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Reduction Sugar of Tuber Paste Flour Additional α-Amylase from *Lc. mesenteroides* EN17-11 and *Fr. fructosus* EN17-20 to Protect People from Diabetes Mellitus

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ABSTRACT

Local tuber flour as wheat flour alternative can be used to produce food with low sugar which good to protect people from Diabetes Mellitus. To know that flour carbohydrate degradation in order to produce tuber flour with low sugar, reduction sugar of tuber paste flour additional α-amylase from *Leuconostoc mesenteroides* EN17-11 and *Fructobacillus fructosus* EN17-20 to protect people from Diabetes Mellitus were researched. Flour used were cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and yam taro (*Colocasia esculenta*) with wheat (*Triticum*) as comparison. The crude α-amylase was characterized. The detection of α-amylase activities and reduction sugar contents used 3,5-Dinitrosalicylic Acid (DNS) methods. Data were analyzed with three replicates. The research results showed that optimum activity of *Lc. mesenteroides* EN17-11 α-amylase was reached at 30°C, pH: 4.5; while that *Fr. fructosus* EN17-20. was 60°C, pH 7.0. In 60 minutes incubation time, *Lc. mesenteroides* EN17-11 α-amylase stability was reached at 25-40°C, pH: 4.5-5.0; while that *Fr. fructosus* EN17-20 was at 40-70°C, pH 5.0-7.0. Reduction sugar contents increase of cassava, sweet potato and yam taro paste flour additional *Lc. mesenteroides* EN17-11 α-amylase were sequently 1.27%, 40.35% and 3.90%; while that *Fr. fructosus* EN17-20 were 34.44%, 52.22% and 55.27%; with that wheat additional *Lc. mesenteroides* EN17-11 was 17.40% and *Fr. fructosus* EN17-20 was 44.53%. Based on the result, it is concluded that the treatment may reduce sugar on cassava and yam taro flour. The low sugar flour might be an alternative diet for diabetic persons.

Keywords*: α-amylase, reduction sugar, Lc. mesenteroides EN17-11, Fr. fructosus EN17-20, local tuber paste flour*

INTRODUCTION

Indonesia had high people with Diabetes Mellitus, [1] so low sugar food was needed to protect people from that disease. Low sugar food can be made from local tuber flour as wheat flour alternative. Tuber flour in powder can be used as low sugar food, such as tuber paste flour. Tuber paste flour additional α-amylase improve quality of that flour and the flour are more to be digested. Tuber local flour, such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and yam taro (*Colocasia esculenta*) were made tuber pasta flour and the other tuber food products, mainly baby food, bread and snack.

Tuber pasre flour with low reduction sugar was good for people to protect from Diabetes Mellitus disease. Some species of lactic acid bacteria (LAB) produced α-amylase, such as *L. fermentum* and *L. plantarum*. Some species of LAB reported producing α-amylase were *L. manihotivorans* LMG 18010T, *L. plantarum*, and *L. fermentum*..[2,3]. The quality increase of flour was conducted by addition of α-amylase to the flour. [4,5,6] Flour additional α-amylase produced glucose and

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maltose due to catalyzing amylose in the flour by the added α-amylase. [5,6,7] In human ulcer, flour additional α-amylase from LAB were more digestible due to hidrolyzing amylose to glucose and maltose. [4,6,7]

The flour type and the concentration of α-amylase used affected the glucose and maltose contents in the flour additional α-amylase.[.4,5,6] The different amylose concentration between those flour depend on the tuber flour different type. [4,5,6] The diferent amylose hidrolysis by α-amylase to glucose and maltose was caused by the α-amylase different concentration. [4,6,7]

Glucose as one of reduction sugar was generally higher concentration produced than maltose in flour additional α-amylase.[5,6,7] Beside, LAB species of *Leuconostoc mesenteroides* and *Fructobacillus fructosus* producing α-amylase which have potency to produce reduction sugar in local tuber flour haven't been known yet. This research focused in reduction sugar of tuber paste flour additional α-amylase from *Leuconostoc mesenteroides* EN17-11 and *Fructobacillus fructosus* EN17-20 for people with Diabet Mellitus.

MATERIALS AND METHOD

Sub-culture *Lc. mesenteroides EN17-11* and *Fr. fructosus EN17-20*: *Lc mesenteroides* EN17-11 and *Fr. fructosus* EN17-20 as indigenous lactic acid bacteria (LAB) identified molecularly and found from traditional fermented nira, Enggano Island, collected Research Center for Biology were sub-cultured in *MRS* (*de Mann Rogosa Sharpe*) media which consist of 0.8% beef extract (Himedia RM002-500G), 1% peptone (Bacto TM211677), 0.4% yeast extract (Bacto TM 212750), 1% glucose (Merck 1.08337.1000), 0.5% natrium aceetate (Merck 1.06268.0250), 0.2% triamonium citrate (Sigma A1332-100G), 0.02% magnesium sulphate monohidrate (Merck 1.05886.0500), 0.005% mangan sulphate tetrahidrate (Merck 1.02786.1000), 0.2% dinatrium hydrogen phosphate dihydrate (Merck 1.06580.0500) 0.1%, and tween 80 (Merck 8.22187.0500),. The LAB sub-cultured were then incubated at 37°C (Isuzu incubator Himawari).

