International Journal of Bio-Technology and Research (IJBTR)
ISSN(P):2249-6858; ISSN(E):2249-796X
Vol. 7, Issue 1, Feb 2017, 1-10

TRANS STELLAR
•Journal Publications •Research Consultancy

α -L-RHAMNOSIDASE: SOURCES, PRODUCTION, PURIFICATION AND CHARACTERIZATION OF THE DEBITTERING ENZYME

POONAM YADAV[1], ANIL KUMAR CHAUHAN[2] & SATENDRA P SINGH[3]

[1,2]*Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India*

[3]*Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India*

ABTRACT

This review discusses the debittering enzyme α-L-rhamnosidase and its role in processing of citrus fruit juices in food industries. α-L-Rhamnosidase [E.C.3.2.1.40] cleaves terminal α-L-rhamnose mainly from a large number of natural products. The enzyme has wide occurrence in nature and it is reported from plants, animals, yeasts, fungi and bacteria. α-L-rhamnosidase play very important role in biotechnology field and its major application in debittering of citrus fruit juices, enhancement of wine aroma and also in pharmaceuticals industry. Different sources, production methods, enzyme assay, purification and characterization are discussed here.

KEYWORDS: Debittering Enzyme, α-L-Rhamnosidase, Rhamnose, Citrus Fruit Juice and Aroma Enhancement

Received: Dec 21, 2016; **Accepted:** Jan 10, 2017; **Published:** Jan 20, 2017; **Paper Id.:** IJBTRFEB20171

Original Article

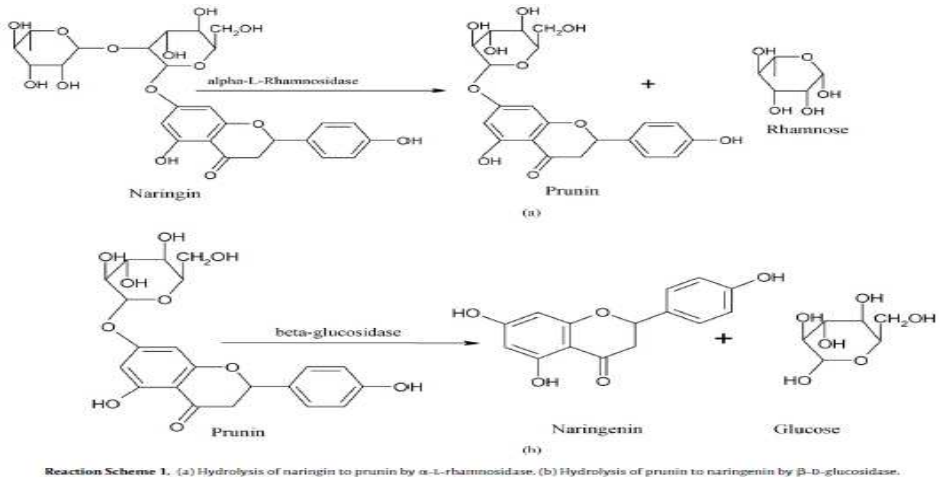
INTRODUCTION

The biotechnological potential of microbial enzymes has drawn a great deal of attention from various researchers worldwide, as they are likely to be biological catalysts in a variety of industrial processes. Many microorganisms have been reported in literature for their potential to produce naringinases; however, little is known about microorganisms that produce α-L-rhamnosidase (EC 3.2.1.40) activity.

Naringinase is a debittering enzyme that is used for the commercial production of citrus juices. It is a multienzyme complex which possesses α -L-rhamnosidase and β-glucosidase active centers (figure 1). α-L-rhamnosidase is extracellular enzymes which remove terminal α -L-rhamnosyl groups at the ends of polysaccharides and glycosides containing L-rhamnose. α-L-rhamnosidase are important industrial enzymes with great significance in biotechnology field and also have application in food and pharmaceuticals industry (Monti D. et al, 2004) as well as several other industrial applications for the bioconversion of natural or synthetic rhamnosides.

