The Design Process for Entrapping Limonin and Naringin in Siam Juice by Cyclodextrin

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Abstract—***Siam juice contain limonin and naringin that produce a bitter taste although these compounds useful as an antioxidant and anticancer. Bitter taste in juice is unfavor for consumer. The purpose of this research was to find the best conditions in reducing bitter taste in Siam juice by using cyclodextrin and cellulose acetate. These research performed at variation of mixing temperature (27; 60 and 80ºC), cyclodextrin concentration (0.1; 0.3 and 0.5% (w/v)) and cellulose acetate (0.2; 0.4 and 0.6% (w / v)). The results showed that the use of cyclodextrin and cellulose acetate were able to trap limonin and naringin. The higher reducing limonin juice was 91.57% by addition cyclodextrin 0.1% at 60ºC, whereas for naringin obtain by addition cellulose acetate 0.5% at 79.69% . At this temperature, the juice quality (vitamin C) can be maintained.***

Keywords— Siam juice, limonin, naringin, cyclodextrin, cellulose acetate.

I. Introduction

Air Orange juice is popular beverage made from freshly squeezed orange. This beverage contains flavonoid and limonoids were be beneficial to resist various diseases. Both of these compounds are antioxidant compounds but could potentially cause a bitter taste in the citrus juice. Limonin compounds commonly referred to as delayed bitterness because the bitter taste can be felt when the citrus fruit stil processed. The compounds that contained in fresh orange is limonoic acid A ring lactone will be entered into orange extract (Puri, 2000).

Various studies have been performed to remove the bitter taste in grapefruit juice , generally using the techniques of separation . In Aghitsni (2008) , the separation is done by filtering microfiltration causing naringin and limonin compounds wasted from orange juice during processing. Fayoux (2008) ever reduce bitter taste in Navel citrus use polymeric film as limonin absorben. From this experiment caused limonin compound wasted from Navel juice. The study about reducing naringin compound was observed by Kasigit (2006). This study used CMC (Carboxy Methyl Cellulose) and naringinase enzim to Siam juice. From this study, naringin enzim can be reduce until 62.1% by using CMC 0.3%. But these study need long incubation time and didn't consider reducing for limonin concentration.

Considering these two compounds are useful for the body then alternative techniques to reduce the bitterness of orange juice while retaining compounds naringin and limonin is by adding cyclodextrin. In addition, orange juice treatment by cyclodextrin as well as compared to treatment with cellulose acetate which has been studied previously. Cyclodextrin selected because of its ability for inclusion into nonpolar part which can mantain critical components in citrus juice. Cyclodextrin is derivatives modified starch that doesn't give a very high calorie as well as the use of sugar in the fruit juice industry. This research use cellulose acetate which also reduce bitter taste in orange juice and then compared with cyclodextrin treatment.

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From figure 1 can be seen that limonoat A-ring lactones present on the cell membrane vesicle from citrus and does not have a bitter taste, when extracted and occur in contact with acidic citrus juice, these compounds are lactone process be limonoat dilakton that has a bitter taste. Changes from monolactone to dilactone limonin occurs at a pH of 5.4% u2013 6.2 and a temperature of 15-45 C. This process is influenced by the activity of the enzyme D-ring lactones limonoid hydrolase.

II. Method

A. Material and Equipment

This research uses Siam orange purchased from the Ciampea Market, Bogor. Moreover, this research used comparison orange sample from comercial that can obtained from one of juice company. Treatment used cyclodextrin and cellulose acetate. Analysis done with chemicals materials such as 0.1 N NaOH, distilled water, benzaldehid, glacial acetic acid, perchloric acid, a solution of phenolphthalein, a yod solution 0.01 N, and diethylene glycol.

The equipment that needed in the study are water bath, shaker, stainless steel knife, lemon juice, sieve 10 mesh, glass tools, scales, centrifuges, bottle jar, cup, bowl. Analysis tools such as refractometer, titration set of tools, oven, pH-meter, viscosimeter Brookfield spindle No. 1, spatula, porcelain cup, saucer aluminum, furnace, 250 ml flask, thermometer, desiccators, test tubes, Erlenmeyer, J2-21 centrifuge models Beckman brand, 6500 UV spectrophotometer Kruss brand.

