Effect of Heat Process for Prebiotic Properties of Taro Starch (*Colocasia Esculenta L. Schott*)

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Abstract: This study investigates the effects of a variety of heat processes on the prebiotic properties of taro starch. The taro starch is treated by the annealing process (24 hours, 50°C), the heat moisture treatment (HMT, moisture 25%, 3 hours, 110°C), and the autoclaving (15 minutes, 121°C) - cooling (24 hours, 4°C) cycles with 1, 2, and 3 cycles. The results show that all treatments improve the prebiotic properties of taro starch. The modified taro starches (MTS) significantly increases their slow digestibility starch (SDS) and resistant starch (RS) content, while the in-vitro digestibility, very rapid digestible starch (VRDS), rapid digestible starch (RDS) are relatively decreased. However, the autoclaving-cooling two cycles (AC-2C) results in the MTS with the best prebiotic properties as shown by its high RS content, low digestibility, high prebiotic effect, high prebiotic index as well as prebiotics activity towards pathogenic bacteria. The AC-2C modified taro starch is very prospective to be used as a prebiotic candidate.

1 INTRODUCTION

Colocasia esculenta L. schott known as taro was one of the tubers from the Araceae family that is rich in consumable starch (Aboubakar *et al.,* 2009; Kaushal *et al.,* 2012; Zhu *et al.,* 2015). The most common consumed parts of taro were corm and cormel, the thickening root which grows in the soil (Deka & Sit, 2016; Yu *et al.,* 2018a). Taro was one of the most cultivated tubers in the tropics and the subtropics, including Southeast Asia, the Caribbean and the North Atlantic Ocean, South and West Africa, Pacific Islands and Polynesia (Aboubakar *et al*., 2009). The utilization of Taro in Southeast Asia was still minimal (Kaushal *et al.,* 2012; Zhu *et al.,* 2015; Deka & Sit, 2016). In the last few years, however, taro cultivation had been increased due to its potential as the functional food which contains up to 70 – 80 gram / 100 gram of starch content, 2 – 6 gram / 100 gram of protein, 0.6 – 0.8 gram / 100 gram of fiber, vitamin, phosphorus, magnesium, and calcium (Kaushal *et al.,* 2012; Zhu *et al*., 2015, Li *et al.*, 2018a). Taro could be widely applied in the food industry and processed into consumable products such as pasta, starch, flour, cereal bar, canned product, chips and beverage powder (Li *et al*., 2018a; Muñoz-Cuervo *et al.,* 2016). The digestibility rate of taro starch was very high and it had been applied to various food products because of its unique structure and small particle of taro starch granules (Kaushal *et al.,* 2012; Muñoz-Cuervo *et al*., 2016). The utilization of native taro starch was still limited as it is high in retrogradation, thermal decomposition, poor in process tolerance, narrow peak viscosity range and resistant towards low shear stress (Demirkesen-Bicak *et al.,* 2018; Yu *et al.,* 2018b). These weaknesses increase the interest of many researchers to modify starch so its functional properties could be improved (Sullivan *et al.,* 2017; Sharlina *et al.,* 2017; Oyeyinka *et al.,* 2018). Starch modification techniques could be carried out by its physical, chemical or enzymatic properties (Hazarika & Sit, 2016). Some physical modification techniques could improve the functional properties of taro starch including the cycle of cooling, autoclaving, HMT and annealing.

Generally, the modification of taro starch was conducted to enhance its functional properties as well

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as its physicochemical characteristics. Setiarto *et al*. (2018) modified the taro flour to improve its prebiotic properties, using the fermentation technique and the autoclaving-cooling cycling. Two cycles of the autoclaving-cooling technique successfully increased the RS content by 2.7-fold (from 4.13% to 11.15%) compared to the control treatment. The modified taro flour resulted shows a better prebiotic effect, index, and activity than the control (the one without fermentation and autoclaving-cooling). This study aims to determine the physicochemical characteristics and prebiotic properties of modified taro starch by implementing annealing, HMT and autoclaving-cooling treatments. The prebiotic properties were assessed during this study to provide the additional information on functional properties for food industry application.

