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BIOTECHNOLOGICAL AND BIO-INDUSTRIAL APPLICATIONS OF ALPHA–L-RHAMNOSIDASE ENZYME

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ABSTRACT

α -L-rhamnosidase is an important biotechnology enzyme that is used in various foods, chemicals and pharmaceutical industries. The α-L-rhamnosidases (α- RHA) belong to a group of glycosyl hydrolases having biotechnological potential in the proesses occurring in industries, they stimulate the breakdown of terminal residues of α-L-rhamnose from many naturally occurring substances present in chemical industries. Flavonoid prunin is produced from Narignine by activity of α-L-rhamnosidase. It has anti-inflammatory and anti-viral activity against DNA or RNA viruses. It can be acquired from plants, animals and different microbial sources, such as (bacteria and yeast). Main sources of α-L-rhamnosidase are microbes, mainly filamentous fungi such as *Aspergillus, Circinella, Eurotium, Fusarium, Penicillium, Rhizopus, and Trichoderma*. The first bacterium α-L-rhamnosidase was screened from the genus *Bacteroides*. The other strains of bacteria that produce α-L-rhamnosidases are the heat-loving bacteria, *Fusabacterium, Pseudoalteromonas, Ralstonia pickettii, Lactobacillus acidophilus*, *Pediococcus acidilactici, Clostridium stercorarium, and Sphingomonas paucimobis*. Yeast rhymosidase is very important because it is produced in short fermentation, with increased shelf life, high thermal stability, the ability to retain flavor of juice and it is non-toxic for human consumption. For α-L-rhamnosidase purification, the centrifuge method is used, and various chemical products, such as ammonium sulphate, NaCl, are used. Finally, molecular weight (MW) of α-L-rhamnosidase has been determined by Gel Filtration Chromatography (GFC) and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Keywords: α-L-rhamnosidase; Food industry; Chemical industry; Pharmacceutical industry

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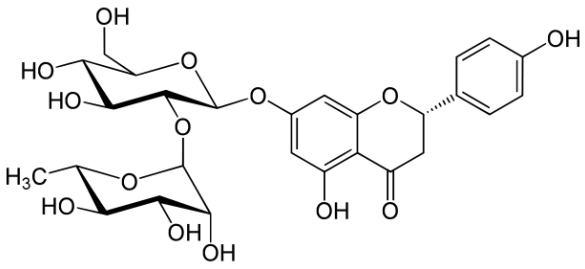
1. INTRODUCTION

Biological transformation of naringin to prunin is performed by the nirginase, which is a multienzyme complex composed of α-L-rhamnosidase (E. C. 3.2.1.40) and β-D-glucosidase [1]. α-L-Rhamnosidase (E. C. 3.2.1.40) belongs to hydrolases, and one of the exo-type enzymes that eliminates peripheral α-L-rhamnosyl groups present at the end of polysaccharides and glucosides. For the purpose, α (1→2) bond association between L-rhamnose and glucose is broken [2].

Structure of naringin:



The Enzymes, narigenase and α-L-rhamnosidase were described from some microbs [1, 3-6], among which *Aspergillus niger* is more significant, reliable and promising source which was industrially practice, because this usable fungi not only appear in the microbial category certified by Food and Drug Administration (FDA) including two active enzymes, narigenase, and α-L-rhamnosidase, and are safe for food and medical use and can also be stimulated to produce some active enzymes for food [7]. In addition, fermentation is easy to expand because this technology is well developed and has been broadly used in the industry for various purposes [7].

The enzyme is applicable for the identification of large molecule's structure, such as sugars, (polysaccharides, glycosides, and glycolipids), to get rid from bitterness of citrus juice [8], for the metabolism of Gellan, to improve the aromas of wine, [9], production of less bitter products [10], for anti-inflammatory and antiviral activity against DNA/RNA viruses to eliminate rhamnose from many steroidal rhamnoses [11] , which are of clinical significance [12-14].

Moreover, α-Rhamnosidases removes the peripheral L-rhamnose of a large number of natural products, for example flavonoids, saponins, and many other naturally occuring glycoside, this enzyme is also used in the food industry to get rid of bitter taste of citrus juices [15-17]. However, the process of decomposition of flavonoids in vitro by α-L-rhamnosidase has initiated a new era of drug development [1, 13, 18, 19].

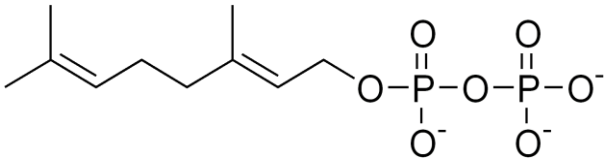
Furthermore, α -L-rhamnosidase has been isolated and purified from microbes like fungi, bacteria and animals [1], and many genes coding this enzyme have been cloned [20-24]. The α-L-rhamnosidases with their various physical and chemical properties are appropriate for various industrial applications. Therefore, there is a scientific need to review the sources of literature that are identified for production of α-L-rhamnosidases with various properties that are appropriate for various applications. According to the latest report, there is α-L-rhamnosidase of *Penicilliumcitrinum* MTCC-3565 [25], which contains the optimum pH in the alkaline pH range. This report also shows that another strain of α-L-rhamnosidase from *penicilliumcorylopholum* MTCC-2011, which contains the optimal pH that is near to the neutral pH range and has been shown to be beneficial for transformation of narigen to prunin and less bitter routine changes to quercetin glucoside. α-L-rhamnosidase from narignine is used in the production of l-rhamnose, which is the pure sterioisomer and can be used as a helical compound in manufacturing of chemicals and as precursors for the formation of aromatic compounds in industries and in different flavors [26]. Rutin and quercitrin are the most common falavonoid glycosides present in human diet, which are described as substrates for rhamnosidases [27, 28]. Interest in enzymes in the scientific community since the extreme has increased over the past 10 years, especially in thermophiles enzymes. These enzymes

are often more visible to industrial processes than their mesophilic counterparts [29, 30]. Once many of the substrates, become poorly soluble at low temperatures such as naringin and rutin, this may be the case in some processes involving α-L-rhamnosidase. Five α-L-rhamnosidase have been cloned and mutated so far, one of *Clostridium Stacorium* [26], two of *AspergillusAcolitus* and two of S. Bacillus GL1[23, 24]. There is a very wide range of synthetic polyhydroxylated piperidines and pyrrolidine [31, 32]. This mimics the residues of individual sugars. When anhydrous group of hydrogen is replaced by hydrogen, oxygen is replaced by a nitrogen ring. In analogues of piperidine of carbohydrates are generally effective inhibitors of the corresponding glycosidases [33] but a significant loss of glycosidase inhibition occurs when the composition of a carbon atom changes in general. Deoxyrhamnojirimycin (DNJ) is very strong inhibitor of many alpha glucosidase as compared to 5-epi-DNJ [34]. As a result, L-Deoxyrhamnojirimycin (LRJ-1), naringinase α-L- rhemnosidase can be predicted by inhibition analogous of L-rhamnose.

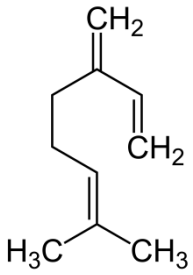
In the invasion of pathogenic fungi of plants, infection of bacteria with bacteriphages and of bacterial biomembranes metabolism, α-L-rhamnosidase is involved [9, 35, 36]. Flavonoid glycosides are transformed into their consumed forms of Alglycons through the activity of bacteria and α-rhamnosidase attributed with β-glucosidase; firstly, α-rhamnosidase activity is attributed to intestinal bacteria [37, 38]. Plant polyphenols, rutin and robinin have been obtained from human intestinal bacteria, which can be analyzed by strains of Bacteroides [39]. The first α-L-rhamnosidase bacteria were purified from Bacteroides JY-6[40]. So far, the genes that symbolize α-rhamosides for specific microorganisms, *clostridium storurium* (rhaA) [26], Bacillus sp. GL1 (rhaA and rhaB) [9, 23], *Sphingomonas paucimobilis* (rhaM) [28, 41] and *Thermomicrobia* sp. [22] have been duplicated. Recently, the crystalline structure of α-L-rhamnosidase of *Bacillus* sp. GL1, an enzyme involved in the breakdown of two generations of Gellan biofilm [42]. In addition, α -L-rhamnosidase is also used by many microbes as carbon and energy source by L-rhamnose release [43]. Monocytes are a class of terpenes with a molecular formula $C_{10}H_{16}$, which consists of two units of isoprene and monoterpene may be acyclic or aromatic. The biochemical modifications of these compounds, for example oxidation or reorganization produce related monoterpenoids.

Acyclic monoterpenes:

When dimethylallyl pyrophosphate chemically combined with isopentenyl pyrophosphate, geranyl pyrophosphate is formed:



Monocyclic terpenes:



2. SOURCES OF ALPHA-L-RHAMNOSIDASE

In numerous microorganisms, plants and animal sources, the α-L-rhamnosidase is obtained from animal tissues [3], Yeast [44], organisms and microbes [40, 45]. Anyway, just that α-L-rhamnosidase are practicable whose procedures depend upon microorganisms [8]. This enzyme has biotechnological uses, *Aspergillus flavipus* MTCC-4644 has great capacity to form α-L-rhamnosidase, and it is the main objective of this study. It has great ability for catalysis at low pH, high temperature and to enhance the media structure for α-L-rhamnosidase generation.

Newly, another strain DB056 of *A. Niger* has been screened that could create a high amount of naringinase (α-L-rhamnosidase and β-D-glucosidase complex), alongside an effective improved scale-up procedure for naringinase generation in a 200 L fermentor in research laboratory of Jimei University, China. Other plant sources of α-L-rhamnosidase are grapefruit leaves [46] , Rhamnus daurica [47], and Fagopyrum esculentum [48]. Turbo cornutus liver and pig liver are the animal source of α-L-rhamnosidases [3, 44]. In microorganisms α-L-rhamnosidase is mainly found in filamentous fungi, for example, *Aspergillus*, *Circinella*, *Eurotium*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Trichoderma* [49]. The generation of α-L-rhamnosidases occur in various strains of fungus, for example, *Acremonium persicinum, Circinella muscae, Emericellanidulans, Fusarium oxysporum, Mortierella alpine, Penicillium oxalicum, Rhizopus arrhizus, Talaromyces flavus* and *Trichoderma harzianum*, by utilizing naringin, rutin, L-rhamnose, hesperidin as persuader [13]. The two most regularly utilized species for its formation with a wine and beer are *Aspergillus niger* and *Penicillium decumbensand* also the activity of enzyme of these species are well characterized [50-53]. The principal bacterial α-L-rhamnosidase was obtained from the genus bacteroids [40]. Some other bacterial strains also produced α-L-rhamnosidases, for example, *thermophilic bacterium* (Birgisson et al., 2004)[22], *Fusabacterium* [54], *Pseudoalteromonas* species, *Ralstonia pickettii* [55], *Lactobacillus acidophilus*[21], *Pediococcus acidilactici* [56], *Clostridium stercorarium* [26], *Sphingomonas paucimobilis* [35], *Bacillus sp*. [9] and *Corticium rolfsii*. From the *Bacillus* sp. four structures from the GH78 rhamnosidase family have been resolute upto date. First crystal structure of protein α-L-rhamnosidase (RhaB) is GL1 accessible at 1.9Å resolution. This is homo dimeric protein, contains 956 residues of amino acid and 106 kDa molecular mass, consisting of core catalytic (α/α) 6 barrel and four ß-sandwich domains [42]. The L-rhamnose complex with the second structure of *Streptomyces avermitilis* (SaRha78A; PDB code 3W5N) α-L-rhamnosidase was experimentally determined, this protein consist of six large monomeric domains [57]. The third homodimeric structure of putative α-L-rhamnosidase from *Bacteroides thetaiotaomicron* VP1-5482 (BT1001; PDB code 3CIH), was determined in an unpublished structural genomics project. Recently, the crystal structure of KoRha, a putative α-L-rhamnosidase from *Klebsiella oxytoca* has been determined at resolution of 2.7 Å with rhamnose which attached to the active site of the catalytic domain. Recently, it is reported that pH scale of α-L-rhamnosidase from *Aspergillus flavus* MTCC-9606, in basic medium is at optimum level [58]. In this correspondence, it is accounted that in the neutral pH range *Penicillium citrinum* MTCC-8897, an α-L-rhamnosidase, has an optimum activity.

