Effect of solvent concentration in sago starch fractionation

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Abstract. The research studied the effect of solvent concentration in the fractionation process from sago starch in order to achieve the amylose fraction. The amylose is aimed to be applied as a functional material such as for edible film. The fractionation process is carried out by utilizing N-butanol as the solvent with a variety of concentration (10, 12.5 and 15%). The highest result is achieved from the concentration of butanol of 15%. It resulted in a yield of 31.19%, a 30.78% solubility, and swelling ability of 2.37%. The spectra from Fourier transform infrared showed the same specific peaks of the obtained amylose. However, each of them shows different intensity means the fractionation process affect the resulted amylose.

1. Introduction
Sago has high carbohydrate content that can be used as a substitute for wheat flour and it is also well used as a thickener that is better than tapioca [1,2]. Starch is a large polysaccharide and is mainly composed of linear amylose and highly branched amylopectin. Amylose is a long, straight, unbranched, and hydrophobic chain polymer with α- (1,4) -D-glucose bonds [3,4]. Amylopectin has the same backbone as amylose, but it also branched by α-(1,6) linkages. Sago starch contains 70-80% more amylopectin, while the amylose content is 15-30%. These components determine the gelatinization, gel stability, solubility, and swelling power. High percentages of amylose give starch less stickiness and low gelatinization temperature. The molecular weights of amylose and amylopectin have been estimated to be about 105 and 108 Da, respectively [5].

Amylose plays an important role in starch gelation, but insight in its main role has been vague by the presence of amylopectin [6,7]. Therefore, this makes amylose content an important quality parameter for starch-based products [8]. Separation or fractionation of amylose and amylopectin has been a field of interest for some decades, but only several successes have been achieved. Schoch described starch separation for the first time [9]. Some authors provided alternative paths and optimized this method, in which the purity of the obtained amylose and amylopectin fractions becomes the main consideration [10–13]. High purity starch fractions are obtained by precipitating the amylose fraction of amylopectin with guest molecules.
Since the previous studies showed that the amylose is able to make inclusion complexes, so that its solubility is reduced in an aqueous dispersion. Then, the insoluble amylose-guest molecule is recovered as a precipitate. Some guest molecules, such as methanol, butanol, and pentasol have been employed to precipitate free amyloses from aqueous dispersions. However, butanol has been extensively used due to its ability in selecting the amyloses compared to other guest molecules. To the best of our knowledge, there is limited study regarding the amylose from fractionation process of sago starch. Therefore, in this study, butanol with varying concentration was used as a guest molecule to create amylose-guest molecule complexes in the fractionation process of sago starch. The obtained amylose fraction from the sago starch were characterized and evaluated their behaviors.

2. Materials and Method

2.1. Materials
Sago (Metroxylon sp.) starch with approximately 34% amylose and 66% amyllopectin was used as the main material. Glycerol (analytical grade) was purchased from Sigma–Aldrich Co., Ltd. (St. Louis, MO, USA). Ethanol and N-butanol technical grade were used. All other reagents used were analytical grade.

2.2. Preparation of sago starch composition
The amylose content was analysed by the spectroscopic method. The solution was measured its absorbance by a UV-Vis spectrophotometer at a wavelength of 625 nm and the levels was determined using the standard curve equation of amylose solution standard. The soxhletation method was used to determine the fat content according to SNI 01-2891-1992.

2.3. Preparation of sago starch fractionation
Sago starch fractionation was carried out by modifying the method of Mizukami et al. [14]. 30 gram of sago starch was mixed with 2 litre of distilled water and then heated to 85°C for 60 min while stirring by a stirrer under nitrogen atmosphere. The water-soluble fraction was separated from the suspension by centrifugation at 8000 rpm for 10 min. The separated water-soluble fraction was added with butanol with variations of 10, 12.5, and 15 % of the water-soluble fraction volume, then incubated for 24 hr. The resulting precipitate is an amylose fraction and then centrifuged at 8000 rpm for 10 min. The precipitate was washed with ethanol 3 times, then washed also with petroleum ether 3 times, followed by drying with CaCl$_2$ then put in a desiccator. The yield of obtained amylose was calculated as follow:

\[
\text{Yield of amylose (\%)} = \frac{\text{weight of obtained amylose}}{\text{initial weight of sago starch}} \times 100\% \quad (1)
\]

