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CARBOHYDRATE DEGRADATION OF TUBER PASTE FLOUR BY THE ADDITION OF α-AMYLASE FROM TWO *Lactobacillus* SPECIES

[Penguraian Karbohidrat Tepung Pasta Umbi dengan Penambahan α-Amilase dari Dua Spesies Lactobacillus]

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ABSTRACT

The quality of Indonesia tuber flour can be improved by α-amylases which hydrolyzes the flour amylose to glucose and maltose. These monosaccharides causes the flour to have better homogeniety similar to wheat flour and easier to digest. This research aimed at investigating carbohydrate degradation of tuber paste flour by the addition of α-amylase from two *Lactobacillus* species. *Lactobacillus* species used were *Lactobacillus bulgaricus* and *L. plantarum* B110, while the flour types were made of local taro (*Colocasia esculenta*), *gadung* (*Dioscorea hispida*) and sweet potato (*Ipomoea batatas*), as well as wheat (*Triticum*) as a reference. Crude α-amylase activity and reducing sugars were detected by the Dinitrosalycylic acid (DNS) method. Data were statistically analyzed with ANOVA. Research results indicated that α-amylase from *L. bulgaricus* and *L. plantarum* B110 have been characterized for their optimum activity and stabilitiy. The reducing sugar content in taro, *gadung*, sweet potato paste flour and wheat paste flour added with α-amylase of *L. bulgaricus* increased by 0.008, 0.006, 0.004 and 0.001%, respectively. Meanwhile, the reducing sugars of the above flours added with amylase from *L. plantarum* B110, increased by 0.008, 0.008, 0.008, and 0.003%, respectively. Increase in reducing sugar contents in carbohydrate degradation of local tuber paste flour added with *L. bulgaricus* α-amylases was higher than that in wheat paste flour with a 0.001% increase. Similarly, the 0.008% increase of sugar content in tuber paste added with *L. plantarum* B110 α-amylase was also higher than that in wheat flour with 0.003% increase. Therefore, local tuber paste flour can be used as an alternative to wheat paste flour.

Keywords: α-amylase, *Lactobacillus bulgaricus*, *L. plantarum* B110, local tube paste flour, reducing sugar

ABSTRAK

Tepung umbi Indonesia meningkat kualitasnya dengan menggunakan α-amilase yang menghidrolisa tepung amilosa menjadi glukosa dan maltosa. Monosakarida ini menyebabkan tepung dengan homogenitas lebih baik hampir seperti tepung terigu dan lebih mudah dicerna. Penelitian ini ditujukan pada penguraian karbohidrat tepung pasta umbi dengan penambahan α-amilase dari dua spesies Lactobacillus. Lactobacillus yang digunakan adalah Lactobacillus bulgaricus dan L. plantarum B110, sedangkan tepungnya adalah tepung lokal taro (*Colocasia esculenta*)*, gadung* (*Dioscorea hispida*)*, ubi jalar* (*Ipomoea batatas*) *dan* terigu (*Triticum*) *yang digunakan sebagai pembanding. Aktivitas α-amilase kasar dan gula reduksi dideteksi dengan metoda Dinitrosalicylic Acid (DNS). Data dianalis secara statistik de-ngan ANOVA. Hasil penelitian menunjukkan bahwa α-amilase dari L. bulgaricus dan L. plantarum B110 su-dah terkarakterisi aktivitas optimum dan stabilitasnya. Kandungan gula reduksi pada tepung pasta taro, gadung, ubi jalar dan terigu dengan penambahan α-amilase L. bulgaricus terkarakterisasi meningkat secara berurutan sebesar 0,008; 0,006; 0,004; dan 0,01%, sedangkan pada L. plantarum B110 meningkat 0,008; 0,008; 0,008; dan 0,003%. Berdasarkan hasil penelitian ini dapat disimpulkan bahwa peningkatan kandungan gula reduksi sebesar 0,004-0,008% hasil degradasi karbohidrat pada tepung pasta umbi lokal dengan penambahan α-amylase L. bulgaricus lebih tinggi dibandingkan pada tepung pasta terigu yang peningkatannya sebesar 0,001%, sedangkan peningkatan gula reduksi sebesar 0,008% pada tepung pasta umbi dengan α-amylase L. plantarum B110 lebih tinggi dibandingkan pada tepung terigu dengan peningkatan sebesar 0,003%, sehingga tepung pasta umbi lokal dapat digunakan sebagai alternatif tepung pasta terigu.*

