Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Review
Volume 13, Issue 4, 2024, 186
https://doi.org/10.33263/LIANBS134.186

Immobilized Naringinase as a Suitable Biocatalyst and an Environment-Friendly Approach for De-bittering of Citrus Juices: Recent Developments and Future Perspectives

Mukesh Yadav [1,*], Nirmala Sehrawat [1], Rachna Nara [1], Anil Kumar Sharma [2,*], Sunil Kumar[3], Amit Kumar [4], Manoj Singh [1], Varruchi Sharma [5], Kuldeep Dhama [6]

1. Department of Bio-Sciences and Technology, M.M.E.C., Maharishi Markandeshwar (Deemed to be University), Mullana 133207, Ambala, Haryana, India
2. Department of Biotechnology, ASBS, Amity University Punjab, Mohali-140306, India
3. Department of Microbiology, Graphic Era University, Dehradun, Uttarakhand, UK, India
4. Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, U.P., India
5. Department of Biotechnology and Bioinformatics, Sri Guru Gobind Singh College Sector-26, Chandigarh (UT), Indi
6. Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, U.P., India

* Correspondence: mukeshyadav7@gmail.com (M.Y.); anibiotech18@gmail.com (A.K.S.);

Scopus Author ID 57714117800

Received: 16.07.2023; Accepted: 12.04.2024; Published: 28.08.2024

Abstract: Naringin is one of the important flavonoids that occurs naturally in citrus fruits and contributes bitterness to citrus fruit juices. Chemical methods can reduce the bitterness in citrus juices, but these methods have several drawbacks. Biologically, the naringin-mediated bitterness can be reduced by naringinase-mediated hydrolysis of naringin. The naringinase (E.C.3.2.1.40) is an enzyme complex that hydrolyses naringin and produces the tasteless product naringenin. The naringinase-mediated treatment of citrus juices is promising for the juice industry in dealing with the natural bitterness of citrus juices. The enzymatic treatment of citrus juices is an environment-friendly approach that fits with sustainable food processing. Enzyme immobilization increases the reusability of enzymes along with improved catalytic efficiency, stability, and reusability. Various immobilization supports have been investigated for naringinase immobilization. Results have supported the promising role of immobilized naringinase in the food industry for de-bittering citrus juice without affecting its health benefits. Also, the end product (naringenin) produced due to naringin hydrolysis has various health benefits. This review summarizes recent trends in the immobilization of naringinase for effective hydrolysis of naringin in citrus juices, which leads to de-bittering of citrus juice, including advanced nanomaterials. This first review article focuses on immobilized naringinase as a biocatalyst for potent industrial applications in de-bittering citrus juice. The advantages of naringinase over chemical methods and the health benefits of the end product have also been discussed.

Keywords: microbial naringinase; naringin; immobilization; citrus juice; bitterness; food industry; sustainable approach; environment friendly.

1. Introduction

A significant number of enzymes are routinely applied in various industrial processes for different product development. The enzymes are useful at the industrial level due to their higher specificity and catalytic efficiency. Further, enzymes can be produced at a faster rate

using suitable microorganisms [1]. Various enzymes have been found useful for various applications in different industries [2,3]. Food enzymes are now well-known for their applications and usability in the industrial sector [4]. These enzymes also have a greater contribution to the enzyme market [5,6]. Microbial naringinase is an important enzyme with significant potential that can be utilized in food industries on a large scale [7-9].

The fruits of the citrus family naturally constitute a well-known flavonoid, Naringin. It is abundantly present in grapefruits [10]. Naringin contributes a bitter taste to citrus fruits and juices. It is also recognized as a fundamental component for causing bitterness in the juices of citrus fruit [11]. This bitterness is undesirable and, therefore, exists as a major concern for the related food industries [12]. Accordingly, the level of naringin should be reduced or removed from the processed products [13]. The naringin-mediated bitterness can be reduced by decreasing the naringin content using chemical methods. Still, the chemical method has several drawbacks and results in inferior quality of the extracted fruit juice [14]. The methods for reducing naringin levels may include adsorptive debittering [15] and β-cyclodextrin treatment [16]. Naringinase enzyme has the potential to hydrolyze the bitter naringin into a tasteless product naringenin and, therefore, has the ability to lessen the citrus juice's bitterness [17,18]. The microbial naringinase has the potential to replace the chemical methods to reduce the bitterness. This is because the production of microbial naringinase is a comparatively efficient, viable, and less costly process. Figure 1 has been shown to demonstrate the proof of concept of the present article.

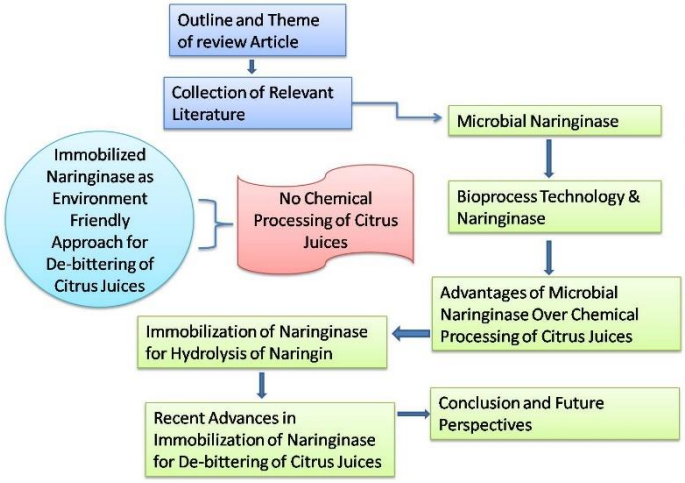

Figure 1. General representation showing the proof of concept of the present article.