In particular, several applications of fungal α-L-rhamnosidase in the food industry, such as the debittering of grapefruit juice in which hydrolysis ofnaringin which is the most bitter flavonoid compound (Soares, 1998), the removal of hesperidin crystals which is present in orange juice, also it help the enhancement of wine aromas by enzymatic hydrolysis of terpenylglycosides (Caldini C, 1994) have been observed. It is also found that the de-rhamnosylated product quercetin-3-glucoside fromrutin (flavonoid compound)improve the antioxidant activity of asparagus juices. α-L-rhamnosidase enzyme has also been used for the studying of structural determination of polysaccharides, glycosides, and glycolipids, gellan and prunin (Roitner M. 1984). Inspite of α-l-rhamnosidases being biotechnologically important enzymes, review article on α-l-rhamnosidases is not more available in the

literature. The α-L-rhamnosidase activities are mainly used for the debitteringof citrus fruit juices which are popularly known as hesperidinases (Monti D, 2004) and naringinases (Romero C, 1984).



Reaction Scheme 1. (a) Hydrolysis of naringin to prunin by α-L-rhamnosidase. (b) Hydrolysis of prunin to naringenin by β-D-glucosidase.

Figure1: Mechanism of Action of α-L-Rhamnosidase and β-D-Glucosidase

SOURCES of ENZYME

Sources of naringinase, an enzyme complex which consist of two subunit, α-L-rhamnosidase and β-D-glucosidase, has been reported in the literaturethat it was firstly isolated from celery seeds (Hall, 1938). There are many sources of enzyme which are discussed below:

Plant Sources

There are limited studies available for the plant source of α-L-rhamnosidase enzyme. Naringinase, firstly reported in grape fruit leaves (Hall, 1938), and also the subunit of enzyme with the name α -L-rhamnosidase and β-D-glucosidase, studied from *Rhamnusdahurica* (Suzuki, 1962). The subunit, α -L-rhamnosidasealone isolated from the seeds of *Fagopyrumesculentum* (Bourbouze et al 1975). However, α– L-rhamnosidases from other plant sources have not been reported till now.

Animal Sources

Animal source of α-L-rhamnosidase enzyme has limited source, there are only two animal sources, viz. *Turbo cornutus* and pig liver have been reported for α-L-rhamnosidase enzyme producing enzyme (Kurosawa et al 1973). The human intestine *Bacteroid* JY-6 and *Fusabacterium*K-60 have been shown to produce α-L-rhamnosidase (Jang and Kim 1996; Park et al 2005).

Microbial Sources

The production of α-L-rhamnosidase by thermophilic anaerobic bacterium *Clostridium stercorarium*has been reported (Zverlov et al 2000). Some*Pseudoalteromonas* species and *Ralstoniapickettii*, which were obtained from the sea water of sub Antarctic environment, show the α-L-rhamnosidase activities in the low temperature range of −1 to 8◦C (Orrillo et al 2007). *Sphingomonaspaucimobilis* and *Bacillus* spGL1 show substantial α-L-rhamnosidase activities in a medium containing gellan as a carbon source (Hashimoto et al 1998). *Corticiumrolfsii* produces α-L-rhamnosidase which is active at low pH (Kaji et al 1973).

Table 1: Sources for α-L-Rhamnosidase Production

Source	Microorganism	References
Plants	Celery seeds(*Apiumgraveolens*)	Hall 1938
	Rhamnusdaurica	Suzuki 1962
	Buckwheat *(Fagopyrumesculentum)*	Bourbouze et al. 1976
	Grape fruit leaves	Ting 1958
Gastropod	*Turbo cornutus*	Kurosawa et al. 1973
Mammal	Pig liver	Qian et al. 2005
Fungi	*Penicillium sp.*	Young et al. 1989
	Aspergillusniger	Manzanares et al. 2001
	A. aculeatus	Manzanares et al. 2001
	A. kawachii	Koseki et al. 2008
	Rhizopusnigricans	Shanmugam and Yadav 1995
	Aspergillusniger (MTCC1344)	Puri et al. 2005
	Penicilliumdecumbens	Mamma et al. 2004
	Aspergillusniger(BCC 25166)	Thammawat et al. 2008
	Penicilliumulaiense	Rajal et al. 2009
	Aspergilluskawachii	Koseki et al. 2008
	Aspergillusflavus MTCC 9606	Vinita et al. 2012
	Acrostalagmusluteoalbus	Natalia et al. 2011
	*Aspergillusclavatonanicus*MTCC-9611	Vinita et al. 2012
	*Penicillium corylopholum*MTCC-2011	Sarita et al. 2013
	Penicilliumpurpurogenum	Maria et al. 2012
Yeast	*Hanshulaanomala, Debaryomyces polymorphus*	McMahon et al. 1999
	Pichiaangusta X349	Yanai and Sato 2000
Bacteria	*Bacteriodes distasonis*,JY-1	Jang and Kim 1996
	Thermomicrobiumroseum	Jang an and Kim 1996
	Clostridium stercorarium	Zverlov et al. 2000
	Bacillus sp. GL1	Hashimoto et al. 2003
	Geothermusvaporicell	Birgisson et al. 2004
	SphingomonasPaucimobilis	Hashimoto et al1998
	Pseudoalteromonas sp.	Mazzaferro et al. 2008
	Lactobacillus ulaiense	Rajal et al. 2009
	Lactobacillus acidophillus	Beekwilder et al. 2009
	Staphylococcus xylosus	Puri et al. 2010;
	*Novosphingobium sp.*PP1Y	Viviana et al. 2014

