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EFFECT OF ETHANOL TREATMENT ON MORPHOLOGICAL AND TECHNICAL PROPERTIES OF CORN STARCH

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ABSTRACT

Solvent exchange is considered an effective method that changes the physicochemical properties of starch, especially the absorption. Ethanol concentration is one of the important influencing factors to form porous pores from the surface to inside starch granules. In this study, the effect of ethanol concentration on technical properties of starch treated by solvents were investigated.

The ratio of ethanol/water was prepared at 1/9, 3/7, 5/5, 7/3 and 10/0 (w/w), respectively. Corn starch was treated by solvent at 8% concentration. Morphology, oil and water holding capacity, solubility, swelling power, viscosity, and transmittance were studied to elucidate the effect of the ethanol concentration on the morphological and technical properties of corn starch treated by solvent. As a result, starch treated by solvent with different concentrations tended to form wrinkles and pore from the surface to the inside of the starch granules during ethanol immersion. Therefore, the oil and water holding capacity, solubility, swelling and viscosity of the solvent-treated starch samples increase as the concentration of ethanol increases. Otherwise, the transmittance of starch glues tends to decrease when the ethanol concentration increases.

***Keywords**: ethanol concentration; physicochemical properties; solvent-exchange; starch; oil holding capacity.*

1. INTRODUCTION

Starch is the second most popular natural compound after cellulose. Therefore, starch is used in many food and non-food products [1-2]. However, the application of natural starch in production faces some limitations, such as high viscosity, easily degenerate, insoluble in cold water... Therefore, modification of starch is implemented to enhance the properties, which does not exist in natural starch. Various methods have been applied to produce modified starches for a variety of applications [3]. In which, starch modification by the simple physical method is inexpensive and avoids the use of chemicals [4]. Among physically modified starches, porous starch receives a lot of attention due to its excellent adsorption capacity [5]. Porous starch is also non-toxic, biodegradable and environmentally friendly [6].

In recent years, besides the enzyme and ultrasonic methods, solvent treatment emerges as a new method for starch modification [7]. However, former researchers focus mainly on the surface area and the adsorption capacity, not the physical and chemical properties of porous starch. Therefore, this study was conducted to investigate the effect of ethanol concentration on physicochemical properties of porous corn starch after solvent treatment.

2. MATERIALS AND METHODS

2.1 Materials

Corn starch used in this study was purchased from ROQUETTE Riddhi Siddhi Company (India).

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2.2 Methods

2.2.1 Treatment of starch by ethanol and solvent

Porous corn starch (PS) was prepared by method of Chang et al. (2011) with modifications. Corn starch (8 g) was dispersed in 100 mL of distilled water. The mixture was stirred and heated at 90°C for 30 minutes to completely gelatinize the starch granules. The starch paste was then cooled at 5°C for 48 hours to form starch gel. After that, the gel was cut into 1 cm × 1 cm × 1 cm cubes and frozen at -10°C for 48 hours. The gel cubes were then soaked in the ethanol/water mixture at room temperature with different volume ratios of ethanol/water: 1/9, 3/7, 5/5, 7/3 and 10/0. The cubes were soaked 3 times, each time for 1 hour. After soaking, cubes were dried at 50°C for 6 hours in an oven and heated to 105°C for the next 2 hours to eliminate excess water and ethanol, forming white solid PS cubes. Then, the PS cubes were crushed and sieved through 80 mesh sieving [8].

PS0, PS1, PS3, PS5, PS7 and PS10 were the abbreviations to distinguish the corn starch samples used in this study. In which, PS0 was a control sample which was not soaked in solvents. PS1, PS3, PS5, PS7 and PS10 were ethanol/water-soaked corn starch samples with concentrations of 1/9, 3/7, 5/5, 7/3, 10/0, respectively.