2.4. Swelling power (SP) and water solubility index (WSI)
Swelling power (SP) of the obtained amylose was determined according to the method of Senanayake et al. [15]. 100 mg amylose was weighed directly into a screw-cap test tube and 10 mL distilled water was added. The capped tubes were placed on a vortex mixer for 10 sec, then incubated at 85 °C water bath with frequent mixing about 10 sec after 5, 15 and 25 min. The tubes were cooled at room temperature and centrifuged at 2000 rpm for 30 min. The supernatant was removed and the remaining sediment in the tube was weighed (\(W_s\)). The supernatant was dried to constant weight (\(W_f\)) in a drying oven at 105 °C. The water swelling power was calculated as follows:

\[
\text{SP (\%)} = \frac{W_s}{[0.1 \times (100\% - \text{WSI})]} \times 100\% \quad (2)
\]

where

\[
\text{WSI} = \frac{W_f}{0.1 \times 100\%} \quad (3)
\]
2.5. **Fourier transform infrared spectroscopy analysis (FTIR)**

FTIR spectra were recorded using FTIR ATR (Tensor II, Bruker, USA). The resolution was 4 cm\(^{-1}\) and there was a total of 40 scans at wave number of 4000-400 cm\(^{-1}\).

3. **Results and Discussion**

3.1. **Sago starch composition**

The sago starch composition is given in Table 1. The moisture content of the sago starch was 11.46% which is typical of commercial starches. The moisture content of most unmodified starches is around 12%. All commercial starches contain small quantities of inorganic materials. The inorganic material generally derived from the way the starch was isolated and also from the used water in the process.

| Component            | Composition (%) |
|----------------------|-----------------|
| Amylose content      | 33.73           |
| Amylopectin content  | 66.27           |
| Moisture             | 11.46           |
| Crude fat            | 1.8             |
| Crude protein        | 0.0017          |

3.2. **Sago starch fractionation**

Sago starch with the content of amylose of about 33% and water content of 11% was prepared in fractionation process. The fractionation process was carried out by utilizing butanol as the solvent to dissolve the amylopectin fraction. This study varied the concentration of butanol namely 10, 12.5, and 15% in order to achieve the highest yield of amylose. Yield is an important parameter in order to know the level of efficiency of a processing process. The greater the yield indicates the more effective and efficient the process carried out. The yield of each variation is shown in Table 2.

| Butanol concentration (%) | Yield of amylose fraction (%) |
|---------------------------|------------------------------|
| 10                        | 25.29                        |
| 12.5                      | 28.24                        |
| 15                        | 31.19                        |

Amylose fractionation in this study was carried out by the modification method. The fractionation process was initially carried out using hot water, because hot water is able to dissolve the starch completely. The starch solubility increases with the increasing temperature of the hot water that used as the solvent. Then the separation was done using butanol as a solvent to dissolve amylopectin. Table 1 shows that the greater the concentration of butanol used, the higher the yield of the obtained amylose fraction from sago starch. The concentration of 15% butanol resulted in the yield of amylose fraction of 31.19%. This result was higher compared to other study which gained the yield of 16.36 and 19.36% for the butanol concentration of 10 and 20% [16]. Other study also showed that the increasing in yield was in line with the increasing of butanol concentration. This is due to the butanol as solvent to dissolve amylopectin fraction so that resulted in high content of amylose. The use of hot water as the initial part
in the fractionation process of starch plays an important role to change the structure and function of starch granules.

Amylose is a starch component that has a straight chain and soluble in water, while amylopectin has a branched chain, not soluble in water but soluble in N-butanol. This is because amylose consists of a straight D-glucose chain that binds to α-1,4. It is also influenced by hydrogen bonds that occur between hydroxyl groups in amylose and water. When starch is heated in hot water at gelatinization temperature, heat energy causes hydrogen bonds in starch to weaken and it provides the water to get into the granules and allows a slight dissolution and exchange of amylose molecules into the water. This emerges due to the presence of excess butanol can dissolve amylopectin, so that amylose can be separated from amylopectin in the form of sediment [10].

