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TABLE CONTENTS

EDITORIAL PREFACE	i
TABLE CONTENTS	ii
 ORIGINAL PAPERS	
Design of Expert System for Hard Skill Competencies-Based Employee's Performance Evaluation <i>Faqih Maulana, Usman Effendi and Arif Hidayat</i>	101
Comparative Study Production of Exopolysaccharide (EPS) by Lactic Acid Bacteria (<i>L. casei</i> and <i>L. plantarum</i>) in Different Media (Dates and Mulberry juice) <i>Elok Zubaidah, Yusry Muqit Suryawira and Ella Saparianti</i>	107
Feasibility Analysis of Cassava and Glucose Syrup as Alternatives Raw Materials for Industrial Nata (Case Study at CV. Agrindo Suprafood, Yogyakarta) <i>Jumeri, Persia Manggala, Ariesta Cintiadewi, Nafis Khuriyati, and Didik Purwadi</i>	113
Selection of <i>Pseudomonas sp.</i> for Lipid and Detergent Degradation <i>Nur Hidayat and Luqman Nur Chandra</i>	121
Characteristics of Crosslink Acylation Tapioca Substituted Nagara Beans (<i>Vigna unguiculata spp cylindrica</i>) Flour <i>Rini Hustiany</i>	125
Assessment of Workers' Body Temperature and Workload in Tomato Production Greenhouse Work <i>Rizky Silalahi, Mirwan Ushada, Mohammad Affan FF, Kotaro Takayama, Noriko Takahashi, and Hiroshige Nishina</i>	133
The Best Solvent and Extraction Time in Pectin Production Made from Waste of Jackfruit (Bark and Straw) <i>Wignyanto, Nur Lailatul Rahmah, and Athika Dyah Margani</i>	141

GUIDELINE FOR PREPARING MANUSCRIPT

FORMAT AND TEMPLATE

Characteristics of Crosslink Acylation Tapioca Substituted Nagara Beans (*Vigna unguiculata spp cylindrica*) Flour

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Abstract

Granules and gels of tapioca will be easily fragile and broken when heated. This study aimed to investigate the characteristics of tapioca cross-linked acylation substituted Nagara bean flour. The method used is the native tapioca cross-linked with Sodium Tripolyphosphat 2 and 5 % and acylated with stearic acid 2 and 4 % under alkaline conditions. Cross-link tapioca then substituted with Nagara beans flour in the ratio 50:50. The highest degree of substitution found in tapioca starch modified with 5 % STPP and 2 % stearic acid, i.e 0.14. The only maximum viscosity of tapioca starch of cross-linked acylation STPP 5 % and stearic acid 2% higher than native tapioca (5220 cP). The breakdown viscosity lower than native tapioca (3651 cP), final and setback viscosity higher than native tapioca (3023 cP and 1454 cP), and pasting temperature higher than native tapioca starch (67.65 °C). When added with Nagara beans flour then the maximum, breakdown, final, and setback viscosity is descended and pasting temperature is 78.5 oC. It is concluded that the granule of tapioca crosslink STTP 5 % and acylation stearic acid 2 % and substituted with Nagara beans flour in the ratio 50:50 is not easily fragile and broken.

Keywords : *Acylation, Beans Nagara, crosslink , stearic acid, Tapioca*

1. INTRODUCTION

Tapioca is starch produced from cassava. The main component of tapioca is starch, 73.3 to 84.9%, consists of amylose is 17% and amylopectin is 83% (Rickard et al. 1991). Starch contains abundant hydroxyl groups. Each anhydroglucose unit contains two secondary hydroxyl groups and the other is a primary hydroxyl group. This hydroxyl group could potentially bind to other compounds, such as sodium tripolyphosphate (STPP) and stearic acid to create a crosslink and acylation.

Native starch granules will be easily fragile and broken when heated. The crosslink starch has viscosity is not easily broken when heated. This is due to the presence of covalent bonds in the bridge crosslinks are not easily broken when heated and granules form remain intact. The crosslink starch granules were more resistant to strain, high temperature and low pH (Rutenberg and Solarek, 1984).

The crosslink method is often combined with other modification methods. Crosslink method combined with hydroxypropylation method on waxy corn and waxy wheat starch (Reddy and Seib, 2000) and the sago starch (Wattanachant et al., 2002) to obtain a paste of starch with stable to heat and freeze-thaw, robust to swelling and the paste is more clear than the native. In addition, the method of crosslinks can also be combined with the method of acetylation on waxy corn and waxy wheat starch (Reddy and Seib, 2000) which also aims to obtain a paste which is resistant to heating.