2.2.2 Morphology of starch granules observed by scanning electron microscope (SEM)

Morphology of the natural and solvent modified starch samples were observed by JSM 7401F scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Corn starch was placed on a specific sample container. It was then coated with a thin layer of gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan). Samples were observed at accelerated voltage of 5 kV and at various magnifications.

2.2.3 Determination of water and oil holding capacity

The oil holding capacity (OHC) was determined by the method of De la Hera et al (2013). This method had some modifications by Sarangapani et al (2016). The centrifuge tube contained 0.1 g of starch and 1.0 mL of walnut oil. The mixture was shaken for 30 minutes using a shaker. Then, the tube was centrifuged at 3000 rpm for 10 minutes. The supernatant was removed slowly, while the centrifuge tube was slanted for 25 minutes allowing the oil to drain out of the tube. OHC was calculated as the number of grams of oil held per gram of starch [9-10].

Water holding capacity (WHC) was determined by the method of Kim et al (2013). A centrifuge tube containing 5% (w/v) suspension of starch in distilled water was shaken for 10 minutes with a shaker. The tube was then centrifuged at 1000 rpm for 15 minutes. The supernatant was removed, but the residue was collected and weighted. WHC was determined by grams of water held per gram of starch. [11]

Specifically, OHC and WHC were calculated by the formulas:

$$\text{OHC (g/g)} = \frac{W_r - W_i}{W_i} \quad (1)$$

$$\text{WHC (g/g)} = \frac{W_r - W_i}{W_i} \quad (2)$$

Where: W_r was the weight of residue after centrifugation (g)

W_i was the weight of initial starch (g)

2.2.4 Determination of solubility and swelling power

Solubility and swelling power of natural and solvent modified starch were determined by the method of Leach, McCowen and Schoch (1959) with some modifications according to Amini et al (2015). The centrifuge tube containing the 1% (w/v) starch suspension was heated in a water bath at 80°C for 30 minutes. The tube was shaken continuously for 1 minute, after every 5 minutes by a vortex. The tube was then cooled to room temperature and centrifuged at 2400×g for 20 minutes. After centrifugation, the residue was collected and weighted. The supernatant was transferred to a petri dish and dried at 105°C to constant mass to determine the solids content in the solution. Solubility (SB) and swelling power (SP) were calculated by equations (3) and (4) [12-14].

$$SB\ (\%) = \frac{m_2}{m_1} \times 100 \qquad (3)$$

$$SP\ (g/g) = \frac{m_3}{m_1 - m_2} \qquad (4)$$

Where: m_1 was the mass of initial starch (g)

m_2 was the mass of remaining solids in supernatant after drying (g)

m_3 was the mass of residue after centrifugation (g)

2.2.5 Determination of viscosity

The viscosity of natural and modified starch paste was carried out according to the method of Kang et al. (1999) with some modifications. The 10% (w/v) starch suspension was heated in a water bath for 30 minutes. Then, it was cooled to 50°C by cold water and kept thermally stable at 50°C in a water bath. The viscosity of starch paste was determined by Brookfield DV-II + Pro viscometer (USA) for 5 hours. This experiment was repeated after every one hour [15].

2.2.6 Determination of transmittance

Transmittance of natural and modified starch was conducted according to the method of Wani et al (2010). Starch suspension (1% w/v) was boiled in a beaker for 30 minutes and then cooled to room temperature. Starch samples were stored at 4°C for 120 hours and measured transmittance at wavelength of 640 nm after every 24 hours for the next 5 days using the Halo VIS 20 Visibility Spectrometer (Dynamica, Switzerland) [16].

2.2.7 Statistical analysis

Data was analyzed by one-way ANOVA in SPSS 22 program (SPSS Institute Inc., Cary, NC, USA) with the Duncan's Multiple Range Test ($p \leq 0.05$) to analyze the significant differences between the mean values.

