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Modification of Cassava Starch (*Manihot utilissima*) Using Precipitation Method with Addition of NaCl

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ABSTRACT

Cassava starch has been widely used as a functional ingredient in many food products with some limitations. To improve its usage, cassava starch has been modified to have a better functionality. A lot of starch modification has been applied including alcoholic treatment or known as precipitation method. This study aimed to evaluate the effect of NaCl concentration during modification using different gelatinization techniques in precipitation methods on the characteristic of cassava starch. The modification of starch was carried out with 3 treatments of gelatinization techniques: using a hot plate and water solvent, a reactor autoclave and water solvent, and a reactor autoclave and water + ethanol mixture as solvent. Each treatment was carried out with addition of 3 levels of NaCl. The result showed that the gelatinisation techniques affected the morphology of starch granules as seen in their SEM images. Both the gelatinisation technique and the presence of NaCl affected the size of starch granules and water and oil absorption capacity of modified starch. NaCl acted as salting-in ions at concentration 0.17 to 0.34 M. When the concentration was increased to 0.51, the salt led to a salting-out property.

Keywords: *Cassava starch, Modification, NaCl, Precipitation.*

1. INTRODUCTION

Cassava starch has been widely used as functional food ingredient such as biocomposites [1], film [2,3], bioplastics [4], bread [5], pasta [6], thickener in yoghurt [7], emulsifier in pickering emulsion [8], etc. The application includes both native and modified starches produced from several methods of modification. Precipitation method starts the modification of starch by dissolution of starch molecules [9]. Starch dissolution can be done by gelatinisation, during which the starch molecules solubilise into gelatinisation media (solvent). Next step in the precipitation method is the addition of a non-solvent chemical which is miscible with the gelatinisation media, in many cases is ethanol, leading to precipitation of the dissolved starch molecules in new form. Precipitation method has been given great attention due to simple procedures and no need for expensive equipment and chemicals [10].

The term *salting in* and *salting out* are familiar when discussing protein solubility. The increase of salt concentration increases the solubility of protein (*salting in*), however when the salt concentration continues to increase, protein solubility is decreased (*salting out*) [11]. In starch gelatinisation, salt plays a similar role to that in protein solubility. To some point, the increase in salt concentration will increase starch solubility. Further increase in salt concentration will decrease its solubility. The presence of salt in starch solvent influences the availability of solvent for gelatinisation. A study showed that the use of 0.1M of NaCl in 6% (w/v) of potato starch solution decreased peak gelatinisation temperature and gelatinisation enthalpy [12] which means that the starch is more easily gelatinised in the present of NaCl. Another study shows that maize and waxy maize starch experience little change in both peak gelatinisation temperature and gelatinisation enthalpy [13]. These findings inform that salt, NaCl in particular, affects starch properties in different ways depending on type of the starch. Therefore, this study was carried out to evaluate the effect of NaCl concentration during modification using different gelatinization techniques in precipitation methods on the characteristic of cassava starch.

2. MATERIALS AND METHOD

2.1. Material

Cassava starch was obtained after extraction of starch from cassava tuber harvested at 5-6 months. Corn oil was bought from a local supermarket. Ethanol 96% was provided by CV Medilab Scientific Prima. Absolute ethanol and NaCl were analytical grades from Sigma Aldrich.

2.2. Experimental Design

The modification of cassava starch was carried out using the following experimental design:

Three gelatinisation techniques were applied:
1. Hotplate
2. Autoclave
3. Autoclave + ethanol

Each gelatinisation technique was performed using 3 levels of NaCl concentration:
1. 0.17M
2. 0.34M
3. 0.51M

These 3x3 experimental units were repeated twice. Hence we had a total 18 experimental units.

2.3. Starch Modification

2.3.1. Modification Using Hot Plate

One gram cassava starch and a certain amount of NaCl were dissolved in 100 gram of distilled water. 1, 2 and 3 gram NaCl was used to produce 0.17M, 0.34M and 0.51M NaCl respectively. This starch-salt suspension was heated on the hotplate at 100°C for 30 minutes with continuous stirring. The starch paste was quickly cooled by slowly adding 500 ml ethanol 96% dropwise and kept stirred for 8 hours using a magnetic stirrer. The mixture was further centrifuged at 2500 rpm for 15 minutes. The precipitate was washed using 15 ml absolute ethanol 3 times, dried in cool dry air in the refrigerator (RH 67%, 14.2 – 17.1°C). Modified starch was weighed and kept in the air tight container at room temperature.

