Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/22153
DOI URL: http://dx.doi.org/10.21474/IJAR01/22153

RESEARCH ARTICLE

PARTIAL PHYSICOCHEMICAL CHARACTERISATION OF NATIVE B-GLUCOSIDASE EXTRACTED FROM MANGO (MANGIFERA INDICA L) KERNELS OF THE GOLDEN GLOBE VARIETY CULTIVETED IN DALOA (CENTRAL WEST, CÔTE D'IVOIRE)

YA Kouame Claude[1],Ouina Toualy Serge Thibaut[1], Zebre Artur Constant [1],Kone Yelakankinonton Clarisse[2], Blei Sika Hortense[1] and Gnanwa Mankambou Jacques[1]

1. Laboratory of Agrovalorization, UFR of Agroforestry, Jean Lorougnon Guede University, BP 150 Daloa, Cote d'Ivoire.
2. Laboratory of Microbiology and Biotechnology, Ecology Research Center, Nangui Abrogoua University, 08 BP 109 Abidjan 08, Cote d'Ivoire.

Manuscript Info

Manuscript History
Received: 10 September 2025
Final Accepted: 12 October 2025
Published: November 2025

Key words:-
Mango Almond, Native, Thermal denaturation, Thermal inactivation

Abstract

The aim of this study was to select new enzymatic sources exhibiting unique activities from mango kernel almonds (Mangifera indica L) grown in the DALOA region (Côte d'Ivoire). A β-glucosidase was thus extracted from the kernel of the Golden Globe variety. Characterization methods, including the determination of temperature and pH, stability at different pH levels and temperatures, thermal inactivation, thermal deactivation, and the effects of chemical agents, were used to understand the behavior of this β-glucosidase. It has an optimal pH of 5.6 with stability in the pH range between 4.00 and 7.00. Its optimal temperature is 90°C and it remains stable between 37 and 95°C. At the optimal temperature, it remains active for 14 hours. The activation energy of this enzyme is 33 kJ·mol⁻¹. It has KM, Vmax, and Vmax/KM values of 0.9973 mM, 1.3132 U, and 1.3166 U/mM, respectively. Although it withstands the effects of ethanol (15%), glucose (100 mM), and ascorbic acid (5 mM), it is strongly inhibited by citric acid (5 mM) and methanol (8%). Disulfide bridge reducers such as β-mercaptoethanol, L-cysteine, and DL-dithiothreitol do not decrease the activity of this enzyme. However, PCMB and DTNB inhibit it. This enzyme is acidophilic, thermophilic, and thermostable.

"© 2025 by the Author(s). Published by IJAR under CC BY 4.0. Unrestricted use allowed with credit to the author."

Introduction:-

B-glucosidase are found in all living organisms where they play a crucial role in the removal of terminal glucosyl residues from saccharides and glycosides [1] [2]. They are responsible for the final degradation of polymers in the plant cell wall as they cleave glucan-based oligo and polysaccharides to release glucose [3] [4]. They are involved in the metabolism of glycolipids and exogenous glycosides in animals, in defense, lignification of plant cell walls, in the activation of phytohormones, and in the release of aromatic compounds in plants [5]. These functions lead to

Corresponding Author:-YA Kouame Claude
Address:-Laboratory of Agrovalorization, UFR of Agroforestry, Jean Lorougnon Guede University, BP 150 Daloa, Cote dIvoire.

numerous industrial applications such as the production of glucose, biofuel, and the synthesis of flavors in wines…[6] [7].The industrial use of these enzymes involves a key property, which is the catalytic efficiency or performance of the enzyme. This performance depends on tree main factors: tolerance to hydrolysis products and by-products, stability across the pH range, and thermal stability [8]. The search for new enzymes that are tolerant and exhibit high activity over a wide pH range and thermal stability could be relevant for improving biotechnological and industrial processes. In this context, β-glucosidases have mostly been sought in thermophilic bacteria, fungi, and other microscopic animals [9] [10]. However, although there are interesting enzymatic sources in plants, the production of plant-derived enzymes is not only limitedby the availability of raw materials but also because they often possess biotechnological and industrial characteristics that are difficult to implement [11].It therefore seems necessary to find new source of β-glucosidases that could meet the requirements for glucose inhibition tolerance, pH and temperature stability as desired by the industry.