When α-L-rhamnosidase along with β-glucosidase remove glycosides terpenols are formed and are accountable for winearoma in industries [59]. In case of orange juice naringin produce bitter taste. The prunin is only 33% bitter as compared to naringin, it is created when end of α -L-rhamnose is cleaved by α-L-rhamnosidase and convert naringin into prunin. So, α -L-rhamnosidase has ability to delete the bittering of citrus fruit juice. Different components of the beverage don't change because the enzymes are specific, and are viable and can take place under milder circumstances so that's the reason enzymatic procedures are preferred. The previously mentioned significant focuses have encouraged the authors to look for α-L-rhamnosidases from recent sources, with the goal to identify the appropriate enzyme for these applications. In the orange peel and agricultural waste, a lot of naringin is present [60], which is a substrate and inducer of α-L-rhamnosidases. High level of α -L-rhamnosidase is secreted from *Aspergillus awamori* MTCC-2879, obtained from sweet potatoes and shows maximum growth on a orange peel medium. Total deglycosylation of substrates is caused by the wild α-L-rhamnosidases [e.g. from microscopic organisms, yeasts, growths, plants, and animals], and are frequently present in a complex with β-D-glucosidase (known as naringinase or hesperidinase). Recently, a recombinant α-L rhamnosidase (alkali and thermo-stable), has been created that can be utilized for preliminary scale

transformation of rutin to isoquercitrin [61]. For reusing an enzyme one of the efficient methods is immobilization. Various kinds of methods like entrapment, encapsulation, cross-linking, adsorption, etc are used for its perfection [62]. Bacteria show an unfimiliar repository of alpha-RHAs, which may reveal novel interesting characteristics. From different microbial sources, the few isolated alpha-RHAs reveals, that optimal pH is one of the primary contrasts present between fungal and bacterial enzymes, as compare to bacterial counterpart's fungal enzyme shows more acidic pH, for which neutral and basic optimal pH values have generally been reported. This feature proposes significant and affective applications for bacterial and fungal enzymes, making bacterial alpha-RHAs appropriate in biotechnological procedures requiring great action in more alkaline solutions, such as, the l-rhamnose is produced by breakdown of naringin or hesperidin [3, 6, 10, 44, 47, 63-67]. Despite finite number of bacterial alpha-RHAs that has been reported until now, information in literature recommend that this activity of enzyme is broadly distributed over a wide range of ecological niches. *Bacteroides* JY-6 an a-RHAs have been recognized and identified in the human intestinal bacterium [40], in cold-adapted *Pseudoalteromonas* species and *Ralstonia pickettii* isolated from the seawater of sub Antarctic environment [55], likewise in soil bacteria such as *Bacillus sp*. GL [9, 23], *Sphingomonas paucimobilis* FP2001[41] and *Sphingomonas sp*. R1 [35] lastly in wine strains of *Oenococcusoeni* [68]. The Lactobacillus species contains α-RHAs which were distinguished and examined for their biotechnological use to de-rhamnosylate flavonoids present in raw materials that are rapidly consumed [21]. What`s more, α-RHAs genes are cloned and separated from *Clostridium stercorarium* and other *thermophilic* bacteria, in most recent new discoveries [26] and a part of phylum of *Thermomicrobia*, for example, from the bacterium PRI-1686 [22]. The crystal structure of *Streptomyces avermitilis* alpha-l-rhamnosidase (SaCBM67) was recently revealed [57]; its structure shows a catalytic carbohydrate-binding site and is different from the two structures of α-l-rhamnosidases (GH78), the BsRhaB extracted from *Bacillus* sp.GL1 [42] and the putative α-L-rhamnosidase BT1001 from Bacteroides t*hetaiotaomicron* VPI-5482 [69]. It is saying that the biotechnological capability of bacterial α-RHAs, whose functional, structural and molecular biological characteristics have not been adequately analysed, is closely related to the procurement of new data on the enzymatic system obtained from new sources of bacteria. An organic solvent, biofilm-shaping marine microbes *Novosphingobiu*m sp. PP1Y, was recently screened from the water present at the surface of a docking bay in the harbor of Poz-zuoli (Naples, Italy), the region was intensely polluted with hydrocarbons [70]. The presence of various genomic features of interest for the biotechnological potential of this microorganism is confirmed by its genome analysis. Strain PP1Y show extremely a novel plenitude among Sphingomonadales of genes encoding for glycosyl hydrolases (53 orfs) [71], which are delivered among 27 unique families. This provoked our interest for searching the presence of α-RHA activities in the crude protein extract of strain PP1Y. Some of animals, plants and microbial sources are shown in table 1.

Table 1. **Sources of α-L-Rhamnosidase Production**

Source	**Specie**	**Part**	**References**
Animals	Pig	Liver	[3]
	Turbo cornutus	Liver	[44]
Plants	Grape fruit	Leaves	[72]
	Rhamnus daurica		[47]
	Fagopyrum esculentum		[48]
Yeast	*Rhamnosidase*		[44]
Bacteria	*Bacteroids*		[40]
	Thermophilic bacterium		[22]
	Fusabacterium		[54]
	Pseudoalteromonas specie		[55]
	Ralstonia pickrttii		[55]
	Lactobacillus acidophilus		[21]
	Pediococcus acidilactici		[56]
	Clostridium stercorarium		[26]
	Sphingomonas paucimobilis		[35]
	Bacillus specie		[23]

	Corticium rolfsii		[73]
Filamentous Fungi	*Pencillium specie* *Aspergillusniger* *A.acculeatus* *A.kawachii* *Rhizopusnigricans* *Aspergillusniger* (MTCC1344) *Pencilliumdecumbens* *Aspergillusniger* (BCC25166) *Penicilliumlaiense* *Aspergilluskawachii* *Acrostalagmusluteoalbus* *Penicillium corylopholum*MTCC-2011		[74] [24] [24] [75] [76] [77] [78] [79] [80] [75] [65] [81]

3. METHODS FOR PRODUCTION OF A-L-RHAMNOSIDASE ENZYME

Historically, α-L-rhamnosidase has been obtained from different plant sources i.e celery seeds and grapefruit leaves [46, 72]; however, processes that depend upon microbial α-L-rhamnosidase are used for practice because it is easily available. In most of the cases methods of production are utilized and only sketchily reported in research papers. An α-L-rhamnosidase is produced by many microorganisms. According to literature, α-L-rhamnosidase from microorganisms are produced by both methods submerged fermentation and solid-state fermentation which are discussed below.

3.1. Submerged Fermentation

A diverse types of microorganisms have been already isolated because of their ability to produce α-L-rhamnosidase. In one study microorganisms crude culture extracts are used to produce α-L-rhamnosidase which have an optimum pH of 5–6 and temperature 60℃ for 4 h with loss of 16% activity only. Enzymatic activity was not lost when culture extracts were not stored at 58℃, and enzyme was not stored at room temperature for one year [46]. The screened enzyme was firstly purified by alcohol precipitation of the culture extracts. One study mainly based upon molds, investigate 96 strains and considered *Aspergillus niger* as the best producer of α-L-rhamnosidase. In 1960, Smythe and Thomas also filed a U.S. patent in which they explained the level of enzyme production which was about 100 U/mL. On the basis of previous research, future research was mainly focused upon enzyme activity and its yield. Shanmugam and Yadav (1995) stated that a fungus strain of *Rhizopus nigricans* was used for extracellular production of α-L-rhamnosidase [76]. They utilize culture having sucrose and rice inoculated with spore suspension (106 spores /mL) for the production of enzyme. The enzymatic activity of α-L-rhamnosidase was noted after 50 h of inoculation. As a result, pH of the medium decreases because of the growth of mycelia. The extracellular culture filtrate containing α-L-rhamnosidase was dialyzed with distilled water at 30℃ over a night. It was observed that when enzyme was tested with *p*-nitrophenyl α-L-rhamnopyranoside as a substrate it follows Michaelis Menten kinetics. The optimum pH of the enzyme was 6.5 and temperature 60-80 C. In all fermentation processes it was observed that, the α-L-rhamnosidase was an extracellular enzyme. Recently, α-L-rhamnosidaseproducing fungal strain was isolated from decaying citrus lemon fruit. 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 by the process of 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. However, yields are greater and contamination is low in submerged fermentation culture. Submerged fermentation has more yield, less chances of contamination and easy to handle.

3.2. Solid-State Fermentation

As compared to submerged fermentation, the solid-state fermentation has been less used for α-L-rhamnosidase production. However, there is great scope for this method of production, as reported by automation capabilities and operating experience with several large-scale solid-substrate fermentation processes [82]. It was reported that α-L-rhamnosidase is also produced by solid-state culture of *Coniothrium diplodiella* [83]. It was reported in a study that the micro-organism responsible for the α-L-rhamnosidase production were grown on soybean cake at 23℃ for 8 days in solid-state fermentation. The enzymes that were crude were further purified. The optimum pH for production of α-L-rhamnosidase was 4.2 and temperature 60–65℃. Sucrose as well as fructose, and to a lesser extent sorbitol along with many other agro-industrial waste like citrus peel, coconut-coir, rice bran etc. are used for inhibiting the production of α-L-rhamnosidase.