2 MATERIALS AND METHODS

2.1 Materials

The main raw material used in this study was the Bogor Taro of Pandan (*Colocasia esculenta*) with eight months harvest age, from Cijeruk Bogor West Java, Indonesia. *Lactobacillus plantarum* SU-LS 36 and EPEC (Entero Pathogenic Escherichia coli) was provided from The Laboratory of Food Microbiology, Research Center for Biology, Indonesian Institute of Science (LIPI).

2.2 Taro (*Colocasia Esculanta*) Starch Extraction Process

Taro starch extraction was performed by applying the technique from Airul et al. (2014) with a few modifications. Taro tuber (*Colocasia esculanta*) was peeled, washed, and soaked in the mixture of 1% NaCl (3: 4) for an hour to remove oxalate crystals. It was then shredded and mixed with distilled water (3: 1) for one minute using a blender (Phillips, Netherland). Double fold cotton cloth was utilized to filter the taro pulp. The obtained taro pulp filtrate was settled overnight to let the starch sink at the base of the beaker glass. Taro pulp was centrifuged with High Speed Centrifuge (Kubota, Japan) at 5000 rpm for 10 minutes to obtain taro starch. Distilled water was used to clean the taro starch three times to remove the supernatant. After that, it was oven dried in at 50oC up to the constant weight. Finally, the dry taro starch was ground using the disk mill (China).

2.3 Modification of Taro Starch

2.3.1 Annealing Treatment

The annealing treatment of taro starch was conducted by the technique from Wang et al. (2018). Twelve grams of taro starch was added to 60 ml of distilled water with the ratio of taro starch: water (1: 5) (b / v) was placed in a polyethylene bag. The annealing treatment was carried out at 50^{o}C for 24 hours by inserting a polyethylene bag which had been tightly closed into a water bath (Hitachi, Japan). Afterward, the taro starch was freeze dried, crushed and filtered using the 100-mesh sieve. The resulting taro starch from the annealing process was then chilled at 4^{o}C prior to further analysis.

2.3.2 Heat-Moisture Treatment

The taro starch modification using HMT was obtained following Deka & Sit (2016). Forty-five grams of taro starch (dry-based) was placed into a glass container, and distilled water was added to it while stirring until the water content reached 25%. Then, the glass container was sealed, balanced for 48 hours at room temperature then heated at 120°C in an electric oven (Shimizu, Japan) for three hours. The heated modified taro starch was then dried at 40°C for overnight, milled and sieved with a 100-mesh sieve.

2.3.3 Autoclaving-Cooling Treatment

The autoclaving-cooling method of taro starch followed the procedure by Setiarto *et al. (*2018). The taro starch was added with aquadest at the ratio of 1: 2, heated in an autoclave (Hitachi, Japan) at 121°C for 15 minutes, then chilled in refrigerator at 4°C for 24 hours. Thereafter, the treated taro starch was dried (70°C, 16 hours) in an oven (Shimidzu, Japan) until the moisture content reached 12%, and milled using a pin disk mill (Shimidzu, Japan). The starch was sieved to obtain the 100-mesh taro starch. The autoclaving-cooling treatment was also completed with two cycles and three cycles.

2.4 In-vitro Digestibility and Digestible Starch Composition Analysis

In-vitro starch digestibility was analyzed by measuring the level of maltose as the product of hydrolysis taro starch by using α-amylase enzyme (Sigma) compared to starch solution. This analysis was performed by referring to a method from Anderson *et al.,* (2002). The absorbance of sample

and blank solutions was determined by Spectrophotometer UV-Vis (Shimadzu UV-1800, Japan) at 520 nm. In this study, the calculation of the starch digestibility (%) is shown in the following formula:

$$\text{Starch digestibility (\%)} = \frac{\text{Maltose content of sample} - \text{Maltose content of blank sample}}{\text{Maltose content of pure starch} - \text{Maltose content of blank pure starch}} \times 100\% \quad (1)$$

The digestible starch composition analysis was conducted in this study by following Englyst *et al.,* (1992) method. There are four types of starch compositions based on their digestibility times. The first type is called very rapid digestible starch (VRDS), which is expressed as the amount of digested starch in the first minute by porcine pancreatin and amyloglucosidase 210 U as explained in the Sigma Cat. No. P7545 and No. A7095, respectively. The second type is called the rapid digestible starch (RDS) which is the amount of digested starch expelled between 1 minute and 20 minutes. The third type is the slow digestible starch (SDS) which is expressed as the amount of digested starch between 20 and 120 minutes. Finally, the resistant starch (RS) is described as non-digestible starch after 120 minutes of analysis.