Kata kunci: *α-amilase, gula reduksi, Lactobacillus bulgaricus, L. plantarum B110, tepung pasta umbi lokal*

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INTRODUCTION

Indonesian local tuber flour in both powder and paste improves in homogenecity almost like wheat flour by using α-amylase to make the flour able to be digested more easily by human intestine. The quality of tuber flour can be improved by the addition of α-amylase. Tuber paste flour were made by using local tubers of taro or *Colocasia esculenta*, *gadung* or *Dioscorea hispida* and sweet potato or *Ipomoea batatas*, and those pasta flour can be used as basic material for producing baby food and many types of tuber cakes.

α-Amylase was produced from bacteria (Moradi *et al.,* 2014) including lactic acid bacteria (LAB). It was reported that there were several types of amylolytic lactic acid bacteria, mainly: *L. plantarum* S 21, *L. fermentum* 04BBA19 and *L. fermentum* Ogi E1 (Fossi *et al.,* 2014; Kapienjai *et al.,* 2015; Santoyo *et al.,* 2003). The addition of α-amylase cause flour to increase in quality (Santoyo *et al.,* 2003; Songré-Ouattara *et al.,* 2009). The α-amylase in flour catalysed amylose to monosaccharides of glucose and maltose (Sharma and Satyanarayana, 2013; Songré-Ouattara *et al.,* 2009). The addition of α-amylase to flour from LAB which hidrolyses amylose to glucose and maltose were more digestible in human intestine (Singh *et al.,* 2015; Kapienjai *et al.,* 2015).

The glucose and maltose contents in flour by the addition of α-amylase depend on the type of flour and the α-amylase concentration used (Savitri and Bhalla, 2013; Kapienjai *et al.,* 2015). Different type of tuber flour contain different amylose concentration (do Esperito-Santo *et al.,* 2014; Songré-Ouattara *et al.,* 2009). Different concentration of α-amylase resulted in diferent amylose hidrolysis by α-amylase to glucose and maltose (Savitri and Bhalla, 2013; Songré-Ouattara *et al.,* 2009).

The contents of glucose and maltose in the tuber paste flour by the addition of α-amylase from *Lactobacillus bulgaricus* and *Lactobacillus plantarum* B110 were not known yet. To make tuber flour increase in homogenecity, the tuber flour could be added with α-amylase from lactic acid bacteria as safe bacteria, with the wheat flour used as a comparison. This research aimed at investigating the carbohydrate degradation of tuber paste flour by the addition of α-amylases from two *Lactobacillus* species namely *Lactobacillus bulgaricus* and *Lactobacillus plantarum* B110.

MATERIALS AND METHODS

Materials

Materials used were flour of taro or *Colocasia esculenta* from farmers at Ratu-Sukabumi harbour, *gadung* or *Dioscorea hispida* and sweet potato or *Ipomoea batatas* from farmers in Simpenan Sukabumi with commercial wheat or *Triticum* (Segitiga Biru, Indonesia) as comparison. *L. plantarum* B110 indigenous as indigenous lactic acid bacteria (LAB) identified molecularly was isolated from traditional fermented vegetable in Bogor, and *Lactobacillus bulgaricus* was obtained from Microbial Culture Collection, Research Centre for Biology, Indonesian Institute of Sciences.