3. RESULTS AND DISCUSSION

3.1 Effect of ethanol concentration on morphology of starch granules

Morphology is one of the important structural properties of starch granules. The starch granular morphology shows the influence of gelatinization and hydrolysis… on the surface and inner structure of the grain [17]. Figure 1 shows morphology of porous corn starch with different rates of ethanol/water immersion. As a result, wrinkles and holes appeared on the surface of the control sample (PS0). This could be explained by the penetration of water molecules into the starch granules during gelatinization and gradual evaporation during freezing and drying processes, leading to the formation of pore structures and wrinkles on the surface of starch granules which also agreed with Bao et al. (2016). In addition, the cause of the above phenomenon might be due to the formation of ice crystals inside the starch granules and the elimination of some amylose and amylopectin molecules of the starch granules during gelatinization [18-19].

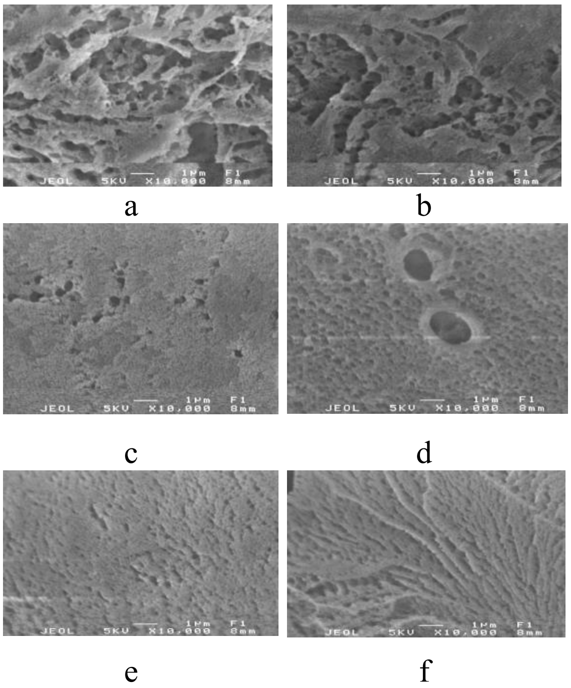

Figure 1. *Morphology of porous corn starch at various concentrations of ethanol/water by SEM*

(a: PS0; b: PS1; c: PS3; d: PS5; e: PS7; f: PS10)

Morphology of solvent-treated corn starch samples at different concentrations of ethanol/water observed by SEM were shown in Figure 1. Concentration of ethanol significantly affected the surface structure of starch granules. Similar to the PS0 sample, the formation of holes in the surface of the solvent treated samples was also related to the concentration of ethanol. The pore structure was formed when ice crystals in frozen gel were replaced by ethanol. First, the natural corn starch granules were destroyed during gelatinization, leading to water penetration into starch granules. Then, during the cooling and freezing process of starch gel, water molecules were fixed and then replaced by ethanol during immersion in ethanol solution. The number of holes formed was proportional to the ethanol concentration. These results were similar to the study of Chang et al (2011), Oliyaei et al (2018), Qian et al (2011). In addition, during the drying process, the pore size of starch granules might be reduced due to the effect of temperature [8] [20-22].

3.2 Effect of ethanol concentration on OHC and WHC of starch granules

Oil holding capacity (OHC) is a technological property that depends on the physical and chemical structure of the starch granule. The ability of the starch granule holding oil is influenced by two factors. (i) The first factor is the capillary blockage of oil molecules in the pores and grooves of starch granules. (ii) The second factor was the ability of the oil molecule to fill the holes between the starch particles [23].

Water absorption (WHC) is the amount of water kept in starch granules. This is a property of starch due to its hydrophilic positions in macromolecular chains. The hydrophilic sites interact with water through hydrogen bonds to hold water molecules [24].

The ability to keep oil and water at room temperature of starch granules treated by the solvent was presented in Table 1. The results showed that the oil and water holding capacity of the starch samples increased proportionally with the concentration of ethanol. The structural change during solvent immersion had a significant effect on the oil and water holding capacity of starch granules [6]. In addition, this phenomenon could also be explained by the process of replacing ice crystals with ethanol. This process created pores from the surface to the inner structure of the starch granules, increased surface area and the capillary absorption power of the starch granules, leading to the ability to hold oil and water significantly improved. This result was similar to the studies of Yu et al (2018), Sujka and Jamroz (2013) [25-26].