Few thermostable α-L-rhamnosidase from the thermophilic bacterium have been reported (Birgisson et al., 2004). Another sources for enzyme like *Saccharomyces cerevisiae,Hanshulaanomala, Debaryomycesploymorphus*were reported but they show low level of α-L-rhamnosidase activities (McMahon et al, 1999). In a study it was found that *Pichiaangusta* X349, a remarkable producer of α-L-rhamnosidase (Yanai, 2000). There were some fungal sources patented and some were kept secrets by the industries. Only two commercial preparations of α-L-rhamnosidase, naringinase and hesperidinase were available and both are from fungal sources. Hesperidinase was from *Aspergillusniger*and *Penicilliums*pecies and naringinase was from *Penicilliumdecumbens* (Romero et al 1985). Another sources of α-L-rhamnosidase enzyme is listed in Table 1.

PRODUCTION of A-L-RHAMNOSIDASE ENZYME

Historically,α-L-rhamnosidasehas been isolated from plant sources like celery seeds (Hall, 1938) and grapefruit leaves (Hall, 1938, Thomas et al., 1958; Ting, 1958); however, for reasons of availability, only processes based on microbial α-L-rhamnosidase are practicable. There are only a few reports on the microbial production of α-L-rhamnosidase. In most of the cases production methods are patented and only sketchily reported in research papers. An α-L-rhamnosidase is produced by many microorganisms, as noted in Table 1. In literature, microbial α-L-rhamnosidase reported to be produced by both method submerged fermentation and solid-state fermentation processing, as discussed below.

Submerged Fermentation

A large number of microorganisms have been already screened for their ability to produce α-L-rhamnosidase. In one study crude culture extracts of microorganisms to obtain α-L-rhamnosidase preparation that had an optimum pH of 5–6 and could be held at 60 ^{0}C for 4 h with only 16% loss in activity. Neither the culture extracts stored at 58 ^{O}C, nor the enzyme stored at room temperature showed any lost in enzyme activity during one year (Thomas et al. 1958). The isolated enzyme was partially purified by alcohol precipitation of the culture extracts. One study focused on molds, explored 96 strains and established *Aspergillusniger* as the best producer of α-L-rhamnosidase (Kishi, 1955). Using these studies as a reference, the Company Tanabe Pharmaceuticals developed a process for producing α-L-rhamnosidaseand it was marketed by name 'Kumitanase.' In 1960, Smythe and Thomas also filed a U.S. patent in which they described the production of enzyme level which was approximately 100 U/mL.On the basis of research done in the past, future studies were focused mainly how to increase the production and the activity of the enzyme.

