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Optimization of Cross-linking Modification on Canna Starch with Sodium Acetate Using Response Surface Methodology

ABSTRACTS

Canna starch is obtained from the extraction of canna tubers. However, the unstable native of canna starch during processing makes its use limited. Crosslinking starch can make starch resistant to shear stress acid resistance and prevent viscosity decrease due to the rupture of starch granules during heating. The purpose of this study was to obtain the optimum conditions of concentration and duration of mixing sodium acetate for cross-linking modification of canna starch with viscosity as the primary response. This study used a factor of sodium acetate concentration and mixing time. The modification of canna starch by cross-linking affects the viscosity. Cross-linking in starch can strengthen the starch granules so that the starch granules are not easily gelatinized. The optimum viscosity condition was obtained from a sodium acetate concentration of 16.21% with a mixing time of 20.71 minutes to get the optimum viscosity of 43.7 cP. The high degree of substitution of sodium acetate in cross-linking modification affects the integrity of starch granules, where starch granules can affect the physicochemical characteristics of starch. The higher the DS value, the lower the amylose content and the solubility. However, increasing the degree of substitution can also increase the viscosity, swelling power, syneresis, and pH of the cross-linked modified starch.

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INTRODUCTION

Canna is one type of tuber that is rarely used, where people only process it by boiling, frying, burning, or steaming [1]. Meanwhile, canna production in Central Java reached 4,941 tons [2]. Canna starch is obtained from the extraction of canna tubers. It contains amylose with a gelatinization temperature of 71.9 to 74.8°C. The crystalline structure of canna starch is type B. Utilization of natural canna starch usually tends to be limited. According to [3], the swelling power level of canna starch is lower than cassava starch or potato starch. The amylose content influences this in canna starch, which affects the water holding capacity during the heating process. In addition, canna starch has physical properties in a significant granule size viscosity level. It tends to be high and unstable when heated has a high retrogradation rate, clear paste form, and is not resistant to low pH and high pressure [4].

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Meanwhile, sometimes the industry requires stable starch to process, from preparation to a wide distributed product. Therefore, it is necessary to improve the properties of starch so that starch can be used widely. One way to improve the properties of starch is by modifying starch. Modification of starch aims to change the chemical or physical properties of starch naturally. The modification of starch is done by cutting the molecular structure using oxidation or substituting chemical groups on starch molecules [5].

According to [6], three methods are used for starch modification: physical, chemical, and enzymatic. The disadvantage of physical modification of starch is the conversion process. This process uses extreme heating and cooling altered the resistant starch (RS) characteristic. Physically treatments also requires more energy. The temperature used in the modification

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process is generally above the melting point of amylopectin crystals.

In contrast, enzymatic modification requires proper conditioning, where the enzyme works specifically. The substrate or part of the substrate must have the right shape with the enzyme's catalytic site apart from environmental factors that influence it [7]. Starch's chemical modification involves esterification, etherification, hydrolysis, oxidation, and cross-linking. Chemical methods with cross-linking reactions can change the hydrophobic properties of starch, starch stability, starch viscosity, and starch resistance to high temperature and frictional forces. An example of a chemical method is hydroxypropylation. This method substitutes the hydroxyl group with hydroxypropyl. Therefore, the attractive forces of the hydroxyl group are reduced during cooling or freezing—the higher the hydroxypropylation, the lower the syneresis [8].

The cross-linking agents commonly used are multifunctional reagents such as phosphorus oxide (POCl₃), sodium trimethyl phosphate, sodium tripolyphosphate, and sodium acetate. In this study, the cross-linking reagent was used in sodium acetate, which was applied to canna starch with response surface methodology. Using a cross-linking agent in sodium acetate in starch modification has several advantages. For example, it is easy to obtain and has economic value compared to other types of cross-linking agents. In addition, research related to the use of cross-linking agents in the form of sodium acetate has a literature review that tends to be minor, so further research is needed on modified starch cross-linking with agents in the form of sodium acetate. Response surface methodology is a combined method of statistical and mathematical techniques used in developing, improving, and optimizing the process so that the modification process obtained will provide the optimum conditions for a response. According to research [9], sodium acetate produced the lowest viscosity of modified starch. Few studies on canna starch modification by cross-linking are available in the literature, and reports on the use of sodium acetate as a cross-linking agent in canna starch were not found.

