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Original Article

Changes of digestible starch composition and improvements of prebiotic properties in modified taro starch by heat treatment

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Abstract

This study investigated the composition of digestible starch and prebiotic properties of taro starch due to different heating treatments. The taro starch has been treated by annealing (24 hrs, 50 °C), heat moisture treatment (moisture 25%, 3 hrs, 110 °C), and autoclaving (15 min, 121 °C) with cooling (24 hrs, 4 °C) for 1, 2, and 3 cycles. Results showed that resistant starch (RS) and slowly digestible starch (SDS) contents in the modified taro starches (MTS) by all heat treatments increased significantly. Furthermore, MTS by autoclaving-cooling two cycles (AC-2C) showed the best prebiotic properties indicated by high resistance in simulated gastric acid (90.19%), high prebiotic effect (2.45), high prebiotic index (1.96) as well as prebiotic activity (0.072) towards *Enteropathogenic Escherichia coli* (EPEC). This MTS also has high RS (21.34%) and SDS (27.17%) content as well as low digestibility (64.41%). Hence, AC-2C MTS is very prospective to be used as the prebiotic source.

Keywords: digestible starch, heat treatment, modified taro starch, prebiotic activity, prebiotic index

1. Introduction

Colocasia esculenta L. schott or taro is the Araceae family which is rich in consumable starch (Aboubakar, Njintang, Scher, & Mbofung, 2009; Zhu, Xiao, Zhou, & Lei, 2015). The most commonly consumed parts of taro were corm and cormel, the thickening roots that grow in the soil (Deka & Sit, 2016; Yu *et al.*, 2018). 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 (Deka & Sit, 2016; Zhu *et al.*, 2015). In the last few years, however, taro cultivation had been increased due to its potential as functional food which has starch content of 70–80 gram/100 gram, protein of 2–6 gram/100 gram, 0.6–0.8 gram/100 gram of fiber, vitamin, phosphorus, magnesium, and calcium (Li *et al.*, 2018; Zhu *et al.*, 2015). Taro could be widely applied in the food industry and converted into products such as pasta, starch, flour, cereal bar, canned product, chips and beverage powder (Li *et al.*, 2018; Muñoz-Cuervo, Malapa, Michaleta, Lebot, & Legendrea, 2016; Sharlina *et al.*, 2017).

Taro starch has a high gelatinization temperature and low breakdown viscosity so that it is relatively more heat-resistant when compared with other tuber starches (Aboubakar *et al.*, 2009). Taro starch has a high setback viscosity, high swelling power, high amylose content, high gel formation strength so that it is more easily retrograded to form a resistant starch structure (Sullivan, Hughes, Cockman, & Small, 2017; Yu *et al.*, 2018). Functional properties of taro starch could be improved by certain physical modification techniques, such as

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the autoclaving-cooling cycle, heat moisture treatment (HMT) and annealing. HMT and autoclaving-cooling cycle techniques were chosen in the taro starch modification process because it was ideal for producing resistant starch type 3 (RS3) (Setiarto *et al.*, 2020). RS3 has potential use as an alternative prebiotic source that can support digestive health by increasing the growth of probiotic bacteria in the colon (Setiarto *et al.*, 2018).

Roberfroid (2007) reported that food can be claimed to have prebiotic properties if it meets the following requirements: a) It is gastric acid-resistant and not hydrolyzed by digestive enzymes; b) It can be a selective substrate for probiotic bacteria growth in the colon; c) It cannot be used for the growth of *Enteropathogenic Escherichia coli*. Evaluation of prebiotic properties in MTS was tested by analyzing its resistance to gastric acid simulated, analysis of prebiotic effects, prebiotic activity and index by measuring the growth of probiotic bacteria (*Lactobacillus plantarum* SU-LS36) and pathogenic bacteria (*Enteropathogenic Escherichia coli*). Generally, the modification of taro starch was conducted to enhance its prebiotic properties as well as its resistant starch content. Setiarto, Jenie, Faridah, Saskiawan, and Sulistiani (2018) reported that modified taro flour with fermentation and autoclaving-cooling two cycle treatment showed improvement in prebiotic properties and a significant increase in resistant starch (RS) levels compared to control treatment. This study aims to analyze the digestibility starch composition and prebiotic properties of modified taro starch by different heat treatments.

