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Effect of lactic acid bacteria fermentation and autoclaving-cooling for resistant starch and prebiotic properties of modified taro flour

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Abstract

This research aimed to increase the resistant starch (RS) content of taro flour by using Lactic Acid Bacteria (LAB) fermentation and autoclaving-cooling method. Taro chips were fermented by mixed culture of *Lactobacillus plantarum* D-240 and *Leuconostoc mesenteroides* SU-LS 67 (1:1) at 37^{0}C for 18 hr. Fermented taro chips were further autoclaved (121^{0}C, 15 mins) and cooled (4^{0}C, 24 hr). The autoclaving-cooling cycle were performed for one and two cycles. Taro chips were then dried (70^{0}C, 16hr), milled and sieved (80 mesh) to obtain the modified taro flour. Prebiotic properties of modified taro flour were evaluated and expressed as prebiotic effect, index and activity. The results showed that fermentation followed by one cycle autoclaving-cooling increased the RS content by 2.8 fold (from 4.13% to 11.45%) compared with control without fermentation. Fermentation of taro chips could be able to reduce the number of autoclaving-cooling cycle to only one cycle. The modified taro flour demonstrated prebiotic effect, index and activity better than control.

Introduction

Resistant starch (RS) is a starch that is not able to be digested by digestive enzymes and it resistant to gastric acid, and thus had allowed it to reach colon to be fermented by probiotic bacteria (Sajilata *et al*., 2006; Zaragoza *et al*., 2010). RS has advantage as prebiotic in comparison to FOS and inulin, as it is able to bind and retain the water content in feces, so it does not cause the constipation and flatulence if it is consumed in large quantities (Ozturk *et al*., 2011; Vatanasuchart *et al*., 2012). Besides, RS is classified as insoluble fiber source and is able to decrease the glycemic index and cholesterol (Okoniewska and Witwer, 2007), preventing the colon cancer, reducing the forming of gallstone, and helping the absorption of mineral (Lesmes *et al*., 2009). FAO recommended consuming RS as much as 15-20 gram daily to gain the health benefit (Huebner *et al*., 2007). Bogor taro is potentially be utilized as RS source, but the natural RS content in taro is relatively low so it needs to be increased by modification of the processing. Bogor taro, as a local commodity, is available in large quantity (68.000 ton), so it can be processed into rich RS flour (Indonesia Center Agency of Statistics, 2014).

The increase of RS content in some diet leads to the increasing of its prebiotic properties (Ozturk *et al.*, 2011). Bogor taro of pandan variety has the highest amylose content ie 25.78% (Rahmawati *et al.*, 2012). The high amylose content is very important for the increasing of RS content by retrogradation process (Vatanasuchart *et al*., 2010). Efforts to increase the RS content in diet have been extensively conducted. The provision of 5 cycles of autoclaving-cooling increased the RS content up to five folds from 2.12% to 10.91% (Sugiyono *et al*., 2009). The combination of acid hydrolysis and 3 cycles of autoclaving-cooling were also able to increase the RS content in the amount of 5.6 fold (Faridah *et al.*, 2013).

In an effort to reduce the amount of cycles of autoclaving-cooling (AC), Jenie *et al.*, (2012) has applied the fermentation of lactic acid bacteria (LAB) before the heating. This led to a double increase of RS content in Tanduk banana flour (from 5.87-6.45% to 12.99-13.71%). The fermentation of mixed culture of *Lactobacillus plantarum* D-240 and *Leuconostoc mesenteroides* SU-LS 67 in this research decreased the taro starch DP value (Degree of Polymerization) in order to obtain the short chain amylose with the DP 19-29 which would be converted into RS by the autoclaving-cooling treatment. Both LAB isolates were chosen because of their high amylase and pullulanase activity, and it was expected to shorten

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the taro fermentation time. The more of autoclaving-cooling cycle applied can increase the RS content of food materials (Zaragoza *et al.*, 2010). However, this led to the amount of production cost, energy, and longer production time. The fermentation treatment of LAB producing amylase and pullulanase is expected to decrease the amount of autoclaving-cooling cycle that the cost and time production can be more efficient.

