The physicochemical characteristics of cassava starch modified by ultrasonication

E M Satmalawati, Y Pranoto, D W Marseno and Y Marsono
Food Science, Faculty of Agriculture Technology Gadjah Mada University, Indonesia
Email: mulatendah@gmail.com, pranoto@ugm.ac.id, djagal@ugm.ac.id, yustimar49@yahoo.co.id

Abstract. In this study, cassava starch was modified using physical modification by ultrasonication. Ultrasonication, known as one of the advanced oxidation methods, is environmentally friendly. Modified starches using ultrasonication are still relatively lacking. This research aims to evaluate the stage of oxidation through carbonyl and carboxyl contents, amylose content, and physicochemical characteristics (swelling power, solubility, paste viscosity, brightness) of ultrasonicated starch. Ultrasonication was carried out in combination amplitude (60% and 80%) and process duration (10, 20, 30, and 40 minutes) consisting of eight combinations. The results showed that ultrasonication did not significantly affect the degree of oxidation. Ultrasonication has more effect on starch depolymerization, mainly on amylose content, solubility, and paste viscosity. Considering the issue of environmentally friendly modification that continues to be campaigned for, ultrasonication can be the method of choice for obtaining starch with the characteristics as needed.

1. Introduction
The availability of carbohydrate sources as the main menu as well as the needs of the food industry is in line with population growth and increasing food processing technology, especially the application of starch in the manufacture of food products to reformulate them and enhance their properties. Starch production has significantly grown recently, increased from 60 million tons in 2007 to 90 million tons in 2016. Cassava is one of the four main sources of starch in the world, second after corn, in addition to other sources of starch (wheat and potatoes) [1]. Cassava has the potential to be developed because of its high carbohydrate content (73.7-84.9%) and the abundance of cassava in Indonesia.

Starch is the most abundant component in several grains (70-80%), roots, and tuber [2]. Starch is included among biopolymer groups. The two main components of starch are amylose (linear chain with few branching points which has a role in the amorphous region of the granules with glucose units in α-(1–4) bonds) and amylopectin (branch component which is responsible in the starch crystalline lamella with glucose units in α-(1–4) bonds in the linear sections and by α-(1–6) bonds in the branching points [3].

The issue of safe food and the environment as a result of the modification process is still being pursued. Chemical modification can leave residue on the product and has the potential to cause environmental pollution problems while many physical modifications are made to avoid the negative effects of using unsafe chemical compounds on the final product. Also, the use of chemical reagents in
the process should be decreased for consumers and the environment’s safety, and lower production costs [4].

Ultrasonication is a starch modification method that causes physical depolymerization. This process reduces the use of chemicals, minimizes waste, and energy consumption. The effects of ultrasonic radiation generate degradation of starch granules with perceptible cracks and pores on the surface but the shape of starch granules does not change [5]. The ultrasound process does not require the addition of oxidants or catalysts and does not produce waste [6]. The Ultrasonication process uses wavelengths of 20-100 kHz which is beyond the range human can hear. The advantages of ultrasonication are higher selectivity and quality, less chemical usage, and processing period. Ultrasonication is also one of the environmentally friendly technologies that are relatively new, fast, and usable in food [7].

Based on the opportunity to increase the use of cassava starch which has certain physicochemical characteristics that can meet the increasing industrial demand by considering food and environmental safety claims, the objective of this study is to modify cassava starch using ultrasound technology. Some physicochemical characteristics of modified starches are evaluated and explained to better understand the level based on different methods of starch modification.

2. Material and methods

2.1. Materials
Starch was obtained from cassava (var. adira-4) with a harvest age of 10 to 11 months obtained in Yogyakarta, Indonesia. Maximum starch yield extraction (30%–35%) was done by adjusting the ratio of cassava pulp to water to 1: 4 (w/w) through the stages that consisted of stripping, grating, dissolving, extracting, filtering, settling, and drying (in a cabinet drier at 50°C for 24 h to yield a 9%–10% moisture level). The starch was then grounded in a mortar, sieved (60 mesh), placed in plastic packaging with silica gel wrapped in aluminum foil, put into a container, and kept at room temperature until further use.

