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Recent Updates on Microbial Naringinase for Debittering of Citrus Juices by Transformation of Flavonoid Naringin

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Naringin is a well-known flavonoid mainly present in citrus fruits and contributes to the bitter flavour of citrus fruit juices. Naringinase is an important enzyme known to hydrolyse naringin into naringenin. Naringenin is a tasteless compound and therefore, naringinase may be used to de-bitter the citrus juices by reducing the level of naringin. This enzyme provides a good alternate to the chemical processing of citrus juices. By using naringinase, bitterness of the citrus juice may be controlled without affecting the beneficial properties of citrus juices. Naringinase from various microbial sources have been reported including bacteria and fungi. Naringinase from fungal sources have been investigated to higher extent as compared to bacterial counterparts. Till date, this enzyme has not been extensively investigated. The enzyme needs to be investigated extensively for its routine use in juice processing industries as well as other related industries. This review presents various microbial sources and production strategies of naringinase for its application in debittering of citrus juices. Recent updates in the area of microbial naringinase production and citrus juice processing has been summarized.

Keywords: Bio-Process Technology; Enzyme Production; Food Processing; Naringinase; Naringin.

Naringin is a flavonoid that naturally found in various citrus fruits (grapefruits, oranges, lemons etc.). Naringin is known to contribute the bitterness to the citrus juices. The delayed start of bitterness in citrus fruit juice affects its consumer acceptability[1]. The naringinase enzyme hydrolyse the naringin and the end product is an unflavoured, flavanone naringenin (non-bitter in taste). Naringinase have two subunits, i.e. α-L-rhamnosidase and also â-D-glucosidase as discussed earlier[2;3]. Hydrolysis of naringin completes in two steps. First, the naringin is hydrolysed by action of á-L-rhamnosidase and lead to formation of prunin along with release of rhamnose. In second reaction, purnin is hydrolysed with action of β-D-glucosidase which leads to production of naringenin (a tasteless flavanone) with release of glucose[4,5,6]. Various industrial uses of microbial naringinase have previously been reviewed by various authors[7,8]. This enzyme has main use in industries which are involved in processing of citrus fruits, where naringin mediated bitterness need to be removed[9,10,11,12,13]. Further,

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the enzyme has been reported to enhance the flavour of wines[14,15]. The by-products of naringin hydrolysis (i.e. naringenin, rhamnose, prunin and glucose) have various biological properties and found useful in different industries[16,17,9]. Besides de-bittering of citru juices, various other applications of naringinase or its component enzymes have been discussed[11,12]. Researchers are still working on industrial production and application of microbial naringinase. The current pace of research in the area of microbial naringinase is not significant and appropriate according to the potential of enzyme. Exploration of new microbial sources in particular bacterial sources is required. Bioprocess approaches, mutant strains and strategies for higher production of naringinase should be improved. This review article focuses on microbial sources, various approaches followed for production of naringinase along with its potent application for debittering of citrus fruit juice and advancement made in various application area. This article will provide important insight into production of bacterial as well as fungal naringinase for citrus juice processing. This enzyme needs to be study more extensively and new microbial sources should be assessed for commercial aspects.

Microbial sources of naringinase

Naringinase has previously been identified in microorganisms such as fungi, yeast, bacteria, and plants. Majority of research has been conducted on fungal sources of naringinase. Naringinase producing fungi have been widely presented in literature while the production of enzyme by bacteria (Table 1) has been reported in few species only[3]. There are limited findings on bacterial naringinase production. Among bacteria, *Enterococcus* sp., *Micrococcus* sp., *Staphylococcus* sp., some probiotic bacterial strains, *Pseudomonas* sp., *Bacillus* sp., and other bacterial strains have been studied for naringinase production (Table 1). Recently, *Paenibacillus stellifer* and *Pseudomonas resinovorans* has also been reported for naringinase production. Among the fungal sources, *Aspergillus* sp. has been studied most extensively (Table 2). The *Penicillium* sp. has also been explored widely for naringinase producing potential (Table 2). The strains of *Aspergillus sp.* showing naringinase activity have been reported among a variety of microbial sources. In terms of various aspects related to naringinase, *Aspergillus* sp. has been the top choice of researchers. Most of the micro-organism capable of producing naringinase was found in soil near citrus plants, irrigation areas and various soil samples, and from decaying citrus fruits[13]. More bacterial sources of naringinase need to be identified for their potential use in food processing industry and their obvious advantages over fungal counterparts.

