1 Independent variable; alkali concentration (0.1 – 0.5 M), extraction time (1.5, 2, 2.5, 3), water ratio 35:1, 40:1, 45:1, 50:1, 55:1).	• Yield; 21.73 – 30.18% • Water content; 10.79 – 11.38% • Ash content; 18.07 – 18.38% • Sulfate content; 18.73–18.82% • Viscosity; 12.1 – 12.6 cP • Gel strength; 516 – 522 g/cm^2	15
4	Distantina et al	2010	Fixed variable; KOH, aquades, extraction temperature 90 °C, drying temp. 60 °C, celite, solvent ratio 30:1 Independent variable; alkali concentration (0.1 - 0.3 N), extraction time.	• Yield; 32 – 49 % • Sulfate content; 3 - 25% • Gel strength; 29 - 80 g/cm^2	20
5	Arfini	2013	Fixed variable ; KCl, extraction temp. 90 – 95 °C, extraction time 3 hour, celite Independent variable ; alkali concentration (1 – 1.5%), precipitation temp. (15 °C, 30 °C), solvent ratio (20:1, 30:1, 40:1).	• Yield; 21.76 - 34.02% • Water content; 6.76 - 9.73% • Ash content; 27.88 - 38.89% • Viscosity; 52.5 - 158.33 cP • Gel strength; 1493.49 - 2202.97 g/cm^2 • Sulfate content; 16.58 -18.62%	12

No	Researcher	Year	Variable	Output (Dependent Variable)	Sample
6	Tunggal and Hendrawati	2015	Fixed variable ; KOH, extraction temp. 90 ^oC, extraction time 1 hour, drying temp. 60 ^oC, solvent ratio 5:1. Independent variable ; alkali concentration (0.1 – 0.9 N).	• Yield; 17.33 – 28.8% • Water content; 8.42 – 11.74% • Ash content; 26.3 – 37.2% • Viscosity; 183.3 – 275.3 cP • Gel strength; 116.9 – 215.82 g/cm^2	5
7	La Ega et al	2016	Fixed variable : KOH, extraction temp. 90 oC, extraction time 30 menit solvent ratio 40:1 Independent variable ; alkali concentration (2% - 12%)	• Yield; 30.02 - 51.68% • Water content; 8.74 - 11.8% • Ash content; 19.42 - 35.38% • Melting point; 24.38 - 36.35 oC • Gelling point; 35.22 - 40.08 oC • Viscosity; 30.02 - 51.68 cP • Gel strength; 442.09 - 556.4 dyne/cm2 • Fat content; 0.13 - 1.76% • Protein content; 0.28 - 3% • Fiber content; 3.69 - 6.23% • Carbohydrate; 49.08 - 60.55%	18

Based on the table 1, there are differences in the treatment and each of these studies gives varying results. The studies have proven the influence of the process on the quality of carrageenan. The alkali concentration is one of the important elements in the process. NaOH concentration as one of the alkali treatment has a significant effect on yield, viscosity, gel strength and gel formation temperature (Widiastuti 2002). The higher of NaOH, then the higher of yield, gel strength and gel formation temperature, but the viscosity is getting lower. NaOH concentration of 5% gave the highest value on yield, gel strength and gel formation temperature, and gave the lowest value on the viscosity (Widiastuti 2002). NaOH concentration also has a significant effect on water content, ash content, gelling point and melting point (Yasita and Rachmawati 2009).

Besides NaOH, KCl and KOH can be used to extract seaweed. The selection of KCl and KOH as NaOH substitutes is (1) easily dissolving in water and (2) colored white. Based on previous studies, KCl concentration did not significantly affect the yield and water content, but significantly affected the viscosity, gel strength, ash content and sulfate content (Arfini 2013). But according to Mappiratu 2009, KCl concentration did not significantly affect water content, ash content, viscosity and gel strength and It significantly affected the yield. The yield increases with increasing KCl concentration to the limit of 0.3 M and after that the changes are small (Mappiratu 2009).

La Ega's research states that the concentration of KOH has a significant effect on viscosity, gel strength, yield, water content, ash content, and some chemical properties that exist in carrageenan. The addition of 12% alkaline solution (KOH) gives the best quality (La Ega et al 2016). The addition of KOH concentration has directly increased the yield, reduce the amount of sulfate content and increase the gel strength. The extraction time to reach the equilibrium in yield is 30 – 40 minutes and 20 minutes in sulfate content (Distantina et al 2010). The KOH concentration and length of the extraction time to produce the best of yield, water content, ash content, viscosity and gel strength were 0.9 N and 60 minutes (Tunggal and Hendrawati 2015).