The choice fell on mango, which is one of the most consumed tropical fruits after banana, pineapple, papaya, and avocado [12]. In Africa, and particularly in Côte d'Ivoire, mango is mainly consumed or marketed fresh and does not keep very long after ripening due to the lack of preservation methods applicable at the local or traditional level [13]. This situation limits the used of mangoes worldwide and results in enormous post-harvest losses. This loss affects both the skins and the seeds of the mango [14]. At the heart of the seed of this fruit is the kernel, which accounts for the largest part of post-harvest losses because it is not consumed at all. Moreover, the kernel is generally abandoned in nature, posing an environmental problem, even though it possesses very interesting properties [15].Searching for enzymes in mango kernel almonds will undoubtedly add greater value to this agricultural by-product.This study aims at the partialbiochemical characterization of β-glucosidase extracted from mango kernels (Mangifera indica L), of the Golden Glob variety. Specifically, it will focus on examining the influence of PH variation, temperature, and some chemical compounds on the enzymatic activity of native β-glucosidase extracted from the kernels of the Golden Globe variety.

Materials and Methods:-

Chemicals and Reagents:

The para-nitrophenyl-glycopyranoside (pNP-glycopyranoside) used was purchased from Sigma Aldrich. All other chemicals and various reagents used were of analytical grade.

Plant Material:

The plant material consists of the kernels of mango seeds from the Golden Globe cultivar, harvested from plantations around the city of DALOA (Ivory Coast). These mango seeds were collected and sun-dried for seven days to prevent moisture and to facilitate easier removal of the kernels from the seeds.

Enzymatic Extraction:

The crude enzymatic extract was obtained according to the method described by Ya [16].

Protein Assay:

The protein concentration was determined by spectrophotometry at 660 nm using the method with bovine serum albumin as the standard [17].

Determination of enzymatic activities:

Under standard conditions, the hydrolytic activity of beta-glucosidase was measured by the release of para-nitrophenol according to the method of Fagbohoun [18]. One unit (U) of enzymatic activity was defined as the amount of enzyme that released one µmol of para-nitrophenol or reducing sugar per minute under standard reaction conditions [19]. The specific activity was expressed in units per mg of protein (U/mg of protein).

Optimal pH and temperature:

The effect of pH on enzymatic activity was determined by performing the hydrolysis of pNP-beta-D-glucopyranoside (5 mM) in two buffers (20 mM) at different pH values (3.6–8.0). The buffers used were sodium acetate (pH 3.6–5.6) and sodium phosphate (pH 5.6–8.0). The pH values of each buffer were determined at 37 °C. The effect of temperature on enzyme activity was determined in 20 mM sodium acetate buffer (pH 5.6) over a temperature range of 30 to 105 °C, using pNP-beta-D-glucopyranoside (5 mM) as the substrate under enzymatic assay conditions [20].

pH and Temperature Stability:-
The pH stability of the enzyme was studied over a pH range of 3.6 to 8.0. The buffers used were the same as those employed in the pH and temperature optimization study. After 1 hour of pre-incubation at 37°C, aliquots were taken and immediately assayed to determine the residual activity of β-glucosidase. Thermal inactivation was determined at 37°C and 90°C in a 20 mM sodium acetate buffer (pH 5.6). Then, aliquots were withdrawn at regular intervals and immediately cooled in ice water. In the thermal denaturation experiments, aliquots of the enzyme solution were pre-incubated at different temperatures ranging from 30 to 80 °C for 15 minutes. The residual activities, determined in all three cases at 37 °C under the enzymatic assay conditions, were expressed as a percentage of the activity of the zero-time control of the untreated enzyme. The Q10 was determined between 75 and 85 °C on the ascending part of the optimal temperature curve for this β-glucosidase. The ratio of the reaction rate at t °C + 10 to the rate at t °C was used to calculate the Q10. Q10 = X10/X, with: X10 = Absorbance at t + 10 °C, X = Absorbance at t °C, and t = Temperature in °C. The effect of temperature is related to the activation energy, as indicated by the relationship proposed by Arrhenius. Thus, plotting the logarithms of residual activities against the inverse of the temperature in Kelvin (1/T K) allowed the graphical determination of the activation energy of this enzyme. The activation part of the activity versus temperature curve was used. The slope of this curve allowed the determination of the activation energy (Ea). Ea = -2.303 x R x slope. R = ideal gas constant (8.286 J/mol/K) [20].

