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The effect of fermentation on physicochemical properties of Cocoyam *(Xanthosoma sagittifolium*) flour using *L. plantarum* bacteria

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Abstract

The cocoyam (Xanthosoma sagittifolium) is contains many carbohydrates that can be used as a source of starch modification. Unmodified starch has a long cooking time, low viscosity, solubility and swelling power. Modification of cocoyam flour was done with lactic acid bacteria (Lactobacillus plantarum). This study aimed to explore the capability of local tubers and to determine the physicochemical characteristics of modified cocoyam flour with lactic acid bacteria. This research method using a randomized block design with two factors. Data were analyzed by the analysis of variance method and a further test of DMRT. The concentrations of L. plantarum used were 3%, 5% and 7% and fermentation time of 48, 72 and 96 h. The best result of this research was obtained using 7% L. plantarum bacteria and fermentation time of 96 h yielding physicochemical characteristics of water content 8.28%, ash 1.05%, swelling power 5.78%, solubility 51.93%, starch 76.47%, amylose 26.28% and color 87.42%. Fermentation significantly increased the swelling power, water content and solubility, while starch and amylose decreased.

Keywords: cocoyam flour, fermentation, Lactobacillus plantarum, local tubers, physicochemical characteristics, starch modification, Xanthosoma sagittifolium

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INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium*) is one of the tubers, which contains high carbohydrate content (30.66 g/100 g). This makes it possible to use cocoyam as a source of modified starch (Ridal, 2003). The natural starch is of limited use in the food industry, hence a technology was developed to modify the flour to obtain higher brightness (clearer), stable viscosity at both high and low temperatures, clearer gel, softer gel texture, low stretch strength, more easily broken starch granules, time and temperature in lower gelatinization, high swelling strength, high solubility, and lower time and temperature for starch granules to break (Koswara, 2009).

Natural starch can be made into modified starch with the desired properties or as needed (Hee-Young, 2005). Fermentation is an ancient method used in the processing of foodstuffs with the aim of increasing shelf life, improving palatability and improving digestibility and enhancing nutritional value (Fadlallah, 2010). Cassava flour was modified with lactic acid bacteria fermentation with 72 hours fermentation time and 5% lactic acid bacteria concentration. The longer the fermentation time, the higher the protein content will be. *Lactobacillus plantarum* lactic acid bacteria produce proteinase enzymes. The increase in protein content is obtained from proteinase enzyme activity produced by microbes present in the fermentation process. A longer fermentation time increases the *L. plantarum* population, thus also increasing the dissolved protein content. The higher the concentrations of the *L. plantarum* bacteria the higher the number of lactic acid bacteria as well. The use of lactic acid bacteria inoculated in fermentation will be able to reduce the growth of pathogenic bacteria (Otegbayo *et al*., 2018). The research objective was to

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Table 1. Physicochemical properties of modified cocoyam (*Xanthosoma sagittifolium*) flour

Concentration of starter (%)	Length of Fermentation (h)	Water content (%)	Ash (%)	Swelling power (%)	Solubility (%)
3	48	$11.96^{g} \pm 0.0576$	$1.30^{a} \pm 0.0001$	$3.49^{a} \pm 0.5417$	$50.17^{a} \pm 0.0008$
	72	$10.76^{f} \pm 0.0511$	$1.29^{a} \pm 0.0001$	$3.80^{b} \pm 0.0607$	$50.53^{b} \pm 0.0017$
	96	$10.24^{e} \pm 0.0187$	$1.28^{a} \pm 0.0004$	$3.73^{c} \pm 0.1967$	$50.59^{bc} \pm 0.0035$
5	48	$9.89^{d} \pm 0.1408$	$1.23^{a} \pm 0.0040$	$4.14^{b} \pm 0.0081$	$50.88^{d} \pm 0.0018$
	72	$9.72^{c} \pm 0.3928$	$1.20^{a} \pm 0.0002$	$4.45^{c} \pm 0.0229$	$50.87^{de} \pm 0.1532$
	96	$9.66^{cd} \pm 0.0600$	$1.20^{a} \pm 0.0003$	$4.62^{a} \pm 0.0781$	$51.08^{de} \pm 0.0018$
7	48	$9.59^{c} \pm 0.4503$	$1.10^{a} \pm 0.0000$	$4.91^{c} \pm 0.0701$	$51.17^{f} \pm 0.0016$
	72	$8.49^{b} \pm 0.0832$	$1.06^{a} \pm 0.0006$	$5.28^{a} \pm 0.2438$	$51.66^{g} \pm 0.0000$
	96	$8.28^{a} \pm 0.2188$	$1.05^{a} \pm 0.0001$	$5.78^{b} \pm 0.0371$	$51.93^{h} \pm 0.0004$