Table 2. Treatment Design

Treatment	X	Y	[C] (%)	t (minute)
1	-1	-1	11,5	10
2	+1	-1	23,5	10
3	-1	+1	11,5	30
4	+1	+1	23,5	30
5	-1,414	0	9,014	20
6	+1,414	0	25,98	20
7	0	-1,414	17,5	4,7
8	0	+1,414	17,5	34,1421
9	0	0	17,5	20
10	0	0	17,5	20
11	0	0	17,5	20
12	0	0	17,5	20
13	0	0	17,5	20

This study aims to optimize the concentration and the mixing time using sodium acetate on the characteristics of canna starch.

Method

Materials

Canna starch was obtained from the local market in Semarang. Several chemicals were used, such as sodium acetate, sodium hydroxide, silicon oxide, ethanol, phenolphthalein indicator, buffer standard at pH 4 and 7, 0.5 M hydrochloric acid, 0.5 M potassium hydroxide, amylose standard from potato starch, 0.2% iodine solution, and 1N acetic acid.

Modification of canna starch

Modified canna starch was prepared following method of [9]. 200g of canna starch was added with 0.2g silicon oxide and mixed for 5 minutes. 20g sodium hydroxide was added, stirred for 20 minutes, and added with sodium acetate and mixed—the concentration of sodium acetate and mixing time, based on the experimental design. The mixture was heated in a water bath maintained at 75°C, stirred continuously for 1 hour, and poured to cool.

Sample analysis

Modified canna starch was analyzed by analysis of the degree of substitution (DS) [10], amylose content [11], viscosity using Viscometer Brookfield DV2T, swelling power, and solubility [12], syneresis [10], and pH.

Experimental design

The formulation and response designs were made using Minitab ver. 19. This stage begins with determining the process conditions used as fixed and modifier variables.

Table 1. Experimental Design

Variables	Levels				
	-α	-1	0	+1	+α
C (%) ^a	9.014	11.5	17.5	23.5	25.98
t (minute) ^b	5.85	10	20	30	34.14

^a C: concentration of sodium acetate; ^b t: mixing time

Statistical analysis

Statistical significance was evaluated by response surface methodology. The response $f(x)$ could be written as $y_i = f(x, y)$. The results of each response were evaluated using multiple regression analysis to develop mathematical models.

Results and Discussion

Table 3. Degree of substitution, amylose content, and viscosity of cross-linked canna starch with sodium acetate

Treatment	[C] (%)	t (minute)	Degree of substitution	Amylose content (%)	Viscosity (cP)
1	11.5	10	0.151 ± 0.013	34.52 ± 0.08	34.3 ± 0.14
2	23.5	10	0.1642 ± 0.003	29.11 ± 0.19	28.85 ± 0.07
3	11.5	30	0.1614 ± 0.007	28.62 ± 0.16	33.5 ± 0.14
4	23.5	30	0.1647 ± 0.003	28.31 ± 0.04	28.2 ± 0
5	8	20	0.1674 ± 0.001	32.72 ± 0.06	36.65 ± 0.07
6	24.9	20	0.1656 ± 0.002	28.56 ± 0.23	27.15 ± 0.07
7	17.5	4.7	0.157 ± 0.003	25.75 ± 0.12	39.05 ± 0.07
8	17.5	32.9	0.1651 ± 0.002	29.05 ± 0.2	42.35 ± 0.07
9	17.5	20	0.1622 ± 0.001	32.43 ± 0.18	48.81 ± 0.02
10	17.5	20	0.1645 ± 0.001	29.74 ± 0.07	44.35 ± 0.07
11	17.5	20	0.1616 ± 0.005	29.98 ± 0.47	37.37 ± 0
12	17.5	20	0.1603 ± 0.003	29.68 ± 0.053	37.2 ± 0
13	17.5	20	0.1649 ± 0.005	29.38 ± 0.11	49.17 ± 0.03

Degree of substitution

The increase in the degree of substitution in cross-linking starch was caused by the addition of many cross-linking agents used. In addition, [13] found that the longer the reaction time, the more the OH groups - corn starch are substituted by phosphate groups. According to [14], distarch phosphate with a phosphate content of less than 0.04% or a degree of substitution less than 0.21. It is included in the modified starch cross-linking. [15] added that the formation of distarch phosphate is one of the most critical requirements in determining starch as food additive or food source. The modified canna starch showed that the modified degree of substitution was less than 0.21. The response surface the 2-dimensional contour of the degree of substitution can be seen in Figure 1.