2.4.1 Analysis of Prebiotic Effect and Prebiotic Index of MTS

The analysis of prebiotic effect and index was conducted by observing the change in the number of *L. plantarum* SU-LS 36 colonies on m-MSRB medium and m-MSRB medium with 2.5% taro starch (native, AC-1C, AC-2C, AC-3C, annealing and HMT). They were determined using the methods by Roberfroid (2007). After incubation process for 24 hours at 37°C, the probiotic cell cultures were enumerated in the MRSA medium. The same procedures were conducted using a commercial prebiotic FOS (fructooligosaccharide) as positive control. The calculations were finished using these following equations:

$$\text{Prebiotic Effect} = \text{Log (cfu/mL) 2.5\% taro starch} - \text{Log (cfu/mL) m-MRSB} \quad (2)$$

$$\text{Prebiotic Index} = \frac{Log\left(\frac{cfu}{mL}\right) 2.5\%\ taro\ starch - Log\left(\frac{cfu}{mL}\right) mMRSB}{Weight\ taro\ starch} \quad (3)$$

2.4.2 Prebiotic Activity Examination of MTS to Diarrhea-Causal-Bacteria

The examination of prebiotic activity was conducted by adding 2% (v/v) of *L. plantarum* SU-LS 36 culture into m-MSRB with 2.5% (w/v) of glucose or 2.5% (w/v) of taro starch (native, AC-1C, AC-2C, AC-3C, annealing and HMT). It was analyzed by referring the method from Huebner et al. (2007). After 0 hour and 24 hours of incubation times, the samples were enumerated in the MRSA medium. The examination was also conducted towards diarrhea-causal-bacteria, *Entero Pathogenic Escherichia coli* (EPEC). The EPEC culture of 2% (v/v) was added into different Erlenmeyer containing m-TSB 2.5% (w/v) of glucose or 2.5% (w/v) taro starch (native, AC-1C, AC-2C, AC-3C, annealing and HMT). The culture was incubated at 37°C, and enumerated in the TSA medium after 0 hour and 24 hours of incubation times. Prebiotic activity value was calculated using this equation:

$$\text{Prebiotic Activity Value} = \left\{\frac{N\ log\left(\frac{cfu}{mL}\right) taro\ starch\ t1 - N \log\left(\frac{cfu}{mL}\right) taro\ starch\ t0}{N\ log\left(\frac{cfu}{mL}\right) Glucose\ t1 - N\ log(cfu/mL) Glucose\ t0}\right\} - \left\{\frac{E\ log\left(\frac{cfu}{mL}\right) taro\ starch\ t1 - E \log\left(\frac{cfu}{mL}\right) taro\ starch\ t0}{E\ log\left(\frac{cfu}{mL}\right) Glucose\ t1 - E\ log(cfu/mL) Glucose\ t0}\right\} \quad (4)$$

Note

N = number of *L. plantarum* SU-LS 36 (log cfu/mL)
t_0 = start of incubation time (0 hour)

E = number of *Entero Pathogenic Escherichia coli* (log cfu/mL)

t_1 = end of incubation time (24 hour)

2.5 Statistical Data Analysis

There were three replications in this experiment, where the statistical analyses were implemented to process the research data. The Duncan statistical test was implied to examine the considerable differences at the level of $p < 0.05$ utilizing the SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Table 1: In vitro digestibility and starch digestibility profile.

