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A-AMYLASE STABILITIES OF *Lactobacillus satsumensis* EN 38-32 AND *Fructobacillus fructosus* EN 17-20 AT STORAGE TEMPERATURES AND TIMES

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Abstract

Lactic acid bacteria produce α-amylase which is used in food and nutraceutical products. This study aims to detect α-amylase stabilities of *Lactobacillus satsumensis* EN 38-32 and *Fructobacillus fructosus* EN 17-20 at storage temperatures and times. The stabilities of α-amylase were determined at 4°C and -20°C, and storage times of 0, 7, 14, 21 and 28 days. The DNS method was used to test α-amylase activity. The relative activity of α-amylase ≥ 50% was expressed as α-amylase activity in stable conditions. Data were analyzed by standard deviation with three replications. The results showed that the α-amylase stabilities of *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 at storage temperatures decreased with increasing storage times. The longer the storage times, the lower the α-amylase stabilities. The relative activities of *L. satsumensis* EN 38-32 α-amylase at storage temperatures for 7- 28 days were in the range of 72.79-93.70% and 72-89.18%, while *Fr. fructosus* EN 17-20 at 56.69-93.11% and 56.70-93.11%. Based on the α-amylase stabilities, it was concluded that *Fr. fructosus* EN 17-20 produced better α-amylase than *L. satsumensis* EN 38-32. So, it is recommended to use *Fr. fructosus* EN 17-20 to produce α-amylase as a biocatalyst rather than *L. satsumensis* EN 38-32.

Keywords: α-Amylase, *Fructobacillus fructosus* EN 17-20, *Lactobacillus satsumensis* EN 38-32, stability, storage

1. Introduction

Microorganisms as a source of enzymes are widely used in the food and non-food industrial sectors in the development of their products. The selection of the right microorganisms is very important in producing the desired enzymes [1], [2], [3]. Lactic acid bacteria are one of the microorganisms that produce various types of enzymes including α-amylase which can be used in food and medicinal and nutraceutical products [4], [5], [6].

Enzyme is widely applied, especially in food, medicinal and nutricical products, because the enzyme process is a biocatalytic process that produces less residue than chemical processes [7], [8]. Enzymes are part of protein molecules necessary for life, including amylase, which is an enzyme that breaks down carbohydrates. Amylase is an enzyme that can be used as a substitute for chemical hydrolysis of carbohydrates [9], [10]. Amylase has great significance in applications in various food products, including bread, snacks and the pharmaceutical industry in the form of

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enzymes [11], [12], [13].

Lactic acid bacteria (LAB) are a group of Gram-positive, anaerobic or facultative cocci, aerobic or rod-shaped (bacillus), can produce amylase which plays a role in carbohydrate fermentation, and are Generally Recognized As Safe (GRAS) [4], [12], [14]. LAB produce α-amylase, including *Lactobacillus fermentum* Ogi E1 [15], and other lactic acid bacteria [5], [16],

The activity of α-amylase is influenced by its specificity, stability, temperature and pH. Selection of α-amylase-producing microorganisms with high activity can be used in industry [17], [18]. Previous research had known the character of α-amylase *Lactobacillus satsumensis* EN 38-32 and *Fructobacillus fructosus* EN 17-20 based on their optimum pH and temperature. The results of α-amylase optimization produced by *L. satsumensis* EN 38-32 obtained an optimum pH of 5.5 and an optimum temperature of 45° C [19]. α-Amylase *Fr. fructosus* EN 17-20 had an optimum pH of 7.0 and an optimum temperature of 60°C [20].

Based on the above, this study is focused on determining the stability of α-amylase produced by *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 at various storage temperatures and times.

2. Methods

a. Materials

The materials used were: 0.8% beef extract media, 0.4% yeast extract, 2% lactose/ glucose, De Man, Rogosa and Sharp agar (MRS) (1% peptone, 0.5% sodium acetate, tri ammonium citrate 0.2%, $MgSO_4$ 0.02%, tween 80 0.1%, agar 1.8%, $CaCO_3$ 0.5%, $MnSO_4$ 0.005%, Na_2HPO_4 0.2%), phosphate buffer solution, phosphate buffer pH 5.0 -8.0, 1000 ppm glucose solution, Dinitrosalicil acid solution (DNS), NaOH, 1% starch solution, Dinitrosalicilic acid (DNS), KN Atartate, *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 (Collection in Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences) and sterile aquadest.

b. Production of α-Amylase-producing Lactic Acid Bacteria (LAB) [21]