The objectives of this research were to improve the RS content of taro flour by fermenting the taro flour with mixed culture of *L. plantarum* D-240 and *Leu. mesenteroides* SU-LS 67 followed by autoclaving-cooling cycle, and to evaluate the prebiotic properties of the taro RS. The evaluation of prebiotic properties in taro flour including the probiotic effect, prebiotic index, and prebiotic activity by measuring the probiotic bacteria growth (*L. acidophilus* and *L. plantarum*) and the pathogenic bacteria EPEC.

Materials and Methods

Materials

The main raw material was Bogor Taro of pandan variety *(Colocasia esculenta)* with the harvest age of 8 months, from Cijeruk, Bogor, West Java. The bacterial cultures of *L. plantarum* D-240 (amylase activity of 2.57 U/mL, pullulanase activity of 2.72U/mL) and *Leu. mesenteroides* SU-LS 67 (amylase activity of 2.50U/mL, pullulanase activity of 2.91U/mL) were used, *L. acidophilus* and EPEC (Entero Pathogenic *Eschericia coli*) were obtained from The Laboratory of Food Microbiology Research Center for Biology, Indonesian Institute of Science.

The preparation of LAB mixed culture

LAB cultures *L. plantarum* D-240 and *Leu. mesenteroides* SU-LS-67 at a ratio of 1:1 as much as 2% (v/v) was inoculated into 50 mL of MRSB medium and incubated at 37°C for 24 hours.

The preparation of taro slices for fermentation

Bogor taro was peeled, washed and sliced into 5 mm thick slices, and soaked in 1% NaCl (3:4) for an hour to remove the oxalate crystals. The taro slices were then washed with distilled water and drained, and ready for fermentation and autoclaving-cooling process.

Fermentation and autoclaving-cooling treatment

The prepared taro slices were treated as follows: fermentation treatment was conducted on the taro slices by inoculating the LAB mixed culture (*Leu. mesenteroides* SU-LS 67: *L. plantarum* D-240) with the ratio of 1:1, containing a total of 108 cfu/mL, 2% (v/v) at the temperature of 37°C for 18 hours. Meanwhile in autoclaving-cooling treatment, the taro chips were added with distilled water at a ratio of 1:2 and then autoclaved (121°C, 15 minutes), followed by cooling it in a refrigerator (4°C, 24 hours). Hereafter, the treated taro chips were dried (70°C, 16 hours) in an oven until the moisture content reached the level of 12%, and then the chips were powdered by using pin disk mill. The powder was sieved to obtain taro flour of 80 mesh. To determine the effect of LAB fermentation and number of cycle for autoclaving-cooling (OC) treatment, the taro flour was grouped. Group A was taro flour without fermentation, i.e.: 1) Code of C (control, without OC); 2) Code of OC-1S (no fermentation, 1 cycle of OC); 3) Code of OC-2S (no fermentation, 2 cycles of OC). Group B was taro flour that was fermented, i.e.: 1) Code of F (fermentation, without OC); 2) Code of FOC-1S (fermentation, with 1 cycle of OC); 3) Code of FOC-2S (fermentation, with 2 cycles of OC).

Chemical analysis of treated taro flour

The six samples of taro flour from the two treatment groups were analyzed in triplicate for total starch, reducing sugar, amylose (Faridah *et al.*, 2013), resistant starch (Goni *et al.,* 1996), total dietary fiber (AOAC, 2005), and the in-vitro digestibility (Anderson *et al*., 2002).

Prebiotic effect and index of MTF (Roberfroid, 2007)

The analysis of prebiotic effect and index was conducted by observing the change in the number of *L. plantarum* and *L. acidophilus* colonies on m-MSRB medium and m-MSRB medium with 2,5% MTF. After incubation for 24 hours at 37°C, the cell cultures were enumerated in MRSA medium. The same procedure was done using commercial prebiotic inulin as positive control.