As we know, naringinase is an enzyme complex that consists of two different catalytic activities, i.e., α-L-rhamnosidase (E.C. 3.2.1.40) activity and also β-D-glucosidase (E.C. 3.2.1.21) activity that works in a sequential manner (Figure 2). This enzyme complex catalyzes naringin hydrolysis, which results in the production of rhamnose, glucose, naringenin, and prunin. Hydrolysis of naringin into naringenin due to naringinase enzyme occurs in two steps: i) [19] hydrolysis of naringin (rhamnoglucoside) leading to production of rhamnose and prunin (glucoside) due to α-L-rhamnosidase activity of naringinase and ii) [20] hydrolysis of prunin into glucose and naringenin due to β-D-glucosidase activity of naringinase [21]. Of these two activities of naringinase, the rhamnosidase activity is considered necessary to eliminate bitterness [22]. Naringinase has the potential to be used at the industrial level to de-bitter citrus juices. Several studies have been carried out on microbial naringinase for hydrolysis of naringin in citrus juices, and results have been found promising [23]. Also, advancements in the immobilization of naringin have increased the potential of this otherwise neglected industrial

enzyme. This review presents overview of naringinase and recent advancements in process development for immobilization of naringinase for hydrolysis of naringin and de-bittering of citrus juices. Microbial naringinase may be an enzyme of interest for food industries in the near future.

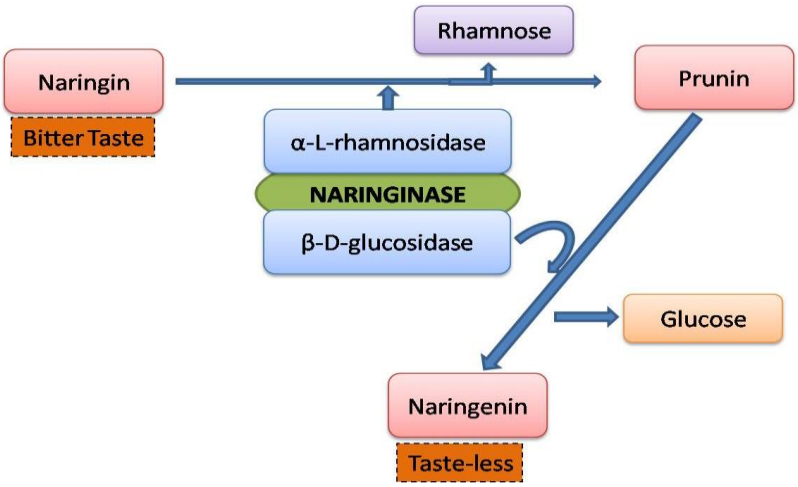

Figure 2. Schematic presentation showing naringinase-mediated hydrolysis of naringin. Naringin contributes bitterness to the citrus juices. Naringinase-mediated hydrolysis of naringin is a two-step process. At first, the α-L-rhamnosidase activity of naringinase hydrolyzes the naringin and results in the production of rhamnose and prunin. In the second step, the prunin is hydrolyzed by the β-D-glucosidase activity of naringinase and results in the production of glucose and naringenin. Naringenin is a tasteless compound; therefore, debittering of citrus juice occurs due to the hydrolysis of naringin into naringenin.

2. Bioprocess Technology and Naringinase.

Advancements and continuous cumulative developments in the area of bioprocess technology have significantly improved the yields of microbial enzymes via fermentation [24]. Besides this, the specificity of the enzyme can be regulated or altered, and its stability can be improved [25]. All these aspects favor the use of enzymes at the industrial level, particularly in the food and pharmaceutical industries [26]. Enzymes of microbial origin have more advantages in comparison to animal and or plant sources [27]. In general, the promising advantages of microbial enzymes include the production at lower costs, use of raw substrates, use of agricultural waste in the form of substrates, easy production in industrial fermenters at large scale, comparatively more probability for genetic modifications, and also the rapid growth of microbes along with choice of microbes. These significant properties of microbial enzymes make them highly suitable biocatalysts for broad applications in industries [28]. In some commercial preparations especially in food industries, chemicals or non-enzymatic based processing of food or food components are associated with some disadvantages or drawbacks. Higher amounts of chemicals, as well as higher energy consumption along with undesired by-products, also impose pessimistic effects on the environment. In specific cases, there is the possibility that these limitations or adverse effects can be abolished to a significant extent by using biological catalysts, i.e., microbial enzymes. Naringinase enzyme complexes have two important catalytic activities: 1) α-L-rhamnosidase as well as 2) β-D-glucosidase activity [29,30]. Due to these activities, this enzyme complex (naringinase) can efficiently hydrolyze the naringin. Naringin is known to contribute bitterness to grapefruit and other citrus fruits. However, the naringin concentration in commercial citrus fruit juices is relatively low and also depends on different factors, but it is able to produce measurable bitterness in the juice as tasted by the consumers [17]. Though naringin has been reported for its various health benefits along

with therapeutic potential, the bitterness in citrus juices is still considered undesirable. This bitterness is an important challenge for the citrus juice industry. The level of naringin can be decreased by some methods, including β-cyclodextrin treatment, adsorptive debittering, and other non-enzymatic methods [15]. The alternate way of reducing naringin in citrus juice is to hydrolyze the naringin by naringinase enzyme. This enzyme-based treatment is considered an efficient method among promising techniques because it improves the organoleptic properties and helps retain citrus juice's health-promoting properties [15].