Production of α-Amylase-producing Lactic Acid Bacteria (LAB) used De Man, Rogosa Sharp (MRS) broth media. The ingredients (2 g yeast extract, 10 g proanalytic starch, 2.5 g sodium acetate, 5 g peptone, 4 g beef extract, 1 g tri ammonium citrate, 0.1 g $MgSO_4$, 0.25 g $MnSO_4$, 1 g $NaHPO_4$ and 0.5 g of tween 80) in 500 mL of distilled water were stirred with a thermomagnetic stirrer, added 9 g of agar and 2.5 g of $CaCO_3$ and stirred again, then the solution was put into an erlenmeyer flask. The Erlenmeyer flask containing the mixture was covered with a gauze plug and sterilized by autoclaving for 15 minutes at 121°C.

c. Sub-culture of *L. satsumensis* EN 38- 32 and *Fr. fructosus* EN 17-20 [20]

2 Ose *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 were sub-cultured in MRSA media using the scratch plate method, then incubated at 37°C for 24 hours.

d. Production of α-Amylase [3]

2% respectively of *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 were inoculated into 500 ml Erlenmeyer flask of production media, and incubated at 24 hours at 37°C. The cell culture was then centrifuged at 9000 rpm, time 10 minutes, temperature 4°C, then the supernatant which is α-amylase was produced.

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e. The standard curve of glucose [22]

A standard 1000 ppm glucose solution was prepared by dissolving 0.1 g of glucose in 100 mL of distilled water and vortexed until dissolved. The solution was diluted into several concentrations (0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm). Each solution was added with 500 µL of DNS solution, vortexed and boiled for 5 minutes. The solution was cooled under running water for 15 minutes. The mixture was added with 1 mL of distilled water and re-vortexed so that the solution became homogeneous, then the solution was measured for its absorbance at λ540 nm by UV-Vis spectrophotometry.

f. α-Amylase Activity [23]

The α-amylase activity was carried out by the Bernfeld method (1955). 50 µL α-amylases of *L. satsumensis* EN 38-32 (pH 5.5) and 50 µL *Fr. fructosus* EN 17-20 (pH: 7.0) were added 50 µL each of 1% starch solution. The solution was vortexed, incubated at optimum temperature of 45°C (*L. satsumensis* EN 38-32) and 60°C (*Fr. fructosus* EN 17-20). The solution was added with 100 µL of DNS reagent, vortexed, heated at 100°C for 5 minutes, 800 µL of distilled water was added, re-vortexed and the absorbance was read at λ540 nm with a UV-Vis spectrophotometer. One unit of α-amylase activity was defined as the amount of enzyme whose reaction yields a product equivalent to 1 µmol of glucose per minute under the conditions of measurement.

The α-amylase activity was expressed in U/mL and calculated by the formula:

$$A - amylase\ activity\ (U/mL) = (Glucose\ content\ x\ 1000\ x\ DF)/(Glucose\ MW)\ x\ \ 1/t$$

Notes : Glucose MW: Glucose Molecular Weight (180.18 g/mol)
DF: Dilution Factor; t: Incubation Time (minutes)

g. α-Amylase Stability [24]

The α-amylase stabilities of *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 were determined by storing α-amylase at 27°C (room temperature), 4°C (cold temperature), -20°C (frozen temperature) within 0, 7, 14, 21 and 28 days of storage. Each sample was then tested for α-amylase activity using the Bernfeld method (1955). The stability of α-amylase occured when α-amylase had a relative activity percentage above 50%

The relative activity of α-amylase was calculated based on the following equation.

$$\boldsymbol{Relative\ activity\ of\ \alpha - amylase}\ (\%) = \boldsymbol{Treatment\ activity}\ (\boldsymbol{(U/mL)})/\ \boldsymbol{Highest\ activity}\ \boldsymbol{(U/mL)x\ 100}\%$$

h. Data Analysis

The data of the research results were analyzed by standard deviation. The data obtained were mean of the three replications

3. Result

a. Stability of *L. satsumensis* EN 38-32 α-Amylase at Various Cold Temperatures and Times

The α-amylase stabilities of *L. satsumensis* EN 38-32 at cold temperatures decreased with increasing storage times (Table 1). The longer the storage times, the lower the α-amylase stabilities. The unit activities of *L. satsumensis* EN 38-32 α-amylase at cold temperatures for 7-28 days were in the range of 1.0211-1.4028 U/mL (Table 1), while the relative activities of *L. satsumensis* EN 38-32 α-amylase at cold temperatures for 7- 28 days were in the range of 72.79-93.70% (Table 1). The stability expressed above 50% relative activities of *L. satsumensis* EN 38-32 α-amylase occurred at cold temperatures for 7- 28 days with the value of 72.79-93.70% (Table 1).