Prebiotic Effect = Log (cfu/mL) 2,5% MTF - Log (cfu/mL) m-MRSB

$$\text{Prebiotic Index} = \frac{\text{Log}\left(\frac{\text{cfu}}{\text{ml}}\right) 2{,}5\ \%\ \text{MTF} - \text{Log}\left(\frac{\text{cfu}}{\text{ml}}\right) \text{mMRSB}}{\text{Weight MTF}}$$

The examination of MTF prebiotic activity to diarrhea-causal-bacteria (Huebner et al., 2007)

The examination of prebiotic activity was conducted by adding 2% (v/v) of *L. acidophilus* or *L. plantarum* culture into m-MSRB with 2,5% (w/v) of glucose or 2,5% (w/v) of MTF. After 0 hour and 24 hours of incubation time, the samples were enumerated in MRSA medium. The examination was

also conducted towards bacteria that cause diarrhea, EPEC. The EPEC culture of 2% (v/v) was added into different Erlenmeyer containing m-TSB with 2,5% (w/v) of glucose or 2,5% (w/v) MTF. The culture was incubated at 37°C, and enumerated in TSA medium after 0 hour and 24 hours of incubation.

$$\text{Prebiotic Activity Value} = \left\{\frac{(N\log\frac{cfu}{mL}\,\text{MTF}\,t1 - N\log\frac{cfu}{mL}\,\text{MTF}\,to)}{N\log\frac{cfu}{mL}\,\text{Glukosa}\,t1 - N\log\frac{cfu}{mL}\,\text{Glukosa}\,to)}\right\} - \left\{\frac{(E\log\frac{cfu}{mL}\,\text{MTF}\,t1 - E\log\frac{cfu}{mL}\,\text{MTF}\,to)}{E\log\frac{cfu}{mL}\,\text{Glukosa}\,t1 - E\log\frac{cfu}{mL}\,\text{Glukosa}\,to)}\right\}$$

Note :

N = number of probiotic bacteria (log cfu/mL)

t_0 = start of incubation time (0 hour)

E = number of EPEC (log cfu/mL)

t_1 = end of incubation time (24 hour)

Statistical analysis

Data was analyzed by using the procedure of Analysis of Variance (ANOVA) with software of SPSS 17.0. To determine the difference between treatments, the least significant different (LSD) with $p \leq 0.05$ was performed.

Results and Discussion

Total starch content

Fermentation, autoclaving-cooling, and the combination of fermentation and autoclaving-cooling treatment significantly affected the total starch content (Table 1) in taro samples. Fermentation treatment (F) significantly affected the total starch content compared to the control sample (C). The decreasing of total starch content in fermented taro flour was caused by the activity of amylase (2.53U/mL) and pululanase (2.81U/mL) from the mixed culture of *L. plantarum* D-240 and *Leu. mesenteroides* SU-LS 67 in hydrolyzing the starch component during the fermentation. The amylase hydrolyzes linear bond of α-1,4 glycosidic in amylose randomly, resulting in dextrin, maltose, and glucose mixture. Meanwhile Vatanasuchart *et al.* (2010) reported that pullulanase hydrolyzed the branch bond of α-1,6 amylopectin in banana starch.

Both OC-1S or OC-2S treatments were differed significantly ($p<0.05$) in the total starch content (Table 1). Autoclaving caused the increasing of starch degradation on the taro chips which led to the increasing of starch damage. The degradation where starch fraction hydrogen bond (amylose and amylopectin) was broken caused by autoclave heating also happened to cassava starch (Zaragoza *et al.*, 2010; Vatanasuchart *et al.*, 2012). Combination of FOC-1S and FOC-2S treatments were differed significantly ($p<0.05$) to the decreasing of total starch. Starch hydrolysis to amylose and amylopectin during fermentation, plus the degradation of starch during the process of autoclave-heating caused the decreasing of total starch content in higher amount. Jenie *et al*. (2012) reported that fermentation process and autoclaving-cooling cycle caused the decreasing of total starch of banana flour. The decreasing of total starch content led to the increasing of reducing sugar and the decreasing of amylose content.

Table 1. The analysis result of total starch, reducing sugar, amylosa and amylopectin content of modified taro flour

Taro flour samples	Total Starch (% bk)	Reducing Sugar (%bk)	Amylosa (% bk)	Amylopectin (% bk)
Without fermentation				
C	80.17±0.60^a	8.75±0.24^a	25.18±0.06^a	58.06±0.68^a
OC-1S	78.34±0.20^b	10.41±0.16^b	27.17±0.06^b	53.21±0.27^b
OC-2S	76.94±0.32^c	12.21±0.75^c	29.99±0.35^c	50.57±0.25^c
With fermentation				
F	75.06±0.58^d	18.57±0.63^d	21.56±0.14^d	55.03±0.84^d
FOC-1S	74.85±0.21^d	15.00±0.21^e	20.63±0.09^e	57.05±0.18^e
FOC-2S	73.84±0.26^e	16.32±0.21^f	22.54±0.14^f	53.77±0.13^b