Figure 1. shows the value of the degree of substitution of modified canna starch with cross-linking, where the responsiveness surface and 2-dimensional contours have not yet obtained the optimum conditions. According to the results of statistical tests using the Minitab application, it can be predicted that the optimal condition of the cross-linking degree of substitution of canna starch is at a sodium acetate concentration of 9.014% with a mixing time of 5.85 minutes will get a DS of 0.15. The following is the equation of statistical test results:

$$\text{Degree of substitution} = 0,1397 + (0,00011x) + (0,001676y) + (0,000029x^2) - (0,000017y^2) - (0,000041xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

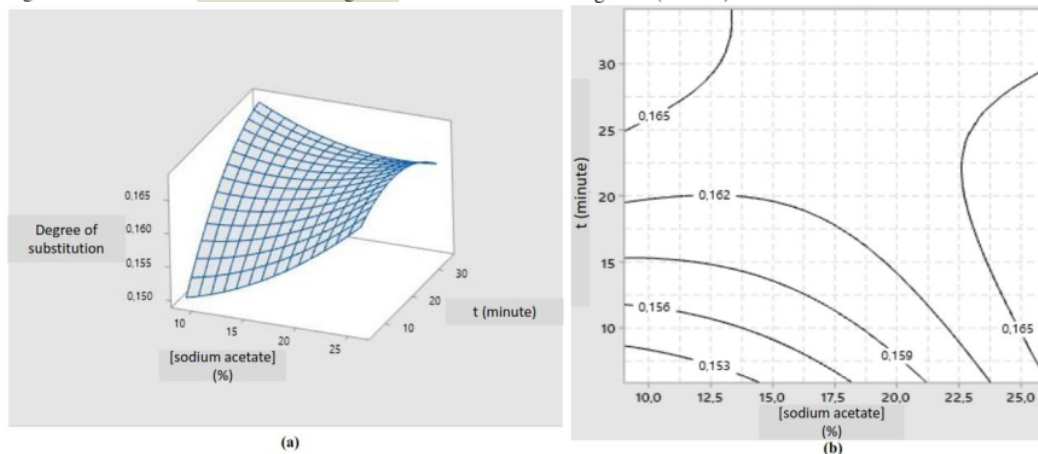


Figure 1. Response surface (a) and contour curve (b) for the degrees of substitution of cross-linked canna starch

Amylose content

The optimal condition to produce the lowest amylose content was after adding 17.5% sodium acetate and mixing for 4.7 minutes. Sodium acetate binds the OH group of amylose, and as a result, the amylose structure changes [9]. Amylose consists of straight chains with functional groups in a more open position. The active group with an open structure is the hydroxyl group (-OH) with atomic C

number 2, accessible and highly reactive to compounds added when modifying starch [16]. The high degree of substitution in cross-linking starch will impact the limited movement of amylose. Steric interactions in the amylose molecule cause the slight movement of amylose. This will result in amylose molecules not being free to join or bind so that the compactness of the granules is reduced as a result of weakened intermolecular hydrogen bonds. In line

with the research of [17], the more groups that substitute for starch, the fewer hydrogen bonds. As a result, the crystalline structure will disappear and become an amorphous structure. The responsive surface of amylose levels in modified canna starch by cross-linking can be seen in Figure 2.

Statistical results using Minitab ver. 19 obtained predictions of optimum conditions, namely sodium acetate concentration as much as 25.98% with a mixing time of

5.87 minutes then will produce amylose content of 24.67%. Where statistical analysis obtained the following equation:

$$\text{Amylose content (\%)} = 41,93 - (1,132x) + (0,059y) + (0,0133x^2) - (0,01141y^2) + (0,0212xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