determine the effect of BAL concentration and fermentation length on physicochemical characteristics of modified cocoyam flour.

MATERIALS AND METHODS

Research design

The materials used in this research were: cocoyams (*Xanthosoma sagittifolium*), distilled water, hydrochloric acid (Sigma, Aldrich), MRS broth (Oxoid, Ltd), *Lactobacillus plantarum* FNCC 0027 strain was obtained from the Department of Food and Agricultural Technology, Gadjah Mada University, sodium hydroxide (Merck), acetic acid (Merck), iodine (Merck). This research was arranged using a randomized block design with two factors. Data were analyzed by the analysis of variance method with the repeated procedure and a further test of DMRT. *L. plantarum* concentrations used were 3%, 5% and 7% and fermentation times of 48 h, 72 h and 96 h. In this study, fermentation was carried out using several concentrations and long-time fermentation to improve the properties of cocoyam flour.

Bacterial rejuvenation

Bacterial rejuvenation began with sterilizing the appliance for 15 minutes using an autoclave. Media (MRS broth) weighed 2.6 g for 50 mL distilled water divided into 10 tubes. The material was homogenized using a magnetic stirrer. Then, it was sterilized for 30 minutes at 121 °C. After the MRS broth was warm, the bacteria were transferred to the MRS broth medium. The last step is incubation for 24 hours before use (Yuliana *et al*., 2014).

Fermentation

The lactic acid bacteria used was *L. plantarum* (FNCC 0027). The bacteria in the agar medium in the test tube were taken using a micropipette of 5000 microns. The bacteria were then transferred to an Erlenmeyer flask containing 10 g cocoyam flour and 100 mL distilled water that had been dissolved with sterilized MRS broth medium. Then, the starter was incubated at 37 °C for 24 h. Cocoyam flour (100 g) was added to 300 mL distilled water until homogeneous. The suspension of cocoyam flour was inoculated with 3%, 5%, 7% starter and fermented for 48 h, 72 h, 96 h. Then, it was washed with distilled water to neutral pH, then placed in an oven at a temperature of 60 °C for approximately 12 hours. Modified cocoyam flour was milled and sieved. Testing included water content, ash (AOAC, 1995), starch, amylose (Kusnandar *et al*., 2018), swelling power and solubility (Deng *et al*., 2013).

RESULTS AND DISCUSSION

The decrease in water content is due to evaporation of free water. Before fermentation, some water molecules form hydrates with other molecules so they are difficult to evaporate, but during the fermentation process microbial enzymes break down carbohydrates and they become simpler so that the bound water is converted to free water (Zubaidah and Irawati, 2013). **Table 1** showed the longer fermentation decreased the ash content as long as the mineral fermentation undergoes leaching in part by water. With the increasing length of fermentation, more minerals experience leaching, so in the process of draining the mineral is removed.