Approaches for fermentative microbial naringinase production

Several studies recorded the production of this important enzyme, naringinase, from different microbial sources. Different media components/ constituents as well as formulations were earlier discussed for microbial naringinase production. Different constituents of media also affect the production of naringinase. In particular, naringin act as a inducer for naringinase production from microbes and its concentration has been shown to have major impact on its expression. Earlier, screening of 348 microbial isolates (fungi) from distinct samples (128) collected from eleven different sources has been reported[18]. Authors described use of synthetic minimal media with naringin (0.1%; w/v) and higher naringinase activity in a period of 23 to 48 hours. Authors identified the isolate PTK-PS10 as *Aspergillus niger* and further optimized the production of naringinase by selected strain in submerged fermentation[18]. Naringinase and rhamnosidase production was found to be affected by $NaNO_3$, rhamnose as well as soya peptone. Similarly, Phukan and Kardong[19] reported the naringinase production from soil bacteria. Four different strains of microbes (MD2, MD6, MD10 and HL13) were selected by screening process, $FeCL_3$ test, and characterized. The nutritional needs of the *Aspergillus oryzae* JMU316 for production of extracellular naringinase enzyme has been investigated[20]. The technique known as one-factor-at-a-time was utilised by authors for the evaluation of various nitrogen and carbon sources for their influence on naringinase synthesis. It was observed that among all other carbon sources, naringin lead to higher production of naringinase. Further, pomelo pericarp powder exhibited significant effects on naringinase production. Among various nitrogen sources tested, peptone was found to be most appropriate for naringinase enzyme synthesis. Uppermost naringinase activity (408.28 IU/mL) was obtained with following media concentrations

(per Litre): peptone (12g), $CaCl_2$ (0.2g), NaCl (0.4g), $MgSO_4 7H_2O$ (2g), K_2HPO_4 (1g) and pomelo pericarp powder (15g).

Besides the above said method, another strategy i.e. RSM (response surface methodology) has also been investigated for higher production of naringinase *Staphylococcus xylosus*[11]. Reportedly, the production of enzyme naringinase was greatly influenced by different media components and their interactions. The 22 factorial CCD (central composite design) was used in a subsequent phase to establish the ideal values for each of the relevant variables. The optimized values of important media components included (w/v): 10.0% of sodium nitrate; 0.50% of naringin; 10.0% of sucrose, with a pH of 5.6. The production of naringinase was expressed as 8.45 U/mL. In a study, Pavithra et al.[6] reported isolation of naringinase producing bacteria from soil samples. The following media components (g/L) were used by authors: KH_2PO_4 0.4g, NH_4NO_3 5g, $ZnSO_4$ 0.01g; KCl 0.2g; $MnSO_4$ 0.01g; $FeSO_4 7H_2O$ 0.01g; Agar 15g; $MgSO_4 7H_2O$ 0.2g; and naringin 1g. In 2011, researchers used *S. xylosus* MAK2 for production of naringinase at stirred tank reactor while using citrus peel powder as source of naringin[21]. The production media was based on peptone, sodium chloride, beef extract, and citrus peel powder. Authors optimized the process parameters in 5L bioreactor with 3.5 L capacity. Fermentation was carried for 40 h (at 30°C) along with aeration level of 1 vvm and 300 rpm agitation. Production of 8.9 IU/ml was achieved at bioreactor level.

Recently, production from *Aspergillus niger* van Tieghem MTCC 2425 has been reported[22]. The production of enzyme was carried using citrus wastes. Authors found that pH, temperature of incubation, and inducer concentration are most important factors affecting production of naringinase. A rotatable CCD (central composite design) was employed to optimize the levels of these factors. Higher production of naringinase (545.2 IU g^{-1}) was recorded using fermentation carried out at 29.8°C along with pH value (4.7) and the concentration of the inducer was 14.9 g L^{-1}.