B. Research Method

This research divide into two part, first, physical and chemical characterization of Siam Citrus without addition of cyclodextrins and celulose acetate. The second, physical and chemical characterization of Siam Citrus with addition of cyclodextrins and celulose acetate.

1) Physical and Chemical Characterization of Siam Citrus without Addition of Cyclodextrins and Celulose acetate

In this study were analyzed fresh orange Siam part of his weight. Initial testing of the fresh juice include yield, moisture content, ash content, vitamin C content, viscosity and total soluble solids (º Brix) Siam citrus juice. Then measured the concentration of limonin and naringin.

Orange juice is made from fresh Siam Citrus extract. Initial screening was conducted to separate the seeds and pulp are still attached. Flowchart of orange juice processing can be seen in Figure 1.

2) Physical and Chemical Characterization of Siam Citrus by additioning cyclodextrins and celulose acetate

a. Additioning cyclodextrin in Siam Juice

The addition of cyclodextrins done in three concentrations of 0.1, 0.3, and 0.5% (w / v) of the amount of juice used. Temperature is used for mixing the cyclodextrin temperature 27, 60 and 80 º C. In the mixing temperature 27 º C, cyclodextrin mixed in fresh orange juice and stir for 5 minutes then pasteurized for 10 minutes at a temperature of 80 º C. Then, the result of the addition of orange juice this cyclodextrin was analyzed. Flowchart of treatment with the addition of cyclodextrins temperature 27 º C are presented in Figure 2.

The addition of cyclodextrins on Siam juice with mixing temperature of 60 ºC after having pasteurized at a temperature of 80 º C. Cyclodextrin treatment at a temperature of 80 º C mixing done simultaneously at the temperature of pasteurization. Mixing is done by stirring ± 5 minutes. Respectively of total treatment with cyclodextrins as much as ± 15 minutes. After cyclodextrin treatment is complete, then analysis the orange juice quality.

b. Siam Juice Treatment with Cellulose Acetate

Mixing of cellulose acetate into orange juice done at concentration 0.2, 0.4 and 0.6 % (w/v) can be seen in Figure 4 . The temperature of mixing at room temperature approximately 27 ^{0}C. Pasteurized orange juice was centrifuged. Orange juice that has been mixed then mixed with centrifugation cellulose acetate with a concentration of 0.2, 0.4, 0.6% (w / v). Mixing is done by stirring using a magnetic stirrer for 45 min at 200 rpm rotation at room temperature. Once the filtering is done with fabric filter to separate the cellulose acetate and juice. Orange juice from screening get mixed back with the results of centrifugation sediment juice with a magnetic stirrer for 10 minutes. After that, juice from treatment with cellulose acetate was analyzed.

Several characteristic from Siam juice that has been analyzed include levels of vitamin C, the acid content (titrated acid total), the degree of acidity (pH), viscosity, total dissolved solids (Brix), naringin and limonin test. Organoleptic test done by comparing the level of bitterness in citrus juice flavors, colour and viscosity by 30 people as untrained panelists, 3 samples of orange juice siklodekstrin treatment and processing the data statistically by Duncan method.

III. Result and Discussion

A. Physical and Chemical Characterization of Siam Citrus without Addition of Cyclodextrins and Celulose acetate

Fresh orange juice showed the value of total dissolved solids and viscosity. From research result, there is no difference for total dissolved solids value as well as before and after pasteurization. The increased of pH pasteurized in orange juice can be caused by reduced ascorbic acid during pasteurization. Decreased levels of vitamin C occurs after the juice is pasteurized at 42.86%. This decrease indicates the oxidation of ascorbic acid during pasteurization.Characteristics of orange juice before the addition of cyclodextrins and cellulose acetate can be seen in Table 1.

Total acid Siam citrus juice did not increase significantly. This is thought to occur during the evaporation of water from pasteurized orange juice so that the overall acid concentration increases per unit volume. This shows that the number of acidic acid compounds contained by a substance which affects the taste and flavor. Limonin and naringin levels tended to increase after pasteurization. This is due to limonin and naringin precursors become more active due to the heating of orange juice during pasteurization. According Mozaffar et al. (2000), A-ring lactone limonoat located on the cell membrane of the vesicles orange and does not have a bitter taste, when extracted and contact with an acidic juice, this compound undergo lactone process be limonoat dilactone which has a bitter taste.

