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RESEARCH ARTICLE

HETEROSIDASE ACTIVITIES EXTRACTED FROM THE SEEDS OF THE PITS OF SIX MANGO (MANGIFERA INDICA L) CULTIVARS

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Abstract

Heterosidases are enzymes capable of releasing bioactive moleculesused in cosmetics,nutrition, medicine and in manyotherfields.For thiswork, the presence of glycosidases withheterosidaseactivitiesweredetectedin the seed of the kernels of six cultivars of mangoes (Mangiferaindica L) cultivatedin the region of DALOA (Côte d'Ivoire) in orderto select new enzymatic sources to original activities. To do this, the crudeenzymaticextractsweretakenfrom the almonds of kernels of the LOCAL, KENT, CAMEROUN, TARDIVE, GREFFE and SUCETTE cultivars of mangoes. Specificactivitiesweredeterminedfromtheseextractsand thencompared. The heterosidaseactivitiestestedwerethose of the β-glucosidase, β-galactosidase, β-fucosidaseand phosphatase activities. All theseactivitieshave been present in thesecultivars.However, thehighestspecificactivitieswerethose of the LOCAL cultivarfollowed in general by those of the GREFFE cultivar. It thereforeemergedfromthisstudythattheseseeds have enzymaticequipment capable of degradingheterosides. Thesedifferent glycosidases couldconstitute important enzymatictools for the valorization of food and non-foodbiomolecules of agricultural rawmaterials and for the development of industry.

Introduction:-

Enzymes are essential catalysts for all biologicalsystems. Theyoperateunderrelativelymildtemperature, pH and pressure conditions (Johannes et al., 2006).They are therefore of undeniablescientificinterestbecausetheircatalyticproperties. Their use in industryismainlylinked to theirspecificities and theircatalytic efficiences. The specificityhelpsprevent the formation of unwanted by-productsthattakes place withchemicalcatalysts (Weil et al., 1998).Efficiency or productivity gain, the reduction in the quantities of secondaryproducts and toxicwaste in favor of researchintobiomoleculeshaving not onlyindustrial applications but which can allow the improvement of the properties of certain molecules. Thesemanyadvantages open the

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industrialfield to the use of enzymes in the sectors of agriculture, medicine, pharmacy, cosmetic, textile, stationery, tannery, enzymaticchemistry (production of antibiotics, aminoacids…) and agro-industry (bakeries, starches, drinks and milk industries…)(Asano, 2003; de Melo et al., 2006 ; Piotrowska and Coper, 2010 ; Fareeha et al., 2011 ; Pandey et al., 2013). Despitetheirabundant use in industry, the enzymes currentlyavailable on the market are generally not very stable and some have a widespecificity.In addition, theiryieldremainslow.The search for new enzymes with high stability, specificwithgoogcatalyticefficiencyisthereforenecessarybecause the yield and catalyticspecificityalsodepend on the nature and origin of the enzyme used(Yoon and Ajisaka, 1996 ; Placier et al., 2009). For thispurpose, enzymes are sought in bacteriat, fungi, animals and plants such as breadfruit and mango, the fruit of MangiferaindicaL. (Garima and Anil, 2021 ; Tiwari et al., 2013 ; Ya et al., 2014 ; Konan at al., 2008 ; Tamires at al., 2011).For this latter case, it must berecognisedthatmanystudies have been carried out on the pulp, skin and seedinside the kernel of this fruit.(Tunchaivaphum et al., 2013 ; Yatnati et al., 2014 ; Asma, 2017 ; Ana et al., 2019 ; Risk, 2019). However, at the level of the kernel seed, enzymaticstudiesremainlimited. We have therefore set ourselves the objective of detecting a certain number of enzymaticactivitiescontained in the seeds of the kernel of six varieties of mangoescultivated in the regionof DALOA Côte d'Ivoire. To achieve the desiredresults, cruds enzyme extracts are first prepared, thenheterosidaseactivities are assayed and finallyspecificactivities are détermined for forbetterinterpretation.