In a study by Shanmugam and Yadav (1995) reported that the extracellular production of α-L-rhamnosidase from a strain of the fungus *Rhizopus*nigricans. They used the culture medium for enzyme production which contained sucrose and rice and inoculated with spore suspension (10^6 spores /mL). The α-L-rhamnosidase activity was observed around 50 h after inoculation. It was observed that pH of the medium decreases because of the growth of mycelia. The extracellular culture filtrate containing α-L-rhamnosidase was dialyzed overnight against distilled water at 30 ^{0}C. It was observed that enzyme followMichaelisMenten kinetics when tested with *p*-nitrophenyl α-L-rhamnopyranoside as a substrate. The pH and temperature optimum of the enzyme were pH 6.5 and 60–80 ^{0}C, respectively. In all reported fermentation processes, the α-L-rhamnosidase was observed as extracellular enzyme. Recently,α-L-rhamnosidaseproducing fungal strain was isolated from decaying citrus lemon fruit (Vinita et al. 2011). The fungal strain which was characterized as *Aspergillusflavus by* MTCC (Microbial type culture collection) Chandigarh. For the purification of α-L rhamnosidase culture was filtrated of from the fungal strain using by ultra-filtration and Ion exchange chromatography on carboxy methyl (CM) cellulose. It was observer that submerged fermentation dominated for the commercial production of α-L-rhamnosidase. Submerged fermentations are relatively easily controlled and scaled up. Moreover, yields are generally higher in submerged culture and the risk of contamination is low. Submerged fermentation has advantages like more yield, less chances of contamination and easy to handle.

Solid-State Fermentation

Compared to submerged fermentation, the solid-state fermentation has been less investigated for production of α-L-rhamnosidase. However, there is substantial amount of scope for this mode of production, as reported by automation

capabilities and operating experience with many other large-scale solid-substrate fermentation processes (Chisti, 1999). Production of α-L-rhamnosidase by solid-state culture of *Coniothriumdiplodiella*was reported (Nomura 1965). In a study the micro-organism for the production of α-L-rhamnosidase were grown on soybean cake at 23 ^{0}C and for 8 days in solid-state fermentation. The crude enzyme was further purified. The optimum pH and temperature for the α-L-rhamnosidase were pH 4.2 and 60–65 ^{0}C, respectively. The α-L-rhamnosidase produced was inhibited considerably by sucrose and fructose, and to a lesser extent by sorbitol. Several other agro-industrial waste like citrus peel, coconut-coir, rice bran etc.

Assaying Methods

The activity of α-L-rhamnosidase was assayed by usingnaringin as a substrate following the Davis (1947) method reported in literature. The reaction solution consisted of naringin dissolved in sodium acetate/acetic acid buffer, pH 4.5, maintained at 60 ^{O}C. Aliquots of the enzyme extract was added to the above solution, after that certain amount of aliquot were withdrawn at regular interval followed by 90% diethylene glycol was and in the last 4 M NaOH was added to stop the reaction. Then, aliquots were withdrawn in cuvette at the intervals of 5 min and were treated in the same manner as mentioned above. The samples were maintained at the room temperature for minimum 10 min, and absorbance of the solutions was measured spectrophotometrically at 420 nm. The absorbance values was converted into the concentrations of naringin values by using a calibration curve drawn with known concentrations of naringin. The rate of naringin hydrolysis was calculated by drawing a graph of concentration of naringin versus time. One unit of enzyme activity (IU) was defined as the amount of enzyme required to hydrolyse 1 µmol min^{-1} of naringin under the above assay conditions.

Another assaying method by Romero et al (1985) was reported in which the synthetic compound*p*-nitrophenyl- α-l-rhamnopyranosidewas used and monitered the liberation of *p*-nitrophenolate ion spectrophotometrically at 400nm, molar extinction coefficient value of 21.44mM^{-1} cm^{-1}. The synthetic substrate, p-nitrophenyl- α-l-rhamnopyranoside, is commercially available but it is more expensive.

Purification and Characterization

The α-L-rhamnosidasefrom the liver of a marine gastropod,*T. cornutus*, has been purified by using ion-exchange column chromatography in which CM cellulose and Sephadex G-150 were used (Kurosawa, 1973). The purification of α-L-rhamnosidasefrom pig liver involved extraction of the enzyme by homogenizing pig liver with buffer, fractional precipitation with ammonium sulphate, dialysis and ion exchange chromatography on DEAE-cellulose (Qianet al., 2005). The purification of α-L-rhamnosidasefrom the*F. esculentum* seeds involved extraction of the crude enzyme, by ammonium sulphate precipitation and ion-exchange chromatography in which columns of Sephadex G-75, DEAE were used (Bourbouze, 1976). The α-L-rhamnosidasefrom the human intestinal bacterium *Bacteroides*JY-6 has also been purified by rapturing the bacterial cells in phosphate buffer followed by ultrasonicator, ammonium sulphate precipitation, column chromatography on DEAE-cellulose etc.