B. Physical and Chemical Characterization of Siam Citrus by additioning cyclodextrins and celulose acetate

1) Total Dissolved Solid

Total dissolved solids in the addition of cyclodextrins tended to increase, whereas the treatment with cellulose acetate tends to remain (Figure 5). The increasing of total solids due to the solubility of cyclodextrin in the process of mixing the juice is pasteurized by heating. Total solids increased with increasing number of cyclodextrin are dissolved. Cyclodextrin has a solubility in water in the α, β and γ cyclodextrins respectively 14.5, 1.8 and 23.2 g/100 ml at room temperature (Madsen, 2000). Cellulose acetate in the treatment of total solids tend to remain as cellulose acetate does not dissolve in orange juice when mixing. Cellulose acetate taking back after stirring. The degree of acidity (pH).

The pH values or degree of acidity associated with deposits of the acid contained in the juice. Changes in its pH can be seen from figure 6. In Figure 6 shows that the addition of cyclodextrins can maintain the pH value is too low so it does not decrease. The ability of cyclodextrins to form inclusion compounds with organic acids which cause the pH can affect the well maintained because of the heating effect of oxidationhe decreasing PH occurred with increasing concentrations of cellulose acetate were added. Cellulose acetate adds acidity because they contain acids that remain in the juice while mixing.

2) Levels of vitamin C

Levels vitamin C is a measure to know how much ascorbic acid contained in juice and. Influence siklodekstrin and cellulose acetate against levels vitamin C can be seen from figure 7. This figure shows that the levels of vitamin C tend to remain by additioning cyclodextrin. The addition of cellulose acetate also showed higher levels of vitamin C are fixed. Contained anomalous levels of vitamin C on the addition of cyclodextrin concentration of 0.5% and 0.2% cellulose acetate concentrations. This is due to ascorbic acid is not oxidized due to the addition of cyclodextrins much done so shortly after pasteurization temperature can be directly tied acids by cyclodextrin.

3) The content of total acid

The amount of total acids expressed as percent citric acid. Total acid can affect pH and flavor. Total acid content of orange juice with the addition of cyclodextrins tend to increase the total value of acid in orange juice 0% addition of cyclodextrins. Highest increase of 24.93% against the use of cyclodextrin with 0.1% concentration. This increase is due to the cyclodextrin as a hydrophobic compound having a polar group capable inclusion with organic compounds such as acids in orange juice. While the use of cellulose acetate lowers the total acid orange juice. The decrease is due to a amount of cellulose acetate can adsorb acid contained in the juice while mixing and filtering. Acids contained in juice by cellulose acetate treatment just little that can be titrated.

4) Viscosity

The change of viscosity by additioning of cyclodextrin and cellulose acetate can be seen in Figure 9. The result shows that the addition of cyclodextrinscan increase the viscosity of orange juice. Whereas treatment with cellulose acetate tend to stay and decreased with increasing amount of cellulose acetate concentration were added. Cyclodextrin in the form of soluble solids in the juice which adds to the total dissolved solids thereby increasing the viscosity. While cellulose acetate is insoluble in orange juice and filtered to separate it from the juice so it does not increase the viscosity of the juice.

5) Limonin levels

Effect of cyclodextrin concentration and cellulose acetate concentration on levels of limonin can be seen in table 2 and 3. Based on these results, so it can be compared to the percentage of binding of limonin levels between the use of cellulose acetate and cyclodextrins.

On the Tables 2 and 3 it can be seen that the concentration of cyclodextrin and temperature of mixing based on quantitative analysis of the most well to bind the content of limonin is at a concentration of 0.1 % with a temperature of mixing 60 º C (after pasteurized) , the concentration of limonin from 51.74 to 4.39 µg ml^{-1} (ppm) . The percentage of decrease is 91.52 % . Limonin concentrations are still found in orange juice under 6 ppm. The concentration at 6 ppm is the threshold for detecting bitterness sensory.

Percentage binding of limonin concentration increased up to 100 % with the addition of cyclodextrin concentration decreased 0.3 and 0.5 % (w / v) at a temperature of 60 º C. mixing While the percentage of cellulose acetate limonin not binding for the cyclodextrin . Compared with cellulose acetate , better cyclodextrin binding compounds limonin . The use of cellulose with 0.6 % concentration levels of limonin only able to bind up to 79.69 % . Limonin by cyclodextrin binding is due to the ability of cyclodextrins to cover particle limonin in orange juice.

