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Dietary fibre production from cassava pulp fibre using *Actinomycetes* cellulase

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Abstract

Cassava pulp is a by-product of tapioca flour manufacturing, which mainly composed of starch and fibre. The purpose of the present work was to produce dietary fibre from cassava pulp fibre using *Actinomycetes* cellulase as a functional food. Crude enzyme was obtained from *Actinomycetes* HJ4(2a) isolates by separation of its biomass through centrifugation. Dietary fibre production was carried out with two substrates, derived from thermal and non-thermal treatments. In the thermal treatment, cassava pulp fibre was heated in an autoclave at 121°C for 1 h before hydrolysis by *Actinomycetes* cellulase. Enzymes at 0.1 U/g were used, and incubations were carried out in 0, 24, and 48 h. Cellulase produced dietary fibre (DF) were done by decreasing insoluble dietary fibre (IDF) value and increasing soluble dietary fibre (SDF) value with reaction time, although there was no significant difference in DF components in the thermal and non-thermal treatments. The functional properties of DF, namely, water holding capacity (WHC), oil holding capacity (OHC), and emulsifying activity (EA) increased with the reaction time, with the thermal treatment being significantly higher ($p < 0.05$) than the non-thermal treatment. Optimum conditions of hydrolysis were shown by thermal treatment in hydrolysis of 48 h in which 9.35% of SDF, 12.08 g/g of WHC, 3.12 g/g of OHC, and 64.53% of EA were produced. In the thermal treatment, a higher amount of hydrolysate by-products, as well as changes in morphological and crystallinity characteristics occurred.

Introduction

Dietary fibre (DF) is a non-starch polysaccharide (cellulose, hemicellulose, pectin, gum, dextrin, chitin, and β-glucan), lignin, and oligosaccharide that can modulate transit time through the intestine, but cannot be digested and fermented (Slavin, 2013; Wichienchot and Wan Ishak, 2017). DF can prevent gastrointestinal diseases such as constipation and digestive cancer (Raupp *et al.,* 2004). Based on its water solubility and fermentability, DF is classified into two categories, namely, (1) soluble dietary fibre (SDF), which is water-soluble and well-fermented (e.g., pectin, gum, and mucilage), and (2) insoluble dietary fibre (IDF), which is insoluble in water and less-fermented (e.g., cellulose, hemicellulose, and lignin) (Dhingra *et al.,* 2012). Both soluble and insoluble fractions together give total dietary fibre (TDF). Both types are known to be associated with specific metabolic and physiological functions in humans.

DF can be produced through the use of either chemical treatment or hydrolytic enzyme. Toxic compounds, such as furfural and HMF (hydroxymethylfurfural), are formed during acid hydrolysis, so detoxification is needed before application in the industry (Pramana *et al.,* 2018). Enzymatic methods have advantages such as broad application in the food industry, production of DF with high biological activity, high SDF yield, lower IDF yield, specificity in reaction, and ability to degrade fibre structure with minimum destruction (Yang *et al.,* 2017). Some enzymes, such as cellulase, xylanase, protease, and hemicellulase can hydrolyse the substrate, hence it is applicable for producing DF (Yoon *et al.,* 2005; Park and Yoon, 2015a; 2015b; Domingo *et al.,* 2015).

Cellulase can be obtained from *Actinomycetes* bacteria, which have extracellular enzymes with cellulolytic activity (Putri *et al.,* 2019). *Actinomycetes* can penetrate cellulose, which is difficult to be degraded by hyphal growth (De Menezes *et al.,* 2008). Soeka *et al.* (2019) reported that *Actinomycetes* from soil has a high cellulase activity (7.3 U/mL at 50°C), and

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maintains such a high activity over large pH and temperature ranges. Several cellulases from *Actinomycetes* are stable at high temperature and pH, and are able to use crops as substrates (Mukhtar *et al.,* 2017). Enzymatic methods advantages are more environmental friendly and healthier than those of the other methods (Yang *et al.,* 2017). Besides, it is more efficient in cost because the cellulases are directly produced from *Actinomycetes* isolates. Putri *et al.* (2019) reported that the *Actinomycetes* HJ4(2a) isolates used in the present work had high cellulolytic index values. The working mechanism of cellulase to produce DF is that it modifies the cellulose structure from crystalline to amorphous. The exoglucanase enzyme attacks crystalline cellulose (Lakhundi *et al.,* 2015) to produce modified cellulose. Cellulolytic bacteria can produce modified cellulose, and thus can be used in DF production (Shi *et al.,* 2014).