In a study the extracellular enzyme α-L-rhamnosidase that hydrolyses naringin for the production of rhamnose and glucose was purified from the culture filtrate of *Aspergillusniger* MTCC 1344 (Manish, 2005). In this study enzyme was purified at several level of purification step which includesammonium sulphate precipitation, ion exchange and gel filtration chromatography which increases the overall recovery and specific activity. The molecular mass of the purified enzyme was calculated bysodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Also the enzyme

optimum pH and temperature was determined. It was observed that α-L-rhamnosidase stable at 37 ^{O}C for 72 h, whereas at 40 ^{O}C the enzyme showed 50% inactivation after 96 h of incubation. Hg^{2+}, SDS, *p*-chloromercuribenzoate, Cu^{2+} and Mn^{2+}showed inhibitory effecton the enzyme activitywhereas, Ca^{2+}, Co^{2+} and Mg^{2+} showed very little inactivation. It was reported in literature that α-L-rhamnosidaseenzyme activity was strongly decreses by rhamnose and increased by $Mg^{2+}$and Ca^{++} ions and remains stable for one year if it was stored at -20 ^{O}C. The purified enzyme have capability to successfully hydrolysenaringin and rutin which is main bitter compound in citrus fruit juices.

A novel alkaline α-L-rhamnosidase from *Acrostalagmusluteoalbus,* an alkali tolerant soil fungus from Argentina was isolated and characterized by Natalia et al, 2011. They develop a more reliable, simple, and inexpensive method for the purification of theα-L-rhamnosidase enzyme and also described the characteristics as well as kinetics of the purified enzyme. In this studyrhamnosewas used as the main carbon source. Molecular weight of the α-L-rhamnosidase from *Acrostalagmusluteoalbus*wasdetermined by SDS–PAGE and isoelectric focusing for the determination of isoelectric point. This enzyme was purified by applying the chromatographicand electrophoretic techniques. In this study, *p*-nitrophenyl α-L-rhamnopiranoside used as asubstrate for the determination of the enzyme activity optimum pH and temperature. The enzyme also follow theMichaelis–Menten kinetics. Divalent cations such as Ca^{2+}, Mg^{2+}, Mn^{2+}, and Co^{2+} and reducingagents such as β-mercaptoethanoland dithiothreitoldid not showed any effect onenzyme activity, whereas this activity was completely inhibited by Zn^{2+}.

APPLICATIONS of ALPHA –L-RHAMNOSIDASE

Anα–L-rhamnosidase has other important potential applications besides debittering of fruit juice. Some of the applications are discussed below:

Preparation of the Antibiotic Chloropolysporin C

The deglycosylation property of novel glycopeptide antibiotic, chloropolysporin from *Faeniainterjecta,* was obtained successfully with α-L-rhamnosidase activity of naringinase. The subunit of chloropolysporins A, B, and C enzymatically converted to deglycosylated derivatives reported by Sankyo, 1988. It was found that combination of Subunit C of chloropolysporin and β-lactam antibiotics wasstrongly effective against methicillin resistant strains bacteria and all the subunit of chloropolysporins showed strong activity against gram-positive bacteria. These antibiotics also inhibit the anaerobic gram-positive *Enterobacteria.* So, chloropolysporin C is the most active component of the chloropolysporin complex.

Preparation of Rhamnose

An α-L-rhamnosidase enzymeused to hydrolyzednaringinfor the production of L-rhamnose. This enzyme mixture is used for the preparation of high α-L-rhamnosidase activity and lower β-D-glucosidase activity (Daniels et al., 1990). Rhamnosidase was also used for organic synthesis in pharmaceuticals and as well as plant protective agent.

Preparation of Prunin by Using Rhamnosidaes

The flavonoid prunin may be produced from naringin using immobilized α-L-rhamnosidase which treated previously with an alkaline buffer. The product which was obtained showed increased yield as well as antiviral activity against DNA and RNA viruses. The prunin showed anti-inflammatory activity and also can be used as sweetening agent

for diabetic persons(Roitner et al., 1984). There were so many natural flavonoids reported which showed antiviral activity against certain RNAand DNA viruses. The flavonoidsnaringin and pruninalso showed antivirus activity against herpes simplex virus type 1 (HSV-1), parainfluenza virus type 3 (Pf-3), polio-virus type 1, and the respiratory syncytial virus (RSV) have been studied in vitro in cell culture (Kaul et al., 1985). The natural glycosideflavonone of naringenin reported to decrease the gastric mucosal ulceration in animal. Naringin was reported to have gastro-protective effect in gastric injury which is induced by ethanol.