4. ROLE OF PROBIOTICS IN CLEAVING RHAMNOGLUCOSIDES FROM *C. JOHNSTONII* EXTRACT

Main components of *C. johnstonii* extract are rutin and narcissin. Narcissin shows faster conversion as compared to rutin and hesperidin. In fact, narcissin is converted by 14% (*L. reuteri*) to 56% (*L. acidophilus*) after 4 days, by 15% (*L. reuteri*) to 87% (*L. fermentum*) after 7 days and greater than 80% by ten strains after 10 days. The glucoside (isorhamnetin-3-glucoside) and aglycone (isorhamnetin) can be observed in parallel. As expected, the relative amount of aglycone to glucoside induces with the duration of incubation. However, narcissin, presence increase the activity of rhamnosidase, so that the conversion of rutin is also increased. In fact, rutin is converted by up to 15% after 4 days (*B. infantis*), up to 51% after 7 days (*L. fermentum*) and up to 78% after 10 days (*L. fermentum*). Activity of Rhamnosidase increased more efficiently in the presence of *L. fermentum, B. longum* ssp. *infantis* and *Lc. lactis*. However, rhamnosidase activity was hardly increased in the presence of *L. reuteri*. The product obtained after hydrolysis are isoquercitrin and quercetin. Quercetin has a longer incubation period due to its increasing amount.

5. EXPRESSION OF RHAMNOSIDASE IN PROBIOTICS

The expression of rhamnosidase is increased by the addition of substrates in *L. acidophilus*. It is observed that incubation time increased the amount of rhamnosidase. The probiotic rhamnosidase having a molecular weight of 80–90 kDa which is like the recombinant rhamnosidase with 90 kDa. The rhamnosidase in the enzyme mixture from *A. niger* had molecular weight of 80 kDa.

6. INDUSTRIAL APPLICATIONS OF A -L -RHAMNOSIDASE ENZYME

In industry, α -L-rhamnosidases contain a wide range of applications. Monoglycosylated flavonoids are produced by rhamnosidases (EC 3.2.1.40). Monoglycosylated flavonoid is an attractive application in the field of biocatalysis of enzymes with regard to the elimination of rhamnose. It is a broad application of rhamnosidases to improve the bioavailability of flavonoids, and has been recently discovered [84]. Flavonoids are widely applied in the case of human diseases, including certain forms of cancer and those related to cardiovascular disease and colds, which have proven to be useful to human health. Flavonoids have also been shown to be useful to human health in other aspects, such as antimicrobial, anti-inflammatory, antidiarrheal, antimicrobial, antioxidant, antiviral, anti-ischemic, estrogenic and radical-scavenging properties. Bitter juices are also a problem, but are also solved by rhamnosidases, which are mainly applied to eliminate bitterness of citrus juices. Narangin, a flavonoid glycoside, causes citrus fruits bitterness, but when the rhamnose is removed, it loses the bitter taste [21]. In Argentina, this type of research has produced great economic importance. In Argentina, 41 % of the world's lemon production, as well as 83 % of industrial lemon production in the southern hemisphere [85]. The narigenine flavonoids also have biological properties that are favourable, for example antioxidant activity, the capability to decrease lipid levels in blood, anti-cancer activity and inhibition of the P450 cytochrome metabolites for selected drugs, so these properties proved to be advantegeous to humans. In addition to its application to reduce the bitter taste of citrus fruit juices, Ramanoside can also be used to

increase the flavors of wine because it has the capability to release volatile terpenes associated with sugar residues [18, 21]. Biological catalyst is used as a process biocatalyst, at the industrial level which is very challenging for enzyme technology field to find it for its applications [86]. α-rhamnosidases is obtained from different types of mammalian tissue and plants, mostly from microorganisms i.e bacteria and fungi [1]. Many bacteria are known to produce α-L-rhamnosidase, but α-L-rhamnosidase activity has also been published so far for microorganism psychrotolerant [86]. Microorganisms have developed many methods for survival and reproduction within a wide range of outlets, throughout evolution, including under harsh environmental conditions. New strategies and mechanisms for new forms of life can be identified. To isolate them they provide a new warning to our basic knowledge of cell biology as well as to the use of microbes in biotechnology applications. Marine biotechnology has produced an unlimited number of new biomarkers that have important essential properties, such as paraffinia, ability to adapt to cold, high tolerance with salt, extreme heat extremes and their suitability for large scale farming, so these properties have wide applications. Microorganisms obtained from sea water habitats have developed an attractive interest in the scientific mind, including members of the genus *Brevundimonas* [87].

6.1. In the food industry

α-L-rhamnosidase has various food applications for beneficial use. Complex structure of the α-L-rhamnosidase and β-glucosidase (naringinase) debitter the taste of citrus juices that can then be used [88]. Hesperetin 7-glucoside is an important introduction to sweetness, it is a product of hesperidin formed by hydrolysis of α-L-rhamnosidase [24]. When free α-rhamnosidases monoterpene is released from terpene glycosides the taste of grape juice and wine is improved [89]. While, α-rhamnosidases can also be used to increase the bioavailability of polyphenols, such as hesperidin [90]. Hesperidin is a substance that appears in studies on animals and *in vitro*, extracted from citrus pith that has many potential health benefits and also develops bone health [91], the effects of reduced fat [92], the properties of antioxidants [93], the effects of heart attack [94], cancer control [95] and anti-inflammatory properties. Hence, these properties proved them beneficial to human health [96].

6.1.1. Sufficient purified enzyme to improve wine smell

The other application of α-L-rhamnosidase is in enhancing the scent of wine. Monoterpenes in glycosidic form is used as precursor for many aromatic components of wine containing α-L-rhamnose [5]. Enzymatic degradation occurs in two steps. When L-Rhamnoside release L-rhamnose α is produced, and then degrades terpenyl glycopyranoside by β-glucosidase to produce volatile terbinol responsible for the smell of wine. To verify the suitability of the purified enzyme to increase the smell, the sample of wine was processed using α-L-rhamnosidase and L-rhamnose is released in the reaction solution using TLC. The stain is not detected rhamnose was released on the TLC plate (results not shown), suggesting that the purified enzyme did not degrade rhamnosidase with the solution present in the wine and, therefore, was not suitable for improving the odor.

6.1.2. Sufficiency of the purified enzyme to get rid of citrus juices

Purification of citrus fruits is also an important application of α-L-rhamnosidase, which destroys the bitterness of citrus fruits [1]. Bitter taste in citrus fruit juices is produced by narignine (4 ', 5, 7 – trihydroxyl flavanone - 7 -glucoside). Then, in order to eliminate the bitterness in the citrus fruit juices, α-L-rhamnosidase degrade the nirginine into the prunin (4, 5, 7-trihydroxy flavononone-7-glucoside) which is only 33 % bitter as compared to Naringin [97, 98]. Some other substances such as glucose, rhamanose, rutin, hesperidine, naringenin and quercetin are found in citrus juices [98]. To verify their suitability to lose bitter taste of citrus juice, it was essential to know the effects of these chemicals on the activity of the purified enzyme. If α-L-rhamnosidase is not inhibited significantly due to previous gradients found in citrus fruit juices, it will be suitable for eliminating the bitter taste of fruit juices. The presence of rhamnose and glucose in citrus fruit juices will not inhibit enzyme activity significantly because they are very weak inhibitors. However, hespridin, naringenin and quercetin, even at a concentration of 1 mm, inhibited the activity of the highly purified enzyme because they are powerful and highly potent inhibitors. Naringin and rutin are substrates of the purified enzyme; their 1mM concentration inhibits the activity of the purified enzyme, which indicate that the enzyme that is purified is not appropriate for removing bitter taste from

citrus fruit juices. Davis method was used for testing; purified enzymes are treated with orange juice and monitor the reduction of the amount of naringin over time in the orange juice. There was no reduction in the concentration of naringin found in orange juice, so it was confirmed that this enzyme was not enough to abolish the bitter taste of citrus juices**:**

Table 2. Applications of α -L -rhamnosidase enzyme in food industries

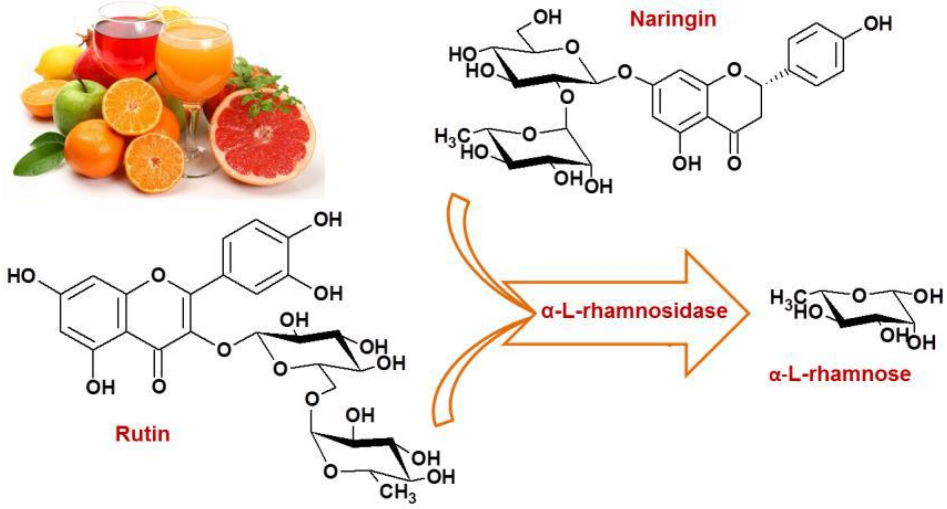
Industry	**Applications**	**References**
Food	Debittering of fruit juice	[99]
	Removal of hesperidin crystals	[24]
	Aroma enhancement in wine	[5]
	Additives	[14]
	Gellan depolymerization	[9]
	Tomato pulp digestion	[21]

6.2. In the chemical industry

6.2.1. Preparation of rhamnose

α-L-rhamnosidase degrades natural glycosides containing terminal L-Rhamnose, which leads to the formation of L-rhamnose [100]. Rhamnose and prunin are the two most important chemicals produced from citrus peel residues through the action of recombinant α-L-rhamnosidease [66]. In the field of enzymatic biochemical stimulation, the synthesis of flavonoids monoglycosyl is an attractive application by eliminating rhamnose radical i.e, rutinosides, as well as the production of the same rhamnose. Biological activity can be improved by flavonoids deglycosylation through improvement of bioavailability [101]. This formation may be related to pharmacokinetics and pharmacodynamics, as well as the complete structure of the molecule. This finding confirms that monoglycosyl flavonoids and monoclonal compounds are readily absorbed, then the original lead compound. Flavonoids have many beneficial effects on human health. These effects include cardiovascular and chronic diseases and some forms of cancer [102-104], as well as antimicrobial, antioxidant, antiviral, anti-thrombocytopenic, anti-ischemic, anti-tumor, anti-inflammatory, anti-allergic, estrogen and radical scavenging, flavonoids proved to be advantageous to human health [105, 106]. Isoquercetin, quercetin and other flavonoids also have useful aspects for human health, for example the protection of low density lipoprotein from oxidation (prevent the formation of atherosclerotic plaque), to change the biosynthesis of eicosanoid (antiprostanoid and anti-inflammatory responses), and avoid the accumulation of platelets (antithrombic effect), improve relaxation of cardiovascular smooth muscle (antihypertensive effects, anti-disruptive effects of systems) [106]. Quercetin has strong health effects related to metabolism, absorption and bioavailability in the human body. In addition, flavonoids, such as prunin have antiviral properties [105]. Flavonoids also act as inhibitors of the reverse transcriptase enzyme, so it is very useful, especially for human health, to control retrovirus infection, such as AIDS. In addition to another application of flavonoids, it also acts as an initial drug formulation. Flavonoids have a potential and strong interest, because of its unlimited properties and applications. The traditional methods of manufacturing flavonoids and saponins often produce secondary reactions because of the mildness and selectivity of the reaction conditions, enzymatic modification is useful. Naringinase is used in pharmaceutical and food industries with high potential. It is an enzymatic

complex also used in deglycosylation of compounds. Naringinase was used to complete the hydrolysis of some glycosides and provides both β-D-glucosidase and α-Lrhamnosidase [107-109].