Indonesia is the fourth largest cassava producing country with a production of 19,046,000 tons in 2017 based on the statistical data of the Food and Agriculture Organization of the United Nations (FAO, 2017). High cassava production is followed by an increase in tapioca production, resulting in an increase in the amount of cassava pulp. In Indonesia, the utilisation of cassava pulp is limited to animal feeds, mosquito coils, sauces, and fermentation substrates (Pramana *et al.,* 2018). Cassava pulp has nutrient content of approximately 50 - 70% starch and 20 - 30% fibre (Chaikaew *et al.,* 2012), thus having potentials for further use. Consisting of cellulose, cassava pulp fibre can be utilised in DF production. Many agricultural by-products from fruits, vegetables, and oilseed have already been used in the production of DF, and so have been cassava pulp through other methods, including acid and enzyme treatments, such as one with amylase, amyloglucosidase, and neutrase (Elleuch *et al.,* 2011; Kachenpukdee *et al.,* 2016; Chirinang and Oonsivilai, 2018; Pramana *et al.,* 2018).

The aims of the present work was to produce DF by investigating the effects of thermal treatment and *Actinomycetes* cellulase hydrolysis time on DF components of insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total dietary fibre (TDF), DF functional properties of water holding capacity (WHC), oil holding capacity (OHC), emulsifying activity (EA), DF crystallinity, and morphological characteristics. Yang *et al.* (2017) stated that thermal treatment can damage fibre, thus enabling production of DF.

Materials and methods

Characterisation of cassava pulp flour and fibre

The cassava pulp used in the present work was retrieved from the waste of a cassava starch mill in Sentul, Bogor, Indonesia; and the *Actinomycetes* HJ4(2a) isolates were retrieved from the collection of the Research Centre for Bioresources and Biotechnology of IPB University. Cassava pulp flour was obtained by drying, grinding, and sifting the cassava pulp through a 40 mesh sieve. Cassava pulp fibre was prepared through the elimination of starch in cassava pulp flour suspension in a 1:25 (b:v) ratio containing 200 ppm of $CaCO_3$ solution. Starch was hydrolysed using 1.75 U/kg of substrate with α-amylase (Termamyl NOVO enzyme) at 95°C for 2 h. Then, the fibres were filtered, washed, dried, skimmed, and sieved through the 40 mesh sieve. There were two types of substrates used for DF production, namely, cassava pulp fibres given the (1) thermal and (2) non-thermal treatments. In the thermal treatment, cassava pulp fibre was heated in an autoclave at 121°C for 1 h, but there was no heating process for the non-thermal treatment. Characterisation of cassava pulp flour was carried out by proximate analysis for the moisture content, ash content, crude fibre, fat, and protein (AOAC, 1999), for carbohydrate content it was carried out by difference, and for the starch content by enzymatic method through the measurement of glucose level from saccharification using α-amylase, amyloglucosidase (AMG), IDF, SDF (methods 993.19 and 991.42; AOAC, 1995), and TDF (IDF and SDF levels added up). Cassava pulp fibre was characterised by moisture and starch components and by IDF, SDF, and TDF levels.

Cellulase production

The crude enzyme from *Actinomycetes* HJ4(2a) isolates was used as a cellulase resource. It was obtained from the separation of biomass by centrifugation. It was collected by rejuvenated isolates grown on 1% liquid cassava pulp fibre for 4 d. The culture was incubated in a shaker at room temperature at a speed of 120 rpm. Production of cellulase was carried out to determine the cellulase activity and the optimum day of enzyme production based on the highest cellulose activity. Cellulase activity was evaluated with CMCase and FPase by the collected crude enzyme filtrate from day 4 to day 11.

DF production through enzymatic hydrolysis

DF production was carried out by two substrates, namely those in thermal and non-thermal treatments. For each use, 20 g of cassava was dissolved in a 0.1 M phosphate buffer at pH 7 at a ratio of 1:30 (w/v). Substrates at 0.1 U/g, based on FPase activity were used, and incubations were carried out in 0, 24, and 48 h at room temperature. The hydrolysate

separated at each time of incubation was washed, dried, and readied for DF analysis. Each treatment was carried out in triplicate.