Treatment	In-vitro digestibility (%)	VRDS (% dry weight)	RDS (% dry weight)	SDS (% dry weight)	RS (% dry weight)
Native taro starch	80.17±0.63^{c}	37.30±0.42^{d}	32.07±0.25^{d}	23.15±0.63^{a}	7.48±0.94^{a}
AC-1C	70.77±0.52^{b}	31.95±0.81^{c}	27.13±0.48^{c}	25.60±0.74^{b}	15.32±0.86^{b}
AC-2C	64.41±0.76^{a}	29.29±0.46^{b}	22.20±0.59^{b}	27.17±0.38^{c}	21.34±078^{c}
AC-3C	62.83±0.82^{a}	27.42±0.35^{a}	21.20±0.72^{b}	28.67±0.55^{c}	22.71±0.21^{c}
Annealing	67.24±0.26^{b}	33.27 ±0.87^{c}	24.02±0.29^{b}	25.27±0.88^{b}	17.44±0.69^{b}
HMT	65.66±0.31^{a}	30.58±0.93^{b}	18.54±0.46^{a}	27.26±0.61^{c}	23.62±0.49^{c}

Note: In-vitro digestibility, VRDS, RDS, SDS and RS content with the different superscript letters within a row were significantly different at $p<0.05$ level

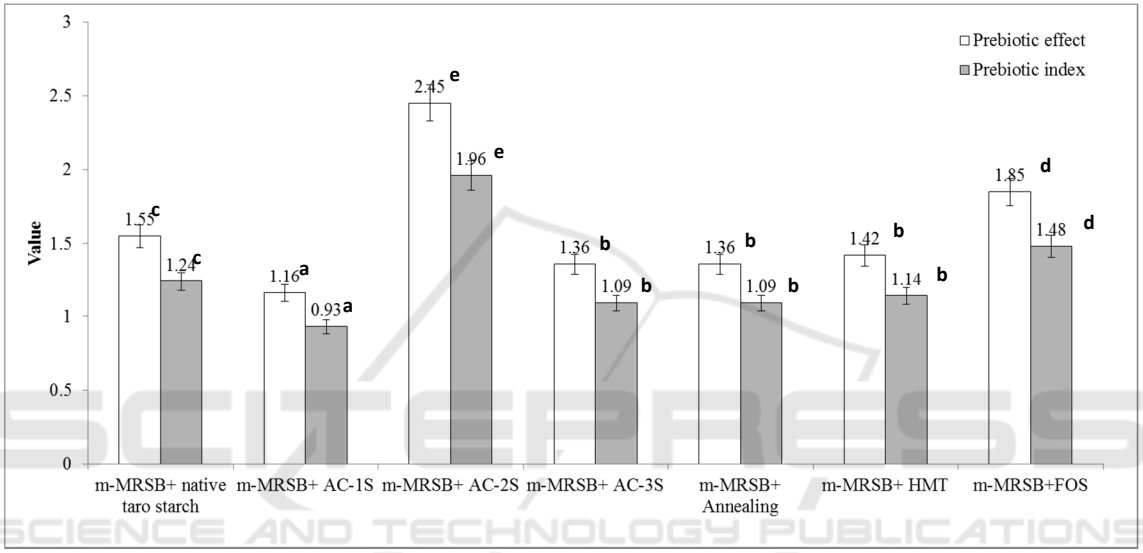

Figure 1: Prebiotic effect and index of native taro starch, modified taro starch by AC-1C, AC-2C, AC-3C, Annealing, and HMT.

Note: Prebiotic effect and index are expressed in the different typescript letters of the bar chart, where the noticeable different occurs at the $p<0.05$ level.