Cyclodextrin compounds have the ability for inclusion compound that has a lower specific gravity . According Tomasik (2004) , cyclodextrins have the ability to interact with a variety of ionic and molecular compounds form a cyclodextrin complex inclusion. Cyclodextrin interact with other compounds to form dynamic balance.

6) Naringin levels

Naringin levels change due to the addition of cyclodextrins and cellulose acetate can be seen in Tables 4 and 5. In general, both of cyclodextrin and cellulose acetate effect on decreased levels of naringin. Sensory threshold to detect bitterness caused by naringin in water reported to be around 20 mg / l (Braddock, 1981).

In Tables 4 and 5 it can be seen that the concentration of various naringin concentrations on cellulose acetate showed a greater binding in orange juice Siam . Binding of naringin concentrations up to 86.50 % with the use of cellulose acetate concentration of 0.6 % (w / v). Cellulose acetate as naringin adsoban can absorb better than the cyclodextrin. Binding of naringin concentration is relatively more stable by using cellulose acetate compared by using cyclodextrin. The threshold to detect bitterness, but consumers have been able to receive this orange juice.

7) Organoleptic test

Based on the bitter taste test, color and flavor by multiple comparison test showed that the treatment was not significantly different. Organoleptic tests for viscosity show the real effect with the addition of cyclodextrins best at concentrations of 0.3 % and 0.5 % . Based on qualitative test that considered the quantitative assay showed that the addition of 0.1 % cyclodextrin on the mixing temperature 60 º C is the best because it can bind limonin below the threshold and received as well as a greater concentration of cyclodextrin.

IV. Conclusion

The use of cyclodextrins and cellulose acetate are capable for binding limonin and naringin compounds in Siam juice. Based on quantitative and qualitative analysis, cyclodextrin concentration and temperature of mixing are the best for binding limonin was at 0.1 % concentration with mixing temperature of 60 º C (after pasteurized) with limonin concentration was 51.74 to 4.39 µg ml-1 with a percentage of 91.57 % binding. Naringin concentration that can be tied at 42.54%.

The largest percentage of limonin binding was 79.69 % by using cellulose acetate 0.6 %. Percentage of naringin binding was 86.5 %. Limonin binding is better by using cyclodextrins than others. But, naringin binding is better by using cellulose acetate than using cyclodextrin. By multiple comparison test, the result showed that there is no significant difference in the color, flavor, and bitter taste. The real difference viscosity of orange juice occured by using cyclodextrin treatment at 99% and 95% confidence interval.

V. Suggestion

Treatment blending cyclodextrin and cellulose acetate can be done to reduce the concentration of limonin and naringin for more leverage . In this study has not been conducted measurements DNS reducing sugar content , this test can be performed for allegedly cyclodextrin still contain high levels of reducing sugars.

Acknowledgement

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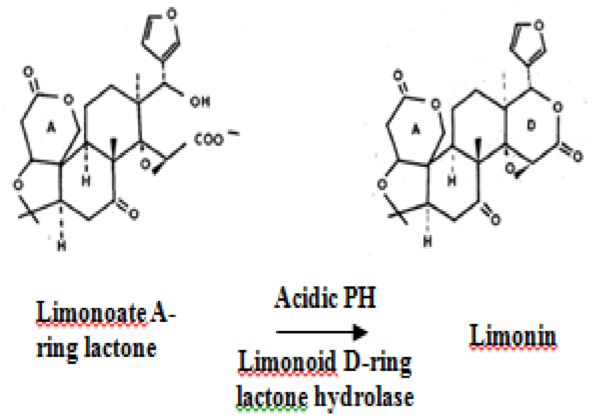

Figure 1. The forming limonin compounds during heating process (Hasegawa, 1975)