2. Materials and Methods

2.1. Materials

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

2.2. Taro (*Colocasia esculanta*) starch extraction

Taro starch extraction was conducted using the modified technique by Airul *et al.* (2014) and Setiarto *et al.* (2020). Taro tuber (*Colocasia esculenta*) 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 (1:3) for one minute using a blender (Phillips, Amsterdam, 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. Taro pulp was centrifuged with High-Speed Centrifuge (Kubota, Tokyo, Japan) at 7,000x *g* for 10 minutes to obtain taro starch. After that, it was dried in the oven at 50 ºC up to the constant weight. Finally, the dry taro starch was ground using the disk mill (Taian City Up International Trade Co. Ltd, Shandong, PR China).

2.3. Modification of taro starch

2.3.1. Annaling treatment

The taro starch annealing treatment was performed by the method from Wang, Reddy, and Xu (2018). Twelve grams of taro starch was poured to 60 ml of distilled water using taro starch: water (1:5) (w/v) ratio was placed in a polyethylene bag. The annealing treatment was conducted by inserting the tightened polyethylene bag into a water bath for 24 hrs at temperature of 50 ºC (Hitachi, Tokyo, Japan). Then it was dried, crushed and sieved using the 100-mesh sieve. The result was then refrigerated at 4 ºC prior to further analysis.

2.3.2. Heat-moisture treatment

The HMT taro starch modification was conducted using the Deka and Sit (2016) method. The dry based taro starch weighted 45 grams was placed into a glass container. It was then poured by the distilled water and stirred until the water content only 25%. It was then tightly closed and left for 48 hrs at room temperature. It was then put in the electric oven (Shimizu, Tokyo, Japan) for three hours at 110 ºC. The result was then desiccated at 40 ºC for overnight, milled, and filtered with a 100-mesh sieve.

2.3.3. Autoclaving-cooling treatment

The autoclaving-cooling of taro starch used the method by Setiarto *et al.* (2018). The aquadest at the 3:1 ratio was poured to the taro starch. It was then heated in an autoclave by Hitachi, Tokyo, Japan at 121 ºC for 15 minutes, and then refrigerated at 4 ºC for 24 hrs. It then was heated at 70 ºC for 16 hrs in an oven (Shimadzu, Tokyo, Japan) until the moisture content up to 12%, and milled using a pin disk mill (Taian City Up International Trade Co. Ltd, Shandong, China). It was filtered to get the 100-mesh starch. The autoclaving-cooling treatment was also conducted by two- and three cycles.

2.4. *In-vitro* digestibility and digestible starch composition analysis

In-vitro digestibility of taro starch was analyzed by measuring the maltose level as the product of hydrolysis taro starch by using α-amylase (Sigma) 100 U compared to starch solution. This analysis was performed by referring to a method from Anderson, Guraya, James, and Salvaggio (2002). Sample absorbance and blank solution were determined by Spectrophotometer UV-Vis (Shimadzu UV-1800, Tokyo, Japan) at 520 nm. In this study, the calculation of the starch digestibility (%) was shown in the following formula:

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

The digestible starch composition analysis was conducted in this study by following Englyst, Kingman, and Cummings (1992) method. There are four types of starch compositions based on their digestibility times. The first type is called very rapidly 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 rapidly digestible starch (RDS) which is the amount of digested starch expelled between 1 minute and 20 minutes. The third type is the slowly 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. The level of glucose within digested supernatant was spectrophotometer UV-Vis (Shimadzu UV-1800, Tokyo, Japan) at 540 nm. The following equations were used in the calculations.