Subculture *Lactobacillus plantarum* B110 Indigenous and *Lactobacillus bulgaricus*

L. plantarum B110 as indigenous lactic acid bacteria (LAB) was identified molecularly and isolated from traditional fermented vegetable in Bogor, with *Lactobacillus bulgaricus* from the Microbial Culture Collection, Microbiology Division, Research Centre for Biology, Indonesian Institute of Sciences. The subcultures of those lactic acid bacteria used *de mann rogosa sharpe* (MRS) media which contain 1% of peptone (Bacto TM211677, United States), 0.8% of beef extract (Himedia RM002-500G, Germany), 0.4% of yeast extract (Bacto TM 212750, United States), 1% of glucose (Merck 1.08337.1000, Germany), 0.1% of tween 80 (Merck 8.22187.0500, Germany), 0.5% of natrium acetate (Merck 1.06268. 0250, Germany), 0.2% of triamonium citrate (Sigma A1332-100G, United States), 0.02% of magnesium sulphate monohydrate (Merck 1.05886.0500, Germany), 0.005% of mangan sulphate tetrahidrate (Merck 1.02786.1000, Germany), and 0.2% of dinatrium hydrogen phosphate dehydrate (Merck 1.0658 0.0500, Germany). The subcultured *L. plantarum* and *L. bulgaricus* were then incubated at 37°C in an incubator (Isuzu incubator Himawari, Y3556528677, Japan).

Tube paste flour

The three local tuber flour of taro or *Colocasia esculenta*, *gadung* or *Dioscorea hispida* and sweet potato or *Ipomoea batatas* were used as materials in production of tube paste flour, and as a comparison, wheat or *Triticum* was used. The tube flour was heated at 70°C, 10 minutes to form paste flour.

Carbohydrate degradation of wheat and local tube paste flour by the addition of α-amylase

The 5 g of each tube flour (wheat, taro, *gadung* and sweat potato) was soluted in 50 mL of aquades, heated, homogenised by thermomagnetic stirrer (Sibata MGH-320, Japan) at 70°C for 10 minutes to form paste flour, added with 1U/mL for each *L. bulgaricus* and *L. plantarum* B110 crude amylase, and incubated in a rotary shaker (V-Tech VTRS-1, Model: Platform Size CM, India) at 37°C for 24 hours.

α-Amylase isolation (Sharma and Satyanarayana, 2013)

Each of *L. plantarum* B110 and *L. bulgaricus* suspension was subcultured into 50 mL of MRSB media and incubated at 37°C for 24 hours in an incubator. *L plantarum* B110 or *L. bulgaricus* crude α-amylase were isolated by subculturing 2% of lactic acid bacteria in 25 mL of sterilised glucose MRSB media (Merck, Germany) was modified with 2% of soluble starch (Merck, Germany) 6 pH, and the incubation was carried out for 24 hours at 37°C by using an incubator, and centrifuged in 9000 rpm for 10 minutes at 4°C (Kubota 5910, Japan). Each crude α-amylase was then tested to investigate its α-amylase activity.

Activity of α-amylase (Bernfeld, 1955)

The activity of α-amylase was measured by using a DNS method. The 50 µl crude α-amylase was mixed with 50 µl of 1% of soluble starch with 5.0-8.0 pH, homogenised by vortex (Sibata MGH-320), incubated in waterbath (Memmert, Japan) at 35-65°C for 10 minutes, added with 100 µl DNS reagen (Sigma D0550-100G, United States), homogenised, heated at 100°C for 5 minutes, poured with 800 µl of aquades and revortexed. After the solution cooled, the absorbance was read at λ540 by using spectrophotometer UV-Vis (Shimadzu UV-1700 Pharmaspec, Japan). The α-amylase activity unit was measured as the amount of enzyme in which the reaction produced the same product with 1 µmol glucose per minute at the condition measured.

α-Amylase activity optimisation in various pH and temperatures (Wang *et al.,* 2018)

Optimisation of α-amylase from *L. bulgaricus* and *L. plantarum* B110 in various pH detected by pH meter (Horiba pH 1100 Scientific, Japan), at incubation time for 10 minutes was conducted at various pH of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The highest α-amylase activity at certain pH indicated the α-amylase optimum activity. Optimisation of those α-amylase activities in various temperatures in 10 minutes incubation was conducted at various temperatures of 35, 40, 45, 50, 55, 60 and 65°C. The highest α-amylase activity at certain temperature indicated the α-amylase optimum activity.