2.3.2. Modification Using Autoclave

One gram cassava starch and NaCl were dissolved in 100 gram of distilled water. 1, 2 and 3 gram NaCl was used to produce 0.17M, 0.34M and 0.51M NaCl respectively. This starch-salt suspension was placed in the autoclave reactor made out of teflon in the inner side and stainless steel in the outside part. The stainless steel was threaded equipped with the lid to make sure that there is no leakage. This autoclave was placed in the oven 140°C for 60 minutes and cooled at room temperature. To the starch paste, ethanol 96% was slowly added dropwise. This mixture was stirred for 8 hours using a magnetic stirrer and centrifuged at 2500 rpm for 15 minutes. The precipitate was washed using 15 ml absolute ethanol 3 times, dried in cool dry air in the refrigerator (RH 67%, 14.2 – 17.1°C). The dried modified starch was weighed and kept in the air tight container at room temperature until further used.

2.3.3. Modification Using Autoclave + Ethanol

The procedures for this modification were similar to the procedures for modification using autoclave in section 2.2.2. The difference was only in the type of solvent used. Instead of using 100 ml distilled water to dissolve starch and salt, this modification used 50ml distilled water + 50ml ethanol 96%.

2.3.4. Yield of Modified Starch

Yield of modified cassava starch was calculated using equation 1 below.

$$\text{Yields} = \frac{weight\ of\ modified\ starch}{initial\ weight\ of\ native\ starch}\ x\ 100\% \qquad (1)$$

2.3.5. SEM

SEM (*Scanning Electron Microscopy*) analysis was performed using SEM JEOL JSM 6510 LA. This analysis was carried out to determine the morphology of the starch granules and their sizes. Before characterization, native starch or the modified starch was placed in the specimen stub with carbon tape and sputter-coated using gold. The analysis was operated at 3 kV.

2.3.6. Granule Size

Measurement of starch granule size was carried out using ImageJ version 1.5.2. The scale at SEM image was fitted to the scale at ImageJ. The measurement was taken for the smallest and largest particles detected.

2.3.7. Water Absorption Capacity (WAC)

Water absorption capacity (WAC) was measured following the method described by Falade and Christopher [14]. A 0.1 gram sample was mixed with 5 ml distilled water using vortex. The mixing was carried out 3 times with 10 minutes pause in between. This mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted carefully and the precipitate was air dried until no runny water could be detected. The humid precipitate was weighed. WAC was calculated using equation 2.

$$\text{WAC (\%)} = \frac{weight\ of\ humid\ precipitate}{initial\ weight\ of\ starch}\ x\ 100\% \qquad (2)$$

2.3.8. Oil Absorption Capacity (OAC)

Oil absorption capacity (OAC) was measured following the method described by Falade and Christopher (2015). The procedure was similar to water absorption capacity in section 2.2.7. The difference was only in the media to mix the sample. Distilled water in procedures for water absorption capacity was replaced by corn oil. OAC was calculated using equation 3.

$$\text{OAC (\%)} = \frac{weight\ of\ precipitate}{initial\ weight\ of\ starch}\ x\ 100\% \qquad (3)$$

3. RESULTS AND DISCUSSION

3.1. Yield of Modified Starch

Yield of modified starch indicates the efficiency of the modification process. The higher the yield is preferred as less starch is lost during modification. Yields of cassava starch modification using several techniques of gelatinisation and several levels of NaCl concentration are presented in Table 1. As seen in Table 1, the highest yield was obtained when modification was carried out using hotplate and 0.17M NaCl.

Table 1. Yield of modified starch using several NaCl concentration and gelatinization technique

NaCl (M)	**Yields (%)**		
	Hotplate	**Autoclave**	**Autoclave + Ethanol**
0.17	81.20±0.07	80.20±0.08	80.28±0.05
0.34	78.24±0.07	73.30±0.10	81.12±0.02
0.51	78.55±0.03	70.96±0.17	51.31±0.10

NaCl in water dissociates into Na^+ and Cl^- ions. These ions might affect the attraction among hydroxyl groups in starch molecules, either strengthen or weaken the hydrogen bonding between the molecules [12]. In the Hofmeister series, Na^+ and Cl^- are both in the middle of the series, therefore the effects of these ions are quite mild [11]. Based on the concentration of NaCl used in this work 0.17 to 0.51M, the increase in NaCl seems to promote starch solubilisation (salting-in), leading to less starch molecules being precipitated. The solubilisation occur due to Na^+ and Cl^- ions that may weaken the hydrogen bonding between the starch molecules [12]. The effect of salting-in is assumed to reach its peak below 0.51M as showed by the fact that the yield was slightly increase at 0.51M. NaCl concentration higher than 0.51M is predicted to give salting-out effect, strengthening the hydrogen bonding between the starch molecules leading to less starch solubilised.

3.2. Morphology and Granular Size of Modified Starch

The morphology of starch was observed using SEM (Figure 1). As seen in Figure 1, gelatinisation technique using hotplate produced more disrupted granules than other techniques. The autoclave reactor was unable to deliver more heat to gelatinise the starch as seen in modified starch using autoclave (Figure 1). These granules remained intact after heating for one hour. Another study showed that cassava starch needs at least 3 hours to gelatinise under similar conditions [15]. The unchanged granules seen more clearly in autoclave + ethanol treatment. While water help starch granules to swell, ethanol prevents the swelling [15,16].