Characterisation of hydrolysis process

The characterisation of hydrolysis process was carried out by measuring the average degree of polymerisation (DP) from hydrolysate, by dividing the total sugars determined with the phenol-sulphuric acid method (Dubois *et al.,* 1956), and by the reducing sugars determined with the DNS method (Miller, 1959), and later by measuring the weight loss after hydrolysis.

Characterisation of DF products

The analysis of DF components included the measurements of IDF and SDF by the enzymatic-gravimetric method (methods 993.19 and 991.42; AOAC, 1995), and TDF by adding up the IDF and SDF.

Analysis of DF functional properties included measurements of water holding capacity (Suzuki *et al.,* 1996), oil holding capacity (Caprez *et al.,* 1986), and emulsifying activity (Yasutmasu *et al.,* 1972). Each treatment was carried out in triplicate.

The DF surface morphology was observed using SEM (EVO MA 10, Carl Zeiss, Germany) at a magnification of 20,000×. Micrographs were taken using a secondary electron (SE) detector at a working distance (WD) of 8.5 mm and EHT of 16 kV.

Analysis of the pattern of the X-ray diffraction spectrum and the crystallinity was performed using an X-ray diffractometer (MAXima_X XRD-7000, Shimadzu, Japan). Measurements were taken at intervals of 2Θ: 5 - 45°, voltage of 40 kV, current of 30 mA, and speed of 2° per minute.

Experimental design and statistical analysis

The experiment was carried out using a randomised block design on thermal and non-thermal treatment groups, with the treatment factor being hydrolysis time (0, 24, and 48 h). Each treatment was carried out in triplicate, and the result was reported as mean ± standard deviation. Analysis of variance (ANOVA) was used for data analysis, and significant differences were analysed by Tukey's advanced test at a significance level of 5% ($p < 0.05$) using SAS 9.4, Cary, NC, US.

Results and discussion

Characteristics of cassava pulp flour and fibre

The composition of the cassava pulp flour and cassava pulp fibre is presented in Table 1. The results of this proximate analysis were used as a basis for the cassava pulp utilisation. Starch and fibre (TDF) were the two main components of cassava pulp flour at 59.21% and 18.57%, respectively. Chaikaew *et al.* (2012) reported that cassava pulp is composed of two primary components of starch (50 - 70%) and fibre (20 - 30%), as well as a small amount of protein, ash, and fat. The content of carbohydrates depends on the production process in the cassava starch industry. High carbohydrate and low ash content, coupled with an easy hydrolysis process, allow for the utilisation of cassava pulp into various types of products (Chaikaew *et al.,* 2012). The fat content of cassava pulp is also low (around 0.12 - 1%), so the rancidity during storage is low (Pramana *et al.,* 2018).

The availability of fibre in cassava pulp allows for its utilisation in DF production. DF is a non-starch carbohydrate, so starch removal is

Table 1. Proximate components of cassava pulp flour and cassava pulp fibre.

Proximate component	Cassava pulp flour (%)	Cassava pulp fibre (%)
Moisture	9.20 ± 0.51	9.98 ± 0.52
Ash	0.88 ± 0.04	
Fat	0.29 ± 0.06	
Crude protein	1.39 ± 1.39	
Crude fibre	16.72 ± 1.69	
Carbohydrate (by difference)	64.44 ± 1.53	
Starch	59.21 ± 0.19	3.49 ± 0.96
Total dietary fibre	18.57 ± 0.93	89.57 ± 2.35
Insoluble dietary fibre	18.02 ± 1.43	86.72 ± 2.45
Soluble dietary fibre	0.55 ± 0.91	2.85 ± 0.08

Proximate components were based on dry basis, except for moisture content.

required. The high starch content in cassava pulp takes the form of granules, some being bound to complex structures that are difficult to separate, and some being free inside the complex structures, making it easy to separate (Saengchan *et al.,* 2015). Separation of starch granules is essential to the effectiveness of starch removal. In the present work, α-amylase was used to hydrolyse starch to sugar residues such as dextrin, maltose, and glucose. This process successfully reduced the starch level by 3.48% and increased the fibre level (TDF) to 89.57% in the cassava pulp fibre. It is probably because cassava pulp contains starch granules that are not bound to complex structures. α-amylase works randomly inside the starch, so it only takes a short time to dissolve the starch (Sunarti *et al.,* 2012). The resulting moisture content was 9.98%, so it was safe for storage. In this step, the highest starch component (59.21%) was hydrolysed using α-amylase to produce simple sugars or glucose syrup, and even further commercial fuel bioethanol. Previously, Chaikaew *et al.* (2012) and Hermiati *et al.* (2011) reported that cassava pulp starch can be used to produce fermentable sugars and glucose. Technology that is easy and environmentally friendly such as enzyme treatment is needed to convert biomass into value-added products.