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Table 1. Stability of α-Amylase from *L. satsumensis* EN 38-32 at Various Cold Temperatures and Times

No.	Storage Time (day)	α-Amylase Activity (U/mL)	Relative Activity (%)
1	0	1.4028 ± 0.0776	100
2	7	1.3145 ± 0.1601	93.70
3	14	1.2775 ± 0.1245	91.06
4	21	1.1664 ± 0.0494	83.14
5	28	1.0211 ± 0.1454	72.79

b. Stability of *L. satsumensis* EN 38-32 α-Amylase at Various Freezing Temperatures and Times

The α-amylase stabilities of *L. satsumensis* EN 38-32 at freezing temperatures decreased with increasing storage times (Table 1). The longer the storage times, the lower the α-amylase stabilities. The unit activities of *L. satsumensis* EN 38-32 α-amylase at freezing temperatures for 7- 28 days were in the range of 0.9670-1.3430 U/mL (Table 2), while the relative activities of *L. satsumensis* EN 38-32 α-amylase at freezing temperatures for 7- 28 days were in the range of 72.00-89.18% (Table 2). The stability expressed above 50% relative activities of *L. satsumensis* EN 38-32 α-amylase occurred at freezing temperatures for 7- 28 days with the value of 72.00-89.18% (Table 2).

Table 2. Stability of α-Amylase from *L. satsumensis* EN 38-32 at Various Freezing Temperatures and Times

No.	Storage Time (day)	α-Amylase Activity (U/mL)	Relative Activity (%)
1	0	1.3430 ± 0.3018	100.00
2	7	1.1977 ± 0.1447	89.18
3	14	1.1664 ± 0.0515	86.85
4	21	1.0011 ± 0.0430	74.54
5	28	0.9670 ± 0.1501	72.00

The α-amylase stabilities of *L. satsumensis* EN 38-32 at cold temperatures were different to the α-amylase stabilities of *L. satsumensis* EN 38-32 at freezing temperatures (Table 1-2). It has been reported that the α-amylase stabilities were affected by the temperatures used for storage of α-amylase [9], [25], [26].

c. Stability of *Fr. fructosus* EN 17-20 α-Amylase at Various Cold Temperatures and Times

The α-amylase stabilities of *Fr. fructosus* EN 17-20 at cold temperatures decreased with increasing storage times (Table 3). The longer the storage times, the lower the α-amylase stabilities. The unit activities of *Fr. fructosus* EN 17-20 α-amylase at cold temperatures for 7- 28 days were in the range of 1.0553-1.8615 U/mL (Table 3), while the relative activities of *Fr. fructosus* EN 17-20 α-amylase at cold temperatures for 7- 28 days were in the range of 56.69-93.11% (Table 3). The stability expressed above 50% relative activities of *L. satsumensis* EN 38-32 α-amylase occurred at cold temperatures for 7- 28 days with the value of 56.69-93.11% (Table 3).

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Table 3. Stability of α-Amylase from *Fr. fructosus* EN 17-20 at Various Cold Temperatures and Times

No.	Storage Time (day)	α-Amylase Activity (U/mL)	Relative Activity (%)
1	0	1.8615 ± 0.0534	100.00
2	7	1.7333 ± 0.0967	93.11
3	14	1.5966 ± 0.1427	85.77
4	21	1.2547 ± 0.0815	67.40
5	28	1.0553 ± 0.0727	56.69

d. Stability of α-Amylase from *Fr. fructosus* EN 17-20 at Various Freezing Temperatures and Times

The α-amylase stabilities of *Fr. fructosus* EN 17-20 at freezing temperatures decreased with increasing storage times (Table 4). The longer the storage times, the lower the α-amylase stabilities. The unit activities of *Fr. fructosus* EN 17-20 α-amylase at freezing temperatures for 7-28 days were in the range of 1.0553-1.8611 U/mL (Table 4), while the relative activities of *Fr. fructosus* EN 17-20 α-amylase at freezing temperatures for 7- 28 days were in the range of 56.70-93.10% (Table 4). The stability expressed above 50% relative activities of *Fr. fructosus* EN 17-20 α-amylase occurred at freezing temperatures for 7- 28 days with the value of 56.70-93.10% (Table 4).