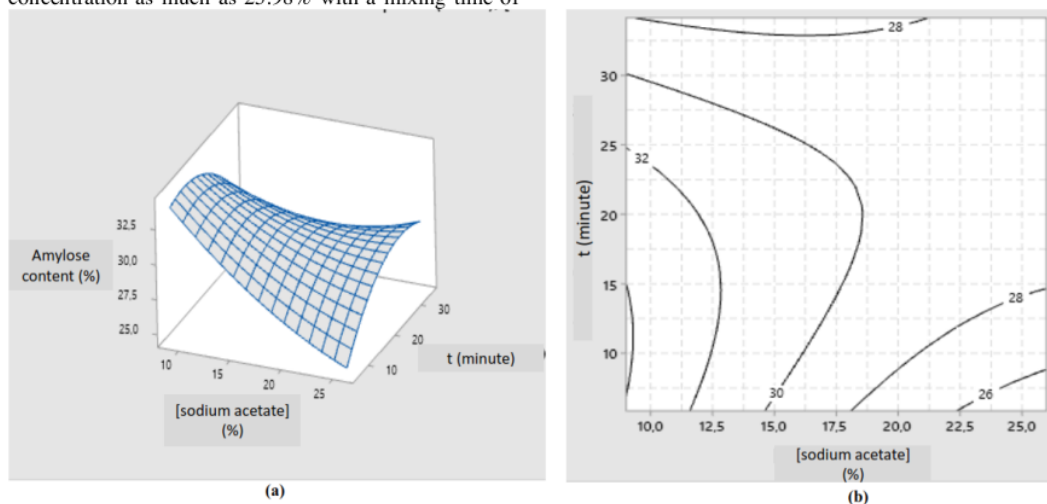


Figure 2. Response surface (a) and contour curve (b) for the amylose content of cross-linked canna starch

Viscosity

The degree of substitution of starch cross-linking also affects the development of starch. The higher the degree of substitution, the more cross-linking agent replaces OH - as a bridge of the molecules that form a network of macromolecules rigid and robust, which causes the starch granules to be difficultly tampered with water the granule may awake [18]. Viscosity correlates with the degree of swelling power and solubility of starch, wherein when the starch granules are heated in an aqueous medium, starch granules will have swelling (swelling) then [19]. It causes the starch granules to swell, and then the size of the starch granules increases, and over time the starch granules break. The rupture of this starch granule causes the dissolving of the amylose in the crystalline part. It then results in the breakdown and instability of the amorphous part, the dissolution of amylose in the granule, which is referred to as the solubility of starch. It can cause the viscosity of the starch to decrease. With the modification of cross-linking, the decrease in viscosity in starch due to granule rupture can be minimized. Cross-linking can increase the integrity of the granules to keep the swollen granules intact [18]. Factors that affect the viscosity of starch are swollen starch granules, the solubility of swollen granules, amylose

mobilization, and the dispersion ability of swollen starch granules [20]. The results of the viscosity analysis of modified canna starch by cross-linking can be seen in Figure 3.

The optimum condition for the highest viscosity was obtained with a concentration of 17.5% sodium acetate and a mixing time of 20 minutes with a viscosity value of 40 cP. In contrast, the statistical analysis results of concentrations can predict the optimum condition of sodium acetate as much as 16.21% with a mixing time of 20.71 minutes to get the optimum viscosity of 43.7 cP. According to the research of [4], the modification of starch by cross-linking has the characteristics of being challenging to gelatinize and more stable during heating. The modified starch by cross-linking can increase the stability of the starch viscosity [20]. Based on statistical data obtained the equation:

$$\text{Viscosity cP} = 18,5 - (6,30x) - (1,07y) + (0,1948x^2) + (0,0261y^2) + (0,0006xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)