The variation in output is based on the differences in treatment during the process. Therefore, we need a method that can conclude these studies specifically the effect of alkali concentration and its relationship to the quality of carrageenan. One of methods that can be used to combine results from the previous studies is a meta-analysis. Meta-analysis is a statistical technique that combines two or more similar studies, so that quantitative data can be obtained from data combination. Meta-analysis is a form of quantitative research that uses numbers and statistical methods to extract information from data. It also can inform the influence of variables on the object under study.

Based on the explanation above, this study was conducted to determine how the effect of alkali concentration on carrageenan quality?

2. Meta-analysis

Meta-analysis is defined as a systematic study using statistical techniques to summarize several studies (Dahlan 2012). Meta-analysis is usually used to assess the effectiveness of clinical interventions by combining several research (Siswanto 2010). Seen from the process, meta-analysis is a retrospective observational study or makes recapitulation of facts without doing experimental manipulation. Through statistical analysis, meta-analysis is a method of combining several studies to get stronger result and conclusion. The objectives of a meta-analysis are as follows (Nindrea 2016):

- To obtain an estimate of effect level; the strength of the relationship or the magnitude of the difference between variables.
- Do data inference in the sample to the population, by testing hypotheses or estimates.
- Control of potential confounding variables.

The research steps with a meta-analysis are as follows (Widhiastuti 2002 and Siswanto 2010):

- Identification of research questions.
- Collecting data through relevant previous study.
- Data evaluation.
- Data extraction from each study.
- Synthesis research with meta-analysis.
- Research results report.

One of the models used in a meta-analysis is the effect study. Effect studies are divided into two types; fixed effects model and random effects model. The fixed effects model is used if data from previous studies are homogeneous and random effects model is used if data from previous studies are heterogeneous. The process flow of a meta-analysis can be seen in Figure 1.

The benefits of a meta-analysis are as follows:

- The results of the study can be generalized.
- Differences in the results of previous studies can be confirmed and given a decision which results are more appropriate.
- The bias in previous studies can be explained scientifically.
- The accuracy of the study results is increasing with the amount of data or studies analyzed.

3. Methodology

Methodology is a way to systematically solve the research problem. The scope of the methodology is wider than research methods. Research methodology has many dimensions and research methods do constitute a part (Kothari 2004). In the methodology, researchers use a variety of different criteria to solve problems. In this study, the research methodology as is follows.

- Problems identification.
- Looking for literature that is relevant to the problem.
- Research question's identification related to the problems.
- Data evaluation for each previous study.
- Find the mean value, standard deviation, standard error, variation and weight for each study. See the equation below.

$$Mean\ (\overline{x}) = \frac{\sum xi}{n} \tag{1}$$

$$S\ Deviation\ Grup = \sqrt{\frac{\sum_{i=1}^{n} ({S_1}^2 x\, \overline{X}_1) + ({S_2}^2\, x\, \overline{X}_2)}{n_1 +\ n_2 - 2}} \tag{2}$$

$$Standard\ error\ (Se) = \frac{S}{\sqrt{n_1 + n_2}} \tag{3}$$

$$Variation\ (V) = {Se_j}^2 \tag{4}$$

$$Weight\ (W) = \frac{1}{V_j} \tag{5}$$

- Find the value of varied between studies (Q), p-value of the whole previous study to find out whether the data are homogeneous or heterogeneous. The data are homogeneous if p-value more than 0.05 (confidence level 95%). If the data homogeneous, use the fixed effect model and if the data heterogeneous, use the random effect model. Then find the effect value of each variable. Y notation is the difference in mean group 1 and mean group 2 of each previous study. See the equation below.

$$Q = \sum_{j=1}^{n} WY^2 - \frac{\left(\sum_{j=1}^{n} WY\right)^2}{\sum_{j=1}^{n} W} \tag{6}$$

$$p\ Value = CHIDIST\ (Q; degree\ of\ freedom\ (df)) \tag{7}$$

$$Ev = \frac{\sum_{j=1}^{n} WY}{\sum_{j=1}^{n} W} \qquad (Fixed\ Effect\ Model) \tag{8}$$

$$Ev = \frac{\sum_{j=1}^{n} WtY}{\sum_{j=1}^{n} Wt} \qquad (Random\ Effect\ Model) \tag{9}$$

$$Wt_j = \frac{1}{Vt_j} \tag{10}$$

$$Vt_j = V_j + t^2 \tag{11}$$

$$t^2 = \frac{(Q - df)}{C} \tag{12}$$

$$C = \sum W - \frac{\sum W^2}{\sum W} \tag{13}$$

- After the Effect value is known, then looking for the confidence interval (CI), Z score (Z') and the effect level (El). The confidence interval and Z score are used to determine the significant effect of alkali concentration on quality. The effect level is used to find out how much influence on it.