Swelling power

Swelling power indicates the water holding capacity of starch, which has generally been used to demonstrate differences between various types of starches (Crosbie, 1991). Swelling volume is the ratio of the sedimented gel to the dry weight of starch. Solubility is the percent amount of starch leached out into the supernatant in the swelling volume determination (Singh *et al*., 2006). **Table 1** shows that the greater the concentration of inoculum starter, the greater the swelling power of modified cocoyam flour. This was probably because during the fermentation process acid attack on starch occurred on the amorphous/less compact part (amylopectin), thus causing a decrease in the proportion of amylopectin and increasing the proportion of amylose. The longer the fermentation, the greater the swelling power of modified cocoyam flour. This was due to the fermentation process of contact between the cocoyam tubers with *L. plantarum* bacteria. The longer the fermentation, the more the bacteria degraded the starch. This condition caused the environmental conditions to become acidic and produces pectinolytic and cellulolytic enzymes that can hydrolyze starch into simple sugars and then be converted to lactic acid. The degraded starch granules will easily absorb water so that when heated they will easily swell, which causes an increase in the value of swelling power.

Table 2. Values of color, amylose and starch of modified cocoyam (*Xanthosoma sagittifolium*) flour

Concentration of starter (%)	Length of Fermentation (h)	Color (%)	Amylose (%)	Starch (%)
3	48	$83.06^{a} \pm 0.0068$	$27.79e \pm 0.0212$	$80.50^{a} \pm 0.0919$
3	72	$83.30^{b} \pm 0.0127$	$27.64^{e} \pm 0.0071$	$80.34^{b} \pm 0.0141$
3	96	$83.53^{c} \pm 0.0354$	$27.62^{d} \pm 0.0071$	$80.20^{c} \pm 0.0071$
5	48	$84.39^{d} \pm 0.0594$	$27.10^{cd} \pm 0.0071$	$79.96^{c} \pm 0.0141$
5	72	$84.49^{de} \pm 0.0153$	$26.99^{cd} \pm 0.0071$	$79.40^{d} \pm 0.7637$
5	96	$84.83^{f} \pm 0.0283$	$26.96^{c} \pm 0.0000$	$77.68^{e} \pm 0.0071$
7	48	$85.85^{g} \pm 0.0373$	$26.57^{b} \pm 0.0071$	$77.53^{f} \pm 0.0141$
7	72	$86.66^{h} \pm 0.0363$	$26.49^{b} \pm 0.0071$	$76.65^{fg} \pm 0.0071$
7	96	$87.42^{i} \pm 0.5265$	$26.28^{a} \pm 0.0071$	$76.47^{g} \pm 0.0566$

Solubility

Table 1 showed that inoculum concentration 3% and fermentation 48 h had the lowest solubility (50.17%), while the treatment with inoculum concentration of 7% and fermentation 96 h had the highest solubility of 51.93%. Degradation of starch produces simpler molecules. These simpler molecules are easier to dissolve in water. The increased duration of fermentation will cause more bacteria to degrade starch, causing increased solubility. The existence of the degradation process caused the cavity to become porous after drying so that more and more water could be absorbed. This causes the starch granules to swell and expand so that the solubility increased. Bello-Pérez *et al*. (2000) reported that the distributions of chain length in the starches cause differences in solubility.

Fermentation as a means to improve properties can be done either with or without the addition of culture. Some of the examples were fermentation of cassava by using *Lactobacillus plantarum* and *Saccharomyces cerevisiae* culture to improve the properties in “mocaf” (Mutia, 2011), fermentation of sweet potato pickle with either *Leuconostoc mesenteroides* (Yuliana *et al*., 2013) or a mixed culture of *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, and with addition of *Lactobacillus plantarum* (Yuliana *et al*., 2014).