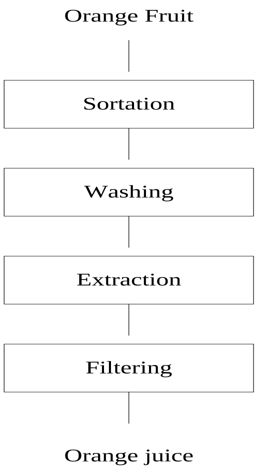

Figure 1. Flowchart orange juice processing

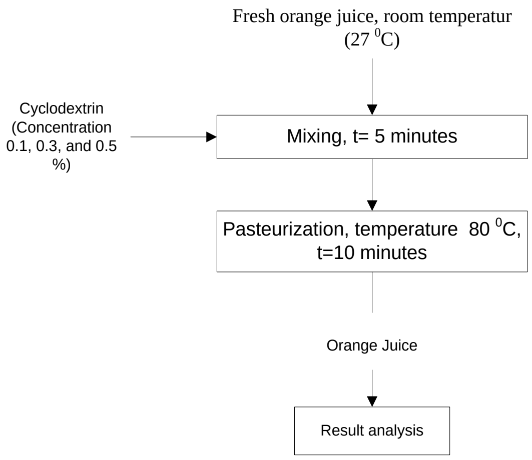

Figure 2. Flowchart orange juice processing by additioning cyclodextrin at room temperature

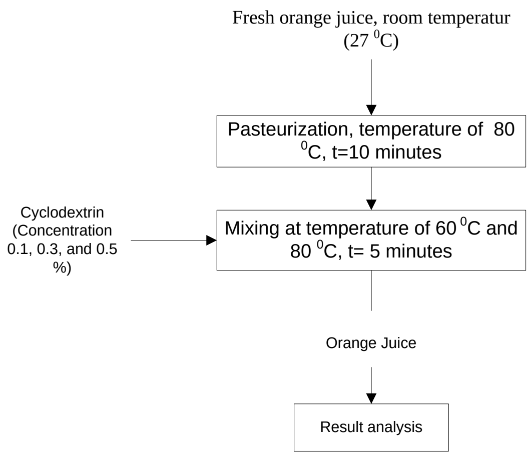

Figure 3. Flowchart orange juice treatment with cyclodextrin at temperature of 60 ^{0}C and 80 ^{0}C