Tube Paste Flour: Tube paste flour was made from tube flour of cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and yam taro (*Colocasia esculenta*) with wheat (*Triticum*) as comparison. The tube flour was heated at 70°C up to formed paste flour

Carbohydrate Degradation of Wheat and Local Tube Paste Flour Additional α-Amylase: The 5 gr of each tube flour (cassava, sweet potato, yam taro, and wheat) was soluted in 50 mL aquadest, heated, homogenized by thermomagnetic stirrer (Sibata MGH-320) up to 70°C up to formed paste flour, added 1U/mL each LAB crude amylase, and incubated by rotary shaker (V-Tech VTRS-1) at 37°C for 24 hours.

α-Amylase Production[2]: Each of LAB suspension was inoculated into 50 mL MRSB media and incubated at 37°C for 24 hours in incubator (Isuzu incubator Himawari). Each of LAB crude α-amylase was found by growing 2% that bacteria into 25 mL sterilized MRSB media glucose (Merck 1.08337.1000) was changed by 2% soluble starch (Merck 1.01252.0100) with pH medium: 6, incubated for 24 hours at 37°C by incubator (Isuzu incubator Himawari), centrifuged at 9000 rpm for 10 minutes at 4 °C (Kubota 5910). Each crude α-amylase from those bacteria was then tested its α-amylase activity.

α-Amylase Activity[8,9]: α-Amylase activity was measured by DNS method. The 50 µl crude α-amylase from each of those bacteria was added into 50 µl 1% soluble starch (Merck 1.01252.0100) in pH 5.0-8.0, homogenized by vortex (Sibata MGH-320), incubated in waterbath (Memmert) at 35°C-65°C for 10 minutes, added 100 µl DNS (Sigma D0550-100G), vortexed, heated at 100°C for 5 minutes, added 800 µl aquades, and revortexed. After cooling solution, the absorbance was read at λ540 by spectrophotometer UV-Vis (Shimadzu UV-1700 Pharmaspec). One unit activity of amylase from each of those bacteria was defined as the amount of enzyme in which its reaction resulted product which equal 1µmol glucose per minute at measured condition.

Optimizaton of α-Amylase Activity in Various pH and Temperature [10]: Optimization of α-amylase from both LAB in various pH detected by pH meter (Horiba pH 1100 Scientific), at 10 minutes" incubation times were conducted at pH: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5. The highest α-amylase activity at certain pH indicated α-amylase optimum activity. Optimization of α-amylase from those bacteria in various temperatures at 10 minutes" incubation times were conducted at 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70°C. The highest α-amylase activity of each from those bacteria at certain temperature indicated α-amylase optimum activity.

α-Amylase Stability in Various pH and Temperature [11]: α-Amylase stability from both LAB were conducted

by measuring α-amylase relative activities at pH: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5 with 60 minutes" incubation times. The ≥ 50% α-amylase relative activity was defined as the α-amylase stability at certain pH range. Those α-amylase stabilities were conducted by measuring α-amylase relative activities at 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70°C. The ≥ 50% α-amylase relative activity was defined as α-amylase stability at certain temperature range.

Reduction Sugar [12,13]: Reduction sugar was measured by DNS method. Reduction sugar (%) was measured by standard curve equation of glucose solution. Carbohydrate degradation in tuber flour of cassava, sweet potato, yam taro and wheat (with and without addition of each LAB crude α-amylase) was centrifuged at 9000 rpm for 10 minutes at 4°C. Then, 100 µl the treated tuber flour was added 100 µl DNS, vortexed, heated at 100°C for 5 minutes, added 800 µl aquadest, and revortexed. The solution was then leaved at a minute, and absorbance was read at λ540 by spectrophotometer UV-*Vis* (Shimadzu UV-1700 Pharmaspec).

Reduction Sugar Concentration (%) = [glucose concentration (mg/mL)/sample weight (mg)] × Volume of reaction total (mL) × 100%(1)

DATA ANALYSIS

Data were analyzed by three replicates every treatments. Mean data were shown in every Table of the teatments results" Tables.