Material and Methods:-

Chemicals and reagents

para-nitrophenyl-glycopyranosides (pNP-glycopyranosides) werepurchasedfrom Sigma Aldrich. All otherchemicals and reagentswere of analytical grade.

Plant material

Mango stones of differentcultivarswereharvestedfrom plantations around DALOA (Côte d'Ivoire). Thesepits of the mangoeswerecollected and dried in the sun for sevendays to avoidhumidity an more easilyremove the seedsfrom the pits. Driedpitswerecracked to obtain the kernels.

Enzyme extraction

30 g of kernelsweregroundwith 80 ml of sodium acetate buffer (20 mM, pH 5.6) containing 0.9% of sodium chloride. The liquide obtainedispooled and centrifuged at 12,000 g for 30 min. The obtainedSupernatantconstituted the crudeextract, whichwasstored at - 20 °C.

Proteinessay

Protein concentration wasdeterminedspectrophotometrically at 660 nm by the method of Lowry et al. using bovine serumalbumin as a standard (Lowry et al.,1951).

Enzyme essay

Under the standard test conditions, hydrolyticactivity of beta-glucosidase, beta-galactosidase, beta-fucosidase or phosphatase againstpNP-beta-D-glucopyranoside, pNP-beta-D-galactopyranoside, pNP-beta-D-fucopyranoside or pNP-phosphate, respectively, weremeasured by the release of para-nitrophenol. An essay mixture (250 µl) containing 75 µl of pNP-glycopyranoside or pNP-phosphate (5 mM) in 20 mM sodium acetate buffer (pH 5.6) with 50 µl enzyme solution, wasincubated at 37 °C for 10 min. The referencecellcontained all reactantsexcept the enzyme. The reactionwasstopped by adding 2 ml of sodium carbonate (2 %, w/v) and the absorbance of the essay solution wasmeasured at 410 nm (Fagbohoun et al., 2012). One unit (U) of enzyme activitywasdefined as the amount of enzyme, whichreleased one µmol of para-nitrophenol or reducingsugar per min under the definedreaction conditions (Bernfield, 1955). Specificactivitywasexpressed as units per mg protein (U/mg of protein).

Statistical analyses

All determinationsreported in thisstudywerecarried out in triplicate. Resultswereexpressed as means ± standard deviation.

Results and Discussion:-

The resultsobtained show the heterosidaseactivitiestested on the seeds of the kernel of the six mango cultivars are indeedpresent in the crudeenzymaticextract. These are β-glucosidase, β-galactosidase, β-fucosidase, and phosphataseactivities. Theseactivities are expressedrespectively as specificactivities(Table I).

Table I :- The heterosidaseactivitiestested on the seeds of the kernels of the six cultivars ofmangoes.

Cultivars	Specificactivities (U/mg)			
	B-glucosidase Activities	**B-galactosidase activities**	**B-fucosidaseactivities**	**Phosphatase activities**
LOCAL	$1,668 \pm 1.10^{-3}$	$1,641 \pm 1.10^{-3}$	$1,491 \pm 7.10^{-2}$	$1,162 \pm 3.10^{-3}$
KENT	$0,421 \pm 2.10^{-2}$	$0,561 \pm 4.10^{-2}$	$0,497 \pm 4.10^{-2}$	$0,286 \pm 1.10^{-2}$
CAMEROUN	$0,186 \pm 1.10^{-3}$	$0,241 \pm 4.10^{-3}$	$0,238 \pm 3.10^{-3}$	$0,175 \pm 1.10^{-2}$
TARDIVE	$0,253 \pm 2.10^{-3}$	$0,281 \pm 3.10^{-3}$	$0,271 \pm 6.10^{-3}$	$0,193 \pm 5.10^{-3}$
GREFFE	$0,172 \pm 1.10^{-2}$	$0,836 \pm 1.10^{-3}$	$0,611 \pm 4.10^{-2}$	$0,486 \pm 7.10^{-3}$
SUCETTE	$0,408 \pm 3.10^{-2}$	$0,489 \pm 1.10^{-2}$	$0,496 \pm 4.10^{-3}$	$0,381 \pm 2.10^{-2}$