Beside to crosslink, so that the starch has hydrophobic and hydrophilic side, the starch can be modified with acylation, one using stearic acid. With the hydrophilic and hydrophobic side, the modified starch can bind materials has a hydrophilic and hydrophobic side, like Nagara bean flour. Hustiany and Mustikasari (2009) stated that the Nagara bean flour contents protein 22.54% wb with polar amino acid 16.28% w/w and non-polar amino acid 8% w/w. The

most found in defatted Nagara bean flour is glutamic acid, amount to 4.75% w/w.

The purpose of this study was to crosslink acylation of tapioca starch with various levels of sodium tripolihsophate to crosslink and stearic acid to acylation; substituting modified tapioca with Nagara bean flour at ratio 50:50, and know the physico-chemical properties of crosslink acylated of tapioca starch consists of degree of substitution, degree of gelatinization, viscosity and chemical structure based functional groups.

2. MATERIAL AND METHODS

2.1. Material

The materials were commercial tapioca starch, Nagara bean, sodium tripolyphosphate technical, stearic acid (Merck) and some other chemicals used to analysis.

2.2. Crosslink Acylation of Tapioca Starch

The crosslink acylation of tapioca starch done by modifying the method Atichokudomchai and Varavinit (2003); Wattanachant et al. (2003), Gonzalez and Perez (2002); Varavinit et al. (2001), Chang et al. (2000), and Hustiany et al. (2005), which is 300 grams of tapioca starch was added with 600 ml of 2% and 5% (w/w dry starch basis) of sodium tripolyphosphate. Then the mixture was added 1.8 g NaOH and 9 g of Na₂CO₃. The starch suspension was stirred at room temperature for 24 hours. The suspension is acylated using stearic acid with 2% and 4% (w/w dry starch basis) and stirred at room temperature for 1 hour. Then the pH of suspension is matched 6.5 with 3M HCl. The suspension was filtered through a Whatman No. 3 and washed with distilled water 3 times (450 ml distilled water each time washing). Then the suspension dried at a temperature of 45°C for 24 hours and 150°C for 2 hours.

2.3. Nagara Bean Flour

Nagara bean sorted and washed before it is immersed in an alkaline solution for 3

hours to reduce the activity of trypsin inhibitors and tannins, and eliminate the hemagglutinin activity. Then, Nagara bean has been drained and removed its skin. Nagara bean dried with oven at a temperature of 50°C until dry, then it is milled with a blender. Nagara bean flour sifted to 60 mesh. Nagara bean flour mixed with crosslink acylation of tapioca starch at ratio of 50: 50.

2.4. Degree of Substitution (Varavinit et al., 2001)

As much as 2 grams of modified starch was added with 50 ml of distilled water containing 25 ml of 0.5 M NaOH. This mixture was stirred with a stirrer at room temperature for 30 minutes. Excess NaOH is titrated with 0.1 M HCl to pH 7 that previously had been added indicators pp 1% as much as 3 drops.

The calculation of degree of substitution (DS) is:

$$DS = 162 M (S - B) / 1000 W$$

where:

W = weight of sample (g)

B = volume of 0.1 M HCl in the blank (ml)

S = volume of 0.1 M HCl in the sample (ml)

M = molarity of HCl (mol / liter)

162 = molecular weight of anhydroglucose (162 g / mol)

DS = number of hydroxyl groups are acylated or crosslinked

2.5. Degree of Gelatinization and Viscosity

Degree of gelatinization and viscosity of starch measured by RVA techmaster 2092435 Newport Scientific brand. Samples (moisture 11%) as much as 3.38 grams and 25.12 grams of water put into the bowl amilograf. The arm sensors mounted and put in bowl. Initial temperature of thermoregulatory set at 40°C.

2.6. Chemical Structure Based Functional Groups (Hustiany et al., 2005)

Changes in the chemical structure of starch can be measured qualitatively using FTIR spectrometer (Fourrier Transformation Infra Red) brand Bruker Tensor 37 series at a wavelength of 400 to 4000 cm^{-1} . The sample was mixed with KBr crystal with ratio was 1:1, 50 mg respectively. This mixture is put into a container to form a pellet. These pellets are inserted into the FTIR to read.