2.2. Sonication process
The ultrasound process uses an ultrasonication probe (Ø7 mm). Starch suspension of 5% (w/v) was kept in a glass cell and homogenized with continuous stirring for 5 minutes before ultrasonication at a constant temperature of 20 ±1°C. The ultrasonication process was carried out using variations of amplitude (60% and 80%) at four different time process (10; 20; 30; and 40 min). After the ultrasonication process, the starch suspension was then precipitated and dried with a dryer at a temperature of 50°C for 24 hours.

2.3. Carbonyl and carboxyl contents
The carbonyl content was examined according to the method by Smith (1967)[8] with slight modifications and the carboxyl content was examined using the method by Chattopadhyay et al. (1997) [9].

2.4. Amylose content
Amylose content was obtained using the method described by Juliano et al. (1981) [10].

2.5. Swelling power and solubility
The swelling power was determined using the procedure described by Adebowale et al. (2002) [11]; sample (1.0 g and had been weighed) was placed into a test tube, which had been cleaned, dried, and weighed first. The sample and tube weight were calculated together as W1. Distilled water (50 ml) was added to the sample until a slurry formed. The mixture was then homogenized and heated at 95°C for 30 min in a boiling bath. The mixture was rested until the temperature reached 30±2°C and then centrifuged (500 rpm, 15 min) until the gel and aliquots were separated. Aliquots (5 ml) were dried at
110°C until a constant weight was achieved. The residue obtained from dried supernatant could be expressed as the solubilized starch in water. Solubility was calculated as g per 100 g of starch on a dry weight basis. The gel and test tube from the above experiment (after centrifugation) with its retained water were quantitatively weighed and taken together as W2. The swelling power was calculated with the equation:

\[
\text{Swelling power (\%)} = \frac{W2 - W1}{\text{weight of starch (dry weight basis)}}
\]

2.6. Paste clarity
Paste clarity was determined using the procedure described by Craig et al. (1989) [12]. Put into a test tube was 0.2 g of starch in 20 mL of distilled water. For 30 minutes the tube was heated in a tub of boiling water, stirred individually every 5 minutes, then cooled at room temperature and stored for a few days to be controlled. Percent transmittance (% T) was determined at a wavelength of 625 nm against water blank and determined using GENESYS 10S UV-VIS Spectrophotometer (USA).

2.7. Pasting properties
Rapid Visco Analyser (RVA 4500 Perten Instrument-Australia) was used to determine pasting properties of the starch with a Standard Analysis 1 profile and the result was expressed in cP (centiPoise) with pasting temperature, peak viscosity, breakdown, final viscosity and setback as parameters. The sample was weighed directly in the aluminum RVA canister, and 25 mL of distilled water was then added to the canister. The temperature of the sample was held at 50°C for 2 min, increased to 95°C in 3.5 min, and then held at 95°C for 2.5 min. The temperature of the sample was decreased to 50°C in 4 min and then held at 50°C for 1 min. The rotating speed was held at 960 rpm for 10 s, and it was held at 160 rpm during the remaining process. All parameters were then recorded.

3. Result and discussion

3.1. Carbonyl and carboxyl contents
Carbonyl and carboxyl content of native and ultrasonication starches are presented in table 1 and table 2. The initial group resulting from the starch oxidation process was carbonyl. Carbonyl was a main functional group formed during the oxidation process (a minor amount of carboxyl groups was also formed). Thus, the degree of oxidation was determined from the formation of the carbonyl and carboxyl groups. The sonication process would produce acoustic cavities in the aqueous mixture causing microbubbles. The microbubbles spreading released high energy which was converted to pressure and temperature. This process generated the degradation of the polymer as well as the acceleration of catalytic reactions [13]. As a result of this treatment, the collapsing microbubbles caught the nearby polymer chain in a high gradient shear field generating the formation of long-chain radicals and breakage of macromolecular C-C bonds[7].