β-glucosidase activities

Fig. 1 represents the different values of specific β-glucosidase activities. This shows that the higestspecificactivityisthat of the LOCAL cultivarfollowed by that of the KENT cultivar. It shouldberememberedthat β-glucosidases fromdifferent sources and havingspecificactivitieshigher or lowerthanthat of the LOCAL cultivar wereextracted and thencharacterized. Wewillquotethat of A. niger URM 6642 (3.7789 U/mg) and that of the crabC. amatum (0.97 U/mg) (Oriente, 2015 ; Ya et al., 2014). This meansthat β-glucosidases can be of animal, plant, microbial and fongalorigin(Bujang et al., 2014 ; Vassao et al., 2018 ; Naz et al., 2010 ; Folasade et al., 2016 ; Yao et al., 2016 ; Méndez-literet al., 2018). This diversity at the level of origins assumes thatthese enzymes are not onlydifferentfromeachother but theyalso express particularitiesduring the precesses of degradation or synthesis of biomolecules. Regarding the role of these enzymes, itshouldbenotedthatthey are involved in the hydrolysis of β-glycosidic bonds between an aglycone which can be an alkyl or aryl group and a glucose molecule or betweentwoglyconeresidues(Padmavathi and Rashmi, 2017). This capacityallowsthem to release or synthesizephenolic compounds and otheralkaloidsfrom plants, aromatic compounds in wines, to allow total saccharification of cellulosicmaterial…(Huajian et al., 2015). Given the multiplicity of roles of these enzymes, itwouldbeinteresting, throughadditionalstudies, to understand not onlytheirroles in the degradation or synthesis of macromolecules in these plant species, but also to elucidatetheir uses in the development of resources of agricultural origins in order to considertheir exploitation for industrialpurposes. Enzymes extractedfrom the LOCALand KENT cultivars possessing the highestspecificactivitiescouldbesubsequentlyconcerned by thesestudies.

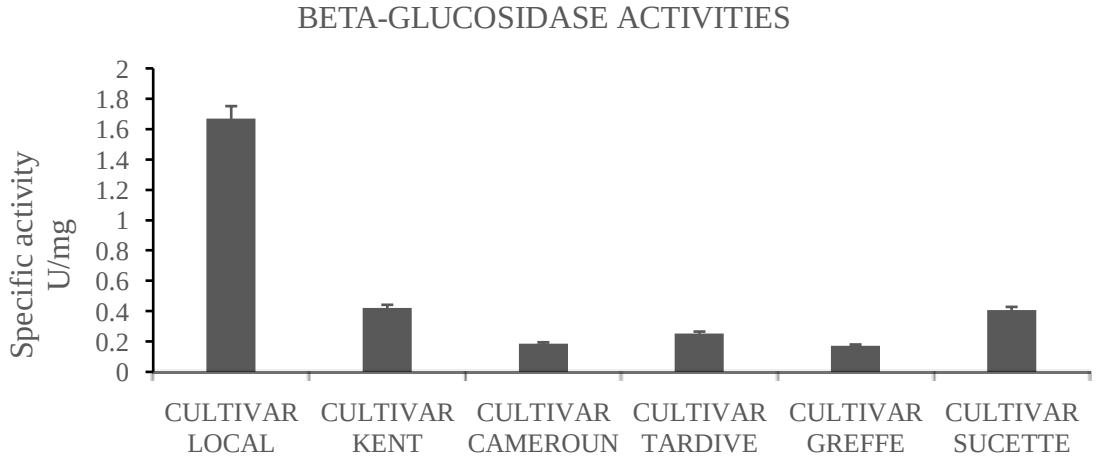

Fig. 1:- The β-glucosidase activitiespresent in the seeds of the kernel of six cultivars of mangoes β-galactosidase activities.