***Table 1**. OHC and WHC of starch granules at different concentrations of ethanol*

Samples	OHC (g/g)	WHC (g/g)
PS0	1.70 ± 0.05^{a}	5.83 ± 0.04^{a}
PS1	1.82 ± 0.04^{b}	6.09 ± 0.04^{b}
PS3	1.86 ± 0.05^{bc}	6.62 ± 0.02^{b}
PS5	1.95 ± 0.05^{c}	6.38 ± 0.02^{c}
PS7	2.15 ± 0.04^{d}	6.57 ± 0.13^{d}
PS10	2.48 ± 0.07^{e}	7.44 ± 0.12^{e}

Data represented mean ± standard deviation. Different alphabet letters in a column statistically indicated significant difference ($p < 0.05$).

3.3 Effect of ethanol concentration on solubility and swelling power

Solubility (SB) and swelling power (SP) represent the degree of interaction between starch chains in the amorphous and crystal regions. This interaction is influenced by factors such as molecular weight, degree of branching, branch length, molecular structure and the ratio of amylose and amylopectin [27]. The solubility and swelling power of the solvent treated starch samples were shown in Table 2.

The results showed that solubility of porous corn starch increased with the number of holes on the surface of starch granules increased (Table 2). This was explained by the fact that when the starch suspension was heated, the crystal structure was broken down, allowing water to penetrate inside the starch. Then, some amylose and amylopectin molecules came out of the starch granules and dissolved in the suspension. Considering porous corn starch, the outflow of amylose and amylopectin was more intense due to the appearance of holes in the surface. This led to an increase in solubility of the porous starch samples [28-29].

The results also indicated that the swelling power of porous corn starch samples tended to increase gradually with the proportion of ethanol (Table 2). This was explained by the formation of the pore structure of starch granules after solvent treatment. An increase in the number of holes allowed starch particles to interact more with water. Then, the swelling power of the solvent-treated starch samples increased [25] [30] which in the agreement with the study of Chang et al (2011) [8].

Table 2. *Solubility and swelling power of corn starch at different ethanol concentration*

Samples	SB (%)	SP (g/g)
PS0	3.50 ± 0.10^{a}	12.34 ± 0.07^{a}
PS1	3.63 ± 0.12^{ab}	13.66 ± 0.16^{b}
PS3	3.76 ± 0.05^{bc}	13.89 ± 0.06^{b}
PS5	3.87 ± 0.06^{c}	13.93 ± 0.08^{b}
PS7	4.10 ± 0.17^{d}	15.70 ± 0.14^{c}
PS10	4.33 ± 0.12^{e}	16.31 ± 0.06^{d}

Data represented mean ± standard deviation. Different alphabet letters in a column statistically indicated significant difference ($p < 0.05$).

3.4 Effect of ethanol concentration on viscosity

Molecular structure of glucan is an important factor affecting the viscosity of starch. Starch containing more short chain glucan molecules results in lower viscosity [31]. The change in viscosity of the solvent treated corn starch is shown in Figure 2.

The study results showed that viscosity of the porous corn starch samples tended to increase gradually with the proportion of water and ethanol. Specifically, the viscosity of the samples grew gradually from the PS0 to PS3 and increased significantly from the PS3 to PS10. This was due to the fact that porous corn starch had a large pore volume and specific surface area. Due to this characteristic, the structure of the porous corn starch granules was not stable. This led to the destruction of starch molecules during gelatinization, creating suitable conditions for water molecules to penetrate and bond with amylopectin molecules [30]. As a result, the viscosity of starch increased which agreed with Fortuna et al. (2000) [32].