Naringinase seems promising, but the required pace in bioprocess technology has not been attained. The enzyme needs to be explored more extensively for its possible commercialization in the citrus juice industry. The enzyme has been neglected compared to other industrial enzymes of microbial origin. Recently, advancement has attained some pace on this enzyme, and more efficient immobilization strategies have been developed with naringinase for application in the de-bittering of citrus juices. Enzyme immobilization has obvious advantages that can improve the functionality and efficiency of enzymes for industrial purposes. The recent findings on naringinase immobilization have been presented here to update workers in the area.

3. Advantages of Microbial Naringinase over Chemical Processing of Citrus Juices

Naringin contributes bitterness to citrus fruits and juices. The naringin content in citrus juice may impart a detectable bitter taste to consumers [31]. Regarding the functional food, biological properties, and therapeutic potential, naringin seems to be an important constituent of citrus juices [32]. However, still, the bitterness in citrus juices is considered undesirable because bitter taste reduces the acceptability of citrus juice among consumers [33]. This bitterness is still an important challenge for the citrus juice industry [34]. The concentration level of naringin can be lowered or decreased by chemical and non-enzymatic processing methods. The alternative way to reduce naringin in citrus juice is to hydrolyze the naringin with the enzyme naringinase [35]. The enzymatic method based on naringinase is a promising tool for treating citrus juice without affecting its health-promoting properties. Cavia-Saiz *et al*. [36] de-bittered the grapefruit juice by physical adsorption with exchange resin and by naringinase enzyme. Then, they compared the antioxidant potential of both juices [36]. After processing it with the enzyme naringinase and exchanging the resin, the authors assessed the de-bittering effects in pasteurized fresh grapefruit juice. As per an earlier report, the amount of residual naringin correlated with bitterness reduction [37]. The juice processed with naringinase and exchange resin was found to have significantly reduced naringin content and a decrease in bitterness. The authors further found that naringinase-treated grapefruit juice showed considerably higher antioxidant potential, greater ability to scavenge free radicals, and more efficient protection of lipid peroxidation and GSH oxidation than the juice treated with exchange resin. The authors stated that “enzymatic debittering with naringinase was more effective than physical adsorption in order to preserve the antioxidant and biomolecule protection capacity of freshly squeezed grapefruit juice” [38]. Microbial naringinase supports several advantages over chemical methods of juice treatment for reducing the amount of naringin [39; 40]. The microbial naringinase can potentially be used to reduce the naringin by hydrolysis in a more natural and biological way. Chemical processing and additives may have limitations on their safe usage. Further, chemical processing methods are not environmental friendly [41]. Enzymatic processing is more safe, natural and environmental friendly. Suitable microbial source of naringinase is the requirement of food industry along with high naringinase

stability and catalytic efficiency [42]. Therefore, this is the need of current time to speed up the research on new microbial sources of naringinase and process developments for de-bittering of citrus juices. Microbes have the potential to produce enzyme at faster rate with high catalytic efficiency and stability [43] .

4. Naringinase Immobilization Methods and Matrices for Naringin Hydrolysis

Naringinase hydrolyzes the flavonoid naringin into naringenin, typically in two catalytic steps. Two different units of naringinase mediate the hydrolysis: i) α-L-rhamnosidase and also ii) β-D-glucosidase. Complete naringin hydrolysis requires both units to be functionally formed. The industrial application of naringinase can be strengthened by its immobilization on the suitable matrix for naringin hydrolysis. The immobilization increases the usage of enzymes, provides stability, and positively affects the catalytic efficiency along with other benefits. Various researchers have reported immobilization of naringinase using different methods and solid supports [44]. Several bio-systems have successively used the immobilized naringinase for de-bittering citrus fruit juices and producing valuable biochemical compounds. Ribeiro has reviewed the immobilization of naringinase. In another study, the naringinase (from *A. niger*) was immobilized in a hollow fiber reactor for hydrolysis of the naringin flavonoid present in grapefruit juice [45]. Naringinase immobilization was investigated on controlled pore glass by Roitner *et al*. [46]. Jimeno *et al*. [47] used naringin as a substrate and reported immobilization of naringinase by diazo coupling with arylamine-derivatized glass. Immobilization of naringinase (from *Penicillium* sp.) on chitin has been reported, followed by successive use for hydrolyzing the p-nitrophenyl-α-rhamnoside or naringin in grapefruit juice in a tubular bioreactor [48]. Tsen *et al*. [49] also immobilized the enzyme naringinase isolated from fungus (*Penicillium* sp.) on the cellulose triacetate support by following the fiber entrapment technique. Immobilization of naringinase using 2% sodium alginate as an appropriate matrix was performed and evaluated by Puri *et al*. [50]. The immobilized naringinase resulted in 82% naringin hydrolysis under specific experimental conditions with fixed units of the enzyme. Similarly, naringinase immobilization in calcium alginate beads has been reported to effectively treat grapefruit juice (83.84% of naringin hydrolysis) under specified conditions [51]. Pedro *et al*. [52] have also recorded the naringinase immobilization using calcium alginate and examined the immobilized enzyme for naringin hydrolysis. In 2008, naringinase immobilization was reported in k-carrageenan beads and k-carrageenan with calcium alginate beads [53]. The authors reported significant loading efficiency of naringinase when immobilization was done in k-carrageenan beads (90% loading efficiency) and k-carrageenan with calcium alginate (80% loading efficiency). Along with the above-mentioned immobilization reports, naringinase has also been reported for its immobilization on glutaraldehyde-coated woodchips [54], Celite by simple adsorption [55], and on a poly (vinyl alcohol) hydrogel-based polymeric matrix, cryo-structured in liquid N_2, and thus obtaining bio-catalytically active beads [56]. The choice of matrix, its concentration, system pH, and enzyme load are among the important factors to be considered during immobilization. In a study, the kinetics of conversion of emulsified di-rhamnolipid into monorhamnolipid and L-rhamnose was studied by Magario *et al*. [57] by using immobilized naringinase isolated from *Penicillium decumbens*. Authors investigated the non-porous micro-magnetic beads for immobilization of naringinase. Besides above mentioned reports, different sol-gel precursors have also been investigated for naringinase immobilization during different times of aging. These gel precursors included were tetramethoxysilane (TMOS), methyl-trimethoxysilane, 3-