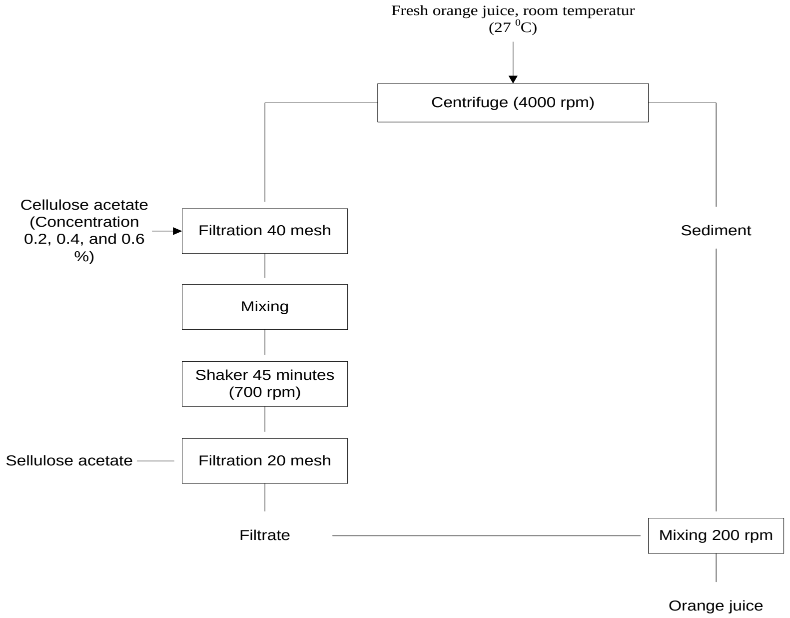

Figure 4. Flowchart Siam juice treatment with cellulose acetate

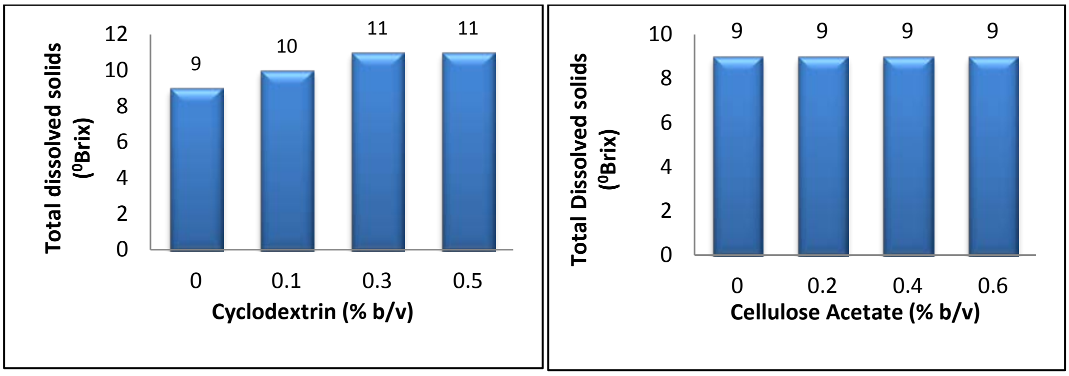

Figure 5. Total dissolved solids on the addition of various concentrations of cyclodextrin and cellulose acetate

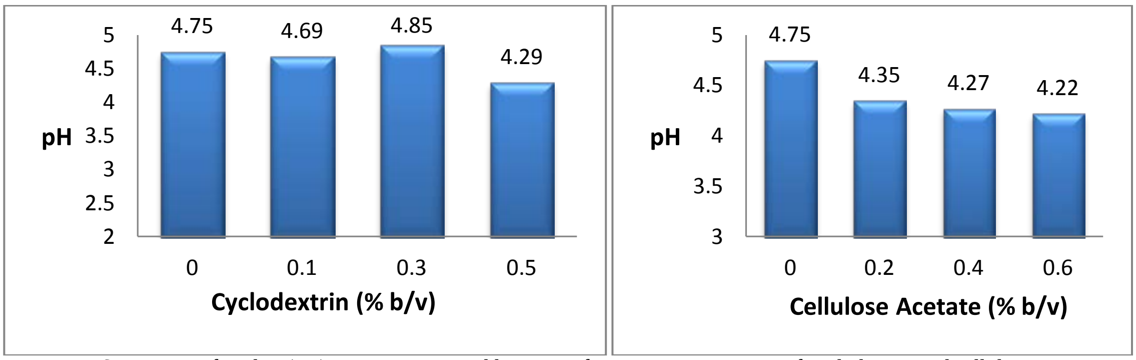

Figure 6. Degree of acidity (pH) orange juice on additioning of various concentration of cyclodextrin and cellulose acetate

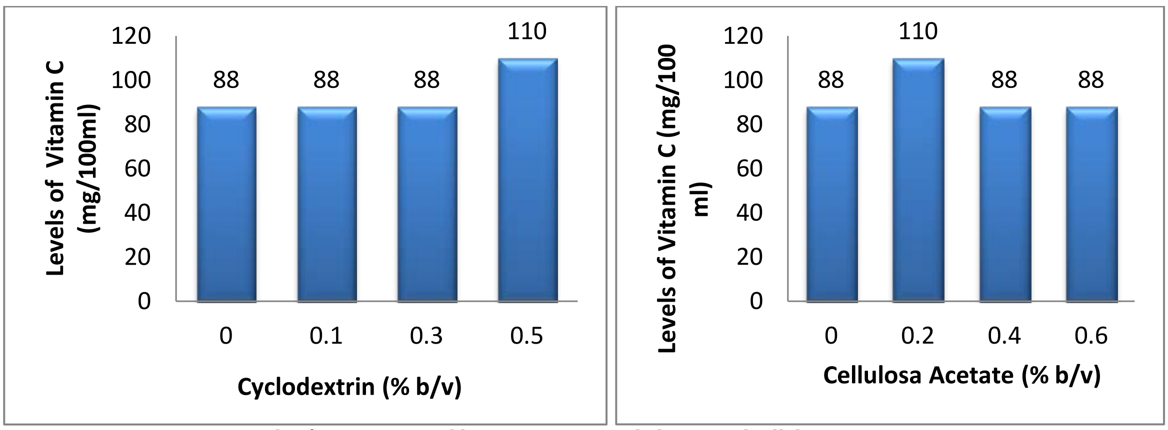

Figure 7. Levels of vitamin C on additioning various cyclodextrin and cellulose acetate concentration