Regardingβ-galactosidase actvities, the most important specificactivityisthat of the LOCAL cultivar followed by that of the GREFFE cultivar (Fig. 2).These values are greaterthanthat(0.115 U/mg) of the thermostable β-galactosidase extractedfromThermussp. A4, but remainmuchlowerthanthat(6,5 U/mg) of β-galactosidase fromBacillus stearothermophilus(Ohtsu et al., 1989 ; Chen et al., 2008). Indeed β-galactosidase not onlyplays an important role in industry but also in treatment of certain biologicaldeficiencies. In industry, itisused in the treatment of whey, in thehydrolysis of lactose in order to increase the sweetening power, to reduceitshygroscopiccapacity and itscrystallizationwhenstoringdairyproducts(Sagib et al., 2017 ; Klein et al., 2010). In the treatmentof deficiency,it

has been reportedthat the presence of excess lactose in the intestine could lead to decreased calcium absorption and tissue (Sagib et al., 2017). Thesefactswouldbe the cause of diarrhea, gas and otherinconveniences(Lukito et al., 2015 ; Felicilda-reynaldo and Kenneally, 2016). A lack of β-galactosidase in the intestine therefore leads to intolerance of milk and itsderivaties by some people. In additional, β-galactosidase is capable of synthesizingglycoconjugates(Mahdian et al., 2016). In general, β-galactosidases are preferablyextractedfrombacteria, yeasts and other fungi and from plants ; mainly in fruits during the ripeningprocess, but rarely in sseds (Seddigh and Darabi, 2014). β-galactosidases have just been detected in the seeds of the kernels of differentmango cultivars, somewith high specificactivities. As specificity, catatyticefficiency, temperature and pH conditions of all enzymes depend on originspecies, β-galactosidasesfromLOCAL and GREFFE cultivars with the highestspecificactivitiescouldbeinvestigated in order to know their performance and interests.

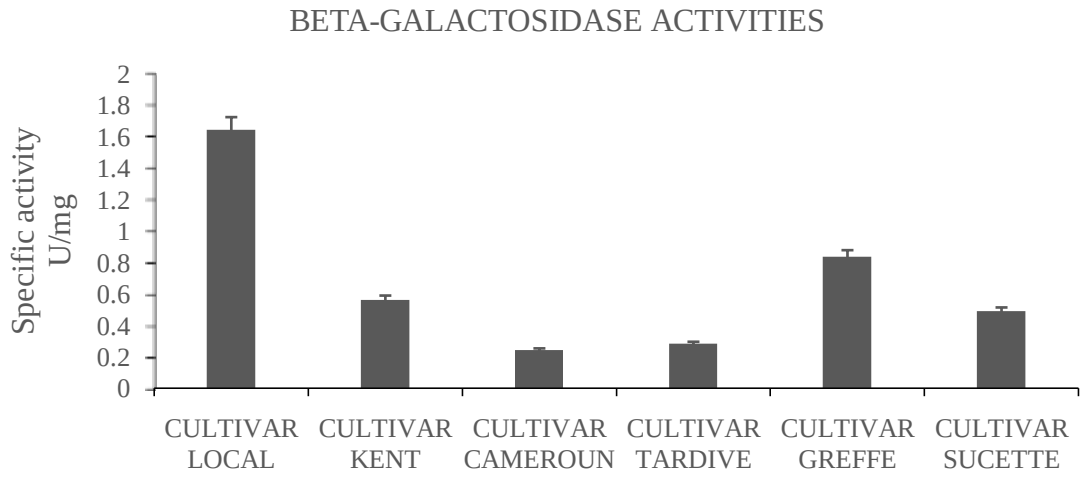

Fig.2:- The β-galactosidase activitiespresent in the seeds of the kernel of six cultivars of mangoes β-fucosidaseactivities.