Kinetic parameters:
The kinetic parameters (KM, Vmax, and Vmax/KM) were determined in a 20 mM sodium acetate buffer (pH 5.6) at 37 °C. Using different concentrations (0.2 to 2.5 mM) of pNP-beta-D-glucopyranoside, KM and Vmax were determined from the Lineweaver-Burk plot [21]. Each experimental point was determined at least in triplicate.

Effects of chemical agents:
To determine the effect of various compounds (cations, EDTA, alcohols, organic acids), which could be potential activators or inhibitors of the crude enzyme, the enzyme solution was pre-incubated at 37 °C for 20 minutes with chemical agents at concentrations of 1 mM [1% (w/v)] or 5 mM [5% (w/v)]. The activity was measured under the enzymatic assay conditions using pNP-beta-D-glucopyranoside (5 mM) as the substrate. Residual activities were expressed as a percentage relative to the control without chemical agents [22].

Statistical analyses:
All determinations reported in this study were performed in triplicate. The results were expressed as means ± standard deviation.

Kinetic parameters:
The kinetic parameters (KM, Vmax, and Vmax/KM) were determined in a 20 mM sodium acetate buffer (pH 5.6) at 37 °C. Using different concentrations (0.2 to 2.5 mM) of pNP-beta-D-glucopyranoside, KM and Vmax were determined from the Lineweaver-Burk plot [21]. Each experimental point was determined at least in triplicate.

Effects of chemical agents:
To determine the effect of various compounds (cations, EDTA, alcohols, organic acids), which could be potential activators or inhibitors of the crude enzyme, the enzyme solution was pre-incubated at 37 °C for 20 minutes with chemical agents at concentrations of 1 mM [1% (w/v)] or 5 mM [5% (w/v)]. The activity was measured under the enzymatic assay conditions using pNP-beta-D-glucopyranoside (5 mM) as the substrate. Residual activities were expressed as a percentage relative to the control without chemical agents [22].

Statistical analyses:
All determinations reported in this study were performed in triplicate. The results were expressed as means ± standard deviation.

Results:-

Effects of pH:-
The optimum hydrolysis pH of β-glucosidase from the Golden Globe variety is 5.6 when the synthetic substrate pNP-β-glucoside is used. It shows maximum activity in 20 mM acetate buffer (pH 3.6 to 5.6). The pH stability of the enzyme is assessed in 20 mM acetate buffer (pH 3.6 to 5.6) and 20 mM phosphate buffer (pH 5.6 to 8.0). It retains more than 80% of its hydrolytic activity in the pH range of 3.8 to 6.9 when pre-incubated at 37°C for 1 hour (Table 1).

Effect of temperature:-

The optimal hydrolysis temperature for the enzyme is 90°C. It retains more than 70% of its hydrolytic activity between 80 and 95°C. The temperature coefficient (Q10) calculated between 75 and 85°C is 3.723 for this beta-glucosidase. Using the Arrhenius plot, the activation energy was found to be 33.24 kJ·mol⁻¹ for this enzyme. The study of thermal stability shows that at 37°C the enzyme retains all of its catalytic activity for more than 15 hours and 50 minutes of pre-incubation in 20 mM acetate buffer at pH 5.6. This hydrolase activity is also retained for 13 hours and 50 minutes at 90°C. Beyond this pre-incubation time, the activity decreases by approximately 48.79%. The study of thermal denaturation shows that at 90°C, this β-glucosidase retains about 100% of its hydrolase activity. Beyond this temperature, a drop in its hydrolytic activities is observed, indicating its denaturation (Table 1).

Kinetic Parameters:

The effect of substrate concentration on enzymatic activity was studied using pNP-beta-D-glucopyranoside. With this substrate, the enzyme followed the Michaelis-Menten equation, exhibiting Michaelis-type kinetics. The values of the Michaelis-Menten constant (KM), the maximum velocity (Vmax), and the catalytic efficiency (Vmax/KM) are 0.997373 mM, 1.31319 U/mg, and 1.31665 U/mM*mg, respectively (Table 1).