The α-Lrhamnosidase Activity for Aroma Enhancement in Wine

The α-L-rhamnosidase activity in combination with β-D-glucosidase and arabinosidase was showed for aroma enhancement in wine preparation. In one study, the enzymes were immobilized on a solid carrier for the developmentof a process for wine aroma enhancement (Caldini et al., 1994).

Steroid Transformation

The fenugreek seeds (*Trigonellafoenumgraecum*) upon enzymatic hydrolysis by α-L-rhamnosidaseproduces sapogenins and diosgenin (a precursor of clinically useful steroid drugs). In this process, the immobilized α-L-rhamnosidase and pectinase could be reused twice without loss of diosgenin yield (Elujoba and Hardman, 1987).

Tomato Pulp Digestion

The α-L-rhamnosidases from *L.* plantarum have been shown to convert flavonoid rutinosides (such as rutin from tomato) into well absorbed glucosides. Such activity implies that probiotic lactobacilli when present in gut microflora may enhance flavonoid bioavailability (Beekwilder et al. 2009).

Debittering Citrus Fruit Juices

Bitterness in citrus fruit juices is mainly due to the presence of compoundnaringin, which may be removed by treating the juice with α-L-rhamnosidases **(**Puri et al. 2008). In this direction, α-L-rhamnosidases has been immobilized on various carrier supports for achieving hydrolysis of naringin and eventually debittering citrus fruit juice (Ribeiro 2011). The immobilized α-L-rhamnosidases on polyvinyl alcohol has been used for the debittering of juices (Busto et al. 2007).In a study the purified recombinantα-L-rhamnosidases enzyme utilized for the treatment of citrus juices, where α-L-rhamnosidases enzyme was immobilized with Ca^{2+} alginate beads for improving the hydrolysis in kinnow fruit juice. This study showed the activity and stability of the free and immobilized enzyme (Puri et al. 2010).

Pro-Drug Therapy

A drug is released from its pro-drug by enzyme action. A system was designed in which drugs of interest were capped with rhamnose and are released by α-L-rhamnosidase enzyme. The carbohydrate structure of rhamnosidase was specifically designed by enzymatic properties based ondeglycosylation and reglycosylation to activate α-L-rhamnopyranosideso thatit may be used in pro-drug therapy. Highly degree ofpro-drug activation was gained by glycosylation which improve the rate of uptake of the protein component from the serum, while it reduces the time for immunological action by exposure. Pro-drugs of doxorubicin and 5-fluorouracil were capped by the other sources than mammalian L-rhamnosyland released by α-L rhamnosidase to its targeted sites (Garnier et al. 2010).

CONCLUSIONS

As per data available, α-L-rhamnosidase is a biotechnologically important enzyme which has potential application in food industries and pharmaceutical companies. These day microbial enzymes has drawn great attention from various researcher worldwide. Commercially viable process for large- scale production of α-L-rhamnosidase by fermentation method is needed in today scenario.

ACKNOWLEDMENTS

One of the authors (PoonamYadav) thanks the Department of Science and Technology (DST), Government of India, for awarding DST-INSPIRE fellowship for financial support.