$$SE = \frac{1}{\sqrt{\sum Wt}} \tag{14}$$

$$CI = Ev \pm (Ztable\ x\ SE) \tag{15}$$

$$Z' = \frac{Ev}{SE} \tag{16}$$

$$Ztable = 1.96\ (confidence\ level\ 95\%)$$

$$El = Ev - SE \tag{17}$$

- Research hypothesis :
    - H_o : Alkali concentration does not affect the quality variable (yield, water content, ash content and viscosity)
    - H_1 : Alkali concentration affects the yield.
    - H_2 : Alkali concentration affects the water content.
    - H_3 : Alkali concentration affects the ash content.
    - H_4 : Alkali concentration affects the viscosity.

- The research hypothesis is based on the confidence interval or Z score (Z'). The confidence interval and Z score (Z') are as follows :
  - If the confidence interval across the zero point, then there is no significant effect.
  - If the confidence interval does not cross the zero point, then it has a significant effect.
  - If Z'≤ Z table (confidence level 95%), then there is no significant effect.
  - If Z' ≥ Z table (confidence level 95%), then it has a significant effect.

- The research hypothesis also based on the effect level of each variable. The effect level criteria are as follows (Asror 2016):
  - effect level ≤ 0.15, can be ignored.
  - 0.15 < effect level ≤ 0.40, low effect.
  - 0.40 < effect level ≤ 0.75, medium effect
  - 0.75 < effect level ≤ 1.10, high effect
  - 1.10 < effect level ≤ 1.45, very high effect
  - 1.45 < highest effect.

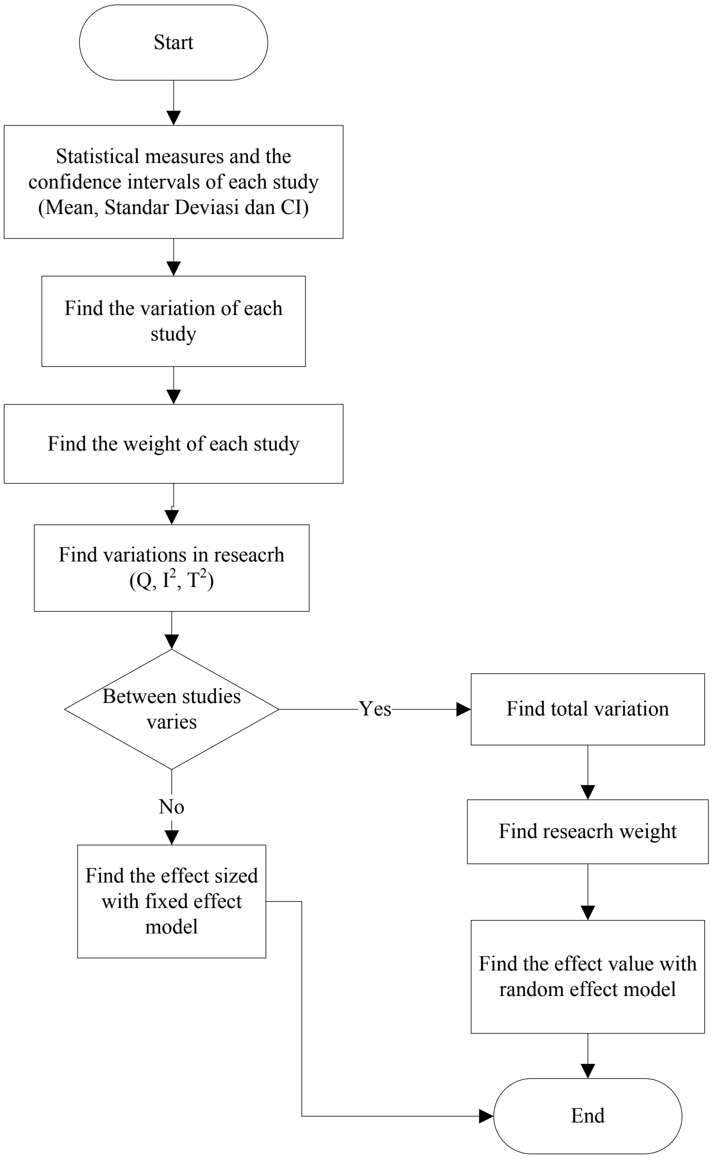

Figure 1. Process Flow of Meta-analysis
Source: Dahlan (2012)

4. Result and Discussion

Based on table 1, only five previous studies were in accordance with this study. This is based on :

- The difference in alkali concentration used in each study between group 1 and group 2.
- The length of extraction time used in each study at least 30 minutes. Based on the results of Distantina et al that the equilibrium conditions in yield were between 30 to 40 minutes.
- The extraction temperature, drying temperature and sediment used in each study has similarities.
- The use of solvent ratio between studies is considered not to affect the results.
- Research uses NaOH and KOH, which will be analyzed further. It is because, Only NaOH and KOH are alkaline, while KCl is neutral.