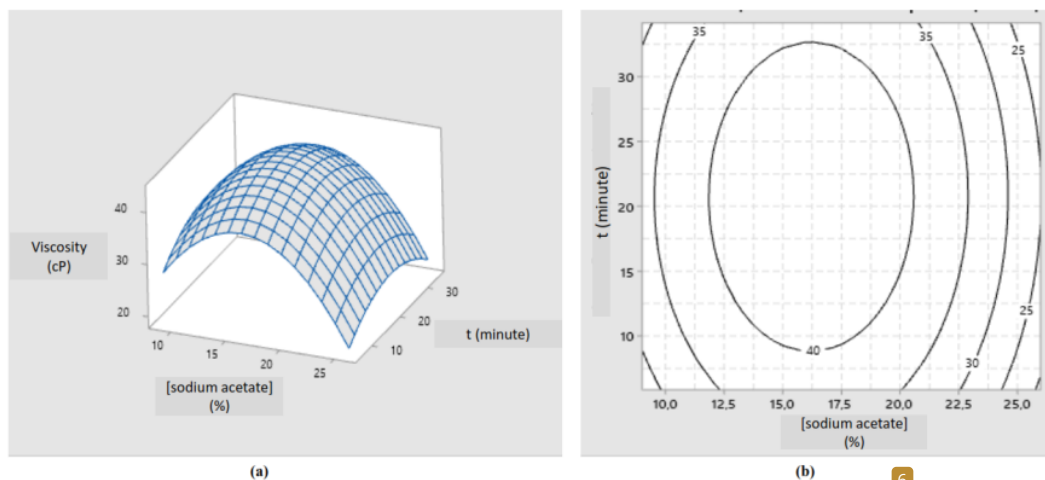


Figure 3. Response surface (a) and contour curve (b) for viscosity of cross-linked canna starch

Table 4. Swelling power, solubility, syneresis, and pH of cross-linked canna starch with sodium acetate

Treatment	[C] (%)	t (minute)	Swelling power (g/g)	Solubility (%)	Syneresis (g)	pH
1	11.5	10	16.4 ± 1.3	0.199 ± 0.002	5.50 ± 0.04	11.92 ± 0.02
2	23.5	10	11.19 ± 0.94	0.164 ± 0.002	5.67 ± 0.17	11.87 ± 0.02
3	11.5	30	12.45 ± 2.6	0.146 ± 0.003	6.72 ± 0.06	11.85 ± 0.03
4	23.5	30	15.34 ± 3.3	0.214 ± 0.011	5.50 ± 0.01	11.83 ± 0.005
5	8	20	15.7 ± 1.9	0.16 ± 0.012	5.53 ± 0.18	11.8 ± 0.04
6	24.9	20	14.19 ± 1.8	0.17 ± 0.013	5.49 ± 0.05	11.82 ± 0.05
7	17.5	4.7	13.13 ± 0.9	0.2 ± 0.01	5.57 ± 0.03	11.85 ± 0.03
8	17.5	32.9	15.49 ± 1.5	0.158 ± 0.017	5.50 ± 0.05	11.82 ± 0.01
9	17.5	20	13.54 ± 3.1	0.169 ± 0.0008	5.52 ± 0.04	11.83 ± 0.02
10	17.5	20	13.79 ± 0.5	0.164 ± 0.004	5.49 ± 0.04	11.77 ± 0.04
11	17.5	20	10.68 ± 0.7	0.169 ± 0.006	5.38 ± 0.12	11.77 ± 0.05
12	17.5	20	14.31 ± 2.2	0.122 ± 0.012	5.47 ± 0.04	11.78 ± 0.03
13	17.5	20	15.85 ± 1.6	0.145 ± 0.014	5.38 ± 0.36	11.73 ± 0.02

Swelling Power

The increase in the swelling power value of the cross-linking starch correlates with the degree of substitution of the cross-linking agent. The higher the concentration of the addition of the cross-linking agent will cause more starch granules to react with the cross-linking agent. In addition, the modification of cross-linking can strengthen starch granules. When the granules are heated in aqueous media, the granules tend to experience maximum swelling but can still maintain granules from breaking. It causes the swelling power of cross-linking modified canna starch to increase. The amylose content in starch also affects the swelling power of modified cross-linking canna starch, where the higher the amylose content in starch, the higher the swelling power. The higher the amylose content in starch, the more water-absorbing capacity. When more

water absorption, the swelling power will also increase. The responsiveness surface and contour of the 2-dimensional swelling power on the concentration and time of mixing canna starch cross-linking can be seen in Figure 4.