α-L-rhamnosidases (α- RHA) stimulate hydrolysis of α-L-rhamnose terminal residues for many of the natural compounds and belong to glycosyl hydrolases [GHs] group [110]. L-rhamnose is present in various forms in plants for example as a component of flavonoid glycosides, terpene glycosides, signal molecules, dyes, and in cell walls as a component of the complex heterogeneous polysaccharide, such as rhamnogalacturonan and arabinogalactan [23, 63, 111-115]. L-rhamnose appears in membrane rhamnolipids and in polysaccharides of bacteria [39, 116, 117].

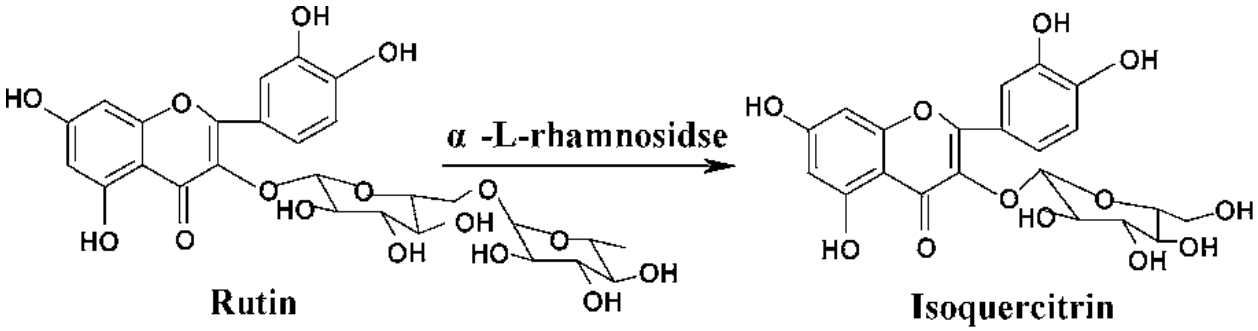
6.2.2. Production of glycolipid

Candida bombicola sophorolipids form glucolipid by *P. decumbens* naringinasa (α-L-rhamnosidase + β-D-glucosidase), which proves that the enzyme can also be beneficial in the synthesis of specific fatty acids [118]. At present, biological catalysis is a versatile and valuable tool for industrial biotechnology as compared to conventional chemical techniques, enzymes that are used as biocatalysts has a great advantage, to obtain a higher rate of reaction, high selectivity of reaction, increased purity of product, and a significant reduction in the production of chemical waste. In industrial processes, by using enzymes a large variety of chemical components are produced [119, 120]. In the past decade, α-L-rhamnosidase as a biocatalyst have attracted considerable attention because of their widespread application in a variety of pharmaceutical, food, and industrial chemical processes [63]. α-L-rhamnosidase belong to glycosyl hydrolase class that breaks the terminal α-rhamnose from wide range of natural products, these products particularly contain flavonoids, and include some terpenyl glycosides [10, 64]. Many other natural glycosides which contain glycolipids and glycopeptide antibiotics have terminal rhamnose [65]. More recently, these enzymes have been the focus of motivation among scientists. For example, α-RHAs are used to increase the biological activity of flavonoids that are useful for human health as dietary supplements or as a direct medicine[63] . In addition, α-L-rhamnose plays a major role in organic synthesis, a chiral intermediate compound for pharmaceutical products that are important chemicals. Using enzymatic activity, α- RHAs produced in hydrolysis reactions of glycosyl compounds that can be recovered from waste materials of industrial food processing (for example, citrus peel), which describes profitable and superb application of biotechnology [66]. Fungi is the main source of α- RHAs. These enzymes are also found in animals, and are obtained from animal tissues, for example from the liver of marine gastropod and the pig [3, 44]. They are also isolated from plants such as *Rhamnusdaurica* and *Fagopyrum esculentum* [47, 67].

6.2.3. Biological transformation of rutin to isoquercitrin

Recombinant α-L-rhamnosidase has the capability to convert the rutin to isoquercitrin. Rutin (quercetin routinoside) is present in fruits, medicinal herbs, vegetables and in many foods derived from

plants [121, 122]. Many activities such as antioxidant effects, antihyperglycemic and neurotoxic effects are introduced through rutin which becomes advantegeous to human health [122, 123]. Isoquercitrin (quercetin -3-b-D-glucoside), a flavonoid, is the rutin product and is obtain by derhamnosylation and rhamnose is the only difference in its structure. Isoquercitrine, rutin and quercetin have some differences in chemical, physical and biological properties, but similarity exists in their structures [124].



By comparing rutin with quercitin isoquercitrin is absorb better, suggesting that its glucose uptake increases its absorption in the small intestine [125]. Quercetin or rutin has an antipoliferative effect less than Isoquercitrin [123, 126]. Isoquercitrin is characterized by a high pharmacological activity as compare to rutin because of its specific antioxidant properties, which play an important role in anti-allergic and anti-aging effects [124, 126]. Isoquercitrin preserves damaged cells by eliminating free radicals, which indicates that it is better than rutin [126]. While isoquercitrin contains many important biological activities, its natural contents are low. It is therefore important to find an effective way to produce it. Isoquercitrin is manufactured by chemical and enzymatic methods. Experience indicates that, the aglycon of rutin is easily glycosylated under mild acidic conditions at appropriate temperatures, but it is difficult to obtain secondary glucoside (isoquercitrin). Therefore, it is better to prepare isoquercitrin by enzymatic degradation of the rutin to acidic hydrolysis. For a large amount of natural glycosides, for example, narignine, a routine, it is important to find an effective way to produce them. By chemical and enzymatic methods, isocercetrine is manufactured. Experience has shown that under mild acid hydrolysis, decreases it is easily immunized at appropriate temperatures, but it is difficult to obtain secondary glucoside (iso quercitrin). Therefore, it is better to prepare isoquercitrin by enzymatic degradation of the routine to acidic hydrolysis. For a large amount of natural glycosides, for example, Narignin, a rutin, hesperidin and terpeneglycoside, binds α-rhamnosidase to the α-rhamnose end. And α-rhamnosidase belongs to the glycosyl hydrolase class, that's why it leads to cleavage actions [21]. This enzyme is also used in the industry to eliminate the bitter taste of citrus fruits by releasing rhamnose from naringin and hydrolysis of terpene, which enhance the aroma of grape juice [5]. According to the recent research, the activity of rhamnosidase produces expensive flavonoids glycosides, isoquercitrine, in an easy and inexpensive process of rutin. Thus, through the method of enzyme hydrolysis, the biological transformation of monoglycosylated isoquercitrin from rutin appears to be a good alternative to get compounds with increased functional properties.

Table 3. Applications of α -L-rhamnosidase enzyme in chemical industries

Industry	**Applications**	**References**
Chemical	Naringin extraction	[108]
	Rhamnose preparation	[66]
	Glycolipid production	[118]

6.3. In the pharmaceutical industry

6.3.1 Preparation of antibiotics

Glycopeptides antibiotic Chloropolisporin C is synthesise by chloropolisporin B through hydrolysis of the enzyme, using Rhase [127]. Activity appears against bacteria, which is beneficial in the treatment of prophylaxis of infections, as well as in the growth factor improvement of animals. *Dioscoria nipphonica* is a recurring herb in China. In Chinese medicine, *dioscoria nipphonica* rhizome is used in the treatment of rheumatic diseases and is used to protect bronchial infections and other respiratory infections as well as viral infections. The Chinese people also use it for various purposes, for example in the treatment of cardiovascular disease and through various mechanisms, reducing the risk of heart disease such as reducing blood lipid levels, ALS or cancer prevention [51, 128-131]. The report, shows that the steroid saponin, the main active ingredient in the rhizome of *dioscorea nipponica* is dioscin. The dioscin in Rhizome of *Dioscoria nipphonica* comprises of three glycosides, after the introduction of the drug, dioscin is hydrolysed by intestinal bacteria and digestive enzymes [132, 133]. Therefore, it is very important to study the transformation of natural products from traditional Chinese medicine into more active ingredients. For the preparation of small saponin and its metabolites, conventional chemical methods such as chemical synthesis, hydrolysis of mild acid or cleavage of alkali produce certain secondary reactions, for example hydration, epimerization, hydroxylation [134, 135] but the enzymatic transformation of saponin is directly moderate. To convert saponin ginseng, Dr. FX Jin achieved enzyme isolation from some microorganisms [136], and has industrial enzyme production. It is reported that liver of animal, for example pig liver, contains a high concentration of dioscin glycosidase, so its hydrolysis is very high. Pig liver contain Diosgenyl-2,4-di-*O*-a -L-rhamnopyranosyl-b -D-glucopyranoside (Dioscin) which was degraded to diosgenyl-*O*-b -D-Glc by the enzyme. The kinetic properties of the enzyme, ie dioscina-L -rhamnosidase, were examined or diocin glycosides, have been systematically purified from pig liver.

6.3.2 Preparation of prunin

Flavonoids prunin are formed from Narignine by using α-L-rhamnosidase activity. Prunin has anti-inflammatory properties and against DNA virus's or RNA viruses, it shows its activity [11]. Microbial glycolyted enzymes play a vital role in many industrial processes and act as bio catalyst, and are therefore of great interest to many researchers around the world. It has been studied that many microorganisms have the capability to produce glycosidase, but the microorganisms that produce α-rhamnosidase. (Rhase, EC 3.2.1.40) activity is unknown. Rhases remove Terminal α-L-rhamnosyl at the ends of polysaccharides and Glycosides composed of L-rhamnanose, so they are exo-type enzymes. Rhases are important industrial enzymes of great importance in current biotechnology and have food applications [5], pharmaceutical preparations [13] and industrial products for the biological transformation of natural or synthetic rhamnosides. Specifically, in the food industry, many technical applications of fungus stages, such as hydrolysis of bitter naringin, to remove bitterness of grapefruit juice [137, 138], to remove crystals of hesperidin in orange juice, and by enzymatic degradation of terpenyl glycosides to

improve the smell of wine [59], was questioned. In addition, the antioxidant property of asparagus juice improves with quercetin- 3 glucoside from flavonoid rutin [139]. By this enzyme the structure of sugars, glycoside and glycolipid can be determined, also used in gellan metabolism [9]; in the formation of pruning [140], which has anti-inflammatory activities and activates both DNA viruses / RNA [11]; It is used to eliminate rhamnose from many of L-rhamnose containing, steroids, such as diosgene deglucoruscin and ginsenosides, whose derhamnosylated clinical products have clinical significance [12-14]. It is an aglycopeptide antibiotic that exhibits anti bacterial activity. It is also useful as a growth promoter of animals and in the treatment and prevention of infections. Using Rhase, the chloropolysporin C compound is synthesized by chloropolysporin B through enzymatic hydrolysis [127]. All of these preparations, currently obtained from the genus *Aspergillus* and *Penicillium*, also contain β-D-glucosidase (Gluse) which could limit their industrial exploitation. In addition, they are and stable and active in acidic pH values, where their substrates are slightly soluble. The use of stable and effective Rhases in alkaline pH values can solve this problem, allowing the analysis of concentrated solutions of substrates in alkaline conditions.