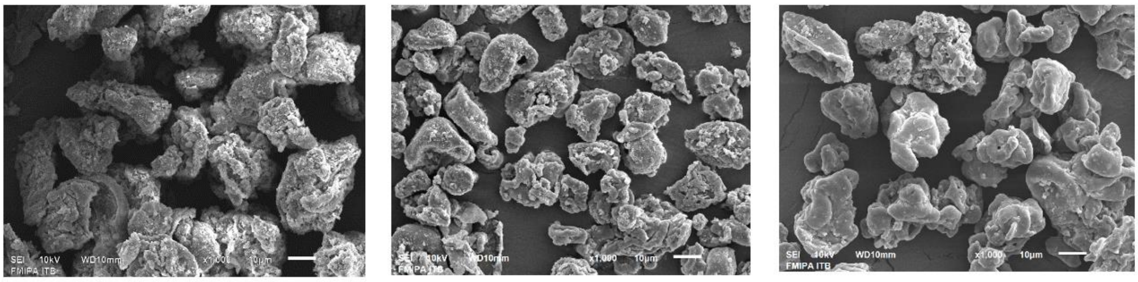
The effect of NaCl concentration was more pronounced in gelatinisation using hotplate. The increase in NaCl concentration to 0.34M caused severe damage to starch granules, but when the concentration was further increased, less damaged granules were observed.

Modification of cassava starch decreased granule size as seen in Table 2. The smallest granule was obtained after modification using a hotplate. Presumably, the starch in the autoclave did not obtain enough heat to break the intermolecular bond between starch molecules in the granules. Therefore, the gelatinisation process was not as complete as the process carried out using hotplate leading to a bigger size of granules.

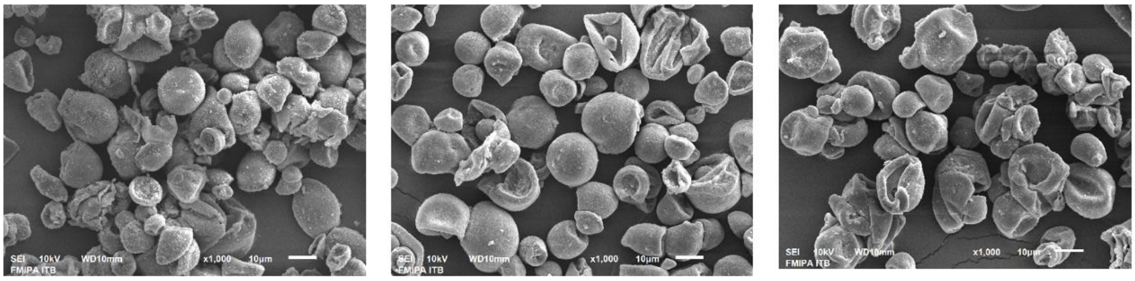
The use of water + ethanol as starch solvent in the gelatinisation using autoclave + ethanol, prevents granules from swelling causing an intact structure and nearly unchanged granule size. However, as NaCl was increased, the granule size was also reduced following a similar pattern in modification using hotplate and autoclave only. NaCl is known as moderately dissolved in ethanol due to its less polar nature when compared to water. The present of ethanol consequently increased NaCl concentration in the water leading to a more pronounced granule size reduction effect (Table 2).

3.3. Water Absorption Capacity (WAC)

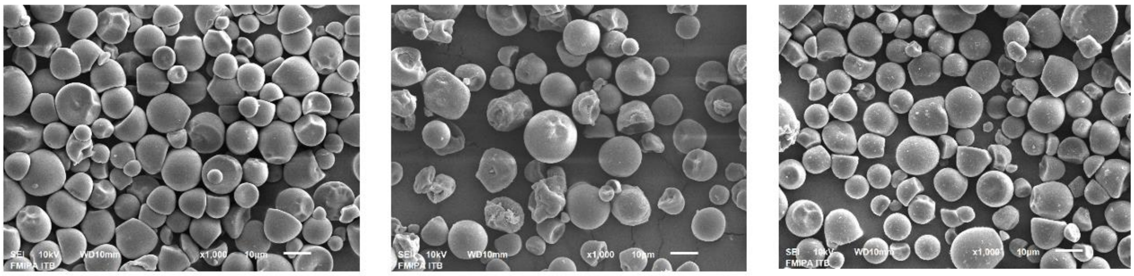
Figure 2 shows the capacity of modified starch to absorb water. The effect of salt concentration on WAC depends on the gelatinisation technique applied for starch modification. In gelatinisation using hotplate, the increase in salt concentration clearly decreased WAC of modified starch. A lower WAC informs that some hydrophilic groups has been missing after modification. This might occurred during retrogradation of starch in the precipitation process using ethanol. Ions from NaCl weaken the hydrogen bonds between molecules of starch which later rearranged to new order during retrogradation [13]. This process might discharge some of the hydrophilic sites in the modified starch.