3. RESULTS AND DISCUSSIONS

3.1. Degree of Substitution

Hydroxyl group (OH) contained in the starch, both on the part of amylose and amylopectin, can be substituted with other groups to change the properties of starch. Each of anhydroglucose unit (AGU), there are four OH groups which can be substituted with other groups, the OH group located at C-2, C-3 and C-4 (a secondary OH group) and C-6 which is the primary OH group. Secondary OH groups, especially the C-2 OH group is more reactive than the primary OH group (Tuschhoff, 1989). OH reactivity of the C-2 is approximately 60-65% (van de Burgt et al. 2000).

Starch is highly hydrophilic when it is heated in water with the presence of OH groups. The hydrophilic properties of starch can be converted into hydrophilic and

hydrophobic with the OH group substituted with other groups, such as stearic acid. Moreover, the starch formed a crosslinking bridge, so that the starch granules are not easily broken by the heating process. Crosslinking formation can be done by substituting the hydroxyl groups of starch with sodium tripolyphosphate (STPP).

A number of substituted OH group termed the degree of substitution (DS). The higher concentrations of stearic acid or STPP, then the higher the degree of substitution (Figure 1). The degree of substitution is 0.04 to 0.14. The highest degree of substitution contained in the modified tapioca starch using STPP 5% and 2% stearic acid, which is 0.14. When stearic acid increased again to 4%, then the substitution of the hydrophilic group nonetheless. According Jarowenko (1989), DS generated by this modified tapioca starch value is low, less than 0.5.

Varavinit et al. (2001) also produce stearic pregelatinization of tapioca starch in ethanol medium with a low DS, ie 0.016 to 0.008. Likewise Miladinov and Hanna (2000) produces esterified corn starch with stearic, propionic, heptanoic and palmitic acid in NaOH medium with a low DS, ie 0.0237 to 0.0071. The substitution is low, due to fraction of water in the reaction between starch with stearic acid or STPP. Fraction of water together with a catalyst to hydrolyze the starch modified ester (Santayonan and Wootthikanokkhan, 2003).

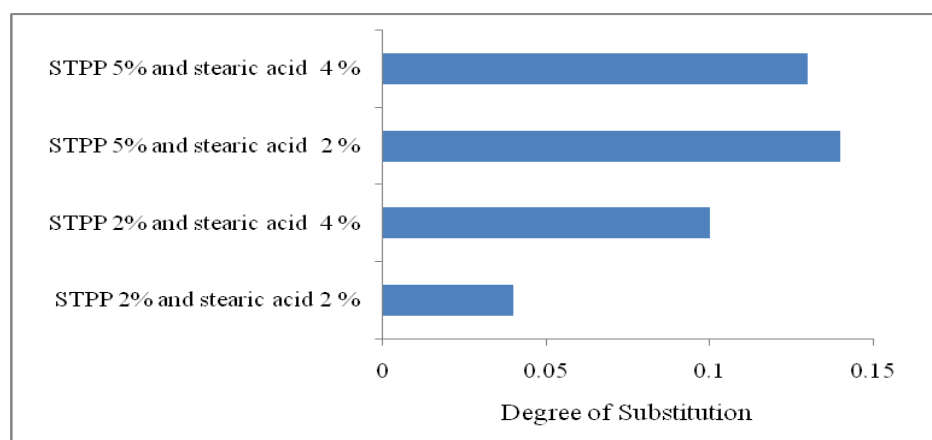


Figure 1. Degree of substitution of various modified tapioca starch with crosslink acylation using STPP and stearic acid

3.2. Identification of Functional Groups

Tapioca starch is a polymer with a monomer is glucose. Starch is composed of amylose and amylopectin. Amylose is a straight-chain glucose polymers with bond α -(1.4), while amylopectin is a straight-chain glucose polymers with bond α -(1.4) and branched chain α -(1.6). Mostly found in tapioca starch (Figure 2) is a OH, CH and CO and aromatic group.

Hydoxyl group is shown at λ 3600 cm^{-1} to 3200 cm^{-1} , CH shown in λ 3200 cm^{-1} to 2800 cm^{-1} and λ 1400 cm^{-1} to 1350 cm^{-1} , the CO group at λ 1300 cm^{-1} to 1000 cm^{-1} and the aromatic group λ 2300 cm^{-1} to 2100 cm^{-1} . The modified tapioca starch with crosslink using STPP and acylation with stearic acid will be an increase in the number of OH groups, CH, CH_2 , CH_3 , CO, and CO carbonyls.