Similarly, isolation of naringinase producing *Bacillus amyloliquefaciens* 11568 was from the soil was reported and results were found promising[23]. Hydrolysis of the neohesperidin, naringin, and some other glycosides was reported by naringinase enzyme. Researchers have reported naringinase production using twelve filamentous fungi in solid substrate fermentation[24]. Authors used orange as well as grapefruit rind in form of substrate containing naringin. The fungal strains (Table 1) included strains of *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. The fungal naringinase was able to hydrolyze the naringin significantly. The highest naringinase activity was obtained from *Aspergillus foetidus* (2.58 U/ml). The production was carried using citrus peel and mineral salt solution containing KH_2PO_4, K_2HPO_4, NH_4Cl, $MgSO_4.7H_2O$, and $FeCl_3$. Production of naringinase from *Aspergillus aculeatus* followed by immobilization on magnetic nanoparticles has been reported[25]. Authors have characterized the immobilized enzyme and presented a new horizon in naringinase research.

Isolation of naringinase producing fungus from spoiled citrus peels has been reported and the efficient naringinase producing fungus was identified as *Aspergillus flavus*[13]. The isolation media reportedly contained naringin (0.2%; w/v) along with yeast extract, glucose and mineral solution ($ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ & $FeSO_4.7H_2O$). Highest naringinase activity was shown by a fungal strain *Aspergillus flavus*. Nutritional requirements of *Aspergillus oryzae* JMU316 for production of naringinase has been reported earlier[20]. Authors optimized the culture media for higher production of naringinase. Naringin was reportedly found to be the most efficient/ effective carbon source followed by pomelo pericarp. Among nitrogen sources, peptone found to be most suitable component. Along with these major components, the media was supplemented with minerals including $CaCl_2$, NaCl, $MgSO_4 \cdot 7H_2O$, and K_2HPO_4. Naringinase production from *Aspergillus niger* has been reported in a systematic manner[26]. Recently, Sindhe and Lingappa[27] isolated various fungal and bacterial strains for production of naringinase. Higher production of naringinase (559 U/ml) was shown by isolate KLA-80 which was later identified as *Aspergillus flavus*. The naringinase enzyme production from *Aspergillus niger* (CECT 2088 and ATCC 16888TM) was also reported[9; 28]. Further, the immobilized fungal naringinase was investigated for debittering of juices[9]. Naringinase production from an isolated strain

of *Aspergillus flavus* has also been investigated by different group of researchers[29]. Isolation of naringinase producing *Bacillus cereus* from soil has been reported[30]. Authors optimized the various parameters for higher production of naringinase including pH, incubation time, temperature, and carbon-nitrogen source. Similarly, naringinase production from procured culture (MTCC, India) *Bacillus amyloliquifaciens* using liquid nutrient media containing naringin (1%) has been reported[31]. Authors investigated naringinase enzyme production and purification where tofu wastewater was used as cost-effective substrate. Researchers have also investigated fermentative naringinase production from *Aspergillus niger* and immobilized the enzyme on grafted gel beads[32]. Interestingly, the immobilization resulted in improved stability (thermo stability) from 50º-70ºC. Similarly, Bodakowska-Boczniewicz and Garncarek[33] reported naringinase production from *Penicillium decumbens* and immobilized on chitosan microspheres activated with glutaraldehyde. Authors reported optimal pH of enzyme as 4.0 for both free as well as immobilized. Further, the higher K_m value was recorded for immobilized enzyme.

Production of naringinase from *Aspergillus tubingensis* UA13 has been reported[34]. Authors optimized the production of enzyme at shake flask level and further at bioreactor level. Among naringin and pomelo peel powder, naringin was found to support higher naringinase production as compared to pomelo peel powder. It was also found that additional carbon sources did not allow further increase in naringinase level. Among various nitrogen sources, yeast extract was selected for further production studies. Authors also studied the effect of inoculums age, inoculums volume, and pH[34]. Further, optimization of process parameters were carried at 5 L stirred tank bioreactor.