In a meta-analysis, each group in each study was combined statistically. This is done to find out whether the variables analyzed affect the object. In this study the variables analyzed were alkali concentration and its effects on carrageenan quality, especially yield, water content, ash content, and viscosity. Therefore, previous studies that will be analyzed further are those that have similarities in terms of the differences in alkali concentration between group 1 and group 2. Based on these, the data range in table 2 uses alkali concentrations of 0.1 N and 0.3 N KOH (Distantina et al and Tunggal et al), 1% and 3% NaOH (Widiastuti), 0.1 % and 0.3% NaOH (Yasita et al), 2% and 4% KOH (La Ega et al).

Table 2. The Data Range of Previous Studies

Researcher	Data Range			
	Yield (%)	Water Content (%)	Ash Content (%)	Viscosity (cP)
Widiastuti	28.05 – 32.26	-	-	45.4 – 110.7
Yasita et al	22.69 – 28.33	11.75 – 15.26	13.24 – 14.57	-
Distantina et al	41.7 – 46.04	-	-	-
Tunggal et al	16.62 – 20.18	10.21 – 12.04	25.19 – 31.9	214.82– 276.29
La Ega et al	34.07 – 37.08	10.8 – 11,49	19.22 – 21.26	29.88 – 31.68

Finding the mean value after the data is known, see equation 1. It can be seen in table 3. Table 4 shows the carrageenan quality standards.

Table 3. Mean of Each Variable from each study

Researcher	Mean			
	Yield (%)	Water Content (%)	Ash Content (%)	Viscosity (cP)
Widiastuti	30.29	-	-	88.2
Yasita et al	25.40	13.48	13.83	-
Distantina et al	43.75	-	-	-
Tunggal et al	18.03	11.22	28.38	243.6
La Ega et al	35.21	11.16	20.94	32.68

Table 4. Carrageenan Quality Standards

Specifications	Commercial carrageenan
Water content (%)	14.34 ± 0.25
Ash content (%)	18.60 ± 0.22
Protein content (%)	2.80
Fat content (%)	1.78

Specifications	Commercial carrageenan
Crude fiber (%)	Max 7.02
Carbohydrate (%)	Max 68.48
Melting point (°C)	50.21 ± 1.05
Gelling point (°C)	34.10 ± 1.86
Viscosity (cP)	Min 5
Gel strength (dyne/cm^2)	685.50 ± 13.43

Source: *A/S Kobenhvsn Pektifabrik* in La Ega (2016)

Almost all studies provide yield above 25%, except Tunggal et al. According to the Indonesian ministry of trade, a minimum yield from seaweed processing to carrageenan is 25% (Arfini 2013). Based on table 4, the water content of each study is outside the quality standard, but according to the FAO standard it is accepted except Yasita et al. The maximum standard of water content is 12% (Arfini 2013). For ash content, there are no studies that correspond to table 4. But based on FAO, all studies are appropriate except Yasita et al. FAO standard for ash content is 15 – 40% (Mappiratu 2009). The ash content is influenced by the presence of salts and other minerals in seaweed. The alkali concentrations (NaOH and KOH) can increase this.

The viscosity was carried out to determine the level of carrageenan thickness. Carrageenan viscosity is influenced by the concentration of alkali during the extraction process. The higher concentration of alkali, the viscosity is lower. This is caused by low sulfate levels in it. One of the alkali functions during the extraction process is to accelerate the elimination process of 6-sulfate from the monomer unit, so it can increase the gel strength. It is inversely proportional to viscosity. Based on table 3, the mean value of viscosity for each study is in accordance with the quality standard.

After the mean value is known, then proceed by looking for variation and the weight of each study by using equation 4 and 5. Variation is the error standard square of each variable in each study. It can be known after calculating deviation standards and error standards for each of previous study. Equation 2 and 3 are used to find out deviation standards and error standards. Weight is the value of each study based on variation. The higher of variation, then the weight is lower. After that, then find out the p-value by using equation 7. It is used to determine the data between studies is homogeneous or heterogeneous, it can be seen in table 5. The p-value can be known after calculating the variance between studies (Q). Equation 6 is used to find it.