The most dominant group in the crosslinked starch STPP 2% and 2% stearic acid is the increased OH and CO group. It is thought the crosslinked tapioca starch formed between starch with STPP. The PO group formed unreadable as CO group. The formation of acylation with stearic acid is

less, because the λ 3200 cm^{-1} to 2800 cm^{-1} and λ 1400 cm^{-1} to 1350 cm^{-1} increased slightly.

The crosslinked acylation of tapioca starch 2% STPP and 4% stearic acid is increasing number of OH, CO and CH groups. This means that the OH groups on the starch substituted with STPP is enough to form crosslink, and the stearic acid is added to increase the substitution of OH groups on the starch. As a result the amount of stearic groups are acylated be increased. So the amount of crosslinking and acylation equilibrium.

The number of OH and CO group on crosslinked acylation STPP 5% and 2% stearic acid of tapioca starch (Figure 3) increase, especially in the CO group. However, only slightly the CH group increase. This phenomenon is similar to croslinked acylation STPP 2% and 2% stearic acid of tapioca starch. But, the number of CO group includes PO group at crosslinked acylation 5% STPP and 2% stearic acid of tapioca starch is more.

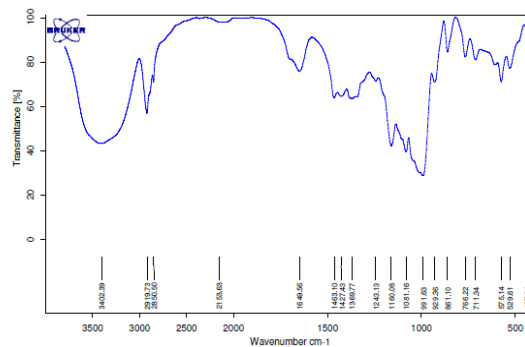


Figure 2. Infra red (IR) spectrum at tapioca starch

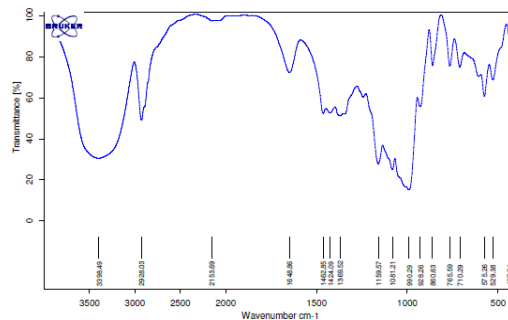


Figure 3. Infra red (IR) spectrum at tapioca starch of crosslinked acylation STPP 5% and stearic acid 2%



Figure 4. Infra red (IR) spectrum at tapioca starch of crosslinked acylation STPP 5% and stearic acid 2% of substituted Nagara bean flour with 50:50 ratio

Interesting phenomenon occurs in tapioca starch of crosslinked acylation 5% STPP and 4% stearic acid. Based on previous analysis, the amount of OH and the CO group should increase, at least the numbers were almost the same as tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid. But the fact is not the case thus, the number of OH, CO and CH group decrease compared to the tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid. This is presumably due to the competition between STPP and stearic acid can substitute for OH groups on tapioca starch.

When the tapioca starch of crosslinked acylation 5% STPP and 2% stearic acid substituted with Nagara bean flour with 50:50 ratio, then the OH, CH, CH₂, CH₃, CO and CO carbonyl group look sharper (Figure 4). Although the percentage is lower than tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid without substituted. This means that the presence of Nagara bean flour substituted, then physically modified tapioca starch still have properties not easily broken and fragile when heated.

In addition, the modified tapioca starch has a high nutritional value with an increase protein content from Nagara bean flour.

3.3. Degree of Gelatinization and Viscosity

Initial temperature of gelatinization in native tapioca starch (Table 1 and Figure 5) and modified tapioca starch (Table 1) ranged between 67.65 to 69.25 °C. That means is temperature to initiate gelatinization on native tapioca starch and modified tapioca starch is more or less the same. Although the initial temperature of gelatinization modified

tapioca starch higher than native tapioca. The final temperature of gelatinization native tapioca starch different with modified tapioca starch.