Hotplate (0.17 M NaCl) Hotplate (0.34 M NaCl) Hotplate (0.51M NaCl)



Autoclave (0.17 M NaCl) Autoclave (0.34 M NaCl) Autoclave (0.51 M NaCl)



Autoclave + ethanol (0.17 M NaCl) Autoclave + ethanol (0.34 M NaCl) Autoclave + ethanol (0.51 M NaCl)

Figure 1. SEM images of modified cassava starch using different gelatinisation techniques at 0.17-0.51 M NaCl

Table 2. Granular size of native and modified starch

Starch	Size (μm)
Native	6.444 s/d 20.094
Modified using hotplate:	
0.17M NaCl	1.197 s/d 10.275
0.34M NaCl	0.850 s/d 11.131
0.51M NaCl	0.802 s/d 9.589
Modified using Autoclave :	
0.17M NaCl	1.921 s/d 14.992
0.34M NaCl	1.367 s/d 14.255
0.51M NaCl	0.946 s/d 14.425
Modified using Autoclave + ethanol:	
0.17M NaCl	4.545 s/d 16.291
0.34M NaCl	2.530 s/d 16.795
0.51M NaCl	1.984 s/d 16.345

In gelatinisation using autoclave, the increase in NaCl concentration from 0.17M to 0.34M increased WAC of the modified starch. Further increase in NaCl concentration to 0.51M decreased WAC. These facts may relate to the state of gelatinised granules which are more incomplete when compared to “hotplate” starch. Therefore, the rearrangement of starch molecules as mentioned above is less pronounced in this “autoclave” starch. A more pronounced rearrangement occurred when NaCl was increased due to salting-out effect [12,13].

Gelatinisation using autoclave with mixture of water and ethanol solvent produced lower WAC although the granules are nearly unchanged (Figure 2). This showed that the rearrangement as discussed above may occur within the intact granule.

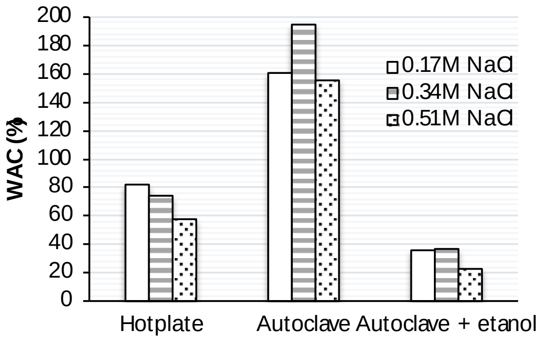


Figure 2. Water absorption capacity (%) of cassava modified starch at different gelatinisation technique and NaCl concentration

3.4. Oil Absorption Capacity (OAC)

OAC denotes the capacity of modified starch to absorb oil. Rearrangement of starch molecules during precipitation may cause some hydroxyl groups to hide. Similar to WAC, both gelatinisation technique and NaCl concentration affect OAC. Similar pattern of OAC in "hotplate" and "autoclave+ethanol" modified starch (Figure 3) suggests that the rearrangement of hydrophilic groups in starch molecules occurred despite the unchanged nature of starch granules.

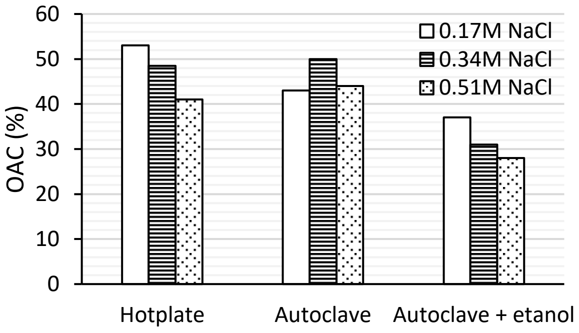


Figure 3. Oil absorption capacity (%) of cassava modified starch at different gelatinisation technique and NaCl concentration

4. CONCLUSION

Both gelatinisation technique and NaCl concentration affect morphology, granule size, WAC and OAC of modified cassava starch. The gelatinization technique using a hot plate with water solvent and 0.51M NaCl produced the smallest modified starch (0.802 µm to 16.795 µm). The gelatinization technique using an autoclave reactor with water solvent and addition of 0.34M NaCl produced the highest water absorption capacity (195%). The gelatinization technique using hot plate with water solvent and addition of 0.17M NaCl produced modified starch with the highest oil absorption capacity (53%).

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