Stability of α-amylase in various pH and temperatures (Sharma and Satyanarayana, 2013 modified)

The α-amylase stability from *L. bulgaricus* and *L. plantarum* B110 was detected by measuring the relative activity of α-amylase in various pH of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 in 60 minute incubation time, at the temperatures of 50°C. The ≥ 50% relative activity of α-amylase was defined as stability of the α-amylase at a certain pH range. Stability of the two α-amylases was also investigated by measuring α-amylase relative activity at various temperatures of 35, 40, 45, 50, 55, 60 and 65°C, with 5.5 pH. The ≥ 50% α-amylase relative activity was defined as stability of α-amylase at a certain temperature range.

Reducing sugar (Miller, 1959)

Reducing sugar was measured by using a DNS method. Reducing sugar (%) was measured by using the standard curve equation of glucose solution. Carbohydrate degradation in tube flour of wheat, taro, *gadung*, and sweet potato (with or without the addition of *L. bulgaricus* or *L. plantarum* B110 crude α-amylase) was centrifuged for 10 minutes at 9000 rpm at 4°C. The 100 µl of tube flour treated was poured into 100 µl DNS reagent, homogenised, and the mixture was heated at 100°C for 5 minutes, poured into 800 µl aquadest, and revortexed. The mixture was then left in a minute, untill the absorbance was read at λ: 540 by using spectrophotometer UV-Vis.

$$\text{RSD (\%)} = \frac{\text{glucose concentration (mg/mL)}}{\text{sample weight (mg)}} \text{ x Volume of reaction total (mL) x100\% ..... (1)}$$

where, RDS= Reducing sugar concentration.

Statistical analysis

Data were statistically analysed using analysis of variance (ANOVA) with completely randomised design (CRD), and further analysis was done using the Duncan test to compare the effects of each treatment. Data analysis was conducted by using SPSS 16.0.

RESULTS AND DISCUSSION

α-Amylase activity optimisation

The results of this research show that *L. bulgaricus* α-amylase activity at pH 5.0-8.0 had a value of 0.243-0.539 U/mL and the *L. bulgaricus* α-amylase optimum activity was reached at 50°C with a value of 0.539 U/mL, with 6 pH, 0.539 U/mL (P<0.05), while the *L. plantarum* B110 α-amylase had a value of 0.403-0.641 U/mL and the *L. plantarum* B110 α-amylase optimum activity was 50°C with a value of 0.533 U/mL, with 7.0 pH, 0.641 U/mL (P<0.05) (Table 1).

Table 1. The activities of α-amylase from *L. bulgaricus* and *L. plantarum* B110 at various pH and temperatures

No.	pH	Activity of α-Amylase (U/mL)		Temperature (°C)	Activity of α-Amylase (U/mL)	
		L. bulgaricus	*L. plantarum* B110		*L. bulgaricus*	*L. plantarum* B110
1	5.0	0.243^{a}±0.018	0.403^{a}±0,278	35	0.254^{a}±0.029	0.214^{b}±0.178
2	5.5	0.468^{b}±0.180	0.439^{a}±0,151	40	0.277^{a}±0.055	0.262^{b}±0.117
3	6.0	0.539^{b}±0.040	0.533^{a}±0.011	45	0.371^{b}±0.084	0.293^{ab}±0.074
4	6.5	0.374^{c}±0.074	0.578^{a}±0.062	50	0.539^{c}±0.041	0.533^{a}±0.011
5	7.0	0.371^{c}±0.191	0.641^{a}±0.421	55	0.280^{a}±0.023	0.227^{b}±0.086
6	7.5	0.243^{a}±0.005	0.505^{a}±0.386	60	0.270^{a}±0.031	0.174^{b}±0.050
7	8.0	0.240^{a}±0.013	0.419^{a}±0.238	65	0.251^{a}±0.025	0.144^{b}±0.036

Note: The different letters in the same column show a significant difference (P<0.05)

The difference in optimum α-amylase activity at different pH and temperature levels between *L. bulgaricus* and *L. plantarum* B110 α-amylases was caused by different species producing α-amylase of the two lactic acid bacteria. It was reported that the different amylolytic lactic acid bacteria species might have resulted in different α-amylase optimum activities of the two bacteria (Santoyo *et al.,* 2003; Tallapragada *et al.,* 2018).