The final temperature of gelatinization of native tapioca starch is 75°C. As for the final temperature of gelatinization tapioca starch of crosslinked acylation STPP and stearic acid are increased. At starch of crosslinked acylation 2% STPP and stearic acid 2%, then the final temperature of gelatinization is 92°C. Even, final temperature of gelatinization starch of crosslinked acylation 2% STPP and stearic acid 4% increase, which is close to 100°C. Neither the final temperature of gelatinization starch of crosslinked acylation STPP 5% and 2% stearic acid and starch of crosslinked acylation STPP 5% and 4% stearic acid also increased, which is 97°C.

Increased final temperature of gelatinization showed that the granule of modified tapioca starch much remains intact, because the number of hydroxyl groups on the starch of crosslinked acylation STPP and stearic acid decreased, so that the starch granules swell and can not be broken at high temperature. This means that the hydroxyl group of tapioca starch has been substituted with STPP or stearic acid. The more STPP and stearic acid were added, the more the hydroxyl group substituted with STPP and stearic acid.

At tapioca starch (Fig. 5) there is a very sharp peak. This means tapioca starch granules easy to broken and fragile. This is supported by the low value of stability. Thus tapioca starch modification in crosslink and acylation to increase stability of granule against heat.

Table 1. Degree of gelatinization, viscosity and stability of tapioca and modified tapioca

Characteristic	Native Tapioca	2% STPP and 2% stearic acid	2% STPP and 4% stearic acid	5% STPP and 2% stearic acid	5% STPP and 4% stearic acid	5% STPP and 2% stearic acid and Nagara bean flour
Peak 1 (cP)	5220	3385	5204	6664	4746	2804
Trough 1 (cP)	1569	2180	2915	5303	3928	2733
Breakdown (cP)	3651	1205	2289	1361	818	71
Final Visc (cP)	3023	5177	7154	8493	8499	4930
Setback (cP)	1454	2997	4239	3190	4571	2197
PeakTime (minute)	5.6	9	6.93	9.07	9.93	118.7
Pasting Temp (°C)	67.65	69.25	68.85	68.85	68.85	78.5
Stability Ratio Hold/Peak	0.3	0.64	0.56	0.79	0.83	0.97

Tapioca starch of crosslinked acylation STPP 2% and 2% stearic acid and tapioca starch of crosslinked acylation STPP 2% and 4% stearic acid still formed peak, although not as sharp peak native tapioca starch. The second granule of modified starch is not easily broken when heated, but easily fragile. This is presumably due to the high hydrophobicity of the modified tapioca starch, but crosslinking is formed is still lacking. As a result, the stability of modified tapioca starch is low.

Different phenomena found in tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid (Fig. 6) and tapioca starch of crosslinked acylation STPP 5% and 4% stearic acid that there is a high peak viscosity at high temperatures. This means that the

granules of two types modified tapioca starch is not easily fragile and broken.

However, the viscosity of tapioca starch of crosslinked acylation 5% STPP and stearic acid 4% lower than tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid. This means that at low viscosity, tapioca starch of crosslinked acylation STPP 5% and 4% stearic acid already gelatinized and slightly to form a film. The viscosity of tapioca starch of crosslinked acylation STPP 5% and stearic acid 2% is higher and the gelatinization process is longer. The viscosity of the tapioca starch of crosslinked acylation 5% STPP and 2% stearic acid of substituted with Nagara bean flour at 50:50 ratio (Figure 7) is decrease and the stability is higher.

Peak 1	5220 cP
Trough 1	1569 cP
Breakdown	3651 cP
Final Visc	3023 cP
Setback	1454 cP
PeakTime	5.6
	minute
Pasting Temp	67.65 °C
Stability Ratio	0.3
Hold/Peak	