The results of statistical processing using Minitab obtained predictions of optimum conditions with a concentration of N-Acetate 25.9853% and a mixing time of 5.8578 minutes resulting in a swelling power value of 9.37552 (g/g). Where statistical analysis obtained the equation:

$$\text{Swelling power: } 30,67 - (1,215x) - (0,603y) + (0,0128x^2) + (0,00142y^2) + (0,0337xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

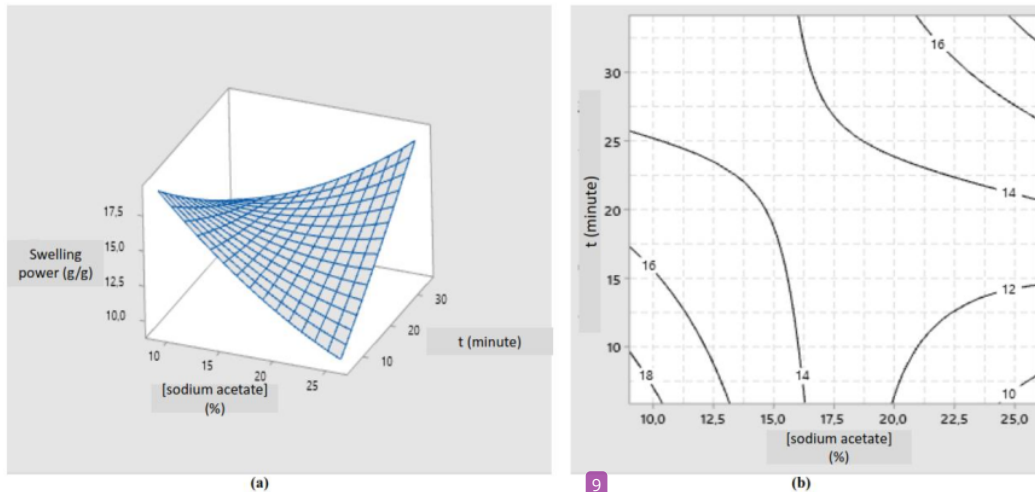


Figure 4. Response surface (a) and contour curve (b) for swelling power of cross-linked canna starch

Solubility

The presence of amylose content causes a food ingredient's high and low solubility index. The higher the amylose content, the higher the solubility index of the material. According to [21], when starch molecules are hydrated, the amylose molecules will spread through the media. The short-chain structure of amylose causes more amylose to dissolve into the environment. On the other hand, when the amylose content tends to be low, the solubility index will be low due to the lack of soluble in water [22]. In addition, the solubility index can be affected by the increasing concentration of sodium acetate added in modifying starch. The presence of a cross-linking agent in sodium acetate can result in depolymerization and weakening of the internal structure of the granules in starch. As a result, the physicochemical properties of cross-linked modified starch using sodium acetate are better than sago starch without cross-linking modification [23]. The capture surface and 2-dimensional contours of the results

of the solubility analysis on the concentration and duration of mixing sodium acetate can be seen in Figure 5

Based on Figure 5, the responsiveness surface and 2-dimensional contours of the cross-linking modified starch solubility showed an increase in line with the sodium acetate concentration. Meanwhile, the more prolonged mixing of sodium acetate showed a decrease in the cross-linking starch solubility. The statistical test results obtained predictions of the optimum conditions for the solubility value. The concentration of sodium acetate was 9.014% with a mixing time of 34.12 minutes to get a solubility of 0.128%.

$$\text{Solubility} = 0,4278 - (0,01517x) - (0,01421y) - (0,000216x^2) + (0,000148y^2) + (0,000429xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