REFERENCES

1. *Beekwilder J, Marcozzi D, Vecchi S, deVos R, Janssen P, Francke C, Hall RD, 2009. Characterization of rhamnosidases from Lactobacillus plantarum and Lactobacillus acidophilus. App Environ Microbiol 75:3447–3454*
2. *Birgisson H, Wheat JO, Hreggvidsson GO, Kristjansson JK, Mattiason B, 2004. Immobilization of a recombinant E. coli producing a thermostablerhamnosidase: creation of a bioreactor for hydrolysis of naringin. EnzMicrobTechnol 40: 1181–1187.*
3. *Bourbouze R, Percheron F, Courtois JE, 1976. α-L-rhamnosidase from Fagopyrumesculentum: purification and some properties. Eur J Biochem 63:331–337.*
4. *Caldini C, 1994. Kinetic and immobilization studies on fungal glycosidases for aroma enhancement in wine. Enzyme MicrobTechnol 16:286–291.*
5. *Chisti Y (1999). Strategies in downstream processing. In: Subramanian G, editor. Bio separation and Bioprocessing: A Handbook, vol. 2. New York: Wiley-VCH. pp. 3–30.*
6. *Hall D, 1938. A new enzyme of the glycosidase type. ChemInd 57:473.*
7. *Hashimoto W, Miyake O, Nankai H, Murata K, 2003. Molecular identification of a α-L-rhamnosidase from Bacillus sp. strain GL1 as an enzyme involved in complete metabolism of gellan. Arch BiochemBiophy 415:235–244*
8. *Hashimoto W, Murata K, 1998. Alpha-L-rhamnosidase of Sphingomonas sp. R1 producing an unusual exopolysaccharide of sphingan. BiosciBiotechnolBiochem 62(6):1068–1074*
9. *Jang IS, Kim DH, 1996. Purification and characterization of alpha-Lrhamnosidase from Bacteroides JY-6, a human intestinal bacterium. Biol Pharm Bull 19:1546–1549*
10. *Koseki T, Mese Y, Nishibori N, Masaki K, Fuhii T, Handa T, Yamane Y, Shiono Y, Murayama T, iefuji H, 2008. Characterization of an α-L-rhamnosidase from Aspergilluskawachii. ApplMicrobiolBiotechnol 80:1007–1013*
11. *Kurosawa Y, Ikeda K, Egami F (1973) a-L-rhamnosidase of the liver of Turbo cornutus and Aspergillusniger. J Biochem 73:31–37*
12. *Lei S, Xu Y, Fan G, Xiao M, Pan S, 2011. Immobilization of naringinase on mesoporous molecular sieve MCM-41 and its application to debittering of white grapefruit. Appl Surface Sci 257:4096–4099*
13. *Mamma D, Kalogeris E, Kekos D, Macris BJ, Christakopoulus P, 2004. Biochemical characterization of the multi-enzyme system produced by P. decumbens grown on ruitn. Food Biotechnol 18:1–18.*

14. *Manish P and Sukirti K, 2005. Purification and characterization of naringinase from a newly isolated strain of Aspergillusniger 1344 for the transformation of flavonoids. World Journal of Microbiology & Biotechnology; 21:753–758.*
15. *Manzanares P, van den Broeck H, de Graaff LH, Visser J, 2001. Purification and characterization of two different α-L-rhamnosidase, RhaA and RhaB from Aspergillusaculeatus. Appl Environ Microbiol 67:2230–2234*
16. *Mazzaferro LS, Orrillo GA, Ledesma P, Breccia JD, 2008. Dose dependent significance of monosaccharides on intracellular α-L-rhamnosidase activity from Pseudoalteromonas sp. Biotech Letts 30:2147–2150*
17. *McMahon H, Zoecklein BW, Fugelsang K, Jasinski Y, 1999. Quantification of glycosidase activity in selected yeasts and lactic acid bacteria. J IndMicrobiolBiotechnol 23:198–203*
18. *Miake F, Satho T, Takesue H, Yanagida F, Kashige N, Watanabe K , 2000. Purification and characterization of intracellular alpha-Lrhamnosidase from Pseudomonas paucimobilis FP2001. Arch Microbiol 173:65–70.*
19. *Miyata T, Kashige N, Satho T, Yamaguchi T, Aso Y, Miake F, 2005. Cloning, sequence analysis and expression of the gene encoding Sphingomonaspaucimobilis FP 2001 rhamnosidase. Current Microbiol, 51:105–109*
20. *Monti D, Pisvejcova A, Kren V, Lama M, Riva S, 2004. Generation of α-L-rhamnosidase library and its application for the selective derhamnosylation of natural products. BiotechnolBioeng 87:763–771.*
21. *Natalia LR, Claudio EV, Roque AH and Sebastián FC, 2011. Purification and characterization of a novel alkaline αLrhamnosidase produced by Acrostalagmusluteoalbus. Journal of Industrial Microbiology & Biotechnology. Volume 38, Issue 9, pp 1515-1522*
22. *Nomura D. 1965. Studies on the naringinase produced by Coniothyriumdiplodiella. The properties of naringinase and the removal of co-existing pectinase from the enzyme preparation. Enzymologia; 29:272–82.*
23. *Orrillo AG, Ledesma P, Delgado OD, Spagna G, Breccia JD, 2007. Cold-active α-L-rhamnosidase from psychrotolerant bacteria isolated from a sub-Antarctic ecosystem. Enzyme MicrobTechnol; 40:236–41.*
24. *Puri M, Kaur A, Singh RS, Kanwar JR, 2008. Immobilized enzymes for debittering citrus fruit juices, In: Busto MD, Ortega N Food Enzymes: Application of New Technologies, Transworld Research Network. Trivandrum, India, pp 91–103*
25. *Puri M, Kaur A, Singh RS, Nahar A, 2010. Response surface optimization for the production of naringinase from Staphlococcusxylosus MAK2. Applied BiochemBiotechnol 162:181–191*
26. *Qian S, Yu H, Zhang C, Lu M, Wang H, Jin F, 2005. Purification and characterization of dioscin-α-L-rhamnosidase from pig liver. Chem Pharm Bull 53:911–914.*
27. *Rajal VB, Cid AG, Ellenrieder G, Cuevas C, 2009. Production, partial purification and characterization of rhamnosidase from Penicilliumulaiense. W J MicrobiolBiotechnol 25:1025–1033*
28. *Roitner M, Schalkhammer T, Pittner F, 1984. Preparation of prunin with the help of inmobilizednaringinase pretreated with alkaline buffer. ApplBiochem Biotech 9:483–488.*
29. *Romero C, Manjon A, Bastida J, Iborra JL 1985. A method for assaying rhamnosidase activity of naringinase. Anal Biochem; 149:566–71.*
30. *Sarita Y, S Yadava and Yadav KDS, 2013. Purification and characterization of α-l-rhamnosidase from Penicilliumcorylopholum MTCC-2011. Process Biochemistry 48:1348–1354.*
31. *Shanmugam V, Yadav KDS (1995) Extracellular production of alpha-rhamnosidase by Rhizopusnigricans. Ind J ExpBiol 33:705–707.*