In a study, *Rhizopus stolonifer* was used for production of naringinase by solid substrate fermentation (SSF)[35]. Paddy husk was used as the base substrate for production of enzyme by SSF. Higher activity of the enzyme was recorded on the 7th day of fermentation. Further, it was observed that naringin at concentration of 0.75 % (w/v) lead to higher production of naringinase. Optimization of the media for naringinase production using *Aspergillus brasiliensis* MTCC 1344 has also been investigated[36]. Cassava waste (CW) was used in form of solid base as well as carbon source for performing the solid substrate fermentation. For solid substrate fermentation, the mineral solution having $NaNO_3$, K_2HPO_4, $MgSO_4$, KCl, $FeSO_4$, and naringin was used to moisten the substrate. The pH was maintained at pH 5.0. Authors optimized the levels of peptone, calcium chloride and maltose. The RSM (Response surface methodology) and PBD (Plackett–Burman design) was used for statistical optimization for naringinase production using *Aspergillus brasiliensis* (MTCC 1344)[36]. In a similar study, extracellular naringinase enzyme production from *Aspergillus niger* (VB07) has been reported[37]. Further, the microbial strain was isolated from the soil collected from citrus fruits market[37]. Higher naringinase yield (17.28 IU/mL) was obtained in a media having optimized levels of naringin, rhamnose, peptone, glycine, and pH 4.5. Isolation of naringinase producing *Micrococcus* sp. from soil samples has been reported[38]. The media used for screening of bacterial cultures included NH_4NO_3, KCl, KH_2PO_4, $FeSO_4.7H_2O$, $ZnSO_4$, $MnSO_4$, $MgSO_4.7H_2O$ and naringin as inducer. Authors also optimized the process parameters for higher naringinase production from *Micrococcus* sp. Similarly, researchers recorded optimization of naringinase production using *Aspergillus niger* MTCC 1344[39]. Among the various carbon sources studied, rhamnose and molasses were found to be effective and lead to higher naringinase production as compared to other tested carbon sources. Peptone was recorded an effective source of nitrogen for naringinase production. Further, authors studied the effect of inoculum age, inoculums size, metal ions, pH and temperature as well. Naringinase production have also been recorded from *Aspergillus niger* (ATCC1015)[40]. Authors studied effect of various carbon sources (naringin, rhamnose, molasses) as well as calcium and magnesium salts on naringinase production using *Aspergillus niger* ATCC1015. Molasses supported microbial growth but did not support the production of naringinase efficiently. Naringinase lead to higher production (8-fold higher) of naringinase along with calcium and magnesium salts. In a similar study, the immobilized naringinase from *Apergillus* sp. isolate Mk156394 was studied for effect on citrus juice[41]. Authors investigated the naringinase:

tannase ratio and incubation temperature as variables while the level of naringin, tannin, total phenolic content and vitamin C were taken as response. The naringinase from *Aspergillus niger* FFCC uv-11 has also been produced and characterized[42]. Authors compared the immobilized and free naringinase for naringin hydrolysis. It was recorded that the naringinase in immobilized form was able to perform over a wide range of pH and also exhibited good thermal stability. *Penicillium decumbens* PTCC 5248 has been reported for naringinase production[43]. Naringin was used as carbon source in the media and able to support naringinase production. Also, rhamnose was found to induce naringinase production. Naringinase production from another strain of *Aspergillus niger* (426) has also been studied[44]. Response surface methodology (RSM) was used for optimal naringinase enzyme production from *Aspergillus niger strain* 426 using SSF (solid substrate fermentation). Three substrates namely, wheat bran, rice bran, and grapefruit peel were assessed for their potential to be used as substrate. The substrates were supplemented with mixture having hesperidin, rutin, and naringin. Carbon source, nitrogen source and inducer play significant role in microbial naringinase production. Patil and Dhake[45] reported production naringinase from *Penicillium purpurogenum* in medium based on corn steep liquor, yeast extract, KH_2PO_4, naringin and $CaCO_3$. Further, authors assessed the partially purified enzyme for debittering of citrus juices. Ribeiro[8] have provided an important review on occurrence of naringinase along with characteristics and its applications. Currently, bacterial naringinase is also in focus of researchers for production aspect, characterization and applications in de-bittering of citrus juices. A yeast culture *Clavispora lusitaniae* has also been shown to produce naringinase[46]. The activity of crude enzyme obtained was 0.0135 (IU/ml). The optimal parameters for naringinase enzyme production were naringin (0.8%), rhamnose (0.6%) with pH of 4. Also, *Candida tropicalis* has been recorded for naringinase production[47]. The microbe was cultured in the medium based on malt extract, yeast extract,