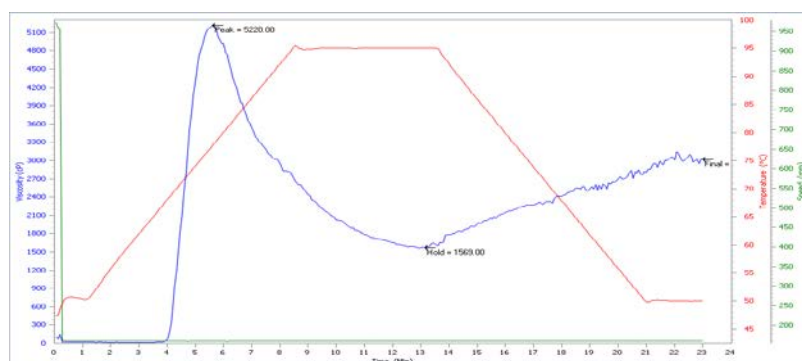


Figure 5. Amilograf of tapioca

Peak 1	6664 cP
Trough 1	5303 cP
Breakdown	1361 cP
Final Visc	8493 cP
Setback	3190 cP
PeakTime	9.07
	minute
Pasting	68.85 °C
Temp	
Stability	0.79
Ratio	
Hold/Peak	

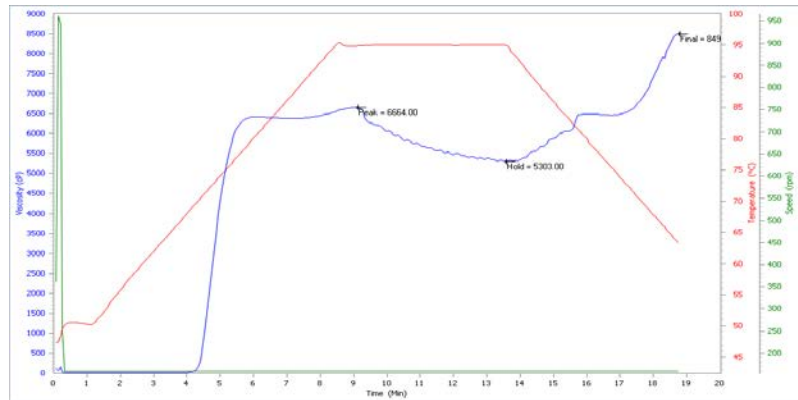


Figure 6. Amilograf of tapioca of crosslinked acylation 5% STPP and 2 % stearic acid

Peak 1	2804 cP
Trough 1	2733 cP
Breakdown	71 cP
Final Visc	4930 cP
Setback	2197 cP
PeakTime	11.87
	minute
Pasting	78.5 °C
Temp	
Stability	0.97
Ratio	
Hold/Peak	

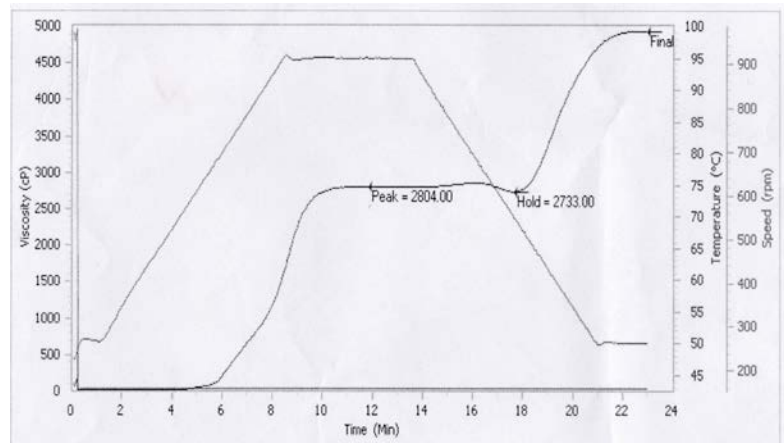


Figure 7. Amilograf of tapioca of crosslinked acylation 5% STPP dan 2% stearic acid of substituted Nagara bean flour with 50:50 ratio

CONCLUSIONS

Modified tapioca starch has a characteristic degree of substitution ranging from 0.04 to 0.14. The highest degree of substitution found in tapioca starch of crosslinked acylation STPP 5% and 2% stearic acid, ie 0.14. Modified tapioca starch is characterized by increased OH, CH, CH₂, CH₃, CO, and CO carbonyls group.

Tapioca starch of crosslinked acylation 2% STPP and stearic acid 2 and 4% had a peak in viscosity, so that the starch easily broken. The tapioca starch of crosslinked acylation 5% STPP and stearic acid 2 and 4% there is no peak, so that the starch is not

easily broken when heated. Final temperature of modified tapioca starch is high, ranging from 93 to 100 °C.

Tapioca starch of crosslinked acylation 5% STPP and 2% stearic acid of substituted Nagara bean flour in the ratio 50: 50 decrease the viscosity of starch and starch is not easily broken and fragile.

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