Table 4. Stability of α-Amylase from *Fr. fructosus* EN 17-20 at Various Freezing Temperatures and Times

No.	Storage Time (day)	α-Amylase Activity (U/mL)	Relative Activity (%)
1	0	1.8611 ± 0.0536	100.00
2	7	1.7326 ± 0.0966	93.10
3	14	1.5959 ± 0.1429	85.75
4	21	1.2537 ± 0.0814	67.36
5	28	1.0553 ± 0.0727	56.70

The α-amylase stabilities of *Fr. fructosus* EN 17-20 at cold temperatures were different to the α-amylase stabilities of *Fr. fructosus* EN 17-20 at freezing temperatures (Table 3-4). It has been reported that the α-amylase stabilities were affected by the temperatures used for α-amylase at storage [25], [26], [27].

The α-amylase unit activities of *Fr. fructosus* EN 17-20 at storage temperatures for 7- 28 days were higher than the α-amylase stabilities of *Fr. fructosus* EN 17-20 at storage temperatures for 7- 28 days (Table 1-4). It has been reported that the α-amylase stabilities were affected by the storage temperatures and the type of microorganism producing α-amylase [10], [25], [26].

Based on stabilities with the values of the unit activities, it was concluded that *Fr. fructosus* EN 17-20 produced better α-amylase at storage temperatures for 7- 28 days than *L. satsumensis* EN 38-32 at the same storage. So, it is recommended to use *Fr. fructosus* EN 17-20 to produce α-amylase which it can be stored for 7-28 days as a biocatalyst rather than *L. satsumensis* EN 38-32.

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4. Conclusion

The α-amylase stabilities of *L. satsumensis* EN 38-32 and *Fr. fructosus* EN 17-20 at storage temperatures decreased with increasing storage times. The longer the storage times, the lower the α-amylase stabilities. At storage temperatures for 7- 28 days, the relative activities of *L. satsumensis* EN 38-32 α-amylase were in the range of 72.79-93.70% and 72-89.18%, while *Fr. fructosus* EN 17-20 at 56.69-93.11% and 56.70-93.11%. It was concluded that *Fr. fructosus* EN 17-20 produced better α-amylase than *L. satsumensis* EN 38-32, based on the α-amylase stabilities. So, it is recommended to produce α-amylase as a biocatalyst to use *Fr. fructosus* EN 17-20 not *L. satsumensis* EN 38-32.

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6. References

[1]. Alariya S, Sethi S, Gupta S, Gupta B. Lal. Amylase Activity of Starch Degrading Bacteria Isolated from Soil. *Archives of Applied Science*. 2013; 5 (1):15-24.

[2]. Djekrif DS, Gillmann L, Bennamoun L, Ait-Kaki A, Labbani K, Nouadri T, Meraihi Z. Amylolytic Yeasts: Producers of α-Amylase and Pullulanase. *Int. J. Life. Sci. Scienti. Res.* 2016; 2(4):339-54.

[3]. Harikrishna, Y., Nanganuru, N., Korrapati., and Satish, M. Studies on The Production of α-Amylase by *Bacillus subtilis*. *IOSR Journal of Engineering*. 2012; 2(5):1053-1055.

[4]. Akihito E, Irisawa T, Yuka FE, Katsumi T, Maret DT, Sanae O, Leon MTD. Characterization and Amanded Description of *Lactobacillus kunkeei* as A Fructophilic Lactic Acid Bacterium. *International Journal of Systematic and Evolutionary Microbiology*. 2012; 62:500–504.

[5]. do Esperito-Santo AP, Mouquet-Rivier C, Humblot C, Cazevieille C, Icard Vemiere C, Socrol C, et al. Influence of Cofermentation by Amylolytic Lactobacillus Strains and Probiotic Bacteria on The Fermentation Process, Viscosity and Microstructure of Gruels Made of Rice, Soy Milk and Passion Fruit Fiber. *Food Res Int*. 2014; 57:104-13.

[6]. Emiliano JQ, Pilar J, Irma C, Jesús T, Javier M, Tomás G. Probiotic Lactic Acid Bacteria. *Food and Nutrition Sciences*. 2014; 5:1765-75.

[7]. Helen HR, Peter OO, Chrinius H, Matthew OI, Judy IA, Evelyn HO. Production of alpha Amylase by *Bacillus cereus* in Submerged Fermentation. *Aceh Int. J. Sci. Technol*. 2014; 3(3):124-130.