$$\text{VRDS (\%)} = \frac{\text{G1 x 0.9 x F}}{\text{W}} \text{ x 100}$$

$$\text{RRDS (\%)} = \frac{\text{(G20 – G1) x 0.9 x F}}{\text{W}} \text{ x 100}$$

$$\text{SRDS (\%)} = \frac{\text{(G120 – G20) x 0.9 x F}}{\text{W}} \text{ x 100}$$

$$\text{RS (\%)} = 100 - \text{VRDS} - \text{RDS} - \text{SDS}$$

with G1: The absorbance of glucose after 1-minute incubation, G20: The absorbance of glucose after 20-minute incubation, G120: The absorbance of glucose after 120-minute incubation, F: 100/ absorbance, W: sample weight, and 0.9 is used to represent an experimental factor to convert monosaccharaides into polysaccharides.

2.5. Prebiotic properties analysis of modified taro starch

2.5.1. Analysis resistance of MTS against simulated gastric acid

MTS was prepared by dissolving modified taro starch into sterile distilled water (1% w/v). Gastric acid simulated is a hydrochloric acid buffer which per gram/liter contains: NaCl (8g/L); KCl (0.2 g/L); $Na_2HPO_4.2H_2O$ (8.25 g/L); NaH_2PO4 (14.35 g/L); $CaCl_2.2H_2O$ (0.1 g/L); $MgCl_2.6H_2O$ (0.18 g/L). The hydrochloric acid buffer was conditioned at pH 2 using HCl 5 M. A total of 5 ml of HCl buffer for each pH treatment was poured to 5 ml of solution, and then incubated in the water bath at 37 ± 1°C during 6 hours. They were analyzed at 0, 0.5, 1, 2, 4, and 6 hours. The percentage (%) of hydrolysis MTS is calculated using equation according to Korakli, Ganzle, and Vogel (2002):

$$\frac{\text{Reducing sugar content (\%drying base)}}{\text{Total sugar content (\%drying base)}} \text{ x 100\%.}$$

Resistance of MTS (%): 100% - Percentage (%) of hydrolysis MTS

2.5.2. Analysis of prebiotic effect and prebiotic index of MTS

L. plantarum SU-LS36 was cultivated in MRS broth (Oxoid Ltd., Hampshire, England) (1:100) (v/v) and incubated (24 hrs, 37 °C). *L. plantarum* SU-LS36 cell biomass was harvested using a high-speed centrifuge 6500 (Kubota, Tokyo, Japan) (5,000 *g*, 20 minutes, 4 °C) until it reached concentration of *L. plantarum* SU-LS36 cell biomass (10^7 CFU g^{-1}). The analysis of prebiotic effect and prebiotic 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 the 24 hours at 37 °C incubation process, 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 equation:

Prebiotic Effect = Log (cfu/mL) 2.5% taro starch – Log (cfu/mL) m-MRSB

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

2.5.3. Prebiotic activity examination 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, Wehling, and Hutkins (2007). At 0 hour and 24 hours of incubation time, samples were calculated 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 (Tryptone Soy Broth) 2.5% (w/v) of glucose or 2.5% (w/v) taro starch (native, AC-1C, AC-2C, AC-3C, annealing and HMT). The cultures were incubated at 37 °C, and calculated in the TSA medium after 0 hour and 24 hours of incubation times. Prebiotic activity value was calculated using the equation:

Prebiotic Activity Value =

$$\left\{ \frac{N\ log\ \text{(cfu/mL) taro starch t1} - N\ log\ \text{(cfu/mL) taro starch t0}}{N\ log\ \text{(cfu/mL) Glucose t1} - N\ log\ \text{(cfu/mL) Glucose t0}} \right\} -$$

$$\left\{ \frac{E\ log\ \text{(cfu/mL) taro starch t1} - E\ log\ \text{(cfu/mL) taro starch t0}}{E\ log\ \text{(cfu/mL) Glucose t1} - E\ log\ \text{(cfu/mL) Glucose t0}} \right\}$$

with 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), and t_1 = end of incubation time (24 hrs).