Cellulase production

Actinomycetes HJ4 (2a) isolate had the highest CMCase activity on the 7th day, which was 0.014 U/mL, and the highest FPase activity on the 8th day, which was 0.025 U/mL. Each cellulolytic bacterium has different cellulase enzyme activity, depending on the complex associated and various factors such as carbon source, cellulose quality, pH value, temperature, inducer, additive media, aeration, and growth time. Based on the highest cellulase production, the optimal time for *Actinomycetes* cellulase enzyme production was attained on the 8th day. The activity in CMC indicates that the isolate has endo-1,4-β-glucanase, while the substrate activity on filter paper indicates that the isolate has endo-1,4-β-glucanase and exo-1,4-β-glucanase. Endo-1,4-β-glucanase works in cellulose chain to produce oligosaccharides or shorter cellulose chains. Exo-1,4-β-glucanase cuts the ends of the crystalline cellulose chain into amorphous cellulose or reducing sugars. In the present work, the cellulase activity produced is lower than the activity of *Actinomycetes* cellulose from soil reported by Soeka *et al.* (2019), which was 7.3 U/mL. Cellulase is able to convert crystalline cellulose to amorphous cellulose to produce DF, which has functional properties as a food ingredient.

Characteristics of the hydrolysis process

The effects of thermal treatment and cellulase hydrolysis time on the hydrolysis process are presented in Table 2. DP (degree of polymerisation) is related to the number of monomer units in the molecule; the smaller the DP value, the more the reducing sugar content (Sunarti *et al.,* 2017). The DP value in the thermal treatment was lower than that in the non-thermal treatment at each hydrolysis time. This DP value shows that enzymes work better to produce reducing sugars in thermal treatments. Thermal treatment can increase porosity and surface area of the fibre and make it more sensitive to cellulase treatment (Sunarti *et al.,* 2012). The high DP value in the present work indicates that there was a small amount of reducing sugars in the hydrolysed molecule. This suggests that the cellulase enzyme has a low ability to degrade fibre into reducing sugars. This might happen because cellulase is able to degrade crystalline cellulose to more amorphous. Yang *et al.* (2017) described that enzymatic hydrolysis degrades fibre structure with minimum destruction. Besides, autoclave treatment has a lower capability to convert fibre into simple sugars as compared to microwave-assisted heating (Sunarti *et al.,* 2012).

Weight loss in cassava pulp fibre expresses the amount of fibre that can be degraded by cellulase to produce DF and hydrolysate by-products. The crystalline bonds are lost, making cellulose soluble in water and usable as food (Lattimer and Haub, 2010). The weight loss in the thermal treatment was

Table 2. Effect of thermal treatment and cellulase hydrolysis time on hydrolysis process.

Parameter	Thermal treatment (h)			Non-thermal treatment (h)		
	0	24	48	0	24	48
DP value	28.14 ± 0.10^{aA}	27.27 ± 0.04^{aA}	23.74 ± 0.06^{bA}	28.72 ± 0.14^{aA}	28.22 ± 0.22^{aB}	23.95 ± 0.21^{bA}
Weight loss(%)	72.3 ± 0.38^{aA}	74.7 ± 0.16^{bA}	78.0 ± 0.45^{cA}	64.5 ± 0.05^{aB}	68.3 ± 0.37^{bB}	71.9 ± 0.35^{cB}

Uppercase letters show a comparison of different treatments (thermal and non-thermal treatments) at the same time (0, 24, 48 h). Lowercase letters show comparison of different times in the same treatment. Different letters in the compared values denote significant difference at $p < 0.05$.

significantly higher ($p < 0.05$) than that in the non-thermal treatment, and significantly increased ($p < 0.05$) with reaction time because cellulase enzymes work better to hydrolyse fibres in thermal treatments. Sunarti *el al.* (2012) reported that temperature and time of heating with autoclave in a pre-treatment process affect weight loss and chemical composition loss of cellulose. Besides, the weight loss percentage of 64 - 78% shows that *Actinomycetes* cellulase is capable of degrading the cassava pulp fibre.