6.3.3 Deglycosylation of flavonoids

Deglycosylation of flavonoids with naringinase in *cleome arabica* leaf extracts (CALE) is an important therapeutic factor in the treatment of chronic conditions [141]. Activity of naringinase rhamnosidase causes successful removal of glucosyl from the antibiotic containing glycopeptide, chloropolyporin from *Faenia interjecta* [127]. L-rhamnose is chiral intermediate in organic synthesis manufactured by activity of rhamnosidase which is very important to be used as a protective agent for plants and as a pharmaceutical product [142]. When β-D-glucosidase is combined with the production and characterization of α-L-rhamnosidasein activity of naringinase *Aspergillus Terrus*, is able to improve the smell of wine making [59]. The preparation of the enzyme from *Penicillium decumbens* is commercially available, and is a fungus that produces Narignanase [74]. For immobilization and transformation of flavonoids, naringinase obtained from *P. Decumbens* has been largely used in many industries [77, 98, 143].

Two significant flavonoids, Naringin (4 ‘, 5, 7'-trihydroxiflavanone-7-rhamnoglucoside) and Naringenin (4’, 5, 7'-trihydroxiflavanone) have strong anti-ulcer, anti-cancer and anti-oxidant activities [144-151]. In addition to their useful properties, they also have some unwanted properties. Such as, at a threshold of 20 µg / ml, Narignin has a stiff and a slightly low bitter taste [108]. Narignin, Naringeninis a structure similar to Prunin (4 ', 5, 7'-trihydroxy-flavanone-7-β-D), which is not easily dissolved in water (Tommasini et al., 2004) -glucoside) [11, 18, 152, 153]. Prunin shows good solubility, strong biological activity, and a slightly bitter taste, which are combined benefits of narigin and naringenin [154]. However, there is naturally prunin in low quantity. The more commercially available Narignine is available as a citrus product. For each product, an effective process has been attempted to convert the Narangine into prunin [155]. A transformation of narinigin to prunin was reported, but they needed severe reaction conditions and complex purification steps. By contrast, because of the simplest process and the lowest production cost of the enzymatic method, it is recommended that enzymatic interaction is controlled with high efficiency and high specificity under milder and more environmentally friendly conditions. According to some investigations [66, 140, 156], the possibility of synthesizing prunin in the enzymatic way, so far due to the lack of industrial catalyst, pruning is not produced commercially. The biological conversion of Narignine to pruning is also performed by the enzyme α-L-rhamnosidese, which breaks down the association between α-1, 2-glycosidic bond of Narningin [1]. Naringinase enzyme is also produced by combining α-L-rhamnosidase with β -D- glucosidase [1]. According to the report, α-L-ramanosidase and Naringinase are present in some microorganisms [1, 3-5, 60], including *Aspergillus niger*, the most promising resource for industrial practice, because these fungi have not only been used in a beneficial manner in the microbial class approved by the FDA and can be stimulated to produce some food-grade enzymes efficiently and have been found to be safe for food and medical use [7], including the α-L-rhamnosidase and naringinase. In addition, fermentation technology is adorable and has been widely used in the industry, making it easy to expand [7].

6.3.5 Flavonoids absorption in Humans

The absorption of flavonoids occurs mainly in the small intestine of the human, where internal glucosidase removes the restricted glucose (or possibly xyloze or arabinose) [157, 158]. For human

glucosides, the terminal rhamnose is not the appropriate substrate. Therefore, non-adsorbent rhamnosylated flavonoids the colon without any change, but local microflora contains α-L-rhamnosidase so, it is hydrolyzed by its activities [159]. In fact, it would be useful, that α-RHA stimulated elimination of terminal rhamnose from rhamnosylated flavonoids as it would improve intestinal absorption of rhamnosylated flavonoids in humans [160, 161]. Absence of α-RHA in humans is harmful was the key to create a new strategy for drug delivery, as described by LEAPT (lectin-directed enzyme activated prodrug therapy) [101, 162]. In the LEAPT system, rhamnosylated prodrug intake, which cannot be treated by mammalian enzymes, allows selective action of the drug site in the cells where the pre-designed α-RHA has been located [163].

Table 4. **Applications of α -L -rhamnosidase enzyme in pharmaceutical industries**

Industry	**Applications**	**References**
Pharmaceutical	Lectin-directed enzyme activated prodrug therapy	[162]
	Steroid biotransformation	[12]
	Ginsenoside production	[13]
	Antibiotics preparations	[127]
	Prunin preparation	[11]
	Flavonoids deglycosylation	[141]

7. DISCUSSION

α-RHA is a group of glycosyl hydrates (GH) that have attracted much attention because of their potential application as vital catalysts in a variety of industrial processes. These enzymes are particularly important for the biological transformation of many natural compounds used in the food and pharmaceutical industry. The enzymatic derhamnosylation can be used by α-RHA, for example, in functional foods and beverages containing molecules with improved health properties. Some examples

include biological transformation of natural steroids, antibiotics, flavonoids and terpenes glycosides responsible for wine flavors.

Moreover, α -L-rhamnosidase is the most viable enzyme that can be obtained from animal, plant and microbial sources. Mainly found in microorganisms such as yeast fungi, bacteria and others. Filamentous fungi such as *Aspergillus, Circinella, Eurotium, Fusarium, Penicillium, Rhizopus* and *Trichoderma* are the main source of α -L-Rhamnosidase. Bacterial strains that produce α-L-rhamnosidases are heat-loving bacteria, *Fusa bacterium, Pseudoalteromonas, Ralstonia pickettii*, *Lactobacillus acidophilus, Pediococcus acidilactici*, *Clostridium stercorarium, Sphingomonas paucimobis* Used to eliminate bitter taste of citrus juices, monoterpenes are released from terpene glycosides to improve grape juice and flavor of wine. In the chemical industry it is used for preparation of chemicals for example in the preparation of DNA, production of special fatty acids i.e glycolipids. In addition to these applications, it also plays vital role in pharmaceutical industries for various preparations of antibiotics that are used against bacteria and other toxins as well as used as a growth promoter for animals. Flavonoid prunin is formed from Narignine using the activity of α-L-rhamnosidase. It has anti-inflammatory and anti-viral activity against DNA / RNA viruses. For α-L- Ramnosidase purification, a centrifuge procedure is used, and various chemical products such as ammonium sulfate and sodium chloride are used. Molecular weight (MW) for α-L-rhamnosidase is determined by GFC and SDS-PAGE. The first crystalline structure of α-L-rhamnosidase RhaB from Bacillus sp. GL1 can be accessed at 1.9 Å accuracy. This protein is homogeneous and consists of four areas of the beta sandwich, a (α / α) of 6 core catalytic barrel, containing 956 amino acid residues and a molecular mass of 106 kilo Dalton [1]. The second structure of *Streptomyces avermitilis* (SaRha78A; PDB code 3W5N) α-L-rhamnosidase was determined in a compound with L-rhamnose, this large, monochromatic protein is composed of Six domains. The third structure, an α-L-rhamnosidase supposedly from *the Bacteroides thetaiotaomicron* VP1-5482 (BT1001; code PDB 3CIH), designed in the project of structural genomics is not published, it is also a homodimer. More recently, the crystalline structure of KoRha, and α-L-rhamnosidase supposed from *Klebsiella oxytoca* with 2.7 Å accuracy was determined with restricted rhamnose at the active site of the catalytic domain. α-L-Rhamnosidases are used for various food, chemical and pharmaceutical applications. It uses Naringinase (containing the activities of α-rhamnosidase and gl-glucosidase) to eliminate bitter taste of

citrus juices. Hesperetin 7- glucoside, a product of hydrolysis of hesperidin by α-L-rhamenosidase, is an important introduction to the production of sweeteners. In addition, there is an increasing interest in using α-rhamnosidases to improve grape juice and wine flavor by releasing monoclonal free radicals from terpenyl glycosides. Many potential beneficial effects of health have been demonstrated in studies on animals and in the laboratoy. These include promoting bone health, the effects of lowering lipid, antioxidant properties and heart protective effects, anti-cancer and anti-inflammatory properties. The recombinant α-L-rhamnosidase has an ability to produce rhamnose and prunin from citrus peel residues at industrial scale. The production of glucolipids from *Candida bombicola* sophorolipids by Ninginin *P.decumbens* (α-Lrhamnosidase + β-D-glucosidase) showed that the enzyme could be beneficial for the production of special fatty acids. Antibiotics of glycopptide chlorophosphyrin C are prepared by enzymatic hydrolysis of chloroporulosporin-B compound using Rhase. It has antibacterial activity, beneficial in the treatment and prevention of infections and as a growth promoter of animals. In humans, absorption of flavonoid occurs mainly in the small intestine, where the associated glucose (or possibly arabinose or xylose) is eliminated by self-glucosidases. Non-absorbent rhamnosylated flavonoid arrives in the colon without any change, where they are broken by α rhamnosidase activity, which are expressed by local bacteria. Thus, to improve intestinal uptake of rhamnosylated flavonoids, their biological availability in humans and elimination of peripheral rhamnose group stimulated by α-RHA would be indeed useful.

8. CONCLUSION

The current study concludes following key points;

1: α-L-rhamnosidase (3.2.1.40) is an exo enzyme that removes the terminal α-L-rhamnosyl at the ends of sugars and glucosides that contain L-rhamnose.

2: The enzyme converts the bitter glucoside naringin into the least bitter prunin by trimming α- (1 → 2) between L-rhamnose and glucose.

3: The production of α-L-rhamnosidase by various mammalian tissues, plants, bacteria and fungi. This enzyme is used to determine the structure of glycolipids, polysaccarides and glycosides, eliminate bitter

taste from citrus juice, improved odors in wine, in the formation of pruning that have anti-inflammatory and antiviral activity against DNA / RNA virus.

4: α-L-rhamnosidase has vast applications in the food, chemical and pharmaceutical industries that are used for human welfare.

5. Alpha-L-rhamnosidase can be purified by the using different methods, such as electric and gel chromatography obtained from different animals and plant sources.