Table1:-Physicochemical properties of native beta-glucosidase extracted from Golden Globe almonds

Physicochemicalproperties	**β-glucosidase activity**
Optimum pH	$5,6 \pm 2.10^{-3}$
pH stability	$3,8 - 6,9 \pm 1.10^{-2}$
Optimum temperature (°C)	90 ± 1.10^{-2}
Thermal stability zone(°C)	$37- 95 \pm 1.10^{-3}$
Temperature coefficient (Q10)	3,723
Activation energy (kJ.mol-1)	33,24
KM (mM)	0,997373
Vmax (U/mg)	1,31319
Vmax/KM (U/mg*mM)	1,31665

Effects of chemical agents :-

Metal ions and EDTA:-

The activating or inhibiting effects of several ions on beta-glucosidase have been explored. This study shows that Na+, Ca2+, Zn2+, Cu2+ ions and EDTA neither inhibited nor activated the enzyme. Meanwhile, K+ and Mg2+ ions act as activators of β-glucosidase activity. Ag+, Ba2+, Pb2+, and Fe2+ ions, on the other hand, are inhibitors. (Figure 1).

Disulfide bond reducers:-

Disulfide bond reducers such as β-mercaptoethanol, L-cysteine, and DL-dithiothreitol have no significant effect on the activity of this enzyme. However, PCMB and DTNB inhibitit (Figure 1).

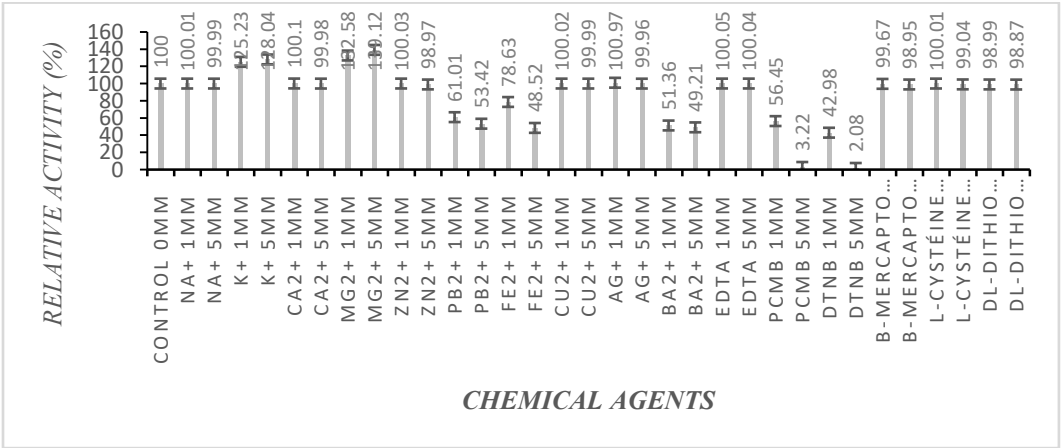

Fig1 :-Effects of chemical agents

Effects of alcohols, organic acids, and glucose:-

Methanol at 15% inhibits the activity of this enzyme. However, ethanol at 15% has no significant effect on the enzyme. (Figure 2)

Fig2 :-Effects of some alcohols

While citric acid (5 mM) reduces the activity of this β-glucosidase by 96%, acetic acid (5 mM), ascorbic acid (5 mM), and glucose (100 mM) have no significant effects on the enzyme (Figure 3).