Component of DF

There was no significant difference between IDF and SDF values in thermal and non-thermal treatments for each hydrolysis time (Table 3). The IDF component decreased, and the SDF component increased, with hydrolysis time. Exoglucanase enzyme attacks crystalline cellulose (Lakhundi *et al.,* 2015) to produce more amorphous cellulose. Endoglucanase enzyme too, attacks cellulose randomly to eliminate the crystalline region. Yang *et al.* (2017) also reported that enzymatic hydrolysis increases the SDF value and decreases the IDF value. The results of hydrolysis show lower IDF value and higher SDF value in the thermal treatment for each hydrolysis time. However, changes in IDF and SDF values were lower in the thermal treatments with increasing hydrolysis time. For example, the decrease in IDF value of 0.4% from hydrolysis at 0 to 24 h in the thermal treatment was lower than the decrease in IDF value of 0.5% from hydrolysis at 0 to 24 h in the non-thermal treatment. It indicates a better cellulase ability to degrade substrate in the non-thermal treatment than in the thermal treatment. The benefits of IDF are that it can increase faecal bulk, lower intestinal transit time, and lower porosity. Meanwhile, SDF can be used to lower LDL cholesterol levels, increase glucose metabolism, and increase insulin response (Elleuch *et al.,* 2011).

High SDF values and low IDF values are beneficial because they increase the physicochemical and physiological properties of DF (Feng *et al.,* 2017). SDF increases the viscosity and stability of drinks and is the most commonly used component because of its greater dispersibility in water as compared to IDF's (Park and Yoon, 2015b). The SDF values in the present work are higher than the SDF values in wheat, barley, oats, rye, and millet of 0.19 to 6.85% (Menkovska *et al.,* 2017), and the SDF value in black bean coats of 7.8% (Feng *et al.,* 2017). SDF has potent antioxidant activity, great fermentation ability, and gel formation ability. Therefore, it is applied in the food industry as thickener, emulsifier, stabiliser, and fat substitute (Feng *et al.,* 2017).

Functional properties of DF

DF increases food functional properties such as water holding capacity (WHC), oil holding capacity (OHC), and emulsifying activity (EA), and it is beneficial when used in food products because it can change textures, stabilise food, emulsify, and prolong shelf life (Elleuch *et al.,* 2011). The effects of thermal treatment and cellulase hydrolysis time on DF functional properties are presented in Table 3. WHC values in the thermal and non-thermal treatments significantly increased ($p < 0.05$) with hydrolysis time. For example, 10.24 g/g (0 h of reaction time) to 12.08 g/g (48 h of reaction time) in the thermal treatment, and 7.80 g/g at 0 h to 9.13 g/g at 48 h in the non-thermal treatment. Moreover, WHC in the thermal treatment was significantly higher ($p < 0.05$) than in the non-thermal treatment. This happened because the change from crystalline cellulose to amorphous cellulose can increase porosity, surface area of fibres, and physicochemical properties of DF (Sunarti *et al.,* 2012; Pramana *et al.,* 2018).

The WHC values in the present work are higher than that in commercial cellulose (4.92 g/g)

Table 3. Effect of thermal treatment and cellulase hydrolysis time on DF chemical composition and functional properties.