The research used *L. plantarum* to ferment cocoyam flour. The physicochemical characteristics of cocoyam (*Xanthosoma sagittifolium*) flour had the value of 2.564 g/g swelling power, 47.66% solubility and 13.02% yield. Characteristics of cocoyam flour compared with cassava flour, the value of swelling power of cocoyam flour was lower, but its solubility was higher. The higher the starter concentration and the longer the fermentation process, the lower the water content in modified cocoyam flour. This was because the length of fermentation increased the activity of bacteria to degrade the compounds contained in the cocoyam flour. This degradation reduced the ability of the starch granules to retain water because it loses carboxyl groups. Thus, the bound water was released into free water. The free water was easy to evaporate during drying so that the water content decreased.

According to Fleche (1985), when the starch molecule was completely hydrated, these molecules began to spread to the media on the outside. Molecules that first came out were molecules that had shorter chains. Hydrolysis of starch granules during fermentation leads to a lower structural rigidity in comparison to fermented sweet potato flour. Shorter starch chains as a result of this hydrolysis process then tend to easily absorb water. Claver *et al.* (2010) reported that when the temperature increases, intermolecular bonds in the starch break, allowing hydrogen-bonding sites to accommodate more water molecules. Water absorbed by each starch granule would make the starch granules swell and increase the swelling (Adeleke & Odedeji, 2010).

During the fermentation, growing bacteria produced pectinolytic and cellulolytic enzymes that can destroy the cell wall of cocoyam so that starch granules are degraded (Subagio, 2007). Degradation of starch causes starch granules to become porous, so they easily absorb water and easily expand when starch is heated. When starch granules absorb water, the granules coincide, causing the swelling power to increase. Yuliana *et al*. (2017) reported fermentation significantly increased the swelling power. Increase in swelling power of flour as a result of fermentation treatment is in conformity with earlier reports for fermented white sweet potatoes (Yuliana *et al*., 2013) and fermented moringa flour (Oloyede *et al*., 2016).

During fermentation, starch granules will be degraded into smaller, water-soluble molecules (Collado *et al*., 2001). The microbial fermentation process produces cellulase enzymes that can degrade cellulose in the cell walls of starch and cause damage to cell walls and starch granules. Then, starch granules are partially hydrolyzed so that they become perforated. Porous starch granules cause water and water-soluble starch molecules (amylose) easily to exit and enter the starch granules resulting in leaching of amylose. Water-soluble starch molecules affected solubility. According to Agusmanto (1995), flour with low solubility and stable values are the best flour for noodles’ use. Starch structural features such as chain length distribution of amylose and amylopectin might cause differences of starch solubility among samples in, e.g., amylose. The higher the temperature the more the starch molecules will leach out of the starch granules.

Table 2 shows that the treatment of inoculum concentration 3% and fermentation 48 h had the highest value of amylose content of 27.79%. The treatment of inoculum concentration of 7% and fermentation of 96 h had the value of the lowest amylose, which was 26.28%. The greater inoculum concentration and the longer fermentation caused the amylose content of modified cocoyam flour to decrease. This was because the more lactic acid bacteria and the fermentation time work to produce more amylase enzyme. The lactic acid bacteria hydrolyzed amylose so that amylose levels decreased. *L. plantarum* produced an amylase enzyme to break down a portion of starch into glucose. The amylase enzyme hydrolyzes the starch by cutting the glycoside bond. The α-amylase enzyme catalyzes endoamotic cycling by releasing short-chain oligosaccharides. The low amount of amylose lost during the heating was due to the large number of well-amylose forms complexes with amylose, amylopectin or fat. Complex bond formation caused the starch to have a bond that was compact and tight, so that the amount of amylose given off was lower.

CONCLUSIONS

The concentration of lactic acid bacteria and incubation time affected the properties of swelling power, solubility, color and amylose composition. The best result of this research was obtained by using *L. plantarum* 7% bacteria and fermentation time of 96 hours, which yielded physicochemical characteristics of water content 8.28%, ash content 1.05%, swelling power 5.78%, solubility 51.93%, starch 76.47%, amylose 26.28% and color 87.42%. There needs to be research for the implementation of food products that are in accordance with the characteristics of this modified cocoyam flour.

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