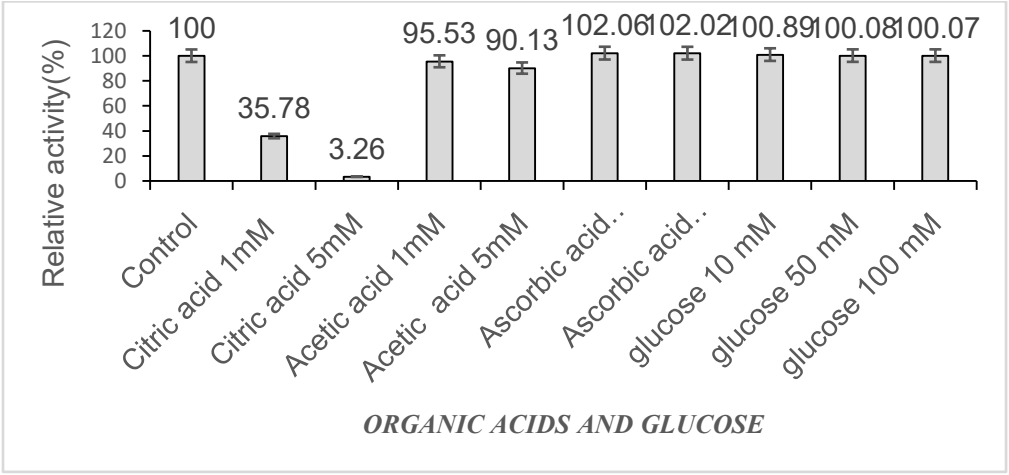

Figure 3:-Effects of some organic acids and glucose

Discussion:-

The pH = 5.6 is identical to that of β-glucosidase with β-xylosidase activity extracted from the digestive juice of the crab Cardisomaarmatum [20]. However, it is higher than the pH = 5.0 of β-glucosidases from the fungus Myceliophthoraheterothallica F.2.1.4 obtained by solid-state and submerged culture [23]. These identical pH values could suggest that these two enzymes have the same amino acid composition, as amino acid composition is one of the factors that greatly influences pH [24]. This pH range corresponds to that reported by Ketudat and Essen [25]. They state that the optimum pH of most β-glucosidases is between pH 4 and 7.5, and that the pH values within this range depend on their sources, amino acid sequences, and cellular locations. A β-glucosidase with a pH of 5.6 was also extracted from the crab Cardisomaarmatum, with a stability range between 4.6 and 6.6 [20]. This stability range is lower than that newly extracted from the Golden Globe variety. This varies from 3.8 to 6.9. The β-glucosidase extracted from the kernels of this mango variety is therefore active in an acidic environment. The enzyme is thus acidophilic. These acidic pH β-glucosidases are particularly widely used in the conversion of plant material into biofuel and in the synthesis of aromatic compounds during wine fermentation [26] [27].

The optimal hydrolysis temperature of the enzyme being 90°C, it can be stated that this enzyme is thermophilic. This characteristic is very common in enzymes of bacterial and fungal sources. It is, moreover, highly sought after

by industry [28][29]. This enzyme is active at 90°C for 13 hours and 50 minutes. It could therefore be described as a thermostable enzyme. Furthermore, hydrolysis catalyzed by a thermostable enzyme has several advantages. At high temperatures, the enzyme maintains its structure and stability, thereby reducing its degradation and also facilitating its transport and storage. High temperature improves the solubility of materials and decreases their viscosity, thereby facilitating enzyme-substrate interactions while accelerating the reaction rate [30].

The activation energy of the enzyme is 33.24 kJ.mol-1. This value is lower than those of beta-glucosidases from Aspergillus niger of form N, Thermotoga maritima, and Cellulomonas biazotea (88 kJ.mol-1), which are respectively 50, 36.92, and 88 $kJ.mol^{-1}$ [31] [32] [33]. The low activation energy value between 75 and 85°C could indicate that at these temperatures, the enzyme still maintains its three-dimensional structure while increasing the reaction rate. Therefore, this enzyme is thermostable as it withstands these high temperatures by lowering the required activation energy.

The phenomenon of denaturation could be explained by the fact that proteins generally have three-dimensional structures that can be easily affected by high thermal treatments [34]. These denaturations are preceded by inactivation phenomena that could occur according to a two-step mechanism [35]. In the first step, we observe a modification of low-energy bonds such as hydrogen bonds, hydrophobic interactions, and Van der Waals interactions that link the different amino acids constituting the catalytic sites.This leads to an initial disorganization of these sites. In the second stage, the changes affect the covalent bonds of the amino acids in the active centers. As the temperature continues to rise, the disorganization becomes irreversible, and we observe denaturation as noted in this study [36].

The KM value (0.997373 mM) is higher than those of the cloned β-glucosidases from Thermotoganaphthophila (0.45 mM) and Thermotoga maritima (0.56 mM) [37]. However, it remains lower than the KM value of the β-glucosidase from Thermotoganaphthophila RKU-10T, which is 1.5 mM [38]. These results suggest that the β-glucosidase extracted from the kernels of Golden Globe mango seeds has a higher affinity for the substrate pNP-β-D-glucopyranoside than the cloned β-glucosidases from Thermotoganaphthophila and Thermotoga maritima, but a lower affinity compared to the KM value of the β-glucosidase from Thermotoganaphthophila RKU-10T.

These minerals that act on the enzyme could indicate that they are cofactors of this enzyme, as they play an important role in the active site of the enzyme. They are therefore essential for the functioning of this β-glucosidase. Ethanol has no effect on this enzyme, whereas it reduces the relative activity of the β-glucosidase extracted from the thermophilic fungus Myceliophthoraheterothalica F.2.1.4 by 15% [23]. This good tolerance to ethanol could be beneficial for wine production, as is the case with certain enzymes of fungal origin that are not necessarily Saccharomyces [39].These high concentrations, which have no effect, could be advantageous for the food and other industries. There are essentially four groups of β-glucosidases, some of which are tolerant to the presence of glucose and others that are activated or inhibited by its presence [40]. These glucose-tolerant or glucose-stimulated β-glucosidases are the most sought after, hence their use as a commercial enzyme cocktail to improve the hydrolysis of plant material [41] [42].