Note: within columns, followed by a common letter do not differ significantly ($p<0.05$) after statistical test with LSD in SPSS 17.0

Reducing sugar content

Fermentation treatment, autoclaving-cooling, and combination of both were differed significantly ($p<0.05$) in the increasing of reducing sugar in taro flour (Table 1). The increasing of reducing sugar caused the decreasing of total starch and amylopectin content (Table 1). Jenie *et al.* (2012) and Nurhayati *et al*. (2014) reported that the increasing of reducing sugar in Tanduk Banana flour more affected by fermentation rather than by autoclaving-cooling. Amylase and pullulunase resulted during the LAB fermentation would hydrolyze the banana starch into reducing sugar, glucose and maltose (Vatanasuchart *et al*., 2010). The increasing of reducing sugar was also affected by the increasing of short chain amylose which was measured as reducing sugar. The increasing of short chain amylose led the increasing of reducing end measured as reducing sugar (Zaragoza *et al*., 2010; Moongngarm, 2013).

Table 2. The analysis result of in vitro digestibility, resistant starch, insoluble dietary fiber, soluble dietary fiber, and total dietary fiber content of modified taro flour

Taro flour samples	*In vitro* digestibility (%)	insoluble (% dietary fiber content bk)	Soluble dietary fiber content (% bk)	Total dietary fiber (% bk)	Resistant Starch (% bk)
Without fermentation					
C	56.42±0.70[a]	5.08±0.01[a]	2.14±0.04[a]	7.21±0.02[a]	4.13±0.05[a]
OC-1S	48.72±0.39[b]	6.75±0.28[b]	3.21±0.11[b]	9.95±0.17[b]	7.92±0.18[b]
OC-2S	32.90±0.30[c]	11.09±0.25[c]	3.70±0.36[c]	14.78±0.61[c]	11.15±0.11[c]
With fermentation					
F	64.93±0.65[d]	3.58±0.21[d]	1.77±0.14[d]	5.35±0.35[d]	3.82±0.11[a]
FOC-1S	35.64±0.48[c]	10.45±0.09[c]	3.83±0.46[c]	14.28±0.55[c]	11.45±0.04[c]
FOC-2S	32.38±0.73[c]	10.46±0.44[c]	3.77±0.02[c]	14.23±0.42[c]	11.76±0.12[c]

Note: within columns, followed by a common letter do not differ significantly ($p<0.05$) after statistical test with LSD in SPSS 17.0

Amylose and amylopectin content

The treatment of OC-1S or OC-2S were differ significantly ($p<0.05$) in increasing the amylose content of taro flour compared with the control. This increasing of amylose caused the amylopectin content decreased significantly ($p<0.05$) (Table 1). It happened as the autoclave-heating caused some of starch fraction (amylose and amylopectin) hydrogen bond unbinded that the amylopectin structure changed from branch to linear (Zaragoza et al., 2010). Amylopectin linearization during the autoclave-heating caused the increasing of amylose content and the decreasing of amylopectin content (Moongngarm, 2013). The fermentation treatment (F), combination of FOC-1S or FOC-2S in taro flour were differ significantly ($p<0.05$) in decreasing the amylose or amylopectin content (Table 1). The main causal factors of amylose and amylopectin content decreasing on the treatment were amylase and pullulanase from mixed culture of LAB which hydrolyzed the amylose and amylopectin during the fermentation, as occurred in banana flour (Jenie *et al*., 2012; Nurhayati *et al.*, 2014).

Resistant starch content

The fermentation treatment (F) did not significantly affect ($p>0.05$) RS content in fermented flour compared with control (C) (Table 2). The decreasing of RS content during fermentation caused by natural hydrolysis of resistant starch, RS2, which is utilized as carbon source for LAB growth (Nurhayati *et al.*, 2014). The first step research (in-press) showed that taro fermentation with mixed culture (*L. plantarum* D-240 and *Leu. mesenteroides* SU-LS 67) for 18 hours resulted in DP value of 27.13 which qualified to form the RS (DP 19-29) compared with single culture *L. plantarum* D-240 (DP value of 34.37) or *Leu. mesenteroides* SU-LS 67 (DP value of 31.27).