aminopropy-ltrimethoxysilane and diglycerylsilane (DGS) [58]. The immobilization matrices and methodologies have proved effective in developing immobilized catalyst based on naringinase. With the advancements in immobilization strategies, various new matrices combinations, immobilization supports, methods and processes have been developed for enzyme immobilization and development of efficient catalyst/ system. Though free naringinase can be used for hydrolysis of naringin in citrus juices, the free enzyme is difficult to recover from citrus juice after treatment. Therefore, immobilized enzymes may be a cost-effective option and have high reusability, efficiency, and stability in terms of industrial purposes.

5. Recent Trends in Immobilization of Naringinase for Naringin Hydrolysis and De-bittering of Citrus Juices

Along with the traditional approaches and suggested improvements, the cross-linked enzyme aggregates (CLEAs) have been investigated as novel biocatalysts for naringin hydrolysis [59]. Several advantages of these enzyme aggregates (CLEAs) have been identified, "as it is simple and amenable to rapid optimization, leading to low costs and short time-to-market processes". The self-immobilization technique of CLEAs provides high volumetric and specific activities. The immobilization of enzymes using CLEAs (cross-linked enzyme aggregates) includes the enzyme precipitation using a suitable salt by salting out method and the addition of suitable non-ionic polymers or a mixture of solvents. Polyethylene glycol (PEG 6000, PEG 8000), ammonium sulfate, or ter-butyl alcohol are among the most commonly used precipitating agents [60]. The CLEAs of naringinase enzyme isolated from *Penicillium decumbens* have been reported [61]. The naringinase-CLEAs having elevated activity and improvement were produced by precipitation with tert-butyl alcohol and cross-linking with glutaraldehyde (at a pH of 4) in a suitable temperature range (7°C - 10°C). The naringinase-CLEAs were investigated and found greatly effective for the hydrolysis of naringin. Further, the authors investigated the naringinase-cross-linked enzyme aggregates complex stability by analyzing six successive reutilizations. The combined effects of naringin concentration and temperature were also assessed on naringinase activity to produce reducing sugars by utilizing response surface methodology (RSM). The naringinase-CLEAs were found to be extremely effective for the hydrolysis of naringin and also seemed promising for industrial purposes.

Anfeng *et al*. [62] have reported immobilization of naringinase using magnetic nanoparticles (Fe_3O_4) that were prepared using the chemical co-precipitation technique. The findings of this study demonstrated that the immobilized Fe_3O_4 nanoparticles exhibited nearly 15 nm size and reduced value for saturation magnetization. The recovery in enzymatic activity increased by nearly 31.9% when the enzyme (0.35 mg/mL) was mixed with glutaraldehyde (3%) at pH 5 and 28°C temperature for 4h. However, the optimum temperature and pH of enzyme naringinase remained unaffected due to immobilization. The authors also found that naringinase-Fe_3O_4 nanoparticles showed greater pH stability but a decline in temperature stability due to immobilization compared to free nanoparticles. Even after the reutilization of eight cycles, the residual activity of the enzyme was retained around 83%. With these findings, it was suggested that immobilization of naringinase also preserves its original activity (>78%) even after storage for 60 days at 4°C.

The immobilization of enzymes can also be done using polyvinyl alcohol (PVA), a synthetic polymer [63]. PVA-alginate beads were reportedly stable regarding thermal, mechanical, and chemical properties. Naringinase isolated from *Penicillium decumbens* was also immobilized and supported over 10 % polyvinyl alcohol-alginate beads at appropriate

temperature (70 °C) and pH 4. The highest activity yield for naringinase recorded was 80% in PVA (10%) -alginate (1%) beads under optimal conditions. The immobilized catalyst was reported to retain 90% of the initial activity even after six weeks of storage in acetate buffer at pH 4 and temperature of 4°C. The results were highly promising and significantly explored the potential of the immobilization strategy used in this study.

In another study, recombinant His-tagged rhamnosidase gene, i.e., ramA, was isolated from *Clostridium stercorarium*, followed by cloning and expression [64]. The enzyme was purified using the affinity resin (Ni^{2+}-NTA) based column chromatography. The purified α-L-rhamnosidase was later immobilized in calcium-alginate beads. Optimum results were obtained with 3% alginate; maximum naringin hydrolysis (67%) was obtained at this concentration. The results indicated an optimal and efficient porosity for retaining rhamnosidase enzyme and maintaining substrate inflow along with reaction product outflow in an exact manner. The authors evaluated naringin hydrolysis in citrus juice using free as well as immobilized rhamnosidase. The effect of enzymatic catalysis on naringin hydrolysis was investigated at two different temperatures (50 and 60°C), where reaction incubation times ranged from 1 to 5 hours [64]. The author's findings revealed that the incubation period of 2 hours resulted in 76% and 67% naringin hydrolysis using free and immobilized enzymes (rhamnosidase), respectively.