Conclusion:-

The effects of pH, temperature, and certain chemical compounds were tested on the native beta-glucosidase extracted from the kernels of the Golden Globe mango variety (Mangifera indica L). These studies showed that this enzyme is acidophilic. This acidophilic characteristic is widely used in wine and biofuel production. It is also thermophilic and thermostable with low activation energy. Therefore, its use is desirable in the degradation of cellulosic material with glucose release. It remains stable between 37 and 90°C, maintaining 100% of its hydrolytic activity for more than 12 hours. She is resistant to the effects of high concentrations of glucose and methanol. Therefore, she could be used in breweries where fermented beverages are produced. All these results could represent major advantages for her use in the processing of dairy products. However, purification studies and more in-depth analyses of thermal stability and thermodynamic parameters will allow a better understanding of all the capabilities of this enzyme.

References:-

1. Buka M., Ayodeji A., and Santhosh P. (2024): Microbial β-glucosidases: Recent advances and applications. Biochemistry, 225: 49-67.https://doi.org/10.1016/j.biochi.2024.05.009.

2. Samhita M. A. S. Vickram T. B., Sridharan1 R. Parameswari M., and Ramesh P. (2016): Screening, production, optimization and characterization of b-glucosidase using microbes from shellfish waste.3. Biotechnology, 6(213): (1-10).DOI 10.1007/s13205-016-0530-7
3. Paula C. G. M., Jose C. Q. J., Lizzy A. A. V., Mauricio B., Eleni G., Luis Antonio M., and Roberto Da S. (2023): β-glucosidase: An overview on immobilization and some aspects of structure, function, applications and cost. Process Biochemistry, 130: 26-29. https://doi.org/10.1016/j.procbio.2023.03.035
4. Amer A., Faiz ul-H. N., Kashfa B., and Aasia B. (2017): Microbial β-Glucosidase: Sources, Production and Applications. Journal of Applied & Environmental Microbiology, 5(1): 31-46.DOI 10.12691/jaem-5-1-4
5. Sauratej S., Maithili D., and Supratim D. (2023): Chapter 5-β-Glucosidase: Structure, function and industrial applications. Glycoside hydrolase Biochemistry, Biophysics, and Biotechnology Foundations and Frontiers in Enzymology, 97-120. DOI:10.1016/B978-0-323-91805-3.00004-6.
6. Aicha A. H., Nico A., Antje C. S., Petr B., and Said B. (2020): Insights from enzymatic degradation of cellulose and hemicellulose to fermentable sugars-a review. Biomass and Bioenergy, 134: 105481. https://doi.org/10.1016/j.biombioe.2020.105481
7. Gopal S., Verma A. K., and Vinod K. (2016): Catalytic properties, functional attributes and industrial applications of b-glucosidases. 3 Biotechnology in springer 6(3): 1-14. https://doi.org/10.1007/s13205-015-0328-z
8. Annette S., Mette L., Peter S. L., and Birgitte K. A. (2013): Fungal Beta-Glucosidases: A Bottleneck in Industrial Use of Lignocellulosic Materials. Biomolecules 3, 612-631.https://doi.org/10.3390/biom3030612
9. Jingjing S., Wei W., Yu Y. and Jianhua H. (2020): A novel glucose-tolerant GH1β-glucosidase and improvement of its glucose tolerance using site-directed mutation. Applied Biochemistry and Biotechnology, 192: 999-1015.https://doi.org/10.1007/s12010-020-03373-z