The results also showed that viscosity of porous corn starch increased gradually in 5 hours of storage. This phenomenon was explained by the degeneration and gel formation of starch [33]. Degradation changed physical properties of starch after gelatinization. This process occurred when starch molecules bound together to form a double helix structure during storage [34]. The increase in the viscosity of starch on cooling was also related to gel formation. As the temperature dropped, amylose tended to clump together as the hydrogen-water bond between the molecules loosened. This led to amylose precipitation at low concentration or the formation of a three-dimensional gel matrix at high concentration (high molecular weight of amylose) [35]. Amylopectin molecules also began to regroup and recrystallize, but very slowly. Based on the recrystallization dynamics of amylose (fast and strong) and amylopectin (slow and weak), there were two stages in the starch gel formation process. In the first stage, amylose formed junction regions that supported the initial structure of the gel (occurring within seconds to minutes) [36]. In the second stage, the amylopectin chains gradually recombined to strengthen the gel (occurring within hours to days) [37]. Therefore, the viscosity of starch increased during storage.

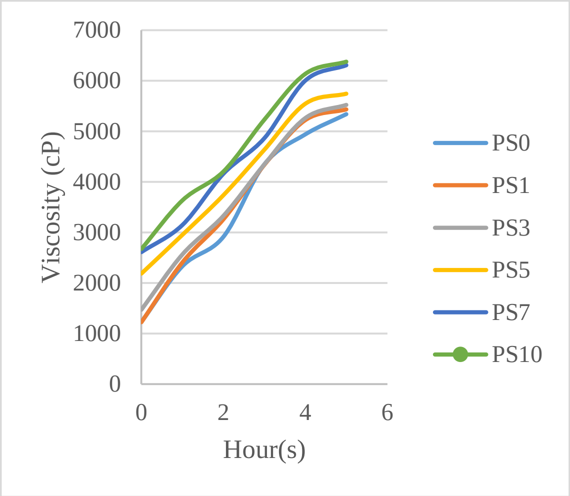

Figure 2. *Viscosity of gelatinized starch*

3.5 Effect of ethanol concentration on transmittance

Transmittance is one of the important factors affecting the optical properties of gelatinized starch [13, 24]. The higher the transmittance is, the more transparent the starch is [38]. The transmittance of starch is influenced by factors such as morphology, size, amylose content and the degree of structural integrity of starch granules [39-41]. The change of transmittance of starch treated by solvent in 5 days of storage was shown in Figure 3. The results showed that the transmittance of the solvent-treated starch decreased during 5 days of storage. This might be due to the degeneration [14] [42]. In addition, the aggregation and recrystallization of amylose and amylopectin molecules also reduced the transmittance during cold storage. The cause of this phenomenon of these two molecules was due to the affinity between the hydroxyl groups [43-44]. Moreover, the transmittance of gelatinized starch decreased when ethanol concentration increased.

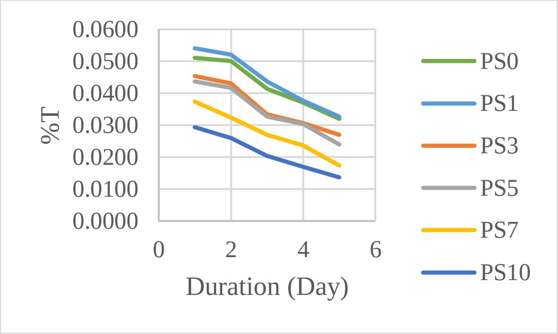

Figure 3. *Transmittance of gelatinized starch during storage*

4. CONCLUSION

To conclude, solvent treatment changed the technological properties of corn starch and significantly modified the structure of starch granules through the formation of pores. Therefore, the starch samples treated with solvents had higher solubility, swelling power, oil and water holding capacity. Those characteristics, including viscosity tended to increase with increasing ethanol concentration. However, the transmittance of gelatinized starch decreased when ethanol concentration increased.

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