Luo *et al*. [65] have suggested silica material (porous) as a better immobilization method for naringinase. The porous silica supports of various pore sizes have been studied as the carrier for naringinase immobilization [65]. Naringinase produced from *A. niger* FFCC uv-11 was immobilized in this study while silica porous materials of different pore diameters (7.7 nm, SBA-15; 80 nm, silica gel; 2 nm, MCM-41) were used. Depending on the higher naringinase activity, SBA-15 was a suitable, efficient carrier material for the immobilization of the naringinase enzyme. The SBA-15 was first modified with the help of 7 % glutaraldehyde at 25°C for 2 hours and then used for immobilizing naringinase. The effect of glutaraldehyde was assessed. An increase in glutaraldehyde concentration from 5% to 7% resulted in a gradual increase in naringinase activity, and at a concentration of 7% glutaraldehyde, the activity of naringinase reached its maximum value. The activity of naringinase after immobilization reached 467.62 U g^{-1} at pH 3.5 and a temperature of 40°C for four hours. The specific activity, activity recovery rate, and binding efficiency of naringinase after immobilization were found as 517.43 U per gram, 87.64%, and 63.66%, respectively, at optimum pH (4.5) and temperature (45°C) required to catalyze the reaction. The immobilized enzyme, naringinase, worked over a broad range of applications and also showed excellent thermal stability compared to free naringinase in terms of naringin hydrolysis. It has also been observed that the enzyme, after undergoing immobilization, retained significant residual activity (61.81%) of naringinase after 8 repeated cycles. The residual naringinase activity retention rate was 80.95% after one month of storage under suitable conditions. This study provided an important development in the naringinase immobilization area and will provide useful insights into naringinase-based applications in industries for fruit juice processing.

Immobilization of tannase and naringinase enzymes produced from *Aspergillus sp*. (mk156394 isolate) was reported by Kumar *et al*. [66]. Crude naringinase was immobilized in sodium alginate (3%). The authors also studied the effect of immobilization of these enzymes on the quality-related characteristics of citrus fruit (*Citrus limetta*) juice. The authors used a statistical approach for optimal juice treatment. Various factors were considered for the optimization of juice treatment using the Box-Benkhen design of Response Surface Methodology (RSM-BBD). The authors optimized the process-related parameters by exploring

the Design Expert 10.0.1 software. According to the RSM-BBD, a total of seventeen experiments were planned for investigation. The ratio of the concerned enzyme (naringinase: tannase) and incubation temperature were taken as the independent variables. The results suggested that the independent variables significantly affected the recorded responses (tannin, naringin, vitamin C, and total phenolic content; TPC). Authors recorded optimal values for these responses as 0.393 mg/ml, 225.367 µg/ml, 34.713 mg/100ml, and 1553.966 mg GAE/L, respectively [66].

The application of high pressure with immobilization has been shown to be effective in naringin hydrolysis and de-bittering citrus juice. Ferreira *et al*. [67] have suggested that an effective, simple, and cheap method for naringinase immobilization and high pressure may be used to reduce the bittering of citrus fruit juices. In this study, immobilization of naringinase was done in calcium alginate beads. The authors evaluated naringin hydrolysis in grapefruit juice and model solution (acetate buffer 0.02 M; pH 4.0). The authors obtained a 50% increase in reducing sugar concentration in the model solution at 37°C temperature and 160 MPa pressure compared to the reaction carried out at atmospheric pressure. The higher concentration of naringenin was attained at 54°C under high pressure (200 MPa), which corresponds to a reduction of naringin (72 %) in the model solution used in this study. Authors correlated the decrease in naringin content with the lessening of the bitterness.

On the other hand, considerable de-bittering (75%) was obtained in grapefruit juice at 37°C under a pressure of 160 MPa for 20 minutes. The decrease of bitterness in citrus fruit juices seems important from a consumer point of view and will help in acceptability by the consumer. The naringinase-mediated reduction of naringin in grapefruit juices may improve their commercial value and maintain the enzyme-regulated health properties of citrus fruits. The application of high pressure with immobilized naringinase hydrolysis of naringin has also been investigated by Ribeiro *et al*. [68]. The modeling of the enzymes for efficient naringin hydrolysis using immobilized naringinase (calcium-alginate beads) under high pressure using response surface methodology (RSM) has been reported for the removal of bitterness from juice [68]. The authors obtained a significantly high conversion of naringin (81%) when naringin hydrolysis was carried out at 60°C under high pressure (205 MPa) for 30 minutes. These findings suggested that the pressure and temperature factors and their combined interactions exert significant effects on the hydrolysis of naringin in model solutions (acetate buffer pH 4.0).

Bodakowska-Boczniewicz and Zbigniew Garncarek [17] have reported the naringinase immobilization on glutaraldehyde-activated chitosan microspheres. Further, they assessed the immobilized enzyme to reduce the bitterness of grapefruit juice. The authors characterized the immobilized enzyme, and the effect of naringinase concentration was also investigated. The authors recorded the maximum activity of naringinase in both states (free and immobilized form) at optimum pH 4.0. The K_M value for the immobilized naringinase was greater than the soluble naringinase. However, the immobilization process did not alter the enzymatic thermal stability. However, the immobilized naringinase exhibited excellent operational stability. The immobilized enzyme system retained 88.1 ± 2.8% of its initial activity after 10 cycles of naringin hydrolysis in fresh grapefruit juice. The results were promising and suggested that immobilized naringinase on chitosan can reduce or remove the bitterness of grapefruit juices and improve other sensory properties. Awad *et al*. [12] reported the covalent immobilization of microbial naringinase on a novel biopolymer that is also thermally stable, which was used for naringin hydrolysis. Isolation of naringinase was done from fungus (*Aspergillus niger)*. Then,

the enzyme was immobilized using grafted gel beads to develop bio-catalytically active beads. Sodium alginate was used as immobilization support. The authors characterized the support for the immobilization of enzymes using TGA and ART-FTIR techniques and also obtained considerable improvement in the thermal stability of the immobilized enzyme over the grafted gel. Moreover, the enzyme packing/ loading capacity was also gradually increased 28-fold. The enzyme loading capacity increased from 32 (U/g gel) to 899 (U/g gel beads), along with retaining 99% of the enzyme immobilization effectiveness and 88% yield of the immobilization. The process of immobilization also resulted in improved thermal stability of the enzyme (from 50 to 70°C). The increased thermal stability of catalysts or enzymes is favorable for industrial processes in food industries. The immobilized enzyme preserved 100% of the immobilized enzyme activity even after 20 cycles, which shows high reusability. The results were very promising and according to the industrial requirements.