10. Tiwari, R., Singh P. K., Singh S., Nain P. K. S., Nain L., and Shukla P. (2017): Bioprospecting of novel thermostable beta-glucosidase from Bacillus subtilis RA10 and its application in biomass hydrolysis. Biotechnology for Biofuels, 10(246): 2-18. DOI 10.1186/s13068-017-0932-8.
11. Sebti, C., and Deghdak, M. (2018): Cellulase production by Trichoderma longibrachiatum cultivated on olive pomace. Master's thesis in Mycology and Fongal Biotechnology, Mentouri Brothers University Constantine 1.1-39.
12. Von M. H. J. (1984). Shrubs and trees of the Sahel, their characteristics and uses. Journal of traditional agriculture and applied botany, 31(3-4): 262-263.
13. Moussa K., Mady C., Aminata T., Marie-Noëlle D.- C. and Aliou G. (2014): Microbiological quality of mango (Mangifera indica L.) slices sold in Dakar (Senegal). International. Journal of Biological and Chemical Sciences, 8(4): 1611-1619.DOI: 10.12691/jfnr-12-1-2
14. Kansci G, Koubala B. B. and Lape I. M. (2003): Effect of ripening on the composition and the suitability for jam processing of different varieties of mango (Mangifera indica). African Journal of Biotechnology, 2: 301-306.https://doi.org/10.5897/AJB2003.000-1061
15. Kabuki T., Hadjime N., Megumi A. and Shigeko U. Kuwabara Y., Dosako S. (2000): Characterization of novel antimicrobial compounds from mango (Mangifera indica L.) kernel seeds.Food chemistry, 71:61-66.
16. Ya K. C., Gnanwa M. J., Blei S. H., Fagbohoun J. B., Kone Y. K. C., Beugre G. A. M. and Kouame L.P. (2021): Heterosidaseactivitises extracted from the seeds of the pits of six mango (Manguifera indica L) cultivars. International Journal of Advanced Rechaerche, 9(06): 800-807.
17. Lowry O. H., Rosebrough N. J., Farr A. L. et Randall R. J. (1951). Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry 193: 265-275.
18. Fagbohoun J.B., Karamoko Y., Dabonne S., Kouadio E. J. P., Due E. A., and Kouame L. P. (2012): Characterization of the dual activity of an endo-beta-D-glycosidase from salivary glands of Macrotermessubhyalinus little soldier against carboxymethylcellulose and xylan, International Journal of Biomolecules and Biomedicine, 2(3): 8-14.
19. Bernfeld P. (1955): Amylase α and beta, In: Methods in Enzymology. Academic Press, 1: 149-58.
20. Claude K. Y., Fankroma M. T. K., Sophie N. G., Soumaila D. and Lucien P. K. (2014): A beta-glucosidase with beta-xylosidase activity from the digestive juice of the land crab (Cardisomaarmatum). Asian Journal of Applied sciences, 2(04).
21. Lineweaver H. and Burk D. (1934): The Determination of Enzyme Dissociation Constants, Journal of the American Chemical Society, 56, 658-666, 1934.
22. Desire Y. A. Y., Dago G., Sebastien L. N. and Lucien P. K. (2009): Purification and biochemical characterization of a specific β-glucosidase from the digestive fluid of larvae of the palm weevil, Rhynchophorus palmarum. Journal of insect science 9 (4): 1-13.
23. Vanessa de C., Teixeira da S., Amanda L. de S. C., Rafael de C. S., Marcello B. S., Eleni G., and Gustavo O. B.-R. (2016): Effect of pH, Temperature, and Chemicals on the Endoglucanases and β-Glucosidases from the