RESULTS AND DISCUSSION

The research results show that *Lc. mesenteroides* EN17-11 α-amylase activities in pH: 4.0-7.5 were in the range 0.101-2.325 U/mL with the optimum activity was reached at pH: 4.5 (2.325 U/mL), and in temperature: 25-70°C were 0.166-1.098 U/mL with the optimum activity was at 30°C (1.098 U/mL) (Table 1-2); while that of *Fr. fructosus* EN17-20 α-amylase in pH: 4.0-7.5 were in the range 0.336-0.0929 U/mL with optimum activity was at pH 7.0 (0.0929 U/mL), and in temperature: 25-70°C were 0.0192- 0.2381 (U/mL) with optimum activity was 60°C (0.2381 U/mL) (Table 1-2).

The different optimum α-amylase activity at a certain pH and temperature between α-amylase from *Lc. mesenteroides* EN17-11 and *Fr. fructosus* EN17-20 was caused the different species of bacteria producing α-amylase between those bacteria. It has been reported that the different optimum α-amylase activity from two lactic acid bacteria may have resulted from the different species of lactic acid bacteria producing α-amylase. [2,3,14]

Table 1: α-Amylase Activities of *Lc. mesenteroides* EN 17-11 and *Fr. fructosis* EN 17-20 in Various pH

pH	α-Amylase Activities (U/mL)*	
	***Lc. mesenteroides* EN 17-11**	***Fr. fructosis* EN 17-20**
4.0	2.000	0.0336
4.5	2.325	0.0405
5.0	2.222	0.0447
5.5	0.555	0.0489
6.0	0.526	0.0558
6.5	0.147	0.0753
7.0	0.118	0.0929
7.5	0.101	0.0750

Note: *: mean data in three replicates

Table 2: α-Amylase Activities of *Lc. mesenteroides* EN 17-11 and *Fr. fructosis* EN 17-20 in Various Temperature

Temperatures	α-Amylase Activities (U/mL)*	
	***Lc. mesenteroides* EN 17-11**	***Fr. fructosis* EN 17-20**
25	0.819	0.0192
30	1.098	0.0209
40	0781	0.0226
45	0.685	0.1192
50	0.588	0.1605
55	0.491	0.1925
60	0.395	0.2381
65	0.236	0.1704
70	0.166	0.1026

Note: *: mean data in three replicates

The *Lc mesenteroides* EN17-11 α-amylase activities in 60 minutes" incubation times in pH: 4.0-7.5 were in range 0.327-2.000 U/mL and the α-amylase relative activities were in range 16.35-100% (Table 3); while that temperature: 25-70°C were 0.210-1.000 U/mL and relative activities were in 16.35-100% (Table 4).

The *Lc mesenteroides* EN17-11 α-amylase stabilities with ≥ 50% α-amylase relative activities in 60 minutes"

incubation times were reached at pH in range of 4.5-5.0 (1.111-2.000 U/mL) with relative activities were 55.55-100% (Table 3), while that at temperature in 25-40°C (0.500-1.000 U/mL) with relative activities were 50.00-100.00% (Table 4).

The *Fr fructosus* EN17-20 α-amylase activities at pH: 4.0-7.5 in 60 minutes" incubation time were in range 0.0055-0.0133 U/mL with relative activities were 41.35-100% (Tabel 3), while that at temperature: 25-70°C were 0.0027-0.0151 U/mL with the activities were 17.88-100% (Table 4).

The *Fr fructosus* EN17-20 α-amylase stabilities with relative activity ≥ 50% in 60 minutes" incubation times were reached at pH in range of 5.0-7.0 (0.0071-0.0133 U/mL) with relative activities were 53.38-100% (Table 3), while that at temperature in 40-70°C were 0.0087-0.0151U/mL with relative activities were 57.62-100% (Table 4)

Table 3: α-Amylase and Relative Activities of *Lc. mesenteroides* EN 17-11 and *Fr. fructosis* EN 17-20 in Various pH in 60 Minutes Incubation

pH	*Lc. mesenteroides* EN 17-11		*Fr. fructosis* EN 17-20	
	α-Amylase activities (U/mL)*	Relative activities (%)*	α-Amylase activities (U/mL)*	Relative activities (%)*
4.0	0.769	38.45	0.0055	41.35
4.5	2.000	100.00	0.0063	47.37
5.0	1.111	55.55	0.0071	53.38
5.5	0.769	38.45	0.0074	55.64
6.0	0.588	29.40	0.0076	57.14
6.5	0.400	20.00	0.0115	86.47
7.0	0.383	19.15	0.0133	100.00
7.5	0.327	16.35	0.0121	90.98

Note: *: mean data in three replicates

Table 4: α-Amylase and Relative Activities of *Lc. mesenteroides* EN 17-11 and *Fr. fructosis* EN 17-20 in Various Temperatures in 60 Minutes Incubation