| Table 1. | Carbonyl level of cassava starch after ultrasound process at various amplitudes and time. |
|----------|--------------------------------------------------------------------------------------------------|
|          | Time (min)                                                                                      |
| Amplitudo | 10’ | 20’ | 30’ | 40’              |
| 60%       | 0.05 ± 0.01aA | 0.06 ± 0.04aA | 0.06 ± 0.02aA | 0.28 ± 0.03bA |
| 80%       | 0.23 ± 0.03bA | 0.22 ± 0.03bA | 0.20 ± 0.02bA | 0.11 ± 0.00aA |

Each value is presented as mean ± standard deviation.
Values with different letters are significantly different (p < 0.05).
a,b: indicates time; A,B: indicates amplitude

The use of ultrasonication in the liquid system causing cavities formation is a phenomenon of bubbles formation, growth, and finally breaking. When ultrasonic waves propagated, the bubbles
oscillated and broke apart, causing heat, mechanical effects (pressure, turbulence, and shear stress) [14], and chemical effects (the formation of free radicals) [15]. Ultrasoundation generated water hydrolysis in oscillating bubbles and free radicals (H⁺ and OH⁻) that could be captured in several chemical reactions in the formed liquids. However, the ability of ultrasonication in oxidizing starch was low, this is indicated by the absence of carboxyl groups (table 2) as a reference for further oxidation of starch. Overall, ultrasonication did not significantly affect the carboxyl group (similar with native).

Table 2. Carboxyl level of cassava starch after ultrasound process at various amplitudes and time

| Amplitude | 10'          | 20'          | 30'          | 40'          |
|-----------|--------------|--------------|--------------|--------------|
| 60%       | 0.007 ± 0.000aA | 0.007 ± 0.001aA | 0.005 ± 0.001aA | 0.006 ± 0.001aA |
| 80%       | 0.007 ± 0.004aA | 0.006 ± 0.002aA | 0.007 ± 0.000aA | 0.005 ± 0.001aA |

Values with different letters are significantly different (p < 0.05).
a,b: indicates time; A,B: indicates amplitudo

3.2. Amylose content
Depolymerization occurred during the starch oxidation process through the hydrolysis of the glycosidic linkage. This is indicated by the increased level of amylose which was the result of breaking the branches of amyllopectin into a straight structure and read as an appearance of amylose. The ultrasound process had a significant effect on starch amylose levels. Depolymerization of amyllopectin and amylose molecules due to the hydrolysis of starch resulted in the formation of smaller size chains [16]. The results of amylose content analysis are shown in table 3.

Table 3. Amylose content (%) of cassava starch after ultrasound process at various amplitudes and time.

| Amplitude | 10'          | 20'          | 30'          | 40'          |
|-----------|--------------|--------------|--------------|--------------|
| 60%       | 37.84 ± 0.00aA | 40.20 ± 0.50bA | 42.50 ± 0.62cA | 44.13 ± 0.28dA |
| 80%       | 39.75 ± 0.31aA | 41.68 ± 0.18bA | 43.85 ± 0.21cA | 41.23 ± 0.25bA |

Values with different letters are significantly different (p < 0.05).
a,b: indicates time; A,B: indicates amplitudo

The results showed that the time of the ultrasonication process had a significant effect on the amylose content. The ultrasound process would produce cavities in the solution and cause microbubbles[13]. The spreading of microbubbles then released high energy which was converted to high temperatures and pressures, this process caused polymer degradation. Cavitation is the breakdown of micro-sized bubbles (microbubbles) and spreads when sound waves pass through the solution. The energy generated from the cavitation process can break covalent bonds in polymeric material [17,18].

3.3. Swelling and solubility
Swelling power and solubility indicate the amount of starch chains interaction in crystalline and amorphous regions. The magnitude of the interaction is affected by the ratio of amylose-amyllopectin, phosphor content, amylose-amyllopectin characteristics such as molecular weight, branching or length, and branch conformation[19].

Swelling power analysis results for all treatments were greater than native (35.39%). The swelling power and solubility of all samples can be seen in table 4 and table 5. The ultrasonication process did not significantly affect swelling power. This can be correlated with the carboxyl group which was not
produced during the process. Carboxyl groups tend to be negatively charged which would provide an electronegative repulsive force, which affects the size of the swelling power.