[8]. Moradi M, Shariati P, Tabandeh F, Yakhcali B, Khaniki GB. Screening and Isolation of Powerful Amylolytic Bacterial Strains. *Int J Curr Microbiol Appl Sci*. 2014; 3(2):758-68.

[9]. Padhiar AR, Kommu S. Isolation, Characterization and Optimization of Bacteria Producing Amylase. *Int. J. Adv. Res. Biol. Sci*. 2016; 3(7):1-7.

[10]. Rameshkumar A, Sivasudha T. Optimization of Nutritional Constitute for Enhance alpha Amylase Production by Using Solid State Fermentation Technology. *International Journal of Microbiology.* 2011; 2(2):143-148.

[11]. Paula MDS, Perola, DOEM. Application of Microbial α-Amylase in Industry. *Brazilian Journal of Microbiology*. 2010; 41:850-861.

[12]. Raj KP, Lakshmi T. Microbial Enzymes Involved in Starch Processing Industries. *Scholarly Journal of Biological Science*. 2015; 4(1)1-3.

[13]. Sharma A, Satyanarayana T. Microbial Acid Stable-Amylases: Characteristics, Genetic Engineering and Applications. *Proc Biochem*. 2013; 48: 201-11.

[14]. Springer. Hlm. 1. Ellie JCG, Kerin LT, Diane MC. Lactobacillus Spesies: Taxonomic Complexity and Controversial Susceptibilities. R. M. Alden Research Laboratory, Culver City and David Geffen School of Medicine at UCLA, Los Angeles, California. *Suplement Article.* 2015; 60:98-107

[15]. Santoyo MC, Loiseau G, Sanoja RR, Guyot JP. Study of Starch Fermentation at Low pH by *Lactobacillus fermentum* Ogi E1 Reveals Uncoupling between Growth and α-Amylase Production at pH 4.0. *Int J Food Microb*. 2013; 80:77-87.

[16]. Tallapragada P, Bhargavi R, Priyanka PR, Niranjan NR, Pavitra PV. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *J Gen Eng and Biotech*. 2018; 16(2018):35763.https://doi. org/10. 1016/j. jgeb. 2018. 03.005.

[17]. Negi S, Banerjee, R. Optimation of Culture Parameters to Enhance Production of Amilase and Protease from *Aspergilus awamori* in A Single Fermentation. *African Journal of Biochemistry.* 2010; 4(3):73-80.

[18]. Wang J, Li J, Lu F. Molecular Cloning and Biochemical Characterization of An α-Amylase Family from *Aspergillus niger*. *Electr J Biotech*. 2018; 32:56-62.

[19]. Khusniati T, Sulistiani, Septiani, D. Characteristics of α-Amilase from *Lactobacillus satsumensis* EN 38-32 and Its Potency in Biodegradation Carbohydrate Pasta Flour of Local Tuber and Cereal. In Indonesian. *Technique Report of Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences*. 2017.

[20]. Khusniati T, Megava TS, Amir M, Nurhayati S, Aminingsih T, Sulistiani. 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. *Indian Journal of Public Health Research and Development*. An International Journal. Scopus IJPHRD Citation Score. 2019; 10 (3): 952-957.

[21]. Man JC, Rogosa M, Sharpe EM. A Medium for The Cultivation of Lactobacili. *J. Appl Bact*. 1960; 23:130-135.

[22]. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *J Analyt. Chem*. 1959; 31:426-8.

[23]. Bernfeld P. Amylases, α and β. Meth. Enzymology. *This Week's Ctation Classic*. 1955; 1(40):14958.

[24]. Saxena R, Singh, R. Amylase Production by Solid-State Fermentation of Agro-Industrial Wastes Using Bacillus sp. *Brazilian Journal of Microbiology*. 2011; 42:1334-42.

[25]. Vengadaramana A, Balakumar S, Vasanthy A. Effect of Temperature, pH, Substrate (Starch) and Glucose on Stability of α-Amylase from *Bacillus licheniformis* ATCC 6346. *Sch. Acad. J. Pharm*. 2014; 3(6):492-495.

[26]. Defaei M, Taheri-Kafrani A, Miroliaei M, Yaghmaei, P. Improvement of Stability and Reusability of α-Amylase Immobilized on Naringin Functionalized Magnetic Nanoparticles: A Robust Nanobiocatalyst. *International Journal of Biological Macromolecules*. 2017; 17:354-60.

[27]. Kanpiengjai A, Lumyong S, Nguyen HT, Haltrich D. Microorganisms and α-Amylase: A Concise Review. *J Mol Cat B-Enzym*. 2015; 120:1-8.

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