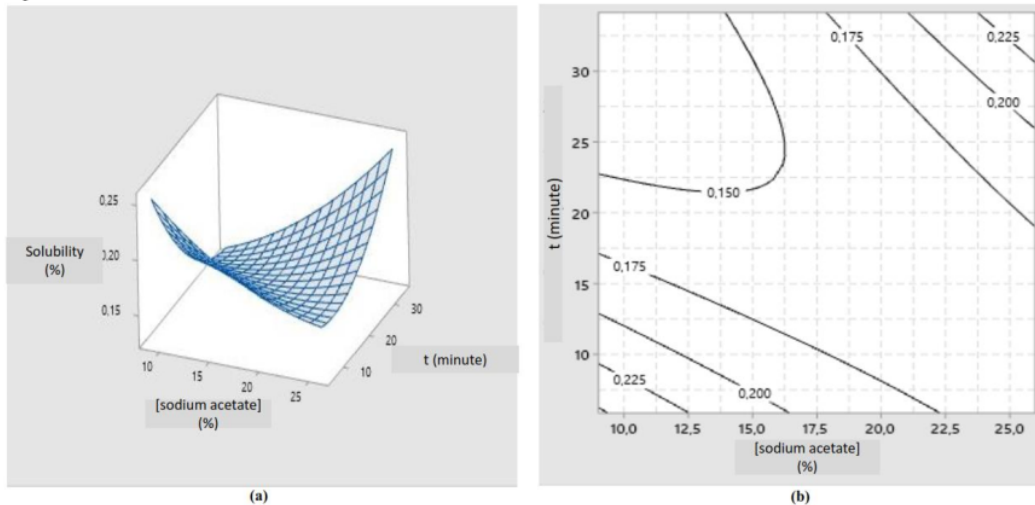


Figure 5. Response surface (a) and contour curve (b) for solubility of cross-linked canna starch

Syneresis

The increase in syneresis during storage is caused by the interaction of amylose and amylopectin chains. It exists and then develops into the junction zone. The previous study [24] stated that the helical structure in amylose could melt during heating and starch granules begin to swell. An increase in viscosity or often called the swelling power phase, occurs. Heating with constant stirring can cause disintegration of the granule structure, increase starch solubility and decrease viscosity after cooling [25]. The retrogradation ability is due to the hydrogen bonds of the amylose molecule. Syneresis occurs when bonds are weakened. The decrease in the percentage of syneresis can be caused by the presence of bonds between amylose and amylose. In addition, the reduced rate of syneresis is also indirectly influenced by the amorphous and crystalline chain structure of the starch. It affects the level of granule

damage during the gelatinization process and the interaction between the starch chains during gel storage [10]. Substitution of modified starch will cause a decrease in the percentage of syneresis. The capture surface and the 2-dimensional contour of the syneresis value of cross-linking modified canna starch to [N-acetate] with mixing time can be seen in Figure 6.

Figure 6 shows the statistical test results of the percentage of syneresis using the Minitab application, which can predict the optimum condition with a sodium acetate concentration of 25.9% and a mixing time of 34 minutes to get a syneresis value of 5.19%. The following is the equation of the statistical test results:

$$\text{Syneresis (g)} = 5,04 - (0,017x) + (0,0635y) + (0,00315x^2) + (0,00125y^2) - (0,00581xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

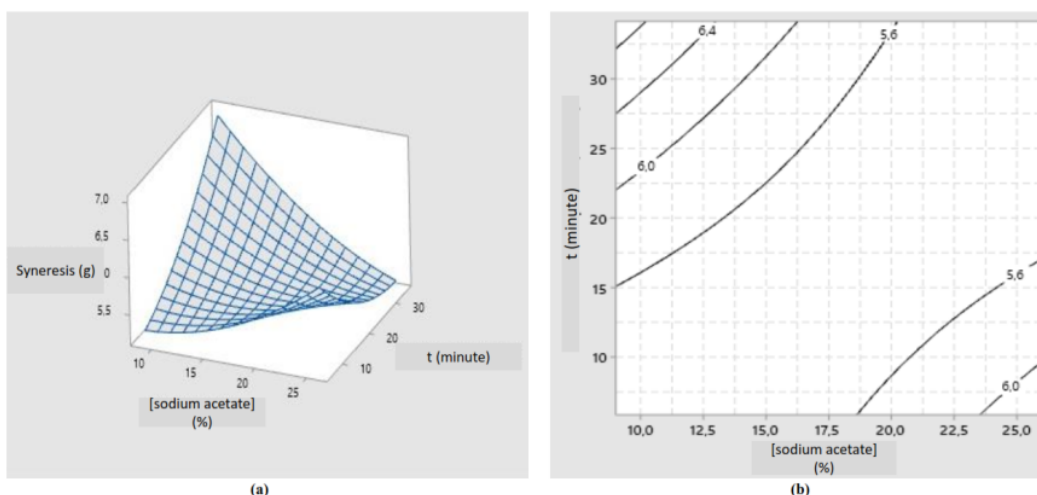


Figure 6. Response surface (a) and contour curve (b) for syneresis of cross-linked canna starch