2.6. Statistical data analysis

This study used three replications where the statistical analyses were conducted with Completely Randomized Design. The Duncan statistical test was used to calculate the considerable changes at the $p<0.05$ level using SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 *In vitro* digestibility of taro starch

The analysis identified 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 can reduce the digestibility of taro starch consistently. Starch digestible had a negative correlation with RS content. This result was similar to the study by Cheng, Chen, and Yeh (2019), Zheng *et al.* (2018) showed that HMT treatment can reduced the in-vitro digestibility of rice starch. Annealing, HMT treatment, and autoclaving-cooling cycles significantly reduced the in-vitro digestion of taro starch ($p<0.05$) (Table 1). HMT and autoclaving-cooling cycles resulted in 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 (Cheng *et al.*, 2019; Zheng *et al.*, 2018). The retrogradation process with HMT and the autoclaving-cooling cycle technique causes amylose and amylopectin in starches to be bonded together in a double helix to form a solid and stable structure by hydrogen bonds (Sajilata, Rekha, & Puspha, 2006). Modified taro starch which is rich in amylose has greater crystallization ability because of the more intensive re-association of hydrogen bonds (Sajilata *et al.*, 2006).

The increasing number of autoclaving-cooling cycles dropped the in-vitro digestibly of taro starch. The treatment of AC-3C showed the lowest digestibility of taro starch (62.83%) compared to other treatments as shown in Table 1. The annealing, autoclaving cooling cycle, and HMT also decreased the starch in-vitro digestibility due to the retrogradation process, hence increased RS and SDS levels (Shah, Masoodi, Gani, & Ashwar, 2016; Chen, Singh, & Archer, 2018; Lovera, Pérez, & Laurentin, 2017; Cheng *et al.*, 2019; Zheng *et al.*, 2018, Shah *et al.*, 2016, Chen *et al.*, 2018). MTS with high RS content had low in-vitro starch digestibility (Shah *et al.*, 2016).

3.2. Digestible starch composition

Annealing, HMT and autoclaving-cooling cycles reduced the levels of VRDS and RDS considerably in comparison with the native taro starch ($p<0.05$), as shown in Table 1. It shows that 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 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 RDS level (18.54%). The VRDS and RDS of the annealing, HMT and autoclaving-cooling cycle treatments indicated a considerate decrease as their internal structures were changed into the SDS and RS. Annealing, autoclaving-cooling cycles, and HMT can increase significantly of SDS and RS levels in MTS ($p<0.05$) (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 in the highest RS levels (23.62%) (Table 1). SDS and RS levels in MTS with AC-2C treatment were not significantly different from AC-3C, HMT and the three were higher than other treatments (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 hrs, 30% moisture content) in corn, pea, and lentil starch. HMT treatment led to the increase of RS of corn, pea, and lentil up to 7.7, 11.2, and 10.4% respectively (Cheng *et al.*, 2019). The RS content of MTS significantly increased after being treated with annealing, autoclaving-cooling cycle and HMT ($p<0.05$) (Table 1). This result was supported by the study from Setiarto *et al.* (2018), Cheng *et al.* (2019), and Zheng *et al.* (2018).

RS3 oat content increased considerably between 25.81–38.88% after autoclaving-cooling process (Shah *et al.*, 2016). RS increase mainly was caused by the retrogradation process of taro starch because of recrystallization of linear amylose and amylopectin would be linked to each other to build double helix bond, so that it formed a solid and stable structure due to hydrogen bond (Shah *et al.*, 2016). During the autoclaving-cooling process, amylose-lipid complexes formation was increased then causes the high level of resistant starch (Shah *et al.*, 2016).