Stability of α-amylase

The activity of *L. bulgaricus* α-amylase at 60 minute incubation time with pH in the range of 5.0-8.0 had a value of 0.044-0.123 U/mL and the relative activity of α-amylases was in the range of 35.684-100% (Table 2); while at the temperature ranging between 35-65°C, it was 0.05-0.08 U/mL with the α-amylase relative activity was in the range of 54.57-100% (Table 3). The stability of *L. bulgaricus* α-amylase with ≥50% α-amylase relative activity in 60 minute incubation time was reached at pH ranging between 5.0-7.0 (0.061-0.123 U/mL) with the relative activities were 50.019-100% (Table 2), while that at temperature in the range of 35-65°C (0.046-0.084 U/mL) with the relative activity between 54.571-100% (Table 3).

The activity of *L. plantarum* B110 α-amylase at 5.0-8.0 pH within 60 minute incubation time was in the range of 0.059-0.117 U/mL with α-amylase relative activity was 50.43-100% (Table 2), while at the temperature of 35-55°C, it was between 0.031-0.100 U/mL with the relative activity between 31.000-100% (Table 3).

The stability of *L. plantarum* B110 α-amylase with relative activity ≥50% in 60 minute incubation time was reached at pH in the range of 5.0-8.0 (0.059-0.117 U/mL) with α-amylase relative activity around 50.43-100% (Table 2), while at the temperature in the range of 35-55°C, it was 0.055-0.100 U/mL with relative activity between 55.000-100% (Table 3).

The different α-amylase stabilities measured based on their relative activity at the range of certain pH and temperatures of α-amylase from *L. bulgaricus* and *L. plantarum* B110 were caused by the different optimum activity of α-amylase from the two lactic acid bacteria species. It was reported that the different species of lactic acid bacteria producing α-amylase might have resulted in the different optimum α-amylase activities from the two lactic acid bacteria species (Kanpiengjai *et al.,* 2015; Santoyo *et al.,* 2003; Shongre-Quottara *et al.,* 2009).

Reducing sugar of wheat and local tube paste flour by the addition of α-amylase

The reducing sugar content of the paste flour of sweet potato, gadung, and taro by the addition of α-amylase from *L. bulgaricus* increased by 0.008, 0.006 and 0.004%, respectively (Table 4), while that of the three paste flours by the addition of α-amylase from *L. plantarum* B110 increased by 0.008% (Table 4). The reducing sugar content of wheat paste flour by the addition of α-amylase from *L. bulgaricus* increased 0.001% (Table 4), while the reducing sugar content of wheat paste flour by the addition of α-amylase from *L. plantarum* B110 increased by 0.003% (Table 4).

The reducing sugar content of paste flour from sweet potato, gadung and taro by the addition of α-amylase from *L. bulgaricus* which increase by 0.008, 0.006, and 0.004%, respectively was higher than that of wheat paste flour which increase by 0.01% (Table 4), and the reducing sugar content of the paste flour of sweet potato, gadung and taro by the addition of α-amylase from *L. plantarum* B110 which increased 0.008% for each was higher than that of wheat paste flour which showed a 0.003% increase.

The tube paste flour by the addition of each α-amylase from *L. bulgaricus* or α-amylase from *L. plantarum* B110 increased the homogenecity of the flour, because of the higher reducing sugar level increases in the tube paste flour by the addition of α-amylase than that of wheat paste flour,

The higher reducing sugar level increases in the paste flour of sweet potato, gadung and taro by the addition of α-amylase from *L. bulgaricus* or α-amylase from *L. plantarum* B110 than that in wheat paste flour was because the content of carbohydrate in sweet potato, *gadung* and taro flour was higher than that in wheat flour.