pH

Measurement of the pH of modified starch tends to be alkaline. In addition, the addition of NaOH as a catalyst in the modification process can also add an alkaline atmosphere to the modified starch. According to the research of [26], the combination of 12% STMP with STPP produces cross-linked starch with a pH of 11.5, having resistant starch properties of 0.4%. Changes in cross-linking starch caused by changes in pH will result in changes in the functional properties of the starch. In addition, reaching 12 was able to significantly reduce levels of cyclic-monostarch monophosphate, monostarch diphosphate, and monostarch monophosphate [27]. Starch cross-linking by processing with alkaline conditions did not show any change in the pasting properties of the starch. Meanwhile, according to [25], starch with alkaline pH was gelatinized did not experience amylose-lipid melting by observed at alkaline pH without disturbing the complex, where there was no significant difference in gelatinization

properties after modified cross-linking treated at pH 9.0 to 12. Surface responsiveness and 2-dimensional contours of pH to the concentration and duration of mixing sodium acetate can be seen in Figure 7.

Figure 8 shows the optimum conditions at 17.5% sodium acetate concentration with a mixing time of 20 to 25 minutes with a pH value of 11.8. Based on the results of statistical predictions, the optimum condition of sodium acetate concentration of 17.9% with a mixing time of 22.14 minutes showed a pH value of 11.77. The high pH of the cross-linking starch was caused by the addition of NaOH to the cross-linking starch. In addition, the difference in the addition of alkaline N-acetate to canna starch causes the starch to have an alkaline atmosphere. The following is the equation of statistical test results:

$$\text{pH} = 12,276 - (0,0308x) - (0,02039y) + (0,000785x^2) + (0,000407y^2) + (0,000125xy)$$

Where x is the concentration of sodium acetate (%); y is mixing time (minute)

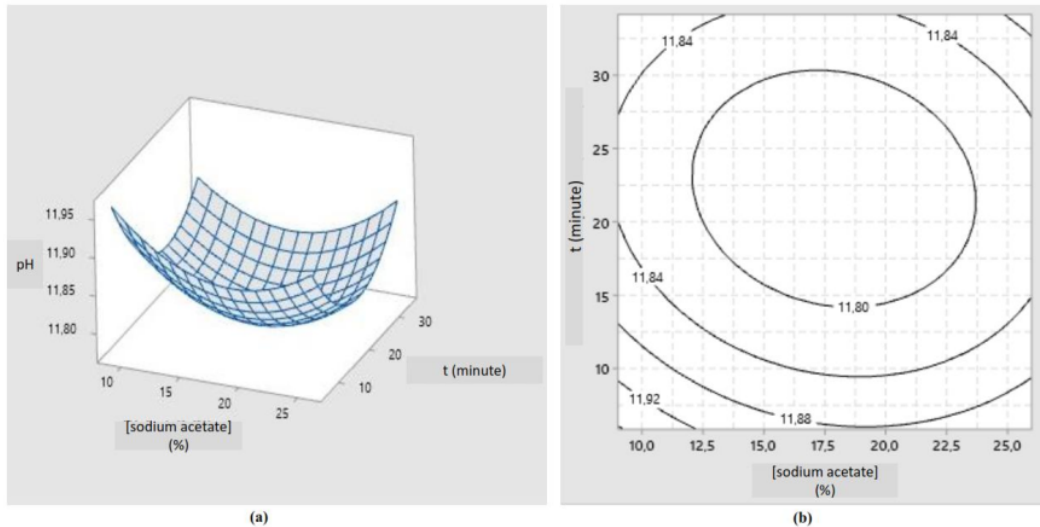


Figure 7. Response surface (a) and contour curve (b) for pH of cross-linked canna starch

Conclusion

Viscosity is the main parameter determining the change in starch properties after being modified by cross-linking. Starch modified by cross-linking can increase the stability of starch viscosity. Cross-linking starch also has the longest time to maintain its viscosity. Cross-linking in starch can strengthen the starch granules so that the starch granules are not easily gelatinized. The optimum viscosity condition was obtained from a sodium acetate concentration of 16.21% with a mixing time of 20.71 minutes to get the optimum viscosity of 43.7 cP. The high degree of substitution of sodium acetate in cross-linking modification affects the integrity of starch granules, where starch granules can affect the physicochemical characteristics of starch. The higher the DS value, the lower the amylose content and solubility. However, increasing the degree of substitution can also increase the viscosity, swelling power, syneresis, and pH of the cross-linked modified starch

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