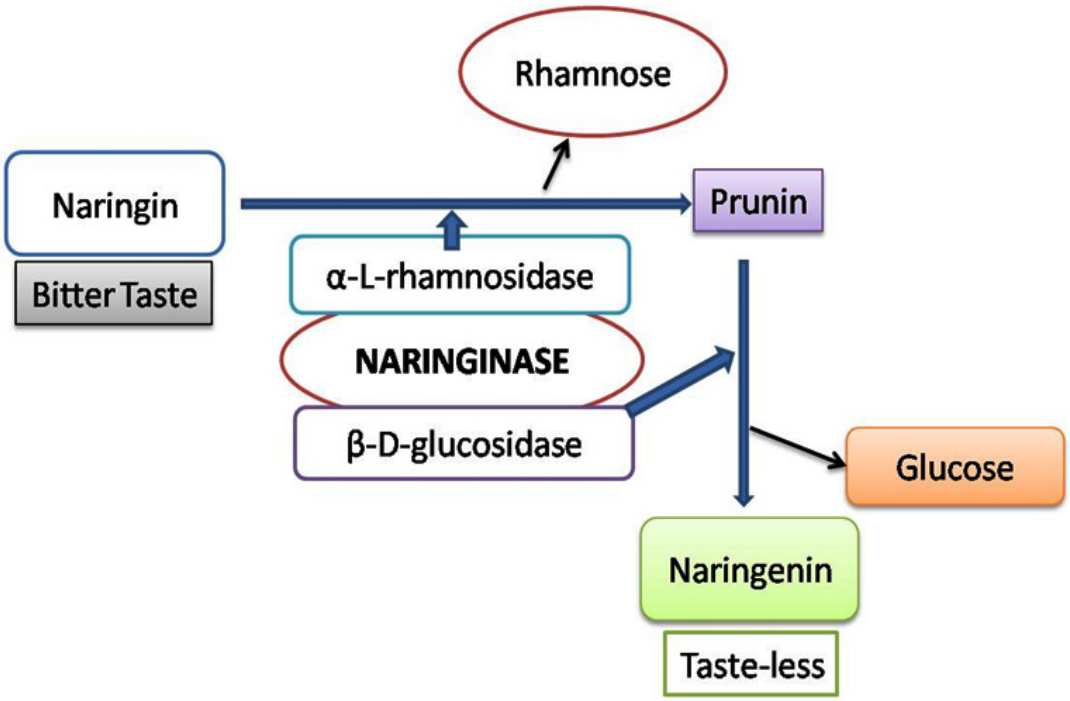

Fig. 1. Naringinase mediated hydrolysis of naringin involves two steps. In first, naringin is hydrolysed to prunin with release of rhamnose by action of á-L rhamnosidase activity while in second step, â-D-glucosidase hydrolyse the prunin into taste-less (therefore, non-bitter) naringenin with release of glucose