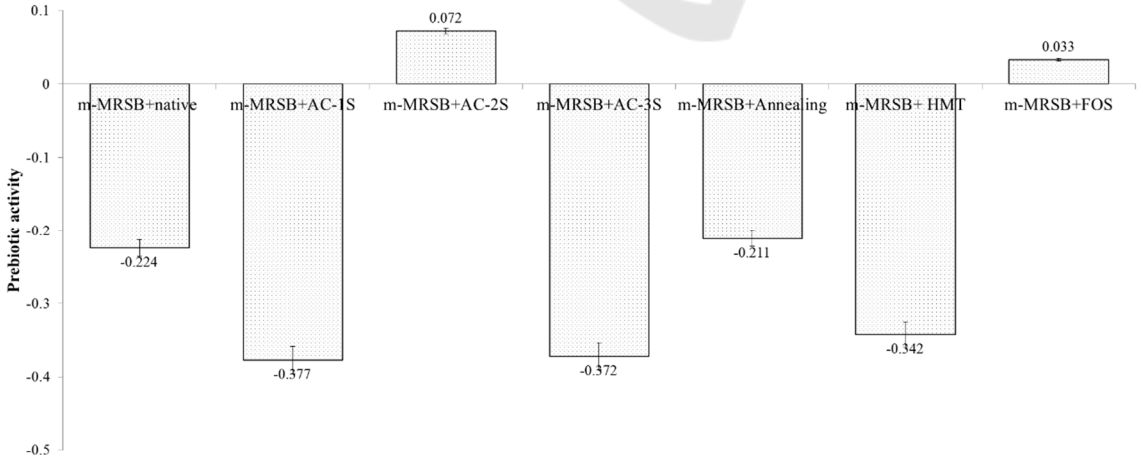

Figure 2: Prebiotic activity of native taro starch, modified taro starch by AC-1C, AC-2C, AC-3C, annealing, and HMT to diarrhea-causal-bacteria.

Note: Prebiotic activity is expressed in the different typescript letters of the bar chart, where the noticeable different occurs at the $p<0.05$ level.

3 RESULTS AND DISCUSSION

3.1 In Vitro and Starch Digestibility Profiles

The analysis identifies that the native taro starch had the highest in-vitro digestibility up to 80.17% compared to modified taro starches (**Table 1**). The improvement of RS content might reduce the digestibility of taro starch consistently. Starch digestibility had a negative correlation with RS content. This result was similar to analysis by Cheng *et al.* (2019), Ashwar *et al.* (2016), Shi & Gao (2011), and Zheng *et al.* (2018) showed that the HMT treatment can reduce the vitro digestibility of rice starch. Annealing, HMT, and autoclaving-cooling cycles significantly reduced in-vitro digestion of taro starch ($p < 0.05$) (**Table 1**). HMT and autoclaving-cooling cycle resulted the formation of double helix structures, the increase of chain bonding between amylose-amylose, amylopectin-amylopectin and amylose-amylopectin, consequently taro starch was more difficult to digest by α-amylase enzymes (Cheng *et al.,* 2019; Zheng *et al.,* 2018).

The increasing number of autoclaving-cooling cycles decreased the in-vitro digestibly of taro starch. The AC-3C treatment showed the lowest digestibility of taro starch (62.83%) compared to other treatments (**Table 1**). The annealing treatment, autoclaving cooling cycle and HMT also reduced the in-vitro digestibility of starch due to the retrogradation process, hence increased RS and SDS levels (Shah *et al.,* 2016; Chen *et al.,* 2018; Lovera & Perez, 2017). MTS with high RS content had low in-vitro starch digestibility (Perera *et al.,* 2010). The in-vitro digestibility reduction due to the retrogradation treatment (e.g. HMT and autoclaving-cooling cycle) was also reported previously by Cheng *et al.* (2019), Shah *et al.* (2016), Ashwar *et al.* (2016), Shi & Gao (2011), Zheng *et al.* (2018), and Chen *et al.* (2018).

Annealing, HMT and autoclaving-cooling cycles reduced the levels of VRDS and RDS significantly ($p<0.05$) compared to the native taro starch (**Table 1**). The more autoclaving-cooling cycles were applied, the lower VRDS and RDS became. Taro starch with autoclaving-cooling of 3 cycles (AC-3C) treatment showed the lowest result of VRDS level (27.42%), followed by AC-2C (29.29%), HMT (30.58%), AC-1C (31.95%) and annealing (33.27%) (**Table 1**). Moreover, taro starch with HMT showed the lowest value of RDS level (18.54%). The VRDS and RDS from annealing, HMT and autoclaving-cooling cycle treatments showed significant decrease as their internal structures were changed into the SDS and RS. This is proved by the significant increase ($p<0.05$) of SDS and RS levels in taro starch after annealing, autoclaving-cooling cycles, and HMT (**Table 1**). The more autoclaving-cooling cycles were applied, the higher SDS and RS become. AC-3C treatment showed the highest SDS levels (28.67%) while HMT resulted the highest RS levels (23.62%) (**Table 1**). These results were relatively higher than the research from Cheng *et al.* (2019) in which the HMT was applied at 120^{o}C condition (2 hours, 30% moisture content) in corn, pea, and lentil starch. HMT treatment led to the increase of resistant starches of corn, pea, and lentil up to 7.7, 11.2, and 10.4% respectively (Cheng *et al.,* 2019).