RS content significantly increased ($p<0.05$) after being treated with autoclaving-cooling treatment. This result was appropriate to Sugiyono *et al.* (2009) and Faridah *et al.* (2013) research on garut starch and to Jenie *et al.* (2012) and Nurhayati *et al.* (2014) research on banana flour. RS content on treatment of OC-1S increased as many as 1.9-fold. Meanwhile treatment of OC-2S increased the RS content more up to 2.7-fold (Table 2). RS increase mainly was caused by the retrogradation of taro flour. During retrogradation, starch molecules such as amylose and amylopectin would be linked to each other in return as double helix that it formed a solid and stable structure due to hydrogen bond (Sajilata *et al.*, 2006; Vatanasuchart *et al.*, 2012). Taro flour with FOC-1S treatment increased the RS content significantly to 2.8-fold compared with control (C) (Table 2). Fermentation process followed by 2 cycles of autoclaving-cooling (FOC-2S) resulted in RS content which did not differ significantly ($p>0.05$) compared with FOC-1S. The FOC-1S treatment was chosen because it was proven to reduce autoclaving-cooling cycle and kept improving the RS content of taro flour.

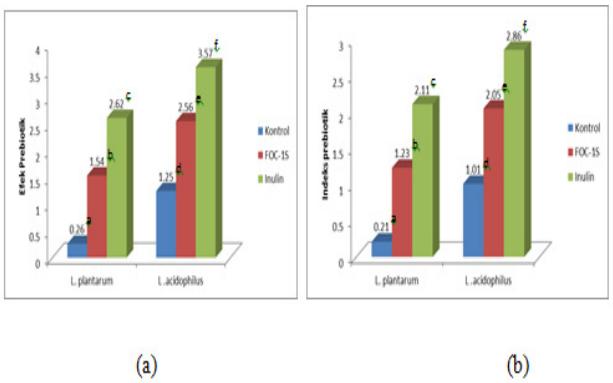

Figure 1. (a) Prebiotic effect, (b) Prebiotic index of Modified Taro Flour (MTF) by Probiotic Bacteria *L. plantarum* and *L. acidophilus*

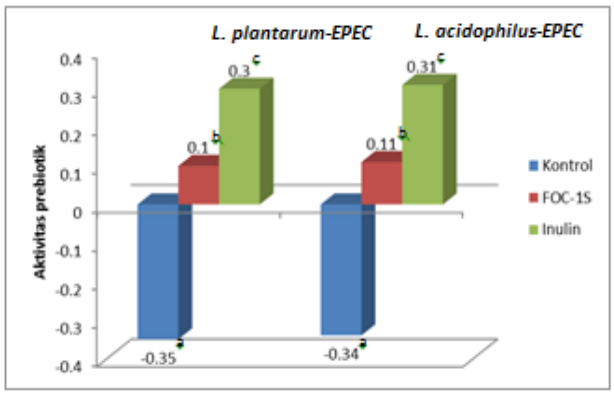

Figure 2. The Prebiotic Activity of Modified Taro Flour (MTF) to *Lactobacillus plantarum*-EPEC and *Lactobacillus acidophilus*-EPEC

Dietary fiber content

The result showed that insoluble dietary fiber composition was higher than the soluble one (Table 2). The increasing of dietary fiber content after the autoclaving-cooling treatment was affected by RS content increasing (Table 2). It was appropriate with Jenie *et al*. (2012) and Nurhayati *et al.* (2014) research on banana flour. As known that RS is classified as insoluble dietary fiber (Zaragoza *et al.,* 2010). The treatment of OC-2S, FOC-1S, and FOC-2S gave the highest total dietary fiber content. The three treatments differed significantly ($p<0.05$) for increasing total dietary fiber twice compared with control (Table 2). The fermentation treatment (F) was differ significantly ($p<0.05$) for decreasing total dietary fiber. Decreasing of the dietary fiber occurred because during taro fermentation, LAB was indicated able to produce cellulase and pullulanase enzyme which are able to hydrolyze fiber component (Lesmes *et al.*, 2009). The main dietary fiber contents in taro are hemicellulose, inulin, raffinose, verbascose, and cellulose (Mbofung *et al*., 2006).