Thermophilic Fungus Myceliophthoraheterothallica F.2.1.4. Obtained by Solid-State and Submerged Cultivation. Biochemistry Research International, 1-9.
24. Shaomin Y. and Guang W. (2019): Predicting pH Optimum for Activity of Beta-Glucosidases. Journal of Biomedical Science and Engineering, 12 (7): 354-367.
25. James R. Ketudat C. and Asim E. (2010): B-glucosidase. Cellular and molecular life sciences, 67: 3389-3405.
26. Neha S., Rishabh R., Sonam J., Karan P., Manish S., Vijay K. T., Rakesh S. S., Vijai K. G., Pranab B. M., Ahamad F. K., and Pradeep K. M. (2019): Microbial Beta Glucosidase Enzymes: Recent Advances in Biomass Conversation for Biofuels Application. Biomolecules, 9(6): 1-23. doi: 10.3390/biom9060220
27. Neelam M., Rekha S., and Veeru P. (2014):Bioethanol Production by A. Niger Produced Cellulase by Solid State Fermentation. International journal of sciences and research, 3(8): 207-810.
28. Yu-Ying H., Zhi-Hua L., Hong-Zhao Z., Qian Z., Meng-Ting L., Peng S., Fei W., Dan Z., Wen-Dong X., and Yi-Rui Y. (2023): Characterization of a thermophilic and glucose-tolerant GH1β-glucosidase from hot spring and its prospective application in corn stover degradation. Frontiers in Microbiology, 14: 1-13. https://doi.org/103339/fmicb.2023.1286682
29. Ramesh C. K., Rishi G., and Ajay S. (2011): Microbial Cellulases and Their Industrial Applications. Enzyme research. 1-10. doi:10.4061/2011/280696
30. Yanchao G., Anke C., Kelin L., and Chaoneng Ji (2024): Structural insights and functional characterization of a novel β-glucosidase derived from Thermotoga profunda.Biochemical and Biophysical Research Communications, 732 (5): 150405.
31. Javed M. R., Rashid M. H., Riaz M., Nadeem H., Qasim M., and Ashiq N. (2018): Physiochemical and thermodynamic characterization of highly active mutated Aspergillus niger β-glucosidase for lignocellulose hydrolysis. Protein & Peptide Letters, 25:208–219.
32. Mehmood M. A., Shahid I., Hussain K., Latif F., and Rajoka M. I. (2014): Thermodynamic properties of the β-glucosidase from Thermotoga maritima extend the upper limit of thermophilicity. Protein and Peptide Letters, 21:1282–1288.
33. Rajoka M. I., Idrees S., Ashfaq U. A., Ehsan B., and Haq A. (2015): Determination of substrate specificities against β-glucosidase A (BglA) from Thermotoga maritima: a molecular docking approach. Journal of Microbiology and Biotechnology, 25:54–59.
34. Stauffer, C. E. (1989): Enzyme assays for food scientists. 1st Ed. New York: Van Nostrand ein hold, 67-78.
35. Daniel, R. M. (1996): The upper limits of enzyme thermal stability. Enzyme and Microbial Technology, 19: 74-79.https://doi.org/10.1016/0141-0229(95)00174-3
36. Ya, K. C., Konan, K. H., Gnangui, S. N. and Kouame, L. P. (2014): Study of thermal stability of beta-glucosidase from the land crab (Cardisomaaumatum) digestive juice: kinetic and thermodynamic analysis. International Journal of Development Research, 4(9): 1836-1840. September 2014 | International Journal of Development Research (IJDR)
37. Akram F, Haq Iu, and Mukhtar H. (2018): Gene cloning, characterization and thermodynamic analysis of a novel multidomain hyperthermophilic GH family 3 β-glucosidase (TnBgl B) from Thermotoganaphthophila RKU-10 T. Process Biochemistry, 66:70-81.https://doi.org/10.1016/j.procbio.2017.12.007
38. Akram F., Haq I., Khan M. A., Hussain Z, Mukhtar H, and Iqbal K. (2016): Cloning with kinetic and thermodynamic insight of a novel hyperthermostable β-glucosidase from Thermotoganaphthophila RKU-10T with excellent glucose tolerance. Journal of Molecular Catalysis B: Enzymatic, 124:92-104. https://doi.org/10.1016/j.molcatb.2015.12.005
39. Fia, G., Olivier V., Cavaglioni A., Canuti V., and Zanoni, B.(2016): Side activities of commercial enzyme preparations and their influence on the hydroxycinnamic acids, volatile compounds and nitrogenous components of white wine: Collateral effects of enzyme treatment on white wine. Australian Journal of Grape and Wine Research, 22: 366-375. https://doi.org/10.1111/ajgw.12232
40.Wenqi Y.,Yaowu S., Rubing W., Huanyu Z., Hongyan J., Jie M., Guoqi Z., Luqi H., Lanping G., Juan W., and Wenyuan G. (2024): Microbial production and applications of β-glucosidase-A Review. International Journal of Biological Macromolecules, 256 (2): 127915. https://doi.org/10.1016/j.ijbiomac.2023.127915
41. Matsuzawa, T., and Yaoi, K. (2016): Screening, identification, and characterization of a novel saccharide-stimulated β-glycosidase from a soil metagenomic library. Applied Microbiology and Biotechnology, 101: 633-646. https://doi.org/10.1007/s00253-016-7803-2
42. Liu, Y., Li, R., Wang, J., Zhang, X., Jia, R., Gao, Y., and Peng, H. (2017): Increased enzymatic hydrolysis of sugarcane bagasse by a novel glucose- and xylose-stimulated β-glucosidase from Anoxybacillusflavithermus subsp. yunnanensis E13T. BMC Biochemistry, 18(4): 2-8. https://doi.org/10.1186/s12858-017-0079-z.