peptone, and glucose with initial pH 6.4-6.8. The growth was carried out at flask level with incubation temperature of 28°C for 5 days. Recently, *Bacillus subtilis* strain BSnari, an isolate from the Red Sea has been reported for naringinase production with activity of 7.09 U/ml[48]. The media was majorly included sucrose (1.5%), citrus peel powder (0.6%) and soybean meal (1%) with pH of 7. The growth was carried at 37ºC. Recently, researchers have isolated 08 microbial strains from 33 yuzu-based fermented foods that shows significant activity of naringinase enzyme[49]. *Aspergillus oryzae* NYO-2 was used for production of prunin and naringenin due to its higher naringinase activity. Authors evaluated the naringinase for reducing the bitterness and further studied the effects of naringenin and prunin in neuroprotection. The results obtained were very promising and suggested the significant applicability of deglycosylated naringin for the purpose of improved sensory and functional properties. Naringinase production from an isolated strain *Bacillus methylotrophicus* has been reported[50]. In this study, authors optimized the naringinase production and found increase in enzyme production (7.46 U/L) by using sucrose-yeast extract as compared to the basic medium. Statistical optimization has been used further for higher production of enzyme and a level of higher production of naringinase (12.05 U/L) was obtained. Naringinase production from *Bacillus methylotrophicus* has also been reported and authors have discussed the role of oxygen transfer rate and reported the greatest oxygen transfer rate (0.035 mMol/L/s) in a 5 L bioreactor while 0.04 mMol/L/s in another bioreactor having capacity of 20 L[51]. Further, the cells were able to produce maximum production of naringinase in terms of naringinase activity of 751 U/L in 5L bioreactor capacity and 778 U/L in 20 L bioreactor capacity, respectively at 34 hours. Bodakowska-Boczniewicz and Garncarek[52] synthesized naringinase from *Aspergillus niger* using naringin, along with powdered albedo, red grapefruit and flavedo segment membranes as stimulators. The carbon sources used was rhamnose while yeast extract and sodium nitrate were used as nitrogen sources. Optimla production was obtained with $NaNO_3$, yeast extract, KH_2PO_4, red grapefruit albedo, naringin, and rhamnose. VinothKumar[53] compared naringinase production form *Aspergillus niger* MTCC 1344 through different inducers including naringin, rhamnose, naringenin, rutin, hesperidin. Higher production of naringinase was obtained in response to naringin as compared to other tested inducers. Authors also optimized the level of inducers for enhanced production of naringinase.

Application of naringinase in debittering of citrus juices

Naringin is an imperative natural flavonoid generally present in citrus fruits. The naringin content may lead to bitter taste of citrus juices detectable by consumers. Though, naringin is well known to provide various health benefits and it has immense therapeutic potential[54,55,56,57]. The bitterness in citrus juices adversely affects its acceptability among consumers[1] and therefore,

Table 1. Bacterial sources majorly studied for naringinase production

Bacterial Source	Reference(s)
Bacillus subtilis BSnari	[48]
Serratia sp.	[6]
Bacillus methylotrophicus	[50]
Streptococcus pasteurianus subsp. AUH-JLD109	[69]
Micrococcus sp.	[38]
Clostridium stercorarium	[70, 10]
Bacillus amyloliquefaciens 11568	[23]
Bacillus cereus	[30]
Staphylococcus xylosus MAK2	[12, 21]]
Bacillus methylotrophicus	[51]
Paenibacillus stellifer RAMCM-44	[71]
Pseudomonas resinovorans RMCSJ-18	[72]

bitterness is considered undesirable in citrus juices. The content of naringin can be reduced by chemical and suitable non-enzymatic processing methods. Alternatively, naringin can be hydrolysed in citrus juice by the action of microbial enzyme, naringinase. Therefore, bitterness in specific juices can be decreased significantly by using enzyme naringinase. This enzyme hydrolyzes the naringin into comparative non-bitter compound naringenin[1]. The naringenin is a tasteless non-bitter compound[7,58,47]. Naringinase production form various microorganisms have been reported but, still the pace of commercial production of this enzyme is lacking[7,24]. Fungal naringinase has been shown to eliminate the bitter flavor in citrus fruit juice contributed by naringin[59,39]. Among the initial studies, immobilized naringinase was used for the purpose of debittering of grapefruit juice[60]. Free and immobilized form of naringinase for naringin hydrolysis has also been studied[32]. Both free enzyme as well as immobilized enzyme leads to 100% hydrolysis of naringin at 60 and 90 min, respectively. In a study on immobilization of naringinase, Bodakowska-Boczniewicz and Garncarek[33] immobilized the enzyme from *Penicillium decumbens* on glutaraldehyde activated chitosan microspheres. Authors recorded shift in optimal temperature of immobilized naringinase as compared to free enzyme. The enzyme preparation was found to retain about 88.1% of its original activity even after 10 runs of naringin hydrolysis. Similarly, immobilized naringinase from *Aspergillus niger* CECT 2088 has been evaluated for debittering of juices[9]. The enzyme was immobilized into a poly(vinyl alcohol) hydrogel based polymeric matrix that was cryostructured in liquid N_2 to obtain beads that are bio-catalytically active. The immobilized enzyme was assessed for hydrolysis of naringin and it was observed that entrapped or immobilized naringinase may be used again through 6 cycles that are runs of 24 hours at 20 °C and was able to retain 36% efficiency for the naringin hydrolysis in simulated juice.