Table 2. The relative activities of α-amylase from *L. bulgaricus* and *L. plantarum* B110 at various pH

No.	pH	Activity of α-Amylase (U/mL)		Relative Activity of α-Amylase (%)	
		L. bulgaricus	*L. plantarum* B110	*L. bulgaricus*	*L. plantarum* B110
1	5.0	0.118	0.101	96.513^{a}±0.025	86.32^{a}±0.340
2	5.5	0.123	0.117	100.000^{a}±0.134	100.00^{a}±0.410
3	6.0	0.061	0.067	50.019^{a}±0.013	57.26^{a}±0.150
4	6.5	0.074	0.059	60.093^{a}±0.178	50.43^{a}±0.227
5	7.0	0.092	0.066	75.203^{a}±0.404	56.41^{a}±0.081
6	7.5	0.052	0.059	42.270^{a}±0.042	50.43^{a}±0.141

Note: The different letters in the same column show a significant difference (P<0.05)

Table 3. The relative activities of α-amylase from *L. bulgaricus* and *L. plantarum* B110 at various temperatures

No.	Temperature (°C)	Activity of α-Amylase (U/mL)		Relative Activity of α-Amylase (%)	
		L. bulgaricus	*L. plantarum* B110	*L. bulgaricus*	*L. plantarum* B110
1	35	0.051	0.069	60.818^{D}±0.124	69.00^{ab}±0.108
2	40	0.050	0.081	60.250^{D}±0.024	81.00^{ab}±0.143
3	45	0.046	0.100	54.571^{D}±0.024	100.00^{a}±0.312
4	50	0.060	0.066	71.607^{D}±0.036	66.00^{ab}±0.026
5	55	0.054	0.055	64.225^{D}±0.072	55.00^{ab}±0.132
6	60	0.084	0.043	100.000^{a}±0.110	43.00^{ab}±0.119
7	65	0.048	0.031	57.411^{D}±0.056	31.00^{ab}±0.106

Note: The different letters in the same column show a significant difference (P<0.05)

Table 4. The increase of reducing sugar contents in the wheat and local tuber paste flour by the addition of α-amylase from *L. bulgaricus* and *L. plantarum* B110

No.	Paste Flour (PF) with and without Addition of α-amylase	α-Amylase from *L. bulgaricus*		α-Amylase from *L. plantarum* B110	
		Reducing Sugar Content (%)	Increase of Reducing Sugar Content (%)	Reducing Sugar Content (%)	Increase of Reducing Sugar Content (%)
1	Wheat paste flour (W-PF)	0.020^{ab}±0.0000	0.001	0.025^{D}±0.0011	0.003
2	W-PF+α-Amylase	0.021^{a}±0.0006		0.028^{a}±0.0000	
3	Sweet potato paste flour (SP-PF)	0.013^{D}±0.0000	0.008	0.015^{e}±0.0006	0.008
4	SP-PF+α-Amylase	0.021^{a}±0.0006		0.023^{c}±0.0000	
5	Taro paste flour (T-PF)	0.015^{c}±0.0006	0.004	0.017^{D}±0.0000	0.008
6	T-PF+α-Amylase	0.019^{D}±0.0000		0.025^{D}±0.0000	
7	Gadung paste flour (G-PF)	0.006^{e}±0.0000	0.006	0.014^{e}±0.0006	0.008
8	G-PF+α-Amylase	0.012^{D}±0.0000		0.022^{c}±0.0000	

Note: The different letters in the same column show a significant difference (P<0.05)

It was reported that the reducing sugar level of flour resulting from the lactic acid bacteria amylase activity in carbohydrate degradation was affected by the carbohydrate contents of the flour (do Esperito-Santo *et al.,* 2014; Kanpiengjai *et al.,* 2015; Santoyo *et al.,* 2003).

CONCLUSION

Carbohydrate degradation shown by the increases of reducing sugar contents which was about 0.004-0.008% in local tuber paste flour by the addition of the characterized α-amylase from *L. bulgaricus* was higher than that of wheat which was 0.001% sugar content, while the increase of reducing sugar contents in the flour by the addition of α-amylase from *L. plantarum* B110 which was 0.008% was higher than that in wheat which was 0.003%, so that local tuber paste flour can be used as an alternative of wheat paste flour.

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