Parameter	Thermal treatment (h)			Non-thermal treatment (h)		
	0	24	48	0	24	48
Insoluble dietary fibre (%)	82.61 ± 0.03^{aA}	82.17 ± 0.16^{abA}	81.27 ± 0.42^{bA}	82.75 ± 0.11^{aA}	82.34 ± 0.13^{abA}	81.46 ± 0.02^{bA}
Soluble dietary fibre (%)	8.15 ± 0.04^{aA}	9.05 ± 0.04^{aA}	9.35 ± 0.02^{aA}	7.87 ± 0.53^{aA}	8.92 ± 0.01^{abA}	9.25 ± 0.01^{bA}
Total dietary fibre (%)	90.76 ± 0.07^{aA}	91.22 ± 0.13^{aA}	90.62 ± 0.41^{aA}	90.62 ± 0.43^{aA}	91.26 ± 0.13^{aA}	90.71 ± 0.02^{aA}
Water holding capacity (g/g)	10.24 ± 0.02^{aA}	10.97 ± 0.05^{bA}	12.08 ± 0.07^{cA}	7.80 ± 0.01^{aB}	8.05 ± 0.07^{bB}	9.13 ± 0.12^{cB}
Oil holding capacity (g/g)	3.01 ± 0.10^{aA}	3.08 ± 0.07^{aA}	3.12 ± 0.12^{aA}	2.68 ± 0.02^{aB}	2.78 ± 0.03^{aB}	3.01 ± 0.05^{bA}
Emulsifying activity (%)	62.18 ± 0.02^{aA}	63.81 ± 0.04^{bA}	64.53 ± 0.09^{cA}	60.07 ± 0.02^{aB}	60.82 ± 0.04^{bB}	62.83 ± 0.04^{cB}

Uppercase letters show a comparison of different treatments (thermal and non-thermal treatments) at the same time (0, 24, 48 h). Lowercase letters show comparison of different times in the same treatment. Different letters in the compared values denote significant difference at $p < 0.05$.

(Chirinang and Oonsivilai, 2018). WHC values in the thermal treatment with hydrolysis times of 24 and 48 h were also higher than those in the previous research of cassava pulp of 7.12, 8.17, and 10.47 g/g (Kachenpukdee *et al.,* 2016; Chirinang and Oonsivilai, 2018; Pramana *et al.,* 2018). They are also higher than those of agricultural waste such as wheat husks and onion waste of 6.7 and 3.4 g/mL (Benítez *et al.,* 2011; Im and Yoon, 2015). WHC is related to the amount of water that can be retained by 1 g of dry fibre under certain conditions such as duration and speed of centrifugation (Elleuch *et al.,* 2011). WHC value is influenced by source of DF, chemical structure of polysaccharides, porosity, ionic form and strength, temperature, pH, particle size, fibre length, processing conditions, stresses upon fibres, and density (Elleuch *et al.,* 2011; Matin *et al.,* 2013; Im and Yoon, 2015). With a high WHC, DF can be used in food products to change textures, form gels or highly viscous solutions of some formulated foods, reduce vaporisation rate, and change freezing rate (Noorlaila *et al.,* 2015; Chirinang and Oonsivilai, 2018).

The OHC value in the thermal treatment was higher than that in the non-thermal treatment. The OHC values of the thermal and non-thermal treatments also increased with reaction time. For example, 3.01 g/g in 0 h increased to 3.12 g/g in 48 h in the thermal treatment, while in the non-thermal treatment from 2.68 g/g in 0 h to 3.01 g/g in 48 h. These values are higher than the OHC of commercial cellulose, which is 2.66 g/g (Chirinang and Oonsivilai, 2018), but lower than those in previous studies on cassava pulp (Kachenpukdee *et al.,* 2016; Chirinang and Oonsivilai, 2018; Pramana *et al.,* 2018). OHC is the amount of oil that can be retained by fibre after mixing, incubation with oil, and centrifugation (Elleuch *et al.,* 2011). The ability of DF to retain oils depend on the surface properties, porosity, thickness, hydrophobic property of the fibre particles, number of lipophilic sites, and capillary appeal (Im and Yoon, 2015; Pramana *et al.,* 2018). Ingredients with high OHC are used as emulsifiers by holding the fat in formulated diets, and as stabilisers for high-fat food products (Elleuch *et al.,* 2011; Matin *et al.,* 2013).