Table 5. The p-Value of Each Variable

No.	Variable	p-Value
1	Yield	2.78655E-07
2	Water content	3.86788E-09
3	Ash content	2.67466E-17
4	Viscosity	2.9142E-125

Based on table 5, the p-value of yield, water content, ash content and viscosity are below 0.05 (confidence level 95%). It means the combined data from previous studies are heterogeneous. Meta-analysis with random effect models is used to determine the effect of each variable, see equation 9. Before using equation 9, the data transformation needs to be done. This is done to homogenize or normalize the data. The data transformation in this study is to homogenize variations. Stages in this process using equations 13, 12 and 11. After that, followed by finding the weight values of each study, see equation 10. The data transformation is complete, then followed by searching for the effect values of each variable. It can be seen in table 6.

Table 6. The Effect Value of Each Variable

No.	Variable	Effect Value
1	Yield	2.2151
2	Water content	1.0172
3	Ash content	2.1532
4	Viscosity	26.1986

After the effect values of each variable is known, then look for the confidence interval and Z score (Z'). This can be seen in table 7. The confidence interval is the range between two values where the mean value is in the middle. It is influenced by the confidence level. The mean value in this confidence interval is the effect values of each variable. To find out the confidence interval of each variable by using equation 14. The standard error (SE) used is the result of equation 15. The Z score is obtained by using equation 16.

Table 7. Confidence Interval and Z Score

No	Variable	Minimum	Maximum	Z Score
1	Yield	1.5339	2.8962	6.3743
2	Water content	-0.4132	2.4477	1.3937
3	Ash content	0.1625	4.1439	2.1200
4	Viscosity	6.2134	46.1836	2.5694

The confidence interval and Z score of each variable showed that the alkali concentration affected the yield, ash content, and viscosity. The effect of alkali concentration on yield is significant, this is indicated by the confidence interval does not cross the zero point and Z score is greater than Z table , therefore H_1 is accepted. This is in accordance with previous research which states that the alkali concentration has a significant effect on yield. The higher of alkali concentration than the higher of the yield produced (La Ega et al 2016). The alkali concentration affects the ash content, the confidence interval is between 0.27 and 3.11 and Z score is greater than Z table. This states that the alkali concentration has a significant effect on ash content, therefore H_3 is accepted. The effect of alkali concentration on viscosity is significant, so H_4 is accepted. High concentrations of alkali can dissolve salts on seaweed, so viscosity can decrease. Besides that, the small sulfate content caused by the high concentration of alkali, therefore the viscosity is low. There is no significant effect of alkali concentration on water content. This is indicated by the confidence interval passes the zero point and Z score is smaller than Z table, so H_o is accepted. After that look for the effect level of each variable by using equation 17, see table 8.

Table 8. Effect Level of Each Variable

No	Variable	Effect level (El)	Information
1	Yield	1.8676	Highest Effect
2	Water content	0.2874	Low effect
3	Ash content	1.1376	Very high effect
4	Viscosity	16.0021	Highest effect

Based on table 8, it is known that alkali concentrations have a high influence on quality variables except water content. The effect of alkali on water content is low, this is different on yield, ash content and viscosity. The effect level of alkali concentration on yield, ash content and viscosity is highest, very high and highest.

5. Conclusion

The conclusions from the results above are as follows :

- The data between studies are heterogeneous, the p-value of each variable is as follows:
  - Yield is 2.78655E-07.
  - Water content is 3.86788E-09.
  - Ash content is 2.67466E-17.
  - Viscosity is 2.9142E-125
- The confidence interval and Z score of each study are as follows:
  - Yield is 1.5339 to 2.8962 and Z score is 6.3743 (significant)
  - Water content is -0.4132 to 2.4477 and Z score is 0.2874 (not significant)
  - Ash content is 0.1625 to 4.1439 and Z score is 2.12 (significant)
  - Viscosity is 6.2134 to 46.1836 and Z score is 2.5694 (significant)
- The alkali concentration affected the three variables of carrageenan quality. The effect level of alkali concentration on each variable is as follows:
  - Yield is 1.8676 (highest effect)
  - Water content is 0.2874 (low effect)
  - Ash content is 1.1376 (very high effect)
  - Viscosity is 16.002 (highest effect)

Based on the confidence interval, Z score and effect level, the alkali concentration had a significant effect on yield, ash content and viscosity, so H_o is not accepted. But on water content it is accepted.

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