3.3. Resistance of MTS against simulated gastric acid

Annealing, autoclaving-cooling cycle, and HMT have a significant effect to increase MTS resistance for hydrolysis by simulated gastric acid fluid ($p<0.05$) in Table 2. The HMT and autoclaving-cooling treatment raised the resistance of MTS to simulated gastric acid fluid because it is able to produce high RS content. Native taro starch is very easily hydrolyzed by simulated gastric acid fluid because it has the highest VRDS and RDS content and the lowest RS content. Treatment of simulated gastric acid fluid (pH 2) with a longer incubation time increased hydrolysis of MTS (Table 2). Based on the research it is known that the MTS of HMT, AC-1C, AC-2C, and AC-3C were resistant for more than 87% to hydrolysis by simulated gastric acid during 2 hour incubation (Table 2). Food can be used as a source of prebiotics if it is 85% resistant to gastric acid hydrolysis hence reached the colon to be fermented by bacteria of probiotic (Cummings & Macfarlane, 2002). In another study Wichienchot, Jatupornpipat, and Rastall (2010) reported that prebiotic sources from pitaya oligosaccharides (dragon fruit) can resist 96% to hydrolysis simulated gastric acid. *Gluconobacter oxydans* NCIMB 4943 produced glucooligo saccharides and was resistant of 98.4% to hydrolysis of simulated gastric acid (Wichienchot *et al.*, 2010).

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: Different superscript letters in the column (treatment) showed significant differences at a level of $p<0.05$ after the Duncan statistical test was applied using the SPSS 18.0 statistical software

Table 2. MTS resistance in the simulation of gastric acid pH 2

Treatment	Resistance percentages of MTS towards simulated gastric acid (%) Incubation time (hours)					
	0	0.5	1	2	4	6
Native taro starch	100±0 %^{a}	85.22±0.18%^{c}	71.29±0.11%^{d}	59.08±0.08%^{f}	40.08±0.23%^{g}	25.25±0.08%^{h}
Annealing	100±0 %^{a}	93.25±0.25%^{b}	87.14±0.09%^{c}	72.68±0.13%^{d}	65.77±0.20%^{e}	53.17±0.12%^{f}
AC-1C	100±0 %^{a}	95.42±0.33%^{b}	92.99±0.16%^{b}	88.75±0.15%^{c}	84.89±0.16%^{c}	76.97±0.17%^{d}
AC-2C	100±0 %^{a}	97.23±0.14%^{b}	95.09±0.10%^{b}	90.19±0.08%^{b}	85.51±0.21%^{c}	82.02±0.14%^{c}
AC-3C	100±0 %^{a}	96.62±0.23%^{b}	93.47±0.21%^{b}	87.62±0.07%^{c}	84.60±0.13%^{c}	81.85±0.22%^{c}
HMT	100±0 %^{a}	96.56±0.15%^{b}	92.52±0.17%^{b}	89.04±0.05%^{c}	84.70±0.25%^{c}	80.44±0.18%^{c}

Note: Different superscript letters in the column (treatment) showed significant differences at a level of $p<0.05$ after the Duncan statistical test was applied using the SPSS 18.0 statistical software

3.4. Prebiotic effect and prebiotic index

The prebiotic effect is the increasing number of absolute probiotic bacteria without considering the prebiotic concentration (Roberfroid, 2007; Huebner *et al.*, 2007). Meanwhile, the prebiotic index is probiotic bacteria population increasing correlated with the prebiotic concentration (Huebner *et al.*, 2007; Roberfroid, 2007). *L. plantarum* SU-LS36 has the potential to be applied to probiotics because it has antibacterial activity, survives in conditions of bile tolerance, has low acidity (pH 3), and grows at 45 °C (Sulistiani, 2018). The highest prebiotic effect and prebiotic 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 prebiotic index were conducted directly to the modified taro starch sample to explain its prebiotic properties. Roberfroid (2007) reported that when the food showed more than 1.0 score of prebiotic effect and prebiotic index it can be grouped as a good prebiotic source.

The AC-2C taro starch was a good prebiotic source because it had more than 1.0 score of prebiotic effect (2.45) and prebiotic index (1.96). This value was higher than fructooligosaccharide (FOS), as commercial prebiotic with prebiotic effect (1.85) and prebiotic index (1.48). The RS content in AC-2C taro starch increased the probiotic growth of *L. plantarum* SU-LS 36 (Figure 1). The prebiotic effect and prebiotic index can be increased by isolating the RS from taro starch or consuming the AC-2C taro starch in the larger quantities (20 gram/day) as a functional food. The RS with 20–30 degree of polymerization had an important role as a prebiotic source, therefore it could be fermented to form the short-chain fatty acids in the colon (especially the butyric acid), using probiotic bacteria assistance (Danneskiold-Samsøe *et al.*, 2019). The increase of butyric acid caused a decrease of pH inside the colon. Therefore this condition inhibited the pathogenic bacteria growth then prevented cancer cells proliferation in the colon (Luo *et al.*, 2017; Sullivan *et al.*, 2017).