7. REFERENCES

[1]. Yadav, V., et al., α-L-Rhamnosidase: a review. Process Biochemistry, 2010. 45(8): p. 1226-1235.

[2]. Magario, I., et al., Deactivation Kinetics and Response Surface Analysis of the Stability of α-l-Rhamnosidase from Penicillium decumbens. Applied biochemistry and biotechnology, 2009. 152(1): p. 29-41.

[3]. Kurosawa, Y., K. IKEDA, and F. EGAMI, α-L-Rhamnosidases of the liver of Turbo cornutus and Aspergillus niger. The Journal of Biochemistry, 1973. 73(1): p. 31-37.

[4]. Manzanares, P., L.H. de Graaff, and J. Visser, Purification and characterization of an α-L-rhamnosidase from Aspergillus niger. FEMS Microbiology Letters, 1997. 157(2): p. 279-283.

[5]. Spagna, G., et al., A simple method for purifying glycosidases: α-L-rhamnopyranosidase from Aspergillus niger to increase the aroma of Moscato wine. Enzyme and Microbial Technology, 2000. 27(7): p. 522-530.

[6]. Puri, M., Updates on naringinase: structural and biotechnological aspects. Applied microbiology and biotechnology, 2012. 93(1): p. 49-60.

[7]. Pel, H.J., et al., Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. Nature biotechnology, 2007. 25(2): p. 221-231.

[8]. Puri, M. and U.C. Banerjee, Production, purification, and characterization of the debittering enzyme naringinase. Biotechnology Advances, 2000. 18(3): p. 207-217.

[9]. Hashimoto, W., et al., Characterization of α-L-rhamnosidase of Bacillus sp. GL1 responsible for the complete depolymerization of gellan. Archives of biochemistry and biophysics, 1999. 368(1): p. 56-60.

[10]. Roitner, M., T. Schalkhammer, and F. Pittner, Characterisation of naringinase from Aspergillus niger. Monatshefte für Chemie/Chemical Monthly, 1984. 115(10): p. 1255-1267.

[11]. Kaul, T.N., E. Middleton Jr, and P.L. Ogra, Antiviral effect of flavonoids on human viruses. Journal of medical virology, 1985. 15(1): p. 71-79.

[12]. Elujoba, A. and R. Hardman, Diosgenin production by acid and enzymatic hydrolysis of fenugreek. 1987.

[13]. Monti, D., et al., Generation of an α-L-rhamnosidase library and its application for the selective derhamnosylation of natural products. Biotechnology and bioengineering, 2004. 87(6): p. 763-771.

[14]. Yu, H., et al., Purification and characterization of ginsenoside-α-L-rhamnosidase. Chemical and pharmaceutical bulletin, 2002. 50(2): p. 175-178.

[15]. Busto, M., et al., Immobilization of naringinase from Aspergillus niger CECT 2088 in poly (vinyl alcohol) cryogels for the debittering of juices. Food chemistry, 2007. 104(3): p. 1177-1182.

[16]. Prakash, S., R.S. Singhal, and P.R. Kulkarni, Enzymic debittering of Indian grapefruit (Citrus paradisi) juice. Journal of the Science of Food and Agriculture, 2002. 82(4): p. 394-397.

[17]. TSEN, H.Y. and G.K. YU, Limonin and naringin removal from grapefruit juice with naringinase entrapped in cellulose triacetate fibers. Journal of food science, 1991. 56(1): p. 31-34.

[18]. Chang, H.-Y., et al., Purification and characterisation of Aspergillus sojae naringinase: The production of prunin exhibiting markedly enhanced solubility with in vitro inhibition of HMG-CoA reductase. Food chemistry, 2011. 124(1): p. 234-241.

[19]. González-Barrio, R., et al., Production of bioavailable flavonoid glucosides in fruit juices and green tea by use of fungal α-L-rhamnosidases. Journal of Agricultural and Food Chemistry, 2004. 52(20): p. 6136-6142.

[20]. Avila, M., et al., Physiological and biochemical characterization of the two α-L-rhamnosidases of Lactobacillus plantarum NCC245. Microbiology, 2009. 155(8): p. 2739-2749.

[21]. Beekwilder, J., et al., Characterization of rhamnosidases from Lactobacillus plantarum and Lactobacillus acidophilus. Applied and environmental microbiology, 2009. 75(11): p. 3447-3454.

[22]. Birgisson, H., et al., Two new thermostable α-L-rhamnosidases from a novel thermophilic bacterium. Enzyme and Microbial Technology, 2004. 34(6): p. 561-571.

[23]. Hashimoto, W., et al., Molecular identification of an α-L-rhamnosidase from Bacillus sp. strain GL1 as an enzyme involved in complete metabolism of gellan. Archives of biochemistry and biophysics, 2003. 415(2): p. 235-243.

[24]. Manzanares, P., et al., Purification and Characterization of Two Different α-l-Rhamnosidases, RhaA and RhaB, from Aspergillus aculeatus. Applied and Environmental Microbiology, 2001. 67(5): p. 2230-2234.

[25]. Yadav, S., et al., Purification and functional characterisation of an α-l-rhamnosidase from Penicillium citrinum MTCC-3565. International journal of food science & technology, 2012. 47(7): p. 1404-1410.

[26]. Zverlov, V.V., et al., The thermostable α-L-rhamnosidase RamA of Clostridium stercorarium: biochemical characterization and primary structure of a bacterial α-L-rhamnoside hydrolase, a new type of inverting glycoside hydrolase. Molecular microbiology, 2000. 35(1): p. 173-179.

[27]. Yanai, T. and M. Sato, Purification and characterization of an α-L-rhamnosidase from Pichia angusta X349. Bioscience, biotechnology, and biochemistry, 2000. 64(10): p. 2179-2185.

[28]. Miake, F., et al., Purification and characterization of intracellular α-L-rhamnosidase from Pseudomonas paucimobilis FP2001. Archives of microbiology, 2000. 173(1): p. 65-70.

[29]. Demirjian, D.C., F. Morís-Varas, and C.S. Cassidy, Enzymes from extremophiles. Current opinion in chemical biology, 2001. 5(2): p. 144-151.

[30]. Hough, D.W. and M.J. Danson, Extremozymes. Current opinion in chemical Biology, 1999. 3(1): p. 39-46.

[31]. Yamashita, T., et al., New Polyhydroxylated Pyrrolidine, Piperidine, and Pyrrolizidine Alkaloids from Scilla s ibirica. Journal of natural products, 2002. 65(12): p. 1875-1881.

[32]. Jespersen, T.M., et al., Isofagomine, a potent, new glycosidase inhibitor. Angewandte Chemie International Edition in English, 1994. 33(17): p. 1778-1779.

[33]. Legler, G., Glycoside hydrolases: mechanistic information from studies with reversible and irreversible inhibitors, in Advances in carbohydrate chemistry and biochemistry. 1990, Elsevier. p. 319-384.

[34]. Fowler, P.A., et al., Synthesis and activity towards yeast α-glucosidase of 1, 5-dideoxy-1, 5-imino-L-iditol (1-deoxy-L-idonojirimycin). Carbohydrate research, 1993. 246: p. 377-381.

[35]. Hashimoto, W. and K. Murata, α-L-Rhamnosidase of Sphingomonas sp. R1 producing an unusual exopolysaccharide of sphingan. Bioscience, biotechnology, and biochemistry, 1998. 62(6): p. 1068-1074.

[36]. Steinbacher, S., et al., Crystal structure of phage P22 tailspike protein complexed with Salmonella sp. O-antigen receptors. Proceedings of the National Academy of Sciences, 1996. 93(20): p. 10584-10588.

[37]. Griffiths, L. and A. Barrow, Metabolism of flavonoid compounds in germ-free rats. Biochemical Journal, 1972. 130(4): p. 1161-1162.

[38]. Macdonald, I.A., J.A. Mader, and R.G. Bussard, The role of rutin and quercitrin in stimulating flavonol glycosidase activity by cultured cell-free microbial preparations of human feces and saliva. Mutation Research Letters, 1983. 122(2): p. 95-102.

[39]. Bokkenheuser, V.D., C. Shackleton, and J. Winter, Hydrolysis of dietary flavonoid glycosides by strains of intestinal Bacteroides from humans. Biochemical Journal, 1987. 248(3): p. 953-956.

[40]. Jang, I.-S. and D.-H. Kim, Purification and characterization of α-L-rhamnosidase from Bacteroides JY-6, a human intestinal bacterium. Biological and Pharmaceutical Bulletin, 1996. 19(12): p. 1546-1549.

[41]. Miyata, T., et al., Cloning, sequence analysis, and expression of the gene encoding Sphingomonas paucimobilis FP2001 α-L-rhamnosidase. Current microbiology, 2005. 51(2): p. 105-109.

[42]. Cui, Z., et al., Crystal structure of glycoside hydrolase family 78 α-L-rhamnosidase from Bacillus sp. GL1. Journal of molecular biology, 2007. 374(2): p. 384-398.

[43]. Twerdochlib, A., et al., L-rhamnose metabolism in Pichia stipitis and Debaryomyces polymorphus. Canadian journal of microbiology, 1994. 40(11): p. 896-902.

[44]. Qian, S., et al., Purification and characterization of dioscin-α-L-rhamnosidase from pig liver. *Chemical and pharmaceutical bulletin*, 2005. 53(8): p. 911-914.

[45]. Manzanares, P., et al., Purification and characterization of an α-l-rhamnosidase from *Aspergillus nidulans*. *Letters in Applied Microbiology*, 2000. 31(3): p. 198-202.

[46]. Thomas, D., C. Smythe, and M. Labbee, ENZYMATIC HYDROLYSIS OF NARINGIN, THE BITTER PRINCIPLE OF GRAPEFRUIT a. *Journal of Food Science*, 1958. 23(6): p. 591-598.

[47]. Suzuki, H., Hydrolysis of flavonoid glycosides by enzymes (Rhamnodiastase) from *Rhamnus* and other sources. *Archives of Biochemistry and Biophysics*, 1962. 99(3): p. 476-483.

[48]. Bourbouze, R., F. Percheron, and J.E. Courtois, α-L-Rhamnosidase de *Fagopyrum esculentum*: Purification et quelques proprietes. *European journal of biochemistry*, 1976. 63(2): p. 331-337.

[49]. Scaroni, E., et al., Hydrolytic properties of crude α-L-rhamnosidases produced by several wild strains of mesophilic fungi. *Letters in applied microbiology*, 2002. 34(6): p. 461-465.

[50]. Custodio, M.V.G., et al., Production and characterization of an *Aspergillus terreus* α-l-rhamnosidase of oenological interest. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 1996. 203(6): p. 522-527.

[51]. Manzanares, P., et al., Construction of a genetically modified wine yeast strain expressing the *Aspergillus aculeatus* rhaA gene, encoding an α-L-rhamnosidase of enological interest. *Applied and Environmental Microbiology*, 2003. 69(12): p. 7558-7562.