32. *Soares NFF, Hotchkiss JH, 1998. Naringinase immobilization in packaging films for reducing naringin concentration in grapefruit juice. J Food Sci 63:61–65*

33. *Suzuki H, 1962. Hydrolysis of flavonoid glycosides by enzymes from Rhamnus and other sources. Arch BiochemBiophys 99:476–483.*

34. *Thammawat K, Pongtanya P, Juntharasari V, Wongvithoonyaporn P, 2008. Isolation, preliminary enzyme characterization and optimization of culture parameters for the production of naringinase isolated from Aspergillusniger BCC25166. Kaestsart J Nat Sci 42:61–72*

35. *Thomas DW, Smythe CV, Labbee MD, 1958. Enzymatic hydrolysis of naringin, the bitter principle of grapefruit. Food Res; 23:591–8.*

36. *Viviana I, Pietro T, Eugenio N, Eduardo P, Alberto D, Antonio T and Annabella T, 2014. α-L-Rhamnosidase activity in the marine isolate Novosphingobium sp. PP1Y and its use in the bioconversion of flavonoids. Journal of Molecular Catalysis B: Enzymatic Vol. 105, 95–103.*

37. *Yadav V, Yadav S, Yadav S, and Yadav KDS, 2012. α-L-Rhamnosidase from Aspergillusclavato_nanicus MTCC-9611 Active at Alkaline pH. Applied Biochemistry and Microbiology, ISSN 0003:6838, Vol. 48, No. 3, pp. 295–301.*

38. *Yanai T, Sato M, 2000. Purification and characterization of anrhamnosidase from Pichiaangusta X349. BiosciBiotechnolBiochem 64:2179–2185*

39. *Young NM, Johnston RAZ, Richards JC, 1989. Purification of the α- L-rhamnosidase of Penicilliumdecumbens and characterisation of two glycopeptide components. Carbohydr Res 191:53–62*

40. *Zverlov VV, Hertel C, Bronnenmeier K, Hroch A, Kellermann J, Schwarz WH, 2000. The thermostable α-L-rhamnosidaseRamA of Clostridium stercorarium: biochemical characterization and primary structure of a bacterial a-l-rhamnoside hydrolase, a new type of inverting glycoside hydrolase. MolMicrobiol, 35(1):173–9.*