3.5. 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 as explained by Huebner *et al.*, (2007). The food had a positively of prebiotic activity (over 0.25) if it was selectively metabolized by probiotic bacteria such as *Bifidobacterium* sp., *L. acidophilus*, *L. plantarum* and it was 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 sources (Figure 2). Setiarto *et al.*, (2018) reported that 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 used 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). The 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 MTS

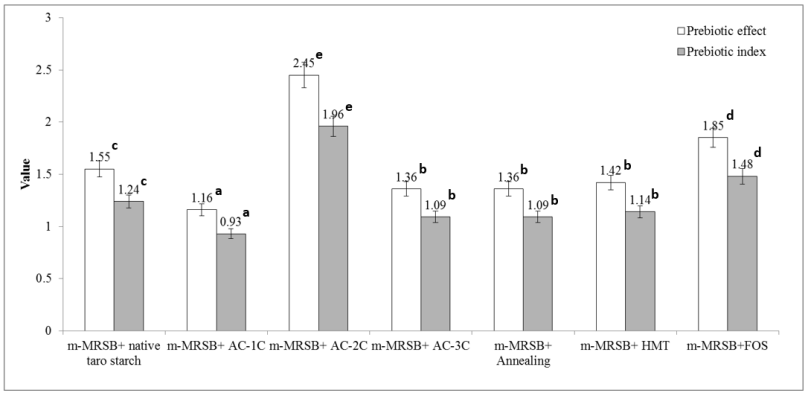

Figure 1. Prebiotic effect and index of native taro starch, modified taro starch by AC-1C, AC-2C, AC-3C, Annealing, and HMT
Note: Different typescript letters on the bar chart indicate where real differences occur at a level of $p<0.05$

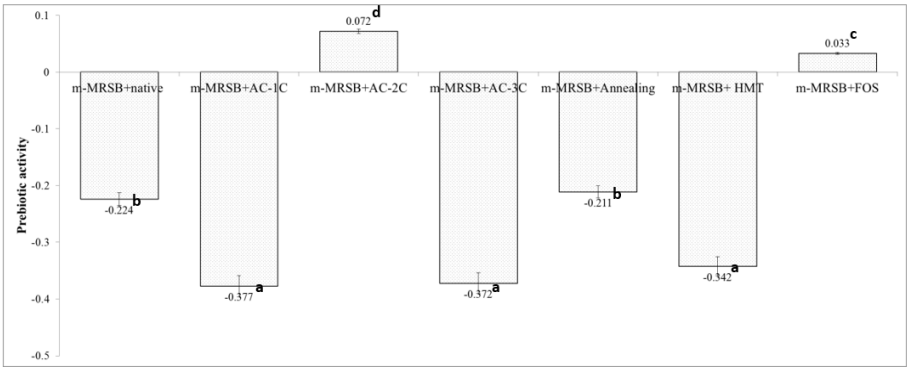

Figure 2. Prebiotic activity of native taro starch, modified taro starch by AC-1C, AC-2C, AC-3C, annealing, and HMT to diarrhea-causal-bacteria, Note: Different typescript letters on the bar chart indicate where real differences occur at a level of $p<0.05$

was the best prebiotic source as it had higher values of prebiotic effect, prebiotic index, and prebiotic 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 of taro starch. MTS with AC-2C showed the potential as a prebiotic source as it indicated from the highest resistance in the simulated gastric acid (90.19%), prebiotic effect (2.45), prebiotic index (1.96) and prebiotic activity (0.072) against *Enteropathogenic Escherichia coli* (EPEC).

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