De-bittering of the grapefruit juice by both physical adsorption with exchange resin and by naringinase enzyme has been investigated by researchers[61]. Authors evaluated the antioxidant potential along with other parameters and compared for both juices. The juice processed with naringinase and exchange resin was found to have significantly reduced content of naringin and decrease in bitterness. Authors further observed that naringinase treated grapefruit juice showed considerably high antioxidant potential, greater ability to scavenge free radicals, more efficient protection of lipid per-oxidation and GSH oxidation as compared to the exchange resin treated

Table 2. Fungal sources used for production of naringinase

Fungal Source	Reference (s)
Aspergillus niger	[58]
Aspergillus niger MTCC 1344	[39]
Aspergillus foetidus	[24]
Aspergillus niger HPD-2	[24]
Aspergillus oryzae SS	[24]
Penicillium caseicolum	[24]
Penicillium chrysogenum NRRL	[24]
Penicillium glaucum	[24]
Penicillium roqueforti I	[24]
Penicillium roqueforti II	[24]
Penicillium roqueforti CNRZ	[24]
Aspergillus aculeatus JMUdb 058	[73, 74]
Aspergillus flavus	[29]
Aspergillus brasiliensis MTCC 1344	[36]
Aspergillus niger	[32]
Aspergillus niger van Tieghem MTCC 2425	[22]
Aspergillus niger strain 426	[44]
Penicillium decumbens PTCC 5248	[43]
Penicillium purpurogenum	[45]
Aspergillus aculeatus	[25]
Aspergillus niger ATCC1015	[40]
Candida tropicalis	[47]
Cryptococcus sp. Jmudeb 008	[75]
Penicillium decumbens	[33]
Aspergillus niger CECT 2088	[9]
*Aspergillus oryzae*JMU316	[20]
Aspergillus niger	[76]
Rhizopus stolonifer	[35]
Aspergillus niger VB07	[37]
Aspergillus niger MTCC 1344	[53]
Apergillus sp. Mk156394	[41]
Aspergillus niger FFCC uv-11	[42]
Aspergillus niger	[26]
Clavispora lusitaniae	[46]
Aspergillus oryzae NYO-2	[49]
Aspergillus flavus	[27]
Aspergillus niger	[18]
*Aspergillus tubingensis*UA13	[34]
Aspergillus niger KMS	[52]