[52]. Orejas, M., E. Ibáñez, and D. Ramón, The filamentous fungus *Aspergillus nidulans* produces an α-L-rhamnosidase of potential oenological interest. *Letters in applied microbiology*, 1999. 28(5): p. 383-388.

[53]. Singh, P., et al., Optimization, production and scale up of debittered kinnow beverage by α-L-rhamnosidase producing yeast. *Emirates Journal of Food and Agriculture*, 2015: p. 548-555.

[54]. Kim, J.-H. and D.-H. Kim, Purification and characterization of quercitrin-hydrolyzing α-l-rhamnosidase from *Fusobacterium* K-60, a human intestinal bacterium. *Journal of microbiology and biotechnology*, 2005. 15(3): p. 519-524.

[55]. Orrillo, A.G., et al., Cold-active α-L-rhamnosidase from psychrotolerant bacteria isolated from a sub-Antarctic ecosystem. *Enzyme and microbial technology*, 2007. 40(2): p. 236-241.

[56]. Michlmayr, H., et al., Heterologously expressed family 51 α-L-arabinofuranosidases from *Oenococcus oeni* and *Lactobacillus brevis*. *Applied and environmental microbiology*, 2011. 77(4): p. 1528-1531.

[57]. Fujimoto, Z., et al., The structure of a *Streptomyces avermitilis* α-L-rhamnosidase reveals a novel carbohydrate-binding module CBM67 within the six-domain arrangement. *Journal of Biological Chemistry*, 2013. 288(17): p. 12376-12385.

[58]. Yadav, V., et al., α-l-Rhamnosidase from *Aspergillus flavus* MTCC-9606 isolated from lemon fruit peel. *International journal of food science & technology*, 2011. 46(2): p. 350-357.

[59]. Caldini, C., et al., Kinetic and immobilization studies on fungal glycosidases for aroma enhancement

in wine. *Enzyme and Microbial Technology*, 1994. 16(4): p. 286-291.

[60]. Puri, M., et al., Molecular characterization and enzymatic hydrolysis of naringin extracted from kinnow peel waste. *International journal of biological macromolecules*, 2011. 48(1): p. 58-62.

[61]. Gerstorferová, D., et al., Recombinant α-L-rhamnosidase from *Aspergillus terreus* in selective trimming of rutin. *Process Biochemistry*, 2012. 47(5): p. 828-835.

[62]. Zajkoska, P., M. Rebroš, and M. Rosenberg, Biocatalysis with immobilized *Escherichia coli*. *Applied microbiology and biotechnology*, 2013. 97(4): p. 1441-1455.

[63]. Manzanares, P., et al., α-L-Rhamnosidases: old and new insights, in *Industrial enzymes*. 2007, Springer. p. 117-140.

[64]. Habelt, K. and F. Pittner, A rapid method for the determination of naringin, prunin, and naringenin applied to the assay of naringinase. *Analytical biochemistry*, 1983. 134(2): p. 393-397.

[65]. Rojas, N.L., et al., Purification and characterization of a novel alkaline α-L-rhamnosidase produced by *Acrostalagmus luteo albus*. *Journal of industrial microbiology & biotechnology*, 2011. 38(9): p. 1515-1525.

[66]. Kaur, A., et al., Hydrolysis of citrus peel naringin by recombinant α-L-rhamnosidase from *Clostridium stercorarium*. *Journal of Chemical Technology & Biotechnology*, 2010. 85(10): p. 1419-1422.

[67]. Bourbouze, R., F. Pratviel-Sosa, and F. Percheron, Rhamnodiastase et α-L-rhamnosidase de *Fagopyrum esculentum*. *Phytochemistry*, 1975. 14(5-6): p. 1279-1282.

[68]. Grimaldi, A., E. Bartowsky, and V. Jiranek, A survey of glycosidase activities of commercial wine strains of *Oenococcus oeni*. *International journal of food microbiology*, 2005. 105(2): p. 233-244.

[69]. Bonanno, J.B., et al., New York-Structural GenomiX Research Consortium (NYSGXRC): a large scale center for the protein structure initiative. *Journal of Structural and Functional Genomics*, 2005. 6(2-3): p. 225-232.

[70]. Notomista, E., et al., The marine isolate *Novosphingobium* sp. PP1Y shows specific adaptation to use the aromatic fraction of fuels as the sole carbon and energy source. *Microbial ecology*, 2011. 61(3): p. 582-594.

[71]. D'Argenio, V., et al., De novo sequencing and assembly of the whole genome of *Novosphingobium* sp. strain PP1Y. 2011, *Am Soc Microbiol*.

[72]. Hall, D.H., A new enzyme of the glycosidase type. *Nature*, 1938. 142(3586): p. 150-150.

[73]. Kaji, A. and T. Ichimi, α-L-Rhamnosidase activity in culture filtrate of *Corticium rolfsii* enzymic activity at low pH. *Agricultural and Biological Chemistry*, 1973. 37(2): p. 431-432.

[74]. Young, N.M., R.A. Johnston, and J.C. Richards, Purification of the α-L-rhamnosidase of *Penicillium decumbens* and characterisation of two glycopeptide components. *Carbohydrate research*, 1989. 191(1): p. 53-62.

[75]. Koseki, T., et al., Characterization of an α-L-rhamnosidase from *Aspergillus kawachii* and its gene. *Applied microbiology and biotechnology*, 2008. 80(6): p. 1007.

[76]. Shanmugam, V. and K. Yadav, Extracellular production of alpha-rhamnosidase by *Rhizopus nigricans*. *Indian journal of experimental biology*, 1995. 33(9): p. 705-707.

[77]. Puri, M., A. Banerjee, and U. Banerjee, Optimization of process parameters for the production of naringinase by *Aspergillus niger* MTCC 1344. *Process Biochemistry*, 2005. 40(1): p. 195-201.

[78]. Mamma, D., et al., Biochemical characterization of the multi-enzyme system produced by *Penicillium decumbens* grown on rutin. *Food Biotechnology*, 2004. 18(1): p. 1-18.

[79]. Thammawat, K., et al., Isolation, preliminary enzyme characterization and optimization of culture parameters for production of naringinase isolated from *Aspergillus niger* BCC 25166. *Agriculture and Natural Resources*, 2008. 42(1): p. 61-72.

[80]. Rajal, V.B., et al., Production, partial purification and characterization of α-L-rhamnosidase from *Penicillium ulaiense*. *World journal of microbiology and biotechnology*, 2009. 25(6): p. 1025-1033.

[81]. Yadav, S., S. Yadava, and K. Yadav, Purification and characterization of α-L-rhamnosidase from *Penicillium corylopholum* MTCC-2011. *Process Biochemistry*, 2013. 48(9): p. 1348-1354.

[82]. Chisti, Y., *Bioseparation and Bioprocessing: A Handbook* - G. Subramanian, editor, Wiley-VCH, New York, 1998 Volume 2 (xviii+ 474 pages) ISBN 3 527 28876 7. *Biotechnology Advances*, 1999. 17(7): p. 599-601.

[83]. Nomura, D., Studies on naringinase produced by *Coniothyrium diplodiella*. I. The properties of naringinase and the removal of co-existing pectinase from the enzyme preparation. *Enzymologia*, 1965. 29(3): p. 272.

[84]. Vila-Real, H., et al., Enzymatic synthesis of the flavone glucosides, prunin and isoquercetin, and the

aglycones, naringenin and quercetin, with selective-l-rhamnosidase and-d-glucosidase activities of naringinase. *Enzyme research*, 2011. 2011.

[85]. Rivas, J.J.N. and A.E.B. Zamora, El complejo agroindustrial limonero de la provincia de Tucumán (Argentina). Ejemplo de producciones no tradicionales y de desaparición de los pequeños productores. *Boletín de la Asociación de Geógrafos Españoles*, 2010.

[86]. Illanes, A., et al., Recent trends in biocatalysis engineering. *Bioresource technology*, 2012. 115: p. 48-57.

[87]. Fritz, I., et al., *Brevundimonas mediterranea* sp. nov., a non-stalked species from the Mediterranean Sea. *International journal of systematic and evolutionary microbiology*, 2005. 55(1): p. 479-486.

[88]. Puri, M., et al., Biochemical basis of bitterness in citrus fruit juices and biotech approaches for debittering. *Critical reviews in biotechnology*, 1996. 16(2): p. 145-155.

[89]. Williams, P.J., et al., Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry*, 1982. 21(8): p. 2013-2020.

[90]. Nielsen, I.L.F., et al., Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. The Journal of nutrition, 2006. 136(2): p. 404-408.

[91]. Kalu, D., et al., A comparative study of the actions of tamoxifen, estrogen and progesterone in the ovariectomized rat. Bone and mineral, 1991. 15(2): p. 109-123.

[92]. Park, Y.B., et al., Interactive effect of hesperidin and vitamin E supplements on cholesterol metabolism in high cholesterol-fed rats. International Journal for Vitamin and Nutrition Research, 2001. 71(1): p. 36-44.

[93]. Miyake, Y., et al., Protective effects of lemon flavonoids on oxidative stress in diabetic rats. Lipids, 1998. 33(7): p. 689.

[94]. Ohtsuki, K., et al., Effects of long-term administration of hesperidin and glucosyl hesperidin to spontaneously hypertensive rats. Journal of nutritional science and vitaminology, 2002. 48(5): p. 420-422.

[95]. Tanaka, T., et al., Suppression of azoxymethane-induced colon carcinogenesis in male F344 rats by mandarin juices rich in β-cryptoxanthin and hesperidin. International Journal of Cancer, 2000. 88(1): p. 146-150.

[96]. Guardia, T., et al., Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Il farmaco, 2001. 56(9): p. 683-687.

[97]. Gray, G.M. and A.C. Olson, Hydrolysis of high levels of naringin in grapefruit juice using a hollow fiber naringinase reactor. Journal of Agricultural and Food Chemistry, 1981. 29(6): p. 1298-1301.

[98]. Manjon, A., et al., Immobilization of naringinase on glycophase-coated porous glass. Biotechnology letters, 1985. 7(7): p. 477-482.

[99]. Puri, M., et al., Immobilized enzyme technology for debittering citrus fruit juices. Food enzymes: Application of new technologies, 2008: p. 91-103.

[100]. Cheetham, P.S. and M.A. Quail, Process for preparing L-rhamnose. 1991, Google Patents.

[101]. Hollman, P.C., et al., The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. Free radical research, 1999. 31(6): p. 569-573.

[102]. Martínez Conesa, C., et al., Treatment of metastatic melanoma B16F10 by the flavonoids tangeretin, rutin, and diosmin. Journal of agricultural and food chemistry, 2005. 53(17): p. 6791-6797.

[103]. Kawai, Y., et al., Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries implication in the anti-atherosclerotic mechanism of dietary flavonoids. Journal of Biological chemistry, 2008. 283(14): p. 9424-9434.

[104]. Bellocco, E., et al., Influence of L-rhamnosyl-D-glucosyl derivatives on properties and biological interaction of flavonoids. Molecular and Cellular Biochemistry, 2009. 321(1-2): p. 165-171.