The in vitro *digestibility*

Fermentation treatment (F) was differ significantly ($p<0.05$) increased the taro flour digestibility compared with control (C) (Table 2). The increasing of digestibility in fermented taro flour (F) was caused by taro starch hydrolysis performed by amylase and pullulanase that produced more digestible short chain amylose, oligosaccharide, maltose, maltotriose, and glucose with high glycemic index. Fermented taro flour (F) could be consumed as it is easily be digested and adsorbed by human body as energy source. Otherwise, OC-1S, OC-2S, FOC-1S, and FOC-2S treatments were differ significantly ($p<0.05$) in decreasing taro flour digestibility compared with control (Table 2). The digestibility decrease in OC treatment was related to the increasing of resistant starch and dietary fiber content due to retrogradation process as in Vatanasuchart *et al*. (2012) research on banana flour and Faridah *et al.* (2013) research on garut starch. This taro flour was an excellent prebiotic source with low glycemic index.

The effect and index of prebiotic

Prebiotic effect is the increasing of probiotic bacteria absolutely without considering the prebiotic concentration. Meanwhile prebiotic index is the increasing of probiotic bacteria population correlated with prebiotic concentration (Roberfroid, 2007). Diet prebiotic effect increasing was differ significantly ($p<0.05$) to the increasing of its prebiotic index as Roberfroid (2007) reported. The highest prebiotic effect and index showed by *L. acidophilus* and *L. plantarum* grown on inulin (Figure 1a and 1b). It occurred as inulin has lower DP value than RS in taro flour FOC-1S does that it is easier utilized for probiotic bacteria growth (Vrese and Marteau, 2007). The examination on prebiotic effect and index were conducted directly to the taro flour sample to explain its prebiotic properties.

Vrese and Marteau (2007) reported that a diet confirmed as a good prebiotic source if it has prebiotic effect and index value over 2.0. Taro flour of FOC-1S was a good prebiotic source as it had prebiotic effect and index value over 2.0 and was higher compared with control. The resistant starch content in taro flour of FOC-1S was able to increase the probiotic growth of *L. acidophilus* and *L. plantarum*. The increasing of prebiotic effect and index could be conducted by isolating the RS from taro flour or consuming the taro flour of FOC-1S in larger quantities (20 gram/day) by applying it as functional diet. *L. acidophilus* had a higher prebiotic effect and index than *L. plantarum* (Jenie *et al*., 2012). It showed that *L. acidophilus* was easier to be grown in prebiotic medium that it is important to increase the number of probiotic

bacteria in colon to keep digestion healthy (Huebner *et al*., 2007), (Lesmes *et al*., 2009).

The prebiotic activity towards diarrhea causal bacteria

The prebiotic activity is prebiotic ability to help the growth of probiotic bacteria which is related to its selectivity towards pathogenic bacteria and compared with glucose (Lesmes *et al*., 2009). A diet will has positive prebiotic activity (over 0.25) if it is metabolized selectively by probiotic bacteria such *L. acidophilus* and *L. plantarum*, but it is not metabolized by pathogenic bacteria such EPEC (Vrese and Marteau, 2007). According to the result, it was known that inulin as commercial prebiotic has the highest prebiotic activity and positive when it was used as growth medium of *L. plantarum*-EPEC (0.30) or *L. acidophilus*-EPEC (0.31). Positive prebiotic activity also produced by taro flour FOC-1S used as growth medium of *L. plantarum*-EPEC (0.10) or *L. acidophilus*-EPEC (0.11) (Figure 2). It showed that taro flour of FOC-1S could be used as prebiotic source because it had higher effect, index, and activity of prebiotic than control treatment (Figure 2).

Conclusion

Taro fermentation with mixed culture of *L. plantarum* D-240 and *Leu. mesenteroides* SU-LS 67 (1:1) (18 hours, 37^0C) followed by one cycle of autoclaving-cooling treatment (FOC-1S) was able to increase the RS content to 2,8 fold compared with control treatment. The treatment of FOC-1S was also proven to reduce amount of autoclaving-cooling cycle applied and producing the RS content which was equal to RS content in OC-2S or FOC-2S. Modified taro flour resulted had better prebiotic effect, index, and activity than control treatment without fermentation and autoclaving-cooling treatment. Advance research is required to be conducted for analyzing the oligosaccharide content in taro flour. Meanwhile, to obtain the prebiotic properties from taro flour RS, its prebiotic properties with the RS isolate need to be evaluated.

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