The value of specificactivity of the β-fucosidaseextratedfrom the LOCAL cultivar isbeyond all the values of the spécificactivities of the other cultivars. This value is more than double that of the specificactivity of the GREFFE cultivar. It isthree times higherthan the values of the respective specificactivities of KENT and SUCETTE cultivars (Fig.3). This high specificactivity value couldbe of interest. However, thereis no reliable and seductive information on the industrial utility of β-fucosidase(Wierzbicka-Woś et al., 2013). In addition, itisoftenassociatedwith β-glucosidase activities(Nunoura et al., 1996). The spécificity of β-fucosidaseisthereforerarely strict. Only a strictly active β-fucosidasewould have beenisolatedfrom the latex of Lactuca savita(Giordani and Noat, 1998). Nevertheless, an in-depthstudy of thisβ-fucosidasefrom the LOCAL cultivar couldconfirm or refutetheseobservations.

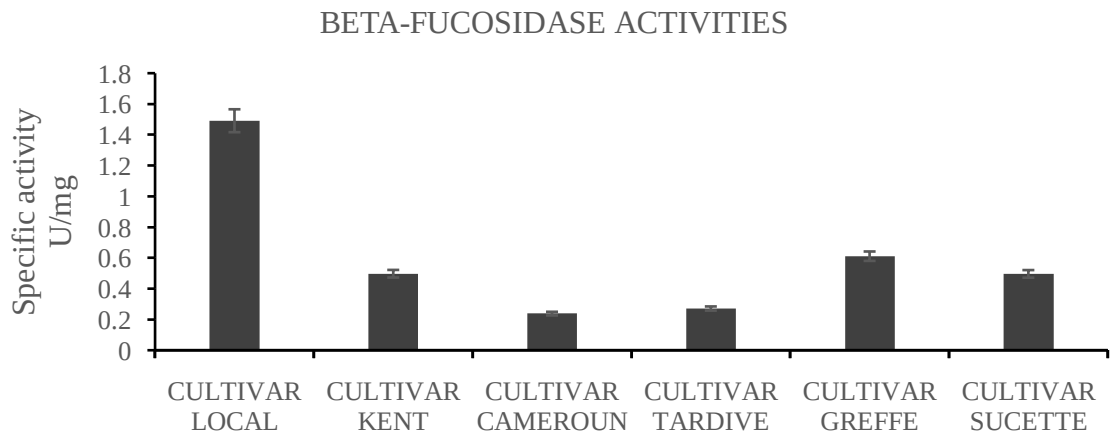

Fig. 3:- The β-fucosidaseactivitiespresent in the seeds of the kernel of six cultivars of mangoes Phosphatase activities.

The phosphatase remainshigher in the LOCAL cultivar with a very high specificactivitywhileitisdeclining in the other cultivars (Fig. 4).Thesephosphatase activitiesweredeterminedunder the experimental conditions at pH = 5,6. It wasspecifiedthatonlyacidphosphatases are active at thispH because in general,they have an optimal pH of lessthanseven(Turner, 2010).When the pH isgreaterthanseven, the phosphatase issaid to bealkaline (Pedro and Luis, 2020).In addition, a nomber of phosphomonosteraseshave an optimum pH between4,5 and 6,5when the para-NitroPhenylphosphatesubstrateisused(Turner, 2010). Phosphatases are agroupe of enzymes that hydrolyse phosphoric ester bonds releasing inorganicphosphates in an acidic medium (5,0 < pH < 6,0) (Kouadio et al., 2006). The are involved in the assimilation of inorganicphosphate, in the mecanism of regulation and production of energy, in the hydrolysis of variousforms of organic phosphate in the soil and in phosphorus pesticides (Kouadio et al., 2009). They are studied in animals, microorganisms and plants (Vance et al., 2003 ; Touhami et al., 2020). An acid phosphatase with a specificactivity of 1,82 U/mg wasextractedfromwheatgerm and immobilized on an agarose gel (Kalita and Ambascht, 2019). This value ishigherthanthoseobtainedfrom the enzymaticextracts of the seeds of kernels of the six cultivars of mangoes.However, phosphatases withspecificactivitieslowerthanthose of the LOCAL and GREFFE cultivars have already been purified and characterized(Kouadio et al., 2006). It wouldthereforebejustified to payparticular attention to the study of the phosphatases revealedfrom the extracts of the seeds of mango kernel becausetheycouldintervene in the conservation and therefore in the valorization of theseseedswhich are untiltodayreleasedinto nature and thusconstitute a source of pollution of ourenvironment.