[105]. Zhang, L., et al., Structural basis for catalytic and inhibitory mechanisms of β-hydroxyacyl-acyl carrier protein dehydratase (FabZ). Journal of Biological Chemistry, 2008. 283(9): p. 5370-5379.

[106]. Tuberoso, C.I.G., et al., Flavonoid characterization and antioxidant activity of hydroalcoholic extracts from Achillea ligustica All. Journal of pharmaceutical and biomedical analysis, 2009. 50(3): p. 440-448.

[107]. Real, H.J.V., et al., High pressure-temperature effects on enzymatic activity: Naringin bioconversion. Food Chemistry, 2007. 102(3): p. 565-570.

[108]. Ribeiro, I.A. and M.H. Ribeiro, Naringin and naringenin determination and control in grapefruit juice by a validated HPLC method. Food Control, 2008. 19(4): p. 432-438.

[109]. Ribeiro, I.A. and M.H. Ribeiro, Kinetic modelling of naringin hydrolysis using a bitter sweet alfa-

rhamnopyranosidase immobilized in k-carrageenan. Journal of Molecular Catalysis B: Enzymatic, 2008. 51(1-2): p. 10-18.

[110]. Henrissat, B., A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemical journal, 1991. 280(2): p. 309-316.

[111]. Mutter, M., Rhamnogalacturonan α-l-rhamnopyranohydrolase. A novel enzyme specific for rhamnogalacturonan regions of pectin. Plant Physiol., 1994. 106: p. 241-250.

[112]. Crick, D.C. and P.J. Brennan, Biosynthesis of the Arabinogalactan-Peptidoglycan Complex of Mycobacterium tuberculosiss. The mycobacterial cell envelope, 2008: p. 25-40.

[113]. Maxwell, E.G., et al., Rhamnogalacturonan I containing homogalacturonan inhibits colon cancer cell proliferation by decreasing ICAM1 expression. Carbohydrate Polymers, 2015. 132: p. 546-553.

[114]. Nguema-Ona, E., et al., Cell wall O-glycoproteins and N-glycoproteins: aspects of biosynthesis and function. Frontiers in plant science, 2014. 5: p. 499.

[115]. Rahim, R., et al., Cloning and functional characterization of the Pseudomonas aeruginosa rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. Molecular microbiology, 2001. 40(3): p. 708-715.

[116]. Gunther, N.W., et al., Production of rhamnolipids by Pseudomonas chlororaphis, a nonpathogenic bacterium. Applied and environmental microbiology, 2005. 71(5): p. 2288-2293.

[117]. Champion, E., et al., Synthesis of l-rhamnose and N-acetyl-d-glucosamine derivatives entering in the composition of bacterial polysaccharides by use of glucansucrases. Journal of Carbohydrate Chemistry, 2009. 28(3): p. 142-160.

[118]. Saerens, K., et al., Production of glucolipids and specialty fatty acids from sophorolipids by Penicillium decumbens naringinase: Optimization and kinetics. Biotechnology Journal: Healthcare Nutrition Technology, 2009. 4(4): p. 517-524.

[119]. Schmid, A., et al., Industrial biocatalysis today and tomorrow. nature, 2001. 409(6817): p. 258-268.

[120]. Bornscheuer, U., et al., Engineering the third wave of biocatalysis. Nature, 2012. 485(7397): p. 185-194.

[121]. Awatsuhara, R., et al., Antioxidative activity of the buckwheat polyphenol rutin in combination with ovalbumin. Molecular Medicine Reports, 2010. 3(1): p. 121-125.

[122]. Chua, L.S., A review on plant-based rutin extraction methods and its pharmacological activities. Journal of ethnopharmacology, 2013. 150(3): p. 805-817.

[123]. You, H.J., H.J. Ahn, and G.E. Ji, Transformation of rutin to antiproliferative quercetin-3-glucoside by Aspergillus niger. Journal of Agricultural and Food Chemistry, 2010. 58(20): p. 10886-10892.

[124]. Valentová, K., et al., Isoquercitrin: pharmacology, toxicology, and metabolism. Food and Chemical Toxicology, 2014. 68: p. 267-282.

[125]. Arts, I.C., et al., The type of sugar moiety is a major determinant of the small intestinal uptake and subsequent biliary excretion of dietary quercetin glycosides. British Journal of Nutrition, 2004. 91(6): p. 841-847.

[126]. Paulke, A., et al., Isoquercitrin provides better bioavailability than quercetin: comparison of quercetin metabolites in body tissue and brain sections after six days administration of isoquercitrin and quercetin. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2012. 67(12): p. 991-996.

[127]. Sankyo, C., Preparation of antibiotic chloropolysporin-C. Japanese Patent, 1988. 63: p. 146.

[128]. Pires, V.S., et al., Saponins and sapogenins from Brachiaria decumbens Stapf. Journal of the Brazilian Chemical Society, 2002. 13(2): p. 135-139.

[129]. Sokolova, L., A. Turovu, and A. Shreter, Dioscorea nipponica: a source of raw materials for the production of polysponm, an aomatherotic preparation. Rart resur, 1968. 4(1): p. 43-50.

[130]. Cai, J., et al., Apoptosis induced by dioscin in Hela cells. Biological and Pharmaceutical Bulletin, 2002. 25(2): p. 193-196.

[131]. Ishihara, M., et al., Combination use of kampo-medicines and drugs affecting intestinal bacterial flora. Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan, 2002. 122(9): p. 695-701.

[132]. Ma, H., Q. Zhou, and B. Wang, Studies on the metabolism of DX by intestinal bacteria and the absorbed components in serum. China Pharm, 2002. 13(4): p. 204-205.

[133]. Akao, T., Metabolic activation of crude drugs components by intestinal bacterial enzymes. J Trad Med, 1992. 9: p. 1-13.

[134]. Han, B.H., et al., Degradation of ginseng saponins under mild acidic conditions. Planta medica, 1982. 44(03): p. 146-149.

[135]. Chen, Y., M. Nose, and Y. Ogihara, Alkaline cleavage of ginsenosides. Chemical and

pharmaceutical bulletin, 1987. 35(4): p. 1653-1655.

[136]. Zhang, C., et al., Purification and characterization of ginsenoside-β-glucosidase from ginseng. Chemical and pharmaceutical bulletin, 2001. 49(7): p. 795-798.

[137] Soares, N., & Hotchkiss, J. (1998). Naringinase immobilization in packaging films for reducing naringin concentration in grapefruit juice. *Journal of Food Science*, *63*(1), 61–65.

[138] Soria, F. F., Cuevas, C., & Ellenrieder, G. (1999). Purification and some properties of α-L-rhamnosidase of *Aspergillus terreus*. *Applied Biological Science*, *5*(2), 109–120.

[139] Sun, T., Powers, J. R., & Tang, J. (2007). Enzyme-catalyzed change of antioxidants content and antioxidant activity of asparagus juice. *Journal of Agricultural and Food Chemistry*, *55*(1), 56–60.

[140] Roitner, M., Schalkhammer, T., & Pittner, F. (1984). Preparation of prunin with the help of immobilized naringinase pretreated with alkaline buffer. *Applied Biochemistry and Biotechnology*, *9*(5–6), 483–488.

[141] Bouriche, H., & Arnhold, J. (2010). Effect of *Cleome arabica* leaf extract treated by naringinase on human neutrophil chemotaxis. *Natural Product Communications*, *5*(3), 1934578X1000500315.

[142] Daniels, L., et al. (1990). *Method for producing rhamnose*. Google Patents.

[143] Romero, C., et al. (1985). A method for assaying the rhamnosidase activity of naringinase. *Analytical Biochemistry*, *149*(2), 566–571.

[144] Parmar, N. (1983). The gastric anti-ulcer activity of naringenin, a specific histidine decarboxylase inhibitor. *International Journal of Tissue Reactions*, *5*(4), 415–420.

[145] Yuting, C., et al. (1990). Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biology and Medicine*, *9*(1), 19–21.

[146] Martin, M., et al. (1994). Antiulcer effect of naringin on gastric lesions induced by ethanol in rats. *Pharmacology*, *49*(3), 144–150.

[147] Gordon, P. B., Holen, I., & Seglen, P. O. (1995). Protection by naringin and some other flavonoids of hepatocytic autophagy and endocytosis against inhibition by okadaic acid. *Journal of Biological Chemistry*, *270*(11), 5830–5838.

[148] So, F. V., et al. (1996). Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices.

[149] Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *The Journal of Nutritional Biochemistry*, *13*(10), 572–584.

[150] Calgarotto, A., et al. (2007). A multivariate study on flavonoid compounds scavenging the peroxynitrite free radical. *Journal of Molecular Structure: THEOCHEM*, *808*(1–3), 25–33.

[151] Ekambaram, G., et al. (2008). Naringenin reduces tumor size and weight loss in N-methyl-N′-nitro-N-nitrosoguanidine–induced gastric carcinogenesis in rats. *Nutrition Research*, *28*(2), 106–112.

[152] Tommasini, S., et al. (2004). Combined effect of pH and polysorbates with cyclodextrins on solubilization of naringenin. *Journal of Pharmaceutical and Biomedical Analysis*, *36*(2), 327–333.

[153] Choi, J. S., Yokozawa, T., & Oura, H. (1991). Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of *Prunus davidiana* stems and its main component, prunin. *Planta Medica*, *57*(03), 208–211.

[154] Puri, M., Marwaha, S., & Kothari, R. (1996). Studies on the applicability of alginate-entrapped naringinase for the debittering of kinnow juice. *Enzyme and Microbial Technology*, *18*(4), 281–285.

[155] Fox, D. W., Savage, W. L., & Wender, S. H. (1953). Hydrolysis of some flavonoid rhamnoglucosides to flavonoid glucosides. *Journal of the American Chemical Society*, *75*(10), 2504–2505.

[156] Soria, F., & Ellenrieder, G. (2002). Thermal inactivation and product inhibition of *Aspergillus terreus* CECT 2663 α-L-rhamnosidase and their role on hydrolysis of naringin solutions. *Bioscience, Biotechnology, and Biochemistry*, *66*(7), 1442–1449.

[157] Day, A. J., et al. (2003). Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology*, *65*(7), 1199–1206.

[158] Wolffram, S., Block, M., & Ader, P. (2002). Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. *The Journal of Nutrition*, *132*(4), 630–635.

[159] Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition*, *130*(8), 2073S–2085S.

[160] Hollman, P. C., et al. (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters*, *418*(1–2), 152–156.

[161] Németh, K., et al. (2003). Deglycosylation by small intestinal epithelial cell β-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, *42*(1), 29–42.

[162] Garnier, P., et al. (2010). Lectin-directed enzyme activated prodrug therapy (LEAPT): Synthesis and evaluation of rhamnose-capped prodrugs. *Journal of Drug Targeting*, *18*(10), 794–802.

[163] Robinson, M. A., et al. (2004). LEAPT: Lectin-directed enzyme-activated prodrug therapy. *Proceedings of the National Academy of Sciences*, *101*(40), 14527–14532.