juice. Authors stated that "enzymatic debittering with naringinase was better in comparison to the physical adsorption in order to preserve the antioxidant potential and biomolecule protection capacity of freshly squeezed juice of grapefruit". Microbial naringinase supports several advantages over chemical methods of juice treatment for reducing amount of naringin. The microbial naringinase can be used potentially to reduce the naringin by its hydrolysis in a more natural and biological way. Researchers have evaluated the naringinase for naringin hydrolysis in orange juice and found that naringinase enzyme at concentration of 1.0g/L lead to 86% reduction in naringin content at 50ºC and 4 hours (incubation period)[62]. In a study, researchers evaluated effect of pressure (high) on immobilized naringinase (sodium alginate beads) mediated naringin hydrolysis in grapefruit juice[63]. This study was carried out in both model solution and grapefruit juice. At pressure of 200 MPa (at 54°C), naringin reduction of 72% was obtained in model solution while at pressure of 160 MPa (at 37ºC), 75% debittering of grapefruit juice was achieved. Researchers have also investigated the naringin hydrolysis property of immobilized (silica material with varied pore size) naringinase produced from *Aspergillus niger* (FFCC uv-11)[42]. Activity of the immobilized enzyme was improved as compared to initial activity of free enzyme. Further, the immobilized enzyme (naringinase) also retained 61.81% of the residual enzyme activity recorded after 08 repeated cycles and showed significantly higher storage life at specified conditions. The purified naringinase from *Aspergillus niger* significantly reduced the naringin and therefore, bitterness in citrus juice at concentration of 0.220 U/ml. It has been reported that resin adsorption resulted in decreased amount of volatile compounds in pummelo juice while naringinase treated juice revealed higher volatile compounds particularly, aldehydes[26]. Further, it was observed that fresh juice and naringinase enzyme treated juices were alike in relation to aroma profiles, while the resin-adsorbed juice was found to have weak intensity of aroma. Application of naringinase for hydrolysis of naringin have also been recorded by Patil and Dhake[45]. The bitterness of citrus juice was reduced by use of partially purified naringinase from another microbe as *Penicillium purpurogenum*. Extreme reduction in naringin content as 74% was found at 1.0g/L concentration of naringinase, and incubation was at 40°C (4 hours). In a study, researchers evaluated both forms of á-L-rhamnosidase (free and immobilized) for hydrolysis of naringin[11,12]. Authors recorded 76% and 67% naringin hydrolysis in Kinnow juice for both form of used enzymes respectively. Ribeiro and Rabaca[63] reported preparation of naringinase-cross linked enzyme aggregates (CLEAs) and these enzyme aggregates were found efficient in naringin hydrolysis. A yeast strain, *Clavispora lusitaniae* has been investigated for reducing the naringin content in citrus juice (Kinnow; *Citrus raticulata* blanco) and development of low alcoholic beverage[46]. The enzyme naringinase produced from *Aspergillus oryzae* NYO-2 has been investigated for naringenin and prunin production[49]. The naringinase was able to convert 19 mM of naringin in to 14.06 mM of prunin and 1.97 mM of naringenin under specific optimal conditions. The bitterness of both naringenin and prunin was also reduced significantly. Further, the role of naringenin and prunin in neuroprotection were also found to be higher as compared to naringin. The purified naringinase from a new isolated strain, i.e. *Bacillus amyloliquefaciens* (11568) was found to hydrolyze the naringin[23]. It was found that naringinase concentration of 4U/mL was enough to eliminate the naringin in citrus juice. Recently, some authors have reviewed the enzyme immobilization and advances in the area for debittering the citrus juices[65]. Microbes are well known for production of various industrial enzymes and metabolites[66,67,68]. Various microbial enzymes and metabolites have been used at commercial level and research works are in continuation. Naringinase is an important enzyme from citrus juice processing perspectives. Various research works suggests that enzymatic treatment of citrus juices for their debittering should be preferred over chemical processing. Various authors and researchers have worked on production and use of free or immobilized enzyme for debittering of citrus juices. The pace of research on naringinase and its utilization for debittering of citrus juices need to be accelerated. This enzyme still seems to be explored more strongly for establishing the process for debittering of citrus juice at industrial scale.

CONCLUSIONS AND FUTURE PERSPECTIVES

Naringinase is an important enzyme with immense industrial potential. It has ability to hydrolyse the naringin. Naringin is the major compound responsible for the bitterness in extracted citrus juices. Naringinase mediated removal of naringin is very potent approach for debittering of citrus juices while maintaining beneficial properties of juices. Naringinase can be produced from various microbial sources including bacteria as well as fungi. The enzymatic (naringinase) treatment of citrus juices suggests several advantages over chemical processing or adsorption-based removal of bitterness. Ongoing research continues to explore the potential applications of naringinase in various other fields also, including medicine, biotechnology, and food sciences. Though, naringinase has been produced from various microbes, characterized and assessed for debittering of juices, but still lacks the required pace in terms of microbial sources, and production aspects. This enzyme seems having immense potential for the citrus juice processing industry and also for those requires transformation of flavonoid naringin for generation of by-products. Extensive research is required on this enzyme as limited literature is available for microbial production and applications for debittering of citrus juices. Its versatile applications make it a valuable tool in the field of biotechnology and contribute to the development of sustainable and efficient processes.

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This study did not involve human participants, and therefore, informed consent was not required.

Authors contributions

Rachna Nara : prepared the draft; Nirmala Sehrawat : prepared the figure and tables; Sunil Kumar, Amit Kumar and Sushil Kumar Upadhyay: reviewed the MS and Mukesh Yadav finalized the MS.

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