The values of EA in the thermal and non-thermal treatments significantly increased ($p < 0.05$) with hydrolysis time, with the value in the thermal treatment being significantly higher ($p < 0.05$) than that in the non-thermal treatment. In the thermal treatment, EA increased from 62.18% (0 h of hydrolysis time) to 64.53% (48 h of hydrolysis time), whereas in the non-thermal treatments it increased from 60.07% (0 h of hydrolysis time) to 62.83% (48 h of hydrolysis time). These values are higher than the EA values in wheat bran, soybean hulls, and barley bran (52.00%) (Matin *et al.,* 2013), but still lower than the EA value in cassava pulp (77.89%) (Pramana et al., 2018). EA is the ability of emulsion to maintain the system after undergoing centrifugal force (Noorlaila *et al.,* 2015). Emulsifying ability is related to the molecular weight and particle size of the emulsifier. In the food industry, high EA levels are useful for the formation of emulsions or for extending the shelf life (Xie *et al.,* 2017). In the present work, the functional value of DF was higher in the thermal treatment, although there was no significant difference between IDF and SDF values. The difference in the values of functional properties is very important in industrial applications. DF production in the present work had high SDF and IDF with high functional properties of WHC, OHC, and EA. High IDF is useful in the food industry for products such as bread, processed meat, low-calorie foods, fat substitutes, high fibre foods, and emulsifying agents (Pramana *et al.,* 2018). Meanwhile, high SDF can increase the viscosity and stability of drinks (Park and Yoon, 2015b).

Surface morphology

The results of the SEM surface morphological analysis of cassava pulp fibre before and after hydrolysis in 48 h with thermal and non-thermal treatments are presented in Figure 1. SEM imaging was performed only 48 h after hydrolysis to show the difference between the two treatments. Starch granules were not seen in the cassava pulp fibre structure before hydrolysis, either with or without thermal treatment (Figure 1a and 1b). This happened because there was a pre-treatment to remove starch, even though the starch content remained at 3.49% (Table 1). α-amylase has a higher starch purification ability (Chirinang and Oonsivilai, 2018). The cassava pulp fibre before hydrolysis with non-thermal treatment was a solid, compact bundle of cellulose fibres containing crystalline cellulose. The cassava pulp fibre before hydrolysis with thermal treatment was also a bundle of cellulose fibres that were degraded by heating. Heating with autoclaves and microwaves can break the crystallinity of fibre to make the fibre more amorphous (Sunarti *et al.,* 2012). Heating makes pores on the surface of cellulosic biomass and provides a surface area that is more easily accessible to cellulase. Fibre degradation could be seen very clearly, and a high porosity level on the cassava fibres was evident as a result of cellulase hydrolysis, both with and without thermal treatment (Figure 1c and 1d). This is in line with Chirinang and

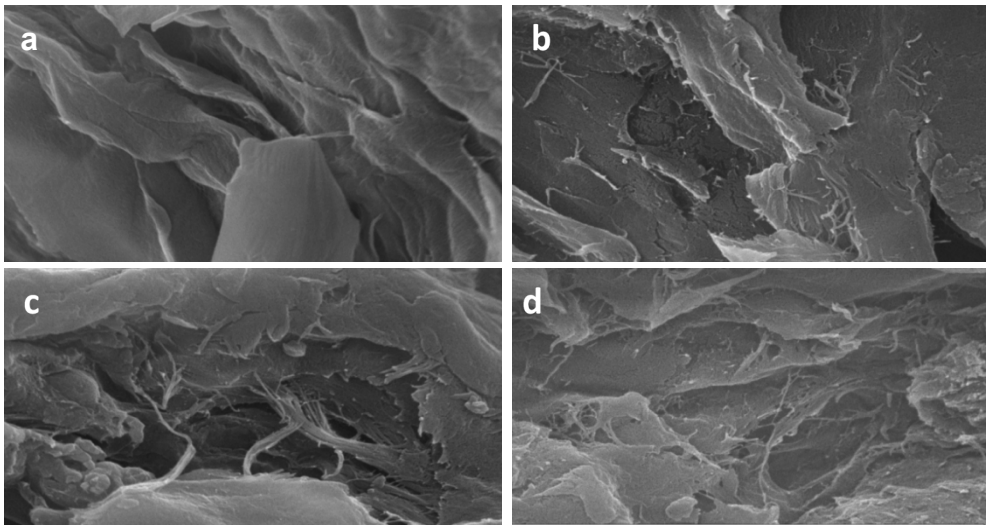

Figure 1. Scanning Electron Microscope (SEM) surface morphological analysis of cassava pulp fibre before hydrolysis with non-thermal treatment (a), thermal treatment (b), cassava pulp after hydrolysis of 48 h with non-thermal treatment (c), and thermal treatment (d).