The solubility of ultrasonicated starches was greater than native, as well as swelling power. Solubility represents the amount of starch molecule that solubilizes at a certain temperature [20]. Solubility increased with the increase of amplitude and the time process. The increase in solubility can be related to the depolymerization and structural weakening of starch granule due to the oxidation process[11]. Ultrasonication starch has an amorphous tendency.

**Table 4.** Swelling power (%) of cassava starch after ultrasound process at various amplitudes and time.

| Amplitude | 10' | 20' | 30' | 40' |
|-----------|-----|-----|-----|-----|
| 60%       | 37.65 ± 0.96aA | 38.36 ± 2.26aA | 38.36 ± 1.02aA | 37.63 ± 0.07aA |
| 80%       | 41.88 ± 2.19aA | 41.07 ± 1.53aA | 43.33 ± 2.65aA | 42.22 ± 0.25aA |

Each value is presented as mean ± standard deviation.
Values with different letters are significantly different (p < 0.05).

**Table 5.** Solubility (%) of cassava starch after ultrasound process at various amplitudes and time.

| Amplitude | 10' | 20' | 30' | 40' |
|-----------|-----|-----|-----|-----|
| 60%       | 0.55 ± 0.016aA | 0.56 ± 0.04aA | 0.53 ± 0.00aA | 0.55 ± 0.00aA |
| 80%       | 0.61 ± 0.04B | 0.75 ± 0.01B | 0.68 ± 0.04 aB | 0.80 ± 0.07B |

Each value is presented as mean ± standard deviation.
Values with different letters are significantly different (p < 0.05).

3.4. Paste clarity
Ultrasoundation starch produced higher clarity than native starch (56.16%) and was more stable during storage. The results of the starch paste clarity analysis are shown in figure 1. Decreased clarity was caused by the formation of gels from the joining of hydroxyl groups between starch chains through hydrogen bonds, this mechanism is called retrogradation.

The clarity of ultrasound starch paste was relatively low, with a maximum number of 66% which was the result of a combination of 60% amplitude treatment at 40 minutes processing time. The clarity of starch paste is influenced by the carboxyl groups formed during the oxidation process. The negatively charged carboxyl group provides a repulsive force to prevent retrogradation [20, 21, 22, 23].

In this study, the ultrasound process could not produce a carboxyl group so that retrogradation could not be prevented, this is indicated by a low clarity value and unstable paste during storage.

3.5. Paste viscosity
One of the parameters in the starch modification is the decrease of viscosity. Ultrasonication causes depolymerized starch through hydrolysis of glycosidic bonds in starch granules, which tends to produce lower starch viscosity as a result of starch molecular weight loss [20, 24, 25]. The results showed the viscosity of the paste decreased. The lowest viscosity was produced from a combination of 80% amplitude treatment at 10 minutes processing time (6159 cP). Paste viscosity of native and ultrasonicated starch can be seen in figure 2a and 2b.
The amplitude and processing time of the ultrasonication process affect the decrease in viscosity. However, in this study, ultrasonication tended to result in a decrease in the paste viscosity which can be related to the presence of starch depolymerization mainly in the chain of amylopectin branches into a straight structure with lower molecular weight and amorphous so that the ability of water absorption increases which results in a decrease in paste viscosity.

The application of ultrasonication to the liquid system generates acoustic cavities in the aqueous mixture and causes bubbles formation, growth, and breaking. Cavitation is the breakdown of micro-sized bubbles (microbubbles) and spreads when sound waves pass through a solution. The energy produced from the cavitation process ruptures the covalent bonds in polymeric substance [18,19]. It was also found that the viscosity and molecular weight of starch produced decreased after ultrasonication [26].

![Figure 1. Paste clarity of ultrasound starches.](image1)

![Figure 2a. Amilograph curve of native and ultrasound starches at 60% amplitudo.](image2a)
Conclusion
Ultrasonication did not significantly affect the degree of oxidation. The low degree of oxidation affected the swelling power and the clarity of starch paste because it was associated with carboxyl groups that were not formed during the ultrasonication process. Ultrasonication had more influence on the degradation of starch polymers which is indicated by significant amylose content, low viscosity, and increased solubility with the production of amorphous starch.

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