Recently, Yu *et al*. [69] reported the synthesis of polyethyleneimine-modified Fe_3O_4 nanoparticles (Fe_3O_4-PEI) followed by immobilization of naringinase (*Aspergillus niger* FFCC uv-11) on this synthesized material. The immobilized naringinase exhibited improved activity that was greater (690.74 U per g-support) than the original activity (406.25 U mL^{-1}) of naringinase under provided conditions including immobilization temperature; 35°C, pH; 5.5, immobilization time duration; 4 h and concentration of glutaraldehyde; 40% (w/v). Also, the immobilization process improved the stability of naringinase against variable pH range (3.5–6.0) and temperature range (40-70°C) applied in this study. The optimum temperature and pH reported for the efficient activity of immobilized enzyme was 60°C and 5.5, respectively. Further, the naringinase enzyme, after immobilization, retained 60.58% of its original activity after ten repeated cycles, indicating the good stability of naringinase in an immobilized state.

Recently, Zheng *et al*. [70] investigated a new strategy of mussel-inspired naringinase immobilization by utilizing the polyethyleneimine/dopamine co-deposition method. Initially, the polyethylenimine/dopamine was coated on the surface of SBA-15 by co-deposition method, and naringinase was immobilized with high activity and operation stability. Authors achieved higher naringinase activity (753.78 U/g carriers). Moreover, naringinase showed a residual activity of 78.91% of the original activity after its storage for 1 month. It also retained 60.79% of activity after eight successive cycles. The authors recognized and suggested the investigated immobilization strategy as a universal and more promising method. This method has several advantages, including high enzyme carrying rate, relative enzymatic activity, recovery rate for enzyme activity, stability under storage, and excellent reusability [70].

Muñoz *et al*. [18] recently studied the combined approach (biochemical and physical) to reduce the bitter components, i.e., naringin and limonin from grapefruit juice. The process was based on the use of heterofunctional supports with glyoxyl groups and alkyl groups. The supports used in the study were butyl-glyoxyl agarose (BGA) and octyl-glyoxyl agarose (OGA). The glyoxyl group of support allowed the covalent immobilization of enzyme naringinase for the hydrolysis of naringin, and alkyl groups were used for the adsorption of limonin. The authors evaluated and compared the debittering of juice (grapefruit) using the soluble enzyme, enzyme-free supports, and immobilized catalysts. The enzyme immobilization in the BGA resulted in a reduction of naringin and limonin concentrations by 54 and 100%, respectively. In comparison, the catalyst immobilized in OGA resulted in a reduction of 74 and 76%, respectively. The final concentration of both bitterness-causing components was obtained under their detection threshold [18].

The immobilization matrices and methods have provided stability and reusability to the naringinase while maintaining efficiency, which is a desired parameter for its industrial use. Various research groups have reported numerous immobilization techniques for naringinase, but still, as compared to other industrial enzymes of microbial origin, naringinase has not been investigated up to the required level. Also, the microbial sources known for naringinase production are limited. New microbial sources and immobilization studies of naringinase from new sources are required for efficient industrial process development. More extensive research is required to speed up the process development for debittering citrus juices on the basis of naringin hydrolysis.

6. Beneficial Aspects of Naringinase

6.1. As a biocatalyst in the food industry.

The immobilization of naringinase has been a very efficient and simplistic approach, especially due to the ease of handling the enzyme and increased productivity and reusability of the biocatalyst [17]. The enzyme has been shown to have optimal activity over an acidic pH range along with a low temperature of the immobilized naringinase, ensuring the suitability of this enzyme in acidic environments, which is quite effective, especially in the case of citrus juice [71]. Moreover, the good part is that the nutritional quality, along with the organoleptic nature of the juice, is also retained. Immobilized naringinase upon hydrolysis has been shown in an earlier study to significantly remove naringin, which is a bitter component of citrus juice. However, naringin has been known to possess many properties from a human health perspective such as anti-cancer, ardiovascular, hepatoprotective, renoprotective, antidiabetic, osteogenic, estrogenic, lipid metabolism and antioxidant properties [72,73]. The decrease in bitterness of the juice is having many beneficial consequences especially from industrial perspective [74], as it may lead to increased processing of fruits out of the total production. Microbes are extensively exploited for naringinase production along with its ease of optimization [75]. It seems possible to improve the juice quality by reducing the naringin content. The ultrasound-aided enzymatic hydrolysis of naringin and limonin seems promising for reducing the bitter compounds along with improved taste and flavor [76]. Naringinase is also known to act on other natural glucosidases, including hesperidin, diosmin, quercitrin, rutin, naringin, and terphenyl glycosides [70], which have many potential implications in the field of healthcare, food sector, and agriculture as they are recognized as antioxidant, anti-inflammatory [77], anti-ulcer, neuroprotective, hypocholesterolemic effects. Improved varieties of naringinase as target enzymes isolated and produced from microbial strains and through optimization of the culture conditions are needed to have a commercially viable naringinase for various industrial applications [1; 35; 78]. Keeping given the above aspects, immobilized naringinase holds promising potential in future food biotechnological and bio-medical applications.