3.3. Swelling power and water solubility
Swelling power is the power of amylose to expand, which occurs due to non-covalent bonds between amylose molecules. Weak hydrogen bonds in the amorphous region will be broken during heating, resulting in water hydration. Amylose will continue to expand so that the viscosity increases to the maximum hydration volume that can be achieved [17]. From data in Table 3, it was stated that the concentration of butanol significantly affected the swelling power and solubility of the amylose fraction of sago starch with a range of 40.18% (butanol 10%) to 30.78% (butanol 15%). These results were lower compared to other study, which the swelling ability reached more than 40% depending on the temperature as its parameter.

| Butanol concentration (%) | Swelling power (%) | Solubility (%) |
|---------------------------|--------------------|---------------|
| 10                        | 3.89               | 40.18         |
| 12.5                      | 3.39               | 37.33         |
| 15                        | 2.37               | 30.78         |

Table 3 shows that the increasing concentration of butanol up to 5% significantly decreases the swelling power and solubility of the amylose fraction of sago starch. This might be due to the utilization of butanol as solvent decreasing the mobility of the water fraction, thereby inhibiting the amylose restructuration in the amorphous phase. The highest value of swelling power and solubility is produced at 10% butanol concentration and the smallest is at 15% butanol concentration.

The amylose restructuration process which is inhibited in the amorphous region during heating and caused the inter- and intra-molecules of hydrogen bonds to become denser, making it difficult for water molecules to enter, since solubility is related to the ease of water molecules to interact with molecules in amylose. The increase in swelling power values is also suggested due to the hydrophilic nature of the amylose so as to bond to hydrogen in water molecules [18]. While the occurring partial hydrolysis during heating in the fractionation process resulted in low molecular weight so that the ability to expand is limited.

3.4. Fourier transform infrared spectroscopy analysis (FTIR)
FTIR analyses were applied in order to study the changes in chemical structure among the obtained amyloses. The changes of functional group in chemical structure can be known from the characteristic of absorption peak. Figure 1 shows the spectra of sago starch, similar as in previous study [19,20]. In the spectra for sago starch the presence of -C-O-C was confirmed by the absorption peaks at 1628-1676 cm⁻¹, the absorption peak at 1436-1479 cm⁻¹ was attributed to -OH bending.
Figure 1. FTIR spectra of sago starch.

Figure 2 shows the FTIR spectrums of biocomposite films. The comparison of each absorption peak from the designated samples is summarized in Table 4.

Figure 2. FTIR spectra of obtained amylose.
Table 4. FTIR characteristic of starch films.

| Samples (cm⁻¹) | Characteristics       |
|---------------|-----------------------|
| SAM 1         | 3253-3304             | OH stretching         |
| SAM 2         | 3248-3344             |                       |
| SAM 3         | 3242-3354             |                       |
| 2908-2937     | 2912-2937             | CH stretching         |
| 1625-1656     | 1627-1660             | C=O group            |
| 1311-1382     | 1348-1354             | OH bending            |
| 1002-1010     | 999-1012              | C=O stretching        |
| 1627-1652     | 1627-1652             |                       |
| 1346-1355     | 1346-1355             |                       |

FTIR spectroscopy was used to examine the influence of butanol concentration in the fractionation process toward the functional group of the obtained amylose from sago starch. This is because if two or more chemicals are mixed, the physical blends towards chemical interactions are reflected by the changes in the characteristic of spectra peaks. The infrared spectra of each amylose are shown in Figure 2. All the results show certain peaks similar to the amylose studied by other groups [21]. It can be seen from all obtained infrared spectra that there was no significant peak change, but difference in intensity of the peak. This might be due to the effect of different concentration of butanol as a solvent for the precipitation of amylose-butanol complexes.

4. Conclusion
The starch separation procedure was successfully synthesized and amylose fraction was obtained from sago starch. The variation of butanol as the solvent resulted in different yield of the obtained amylose. This also implied in the swelling ability and water solubility of each obtained amylose. All amyloses separated from the sago starch had the same functional group with different intensity.

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