3.2 Prebiotic Effect and Prebiotic Index

The prebiotic effect is the increasing number of the absolute probiotic bacteria without considering the prebiotic concentration (Roberfroid, 2007; Huebner *et al*., 2007). Meanwhile, the prebiotic index is the increasing of probiotic bacteria population correlated with the prebiotic concentration (Roberfroid, 2007; Huebner *et al.,* 2007). The highest prebiotic effect and index were noticeable in *L. plantarum* SU-LS 36 in the AC-2C treatment (**Figure 1**). The RS in AC-2C taro starch accommodated the growth of probiotic bacteria. The examination on prebiotic effect and index were conducted directly to the taro starch sample to explain its prebiotic properties. Huebner et al. (2007) reported that a diet was a good prebiotic source if it had more than 1.5 prebiotic effect and index (**Figure 1**).

The AC-2C taro starch was a good prebiotic candidate if it had more than 1.5 prebiotic effect and index. This value was higher than the fructooligosaccharide (FOS), as commercial prebiotic. The resistant starch content in AC-2C taro starch increased the probiotic growth of L. plantarum SU-LS 36 (**Figure 1**). To increase the prebiotic effect index could be achieved by isolating the RS from taro starch or consuming the AC-2C taro starch in the larger quantities (20 gram/day) as a functional diet. The RS with 20 – 30 degree polymerization played an important role as a prebiotic source, therefore it could be fermented to form the short chains fatty acids (especially the butyric acid) in the colon, using probiotic bacteria assistance (Danneskiold-Samsøe *et al.,* 2019). The increase of butyric acid caused the decrease of pH inside the colon. Therefore this condition inhibited the pathogenic bacteria growth and prevented the proliferation of cancer cells in the colon (Sullivan *et al.,* 2017; Luo *et al.,* 2017).

3.3 Prebiotic Activity to Diarrhea-Causal-Bacteria

The prebiotic activity is the prebiotic capability to grow probiotic bacteria, which is related to its selectivity towards pathogenic bacteria over glucose (Vrese & Marteau, 2007). A diet had positive prebiotic activity (over 0.25) if it was selectively metabolized by probiotic bacteria's such as *Bifidobacterium* sp., *L. acidophilus* and *L. plantarum*, and not metabolized by pathogenic bacteria such as EPEC (Vrese & Marteau, 2007). Native taro starch, AC-1C, AC-3C, annealing and HMT had negative prebiotic activity values. These mean that they were not potential as prebiotic candidates (Figure 2). The AC-2C treatment was capable to produce a resistant starch with a degree of polymerization (DP) of around 20-30. Resistant starch was a selective and specific prebiotic source for probiotics *L. plantarum* SU-LS 36.

Furthermore, *L. plantarum* SU-LS 36 probiotics utilized the resistant starch from the AC-2C MTS as a carbon source for its growth. Meanwhile, EPEC could not use it as a source of nutrition for its growth. The AC-2C MTS had the highest prebiotic activity and it was a positive growth medium for *L. plantarum*- EPEC (0.072) (Figure 2). Positive prebiotic activity was also produced by fructooligosaccharide (FOS) as a commercial prebiotic growth medium for L. plantarum-EPEC (0.033) (Figure 2). Finally, the AC-2C MTC was the best prebiotic candidate as it had the higher values of prebiotic effect, index, and activity than any other treatments.

4 CONCLUSIONS

Annealing, autoclaving-cooling cycle, HMT decreased in-vitro starch digestibility, VRDS, RDS significantly. On the other hand, all treatments significantly increased SDS and RS levels from taro starch. MTS with AC-2C had the potential as a prebiotic candidate as it had the highest prebiotic effect, index and activity against EPEC.

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