6.2. Health benefits of naringenin: the end product of naringinase mediated hydrolysis of naringin.

Naringinase hydrolyzes the naringin and ultimately produces the naringenin as the end product. Naringenin is a colorless and tasteless flavanone [79]. Therefore, citrus juices effectively treated with microbial naringinase do not develop a bitter taste due to the hydrolysis of naringin into naringenin [80]. Naringenin has been investigated for therapeutic properties

and has been found promising [81]. It is now well known for providing several health benefits along with immense therapeutic potential, including hepatoprotective, anti-atherogenic, anti-inflammatory, and anti-mutagenic effects [82]. Naringenin has been reported for its antioxidant, anti-inflammatory, immuno-modulatory, and antiviral properties [83]. Also, it has been found effective in fatty acid metabolism [84]. Naringenin has also been reported for anti-proliferative and anti-cancer effects. Antiviral properties of naringenin have also been reported. It has been tesetd against hepatitis C virus (HCV), Zika virus (ZIKV), Dengue virus (DENV) and Chikungunya virus (CHIKV) [85,86]. The inhibitory effect of naringenin against COVID-19 has been studied [87,88]. The reviewed data indicated that naringenin might show therapeutic effects against COVID-19 through the inhibition of COVID-19 main protease [89,90], 3-chymotrypsin-like protease (3CLpro), and also reduction of angiotensin-converting enzyme receptors activity [91]. Naringenin consumption has been associated with reduced ACE2 expression in rats, and the compounds that reduce the ACE2 expression may provide an alternative or adjuvant therapy in COVID-19 [92]. Though hydrolysis of naringin by immobilized naringinase leads to reduced levels of naringin, it produces naringenin that may benefit consumer health. As discussed in the previous section, chemical processing affects the beneficial properties of citrus juices. Therefore, using immobilized naringinase seems promising to remove bitterness from citrus juices while maintaining the health benefits of citrus juice [87].

7. Conclusion and Future Perspectives

The gradual inception of bittering of citrus fruit juice is a very important drawback for their use and storage. Naringin is one of the important citrus flavonoids that majorly contribute to the onset and progression of bitterness in citrus juices. The microbial origin of naringinase has been reported to effectively hydrolyze the naringin into the tasteless compound and also reduce the naringin content of citrus juice. Moreover, the resultant products of the hydrolyzing reaction do not contribute to bitterness. Naringinase is useful in the food industry, particularly in the citrus juice industry, for de-bittering citrus juices without affecting their beneficial properties. Naringinase-mediated removal of the bitterness of citrus fruit juice can be an efficient, environment-friendly, and more promising strategy for the near future. Also, the enzymatic treatment of juices improves their taste and enhances their flavor. Enzymatic treatment is environmentally friendly and more natural than chemical processes, which have several drawbacks. Chemical processing may also have a negative impact on juice properties, while the enzymatic treatment maintains the beneficial properties of juices. Enzyme immobilization is an important aspect of industrial biotechnology that has also been found to be an effective strategy for naringinase. Immobilized naringinase-based catalytic systems can potentially be used commercially in de-bittering citrus juices. Advancements in immobilization and the development of effective catalysts are major demands of the industry that need to be fulfilled by extensive research. Along with an effective immobilization matrix, strategy, and process, new microbial sources are also required for future applications of naringinase. The naringinase research has not attained the required pace; therefore, research gaps must be filled quickly for this promising industrial enzyme.

Funding

No funding was received for this work.

Acknowledgments

Authors (MY, NS, RN, MS) are thankful to the Head, Department of Bio-Sciences and Technology, MMEC, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, India.

Conflict of Interest

There are no financial or non-financial competing interests regarding submitting and publishing the article in Letters in Applied Nanobioscience.