Oonsivilai's (2018) report, that enzyme hydrolysis increases fibre porosity. In heat-treated hydrolysed fibres (Figure 1d), degradation can be seen because of the thermal treatment, which prepares the structure of the fibre and makes it easier for the penetration of cellulose during the hydrolysis process. The IDF and DP values also decreased, and so did the SDF values, but the functional properties (WHC, OHC, and EA) increased from cellulase hydrolysis, both with or without thermal treatment.

X-ray diffraction

The X-ray diffractograms of cassava pulp fibre before hydrolysis and 48 h after hydrolysis with and without thermal treatment are shown in Figure 2. The cassava pulp fibre before hydrolysis with non-thermal treatment had high peaks at 2θ at 16°, 17°, and 23° (Figure 2a). Earlier reports mentioned that a cassava pulp spectrum had high peaks at 15°, 16°, 17°, 18°, and 23° (Hermiati *et al.,* 2011; Pramana *et al.,* 2018). This might happen because the removal of starch with α-amylase was successful, so that there was no fibre crystallinity at 15° and 18°. The percent crystallinity (30.73%) in the present work is also smaller than reported by Farias *et al.* (2014) (35 - 45%). The hydrolysed cassava pulp fibres from the non-thermal treatment had a high peak at 23°, while at 16° and 17°, the crystallinity decreased with percent crystallinity of 19.49% (Figure 2b). It shows that enzyme not only degrades crystalline cellulose to amorphous cellulose but also increases the SDF value and functional properties (WHC, OHC, and EA), and decreases the IDF and DP values. The cassava pulp fibre before hydrolysis with thermal treatment had high peaks at 16°, 17°, and 23° with a crystallinity percentage of 22.91% (Figure 2c). Thermal process breaks down crystalline cellulose into amorphous cellulose and loosens the structure to make the enzyme work easier. The IDF and DP values were lower and the SDF value and functional properties (WHC, OHC, and EA) were higher in the hydrothermal treatment. The peak of the hydrolysed cassava pulp fibre with thermal treatment at 16°, 17°, and 23° decreased with a crystallinity percentage of 18.49% (Figure 2d). Klemm *et al.* (2005) reported that the spectrum peaks that characterise cellulose are 16° and 23°. In the present work, the percentage of crystallinity decreased after hydrolysis because cellulase could break down the crystalline cellulose into amorphous cellulose. However, thermal treatment had the lowest crystallinity percentage because the tenuous fibre structure made the enzyme work easier. Hydrolysis in 48 h with hydrothermal treatment produced the highest SDF value, highest functional properties (WHC, OHC, and EA), and lowest IDF and DP values.

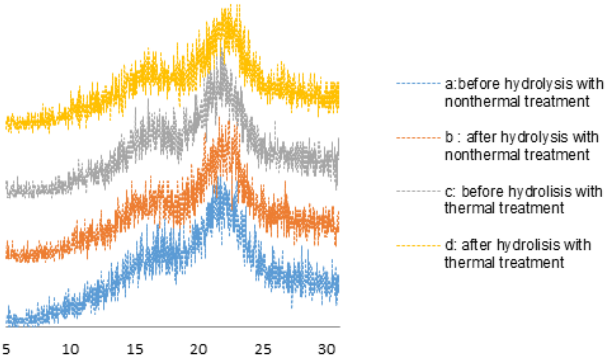

Figure 2. XRD diffractogram shows cassava pulp fibre before and after hydrolysis of 48 h in thermal and non-thermal treatments.

Conclusion

Dietary fibre can be produced from cassava pulp fibre using *Actinomycetes* HJ4(2a) cellulase with thermal and non-thermal treatments. Enzymes could produce DF by decreasing the IDF value and increasing the SDF value along with increasing hydrolysis time, although there was no significant difference of DF components between the thermal and non-thermal treatments. The functional properties of DF (WHC, OHC, and EA) were higher in the thermal treatment than in the non-thermal treatment, and they increased with hydrolysis time. Optimum conditions of hydrolysis were shown in the thermal treatment in hydrolysis of 48 h. Besides, the DP value during the hydrolysis process was lower, and the morphological and crystallinity characteristics changed more in the thermal treatment. This shows that a combination of enzymatic hydrolysis with *Actinomycetes* HJ4(2a) cellulase and thermal treatment can break down crystalline cellulose into amorphous cellulose, and increase the SDF component and DF functional properties for functional foods.

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