Temperatures	*Lc. mesenteroides* EN 17-11		*Fr. fructosis* EN 17-20	
	α-Amylase activities (U/mL)*	Relative activities (%)*	α-Amylase activities (U/mL)*	Relative activities (%)*
25	0.714	71.40	0.0027	17.88
30	1.000	100.00	0.0040	26.49
35	0.750	75.00	0.0063	41.72
40	0.500	50.00	0.0087	57.62
45	-	-	0.0111	73.51
50	0.454	45.40	0.0116	76.82
55	0.408	40.80	0.0138	91.39
60	0.362	36.20	0.0151	100.00
65	0.237	23.70	0.0117	77.48
70	0.210	21.00	0.0098	64.90

Note: *: mean data in three replicates

The different α-amylase stabilities in certain pH and temperature range between α-amylase from *Lc mesenteroides* EN17-11 and *Fr. fructosus* EN17-20 were caused the different optimum α-amylase activity from two spesies of those bacteria. It has been reported that the different optimum α-amylase activity from two lactic acid bacteri species may have resulted from the different species of lactic acic bacteria producing α-amylase.[3,7,9]

The reduction sugar contents“ increases of the cassava, sweet potato, and yam taro paste flour additional *Lc. mesenteroides* EN17-11 α-amylase were sequently 1,27%, 40.35% and 3.90% (Table 5), while that *Fr. fructosus* EN17-20 α-amylase were 34.44%, 52,22% and 55.27%; (Table 6). The reduction sugar content increase of wheat paste flour additional *Lc. mesenteroides* EN17-11 was 17.40% (Table 5), while that *Fr. fructosus* EN17-20 α-amylase was 44.53% (Table 6).

The reduction sugar contents” increases of the tuber paste flour additional *Lc mesenteroides* EN17-11 α-amylase of cassava (1.27%) and yam taro (3.90%) were lower than that wheat paste flour (40.35%) (Table 9), and the reduction sugar contents increases of the cassava paste flour additional *Fr. fructosus* EN17-20 α-amylase (34.44%) were lower than that wheat paste flour (44.53%).

The lower reduction sugar contents” increases from the cassava and yam taro paste flour additional *Lc mesenteroides* EN17-11 α-amylase and that from the cassava additional *Fr. fructosus* EN17-20 than that wheat paste flour were because the carbohydrate degradation of the cassava and yam taro flour (additional *Lc mesenteroides* EN17-11 α-amylase) and that of cassava flour (additional *Fr. fructosus* EN17-20 α-amylase) were lower than that of wheat flour. It has been reported that the tuber flour reduction sugar resulted due to carbohydrate degradation of tuber flour.was affected by lactic acid bacteria amylase activities in that carbohydrate. [4,5,7]

Table 5: Reduction Sugar Contents of Tuber Pasta Flour With and Wthout Addition of *Lc. mesenteroides* EN 17-11 α-Amylase

Paste flour type	Reduction sugar (%)*	Reduction sugar increase (%)
Cassava	0.130	1.27
Control	0.128	
Sweet potato	0.587	40.35
Control	0.350	
Yam Taro	0.437	3.90
Control	0.420	
Wheat	0.440	17.40
Control	0.363	

Note: *: mean data in three replicates

Table 6: Reduction Sugar Contents of Tuber Pasta Flour With and Wthout Addition of *Fr. fructosus* EN17-20 α-Amylase

Paste flour type	Reduction sugar (%)*	Reduction sugar increase (%)
Cassava	0.241	34.44
Control	0.158	
Sweet potato	0.722	52.22
Control	0.345	
Yam Taro	0.237	55.27
Control	0.106	
Wheat	0.256	44.53
Control	0.142	

Note: *: mean data in three replicates

Based on those reduction sugar contents increase in the treated tuber paste flour, it is concluded that cassava and yam taro paste flour additional *Lc. mesenteroides* EN17-11 α-amylase with the 1.27% and 3.90% reduction sugar increase, respectively, and cassava paste flour additional *Fr. fructosus* EN17-20 α-amylase with the 34.44% reduction sugar increase were the tuber paste flour with low reduction sugar.

It is recommended that this low reduction sugar tuber paste flour can be consumed as one of low sugar food, in order to the society were protected from Diabetes Mellitus Diseases.

CONCLUSION

The research showed the addition of α-amilase EN17-11 and EN17-20 resulted in sugar reduction. There was difference in optimum condition between both treatment to reduce sugar in term of pH and temperature. However, based on the reduction of sugar content in the treated tuber paste flour, it is suggested that cassava and yam taro with low sugar might be used as an alternative diet for diabetic persons. In the long term goal, the use of low sugar tuber paste flour is also expected to protect people from diabetes mellitus.

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Ethical Clearance: This study was conducted according to the guidelines laid down in the Declaration of Helsinki

Conflict of Interest: This research wasn't have conflict of interest

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