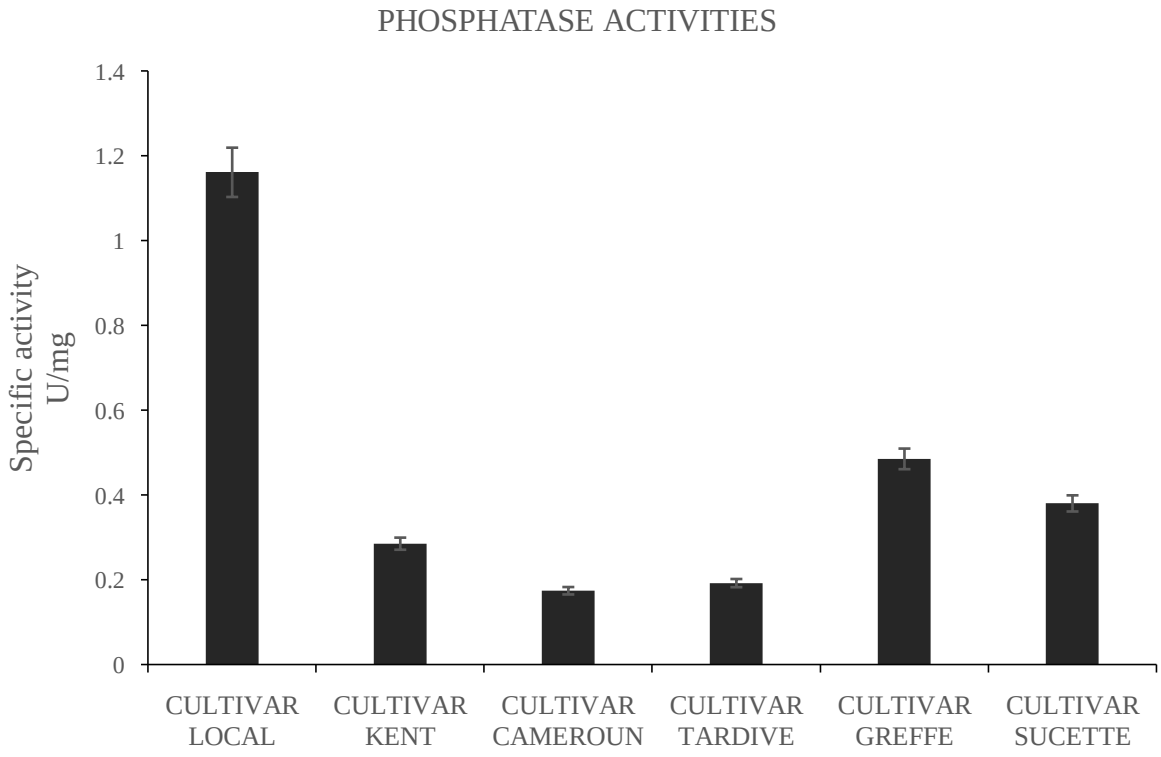

Fig. 4:- The phosphatase activitiespresent in the seeds of the kernel of six cultivars of mangoes.

Conclusion:-

This showedstudyfinaly the existence of severalenzymaticactivitieswhich are the β-glucosidase, β-galactosidase, β-fucosidase and phosphatase activities in the seeds of the kernelsof the mangoes of the LOCAL, KENT, CAMEROUN, TARDIVE, GREFFE and SUCETTE cultivars. The LOCAL cultivar, compared to others, exhibits the highers values of specificactivity. The presence of all glycosidase activitiesin theseseeds places us on the potentialthatwouldbeavailable to us if these enzymes wereused in the development of agricultural resources. However, all these enzymes withtheseactivities must first bepurified and characterized in order to identifytheir full potential.In addition, thisstudyis not exhaustive, whichmeansthatotherenzymaticactivitiescouldstillbedetected in theseseeds of kernels.

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