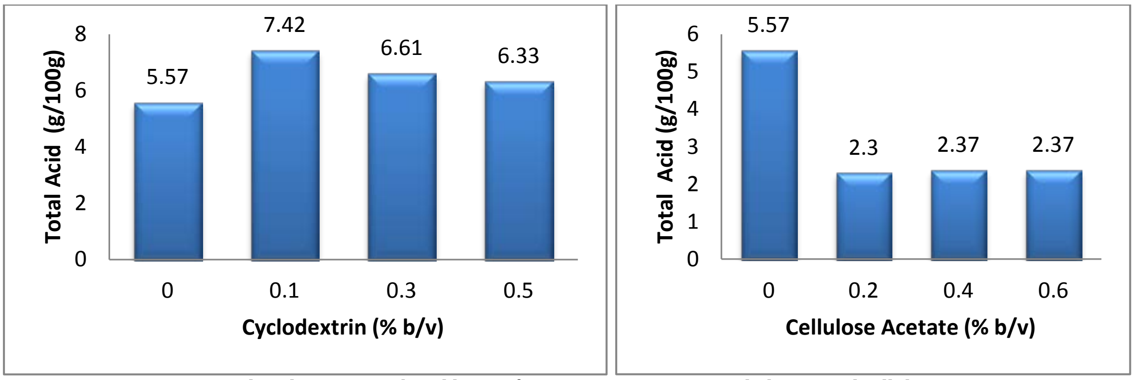

Figure 8. Total acid content on the addition of various concentration cyclodextrin and cellulose acetate

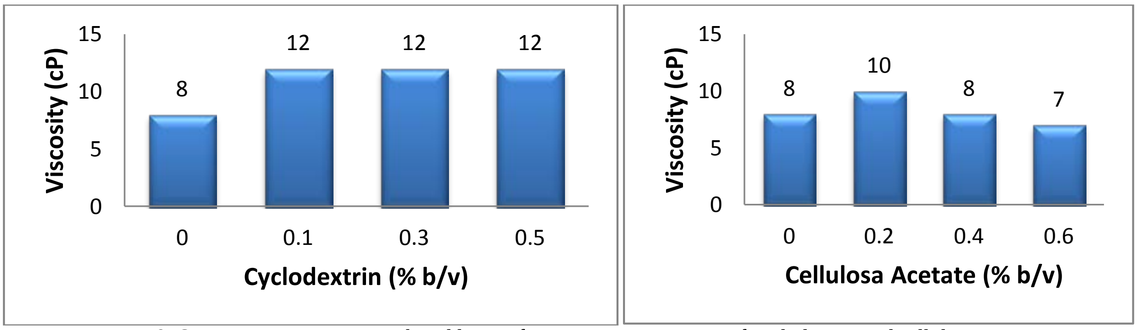

Figure 9. Orange juice viscosity on the addition of various concentrations of cyclodextrin and cellulose acetate

Table 1.
Characteristic of Siam juice before pasteurization) and pasteurization without treatment

Parameters	value	
	Fresh	Pasteurized
Total dissolved solids (ºBrix)	9	9
PH	4.6	4.75
Level of vitamin C (mg/100 ml material)	154	88
Acid content (% citrate acid)	5.25	5.57
Viscosity (cP)	8	8
Limonin concentration (µg/ml)	26.96	51.74
Narigin concentration (µg/ml)	230.2	268.2
Water content (% b/b)	92.07	91.57
Ash content (% b/b)	0.35	0.36

Table 2.
Limonin binding at various concentrations of cyclodextrin

No	B	C	D
1	0	51.74	0.00
2	0.1, 60	4.39	91.52
3	0.3, 60	0	100.00
4	0.5, 60	0	100.00

Table 3.
Limonin binding at various concentrations of cellulose acetate

No	A	C	D
1	0	51.74	0.00
2	0.2	15.41	70.22
3	0.4	15.00	71.01
4	0.6	10.51	79.69

Table 4.
The binding of Naringin at various cellulose acetate concentration

No	A	E	F
1	0	268.2	0.00
2	0.2	82.20	69.35
3	0.4	46.20	82.77
4	0.6	36.20	86.50

Table 5.
The binding of Naringin at various cyclodextrin concentration

No	B	E	F
1	0	268.2	0
2	0.1, 60	154.2	42.54
3	0.3, 60	184.2	31.34
4	0.5, 60	128.2	52.24