References

1. Yadav, M.; Sehrawat, N.; Sharma, A.K.; Kumar, V.; Kumar, A. Naringinase: microbial sources, production and applications in food processing industry. *J. Microbiol. Biotechnol. Food Sci.* **2021**, *8*, 717-720, https://doi.org/10.15414/jmbfs.2018.8.1.717-720.
2. Mahto, R.B.; Yadav, M.; Sasmal, S.; Bhunia, B. Optimization of process parameters for production of pectinase using *Bacillus subtilis* MF447840.1. *Recent Pat. Biotechnol.* **2019**, *13*, 69-73, https://doi.org/10.2174/1872208312666180917094428.
3. Kaur, D.; Sharma, V.; Joshi, A.; Batra, N.; Ramniwas, S.; Sharma, A.K. Pectinases as promising green biocatalysts having broad-spectrum applications: Recent trends, scope, and relevance. *Biotechnol. Appl. Biochem.* **2023**, *70*, 1663-1678, https://doi.org/10.1002/bab.2464.
4. Mahto, R.B.; Yadav, M.; Muthuraj, M.; Sharma, A.K.; Bhunia, B. Biochemical properties and application of a novel pectinase from a mutant strain of *Bacillus subtilis*. *Biomass Convers. Biorefin.* **2023**, *13*, 10463-10474, https://doi.org/10.1007/s13399-021-02225-y.
5. Ângelo Samir Melim, M.; Tathiana Souza, M.-M.; Érika Veríssimo da Costa, F.; Bianca Waruar Paulo, L.; Gisela Maria, D.-O. Enzymes in Bakery: Current and Future Trends. In Food Industry, Innocenzo, M., Ed.; IntechOpen, Rijeka, **2013**; 278-321, https://doi.org/10.5772/53168.
6. Singh, R.S.; Yadav, M. Single-Step Purification and Characterization of Recombinant Aspartase of *Aeromonas media* NFB-5. *Appl. Biochem. Biotechnol.* **2012**, *167*, 991-1001, https://doi.org/10.1007/s12010-012-9589-8.
7. Sarup Singh, R.; Yadav, M. Current trends and developments of aspartase. *Curr. Biotechnol.* **2012**, *1*, 135-147, https://doi.org/10.2174/2211550111201020135
8. Singh, R.S.; Yadav, M. Enhanced production of recombinant aspartase of *Aeromonas media* NFB-5 in a stirred tank reactor. *Bioresour. Technol.* **2013**, *145*, 217-223, https://doi.org/10.1016/j.biortech.2012.11.041.
9. Singh, R.S.; Singh, R.P.; Yadav, M. Molecular and biochemical characterization of a new endoinulinase producing bacterial strain of *Bacillus safensis* AS-08. *Biologia* **2013**, *68*, 1028-1033, https://doi.org/10.2478/s11756-013-0259-2.
10. Puri, M.; Banerjee, U.C. Production, purification, and characterization of the debittering enzyme naringinase. *Biotechnol. Adv.* **2000**, *18*, 207-217, https://doi.org/10.1016/s0734-9750(00)00034-3.
11. Hasegawa, S. Limonin Bitterness in Citrus Juices. In Flavor Chemistry: Thirty Years of Progress, Teranishi, R., Wick, E.L., Hornstein, I., Eds.; Springer US, Boston, MA, **1999**; 89–106, https://doi.org/10.1007/978-1-4615-4693-1_9.
12. Awad, G.E.A.; Abd El Aty, A.A.; Shehata, A.N.; Hassan, M.E.; Elnashar, M.M. Covalent immobilization of microbial naringinase using novel thermally stable biopolymer for hydrolysis of naringin. *3 Biotech* **2016**, *6*, 14, https://doi.org/10.1007/s13205-015-0338-x.
13. Yusof, S.; Ghazali, H.M.; King, G.S. Naringin content in local citrus fruits. *Food Chem.* **1990**, *37*, 113-121, https://doi.org/10.1016/0308-8146(90)90085-i.
14. Puri, M.; Banerjee, A.; Banerjee, U.C. Optimization of process parameters for the production of naringinase by *Aspergillus niger* MTCC 1344. *Process Biochem.* **2005**, *40*, 195-201, https://doi.org/10.1016/j.procbio.2003.12.009.
15. Ribeiro, M.H.; Silveira, D.; Ferreira-Dias, S. Selective adsorption of limonin and naringin from orange juice to natural and synthetic adsorbents. *Eur. Food Res. Technol.* **2002**, *215*, 462-471, https://doi.org/10.1007/s00217-002-0592-0.

16. Mongkolkul, P.; Rodart, P.; Pipatthitikorn, T.; Meksut, L.; Sa-nguandeekul, R. Debittering of Tangerine *Citrus Reticulata* Blanco Juice by β-Cyclodextrin Polymer. *J. Incl. Phenom. Macrocycl. Chem.* **2006**, *56*, 167-170, https://doi.org/10.1007/s10847-006-9078-1.
17. Bodakowska-Boczniewicz, J.; Garncarek, Z. Immobilization of Naringinase from *Penicillium decumbens* on Chitosan Microspheres for Debittering Grapefruit Juice. *Molecules* **2019**, *24*, 4234, https://doi.org/10.3390/molecules24234234.
18. Muñoz, M.; Holtheuer, J.; Wilson, L.; Urrutia, P. Grapefruit Debittering by Simultaneous Naringin Hydrolysis and Limonin Adsorption Using Naringinase Immobilized in Agarose Supports. *Molecules* **2022**, *27*, 2867, https://doi.org/10.3390/molecules27092867.
19. Chandler, B.; Nicol, K. Some relationships of naringin: their importance in orange juice bitterness. *CSIRO Food Res Quart* **1975**, *35*, 79-88.
20. Habelt, K.; Pittner, F. A rapid method for the determination of naringin, prunin, and naringenin applied to the assay of naringinase. *Anal. Biochem.* **1983**, *134*, 393-397, https://doi.org/10.1016/0003-2697(83)90314-7.
21. Ribeiro, M.H. Naringinases: occurrence, characteristics, and applications. *Appl. Microbiol. Biotechnol.* **2011**, *90*, 1883-1895, https://doi.org/10.1007/s00253-011-3176-8.
22. Puri, M.; Marwaha, S.S.; Kothari, R.M.; Kennedy, J.F. Biochemical Basis of Bitterness in Citrus Fruit Juices and Biotech Approaches for Debittering. *Crit. Rev. Biotechnol.* **1996**, *16*, 145-155, https://doi.org/10.3109/07388559609147419.
23. Gupta, A.K.; Rather, M.A.; Mishra, P. Design and development of laboratory scale batch type device for debittering of bitter citrus juice. *J. Food Process Eng.* **2023**, *46*, e14265, https://doi.org/10.1111/jfpe.14265.
24. Falch, E.A. Industrial enzymes — Developments in production and application. *Biotechnol. Adv.* **1991**, *9*, 643-658, https://doi.org/10.1016/0734-9750(91)90736-F.
25. Poulsen, P.B.; Buchholz, K. History of enzymology with emphasis on food production. In Handbook of food enzymology; CRC Press, **2002**; 26-35.
26. Petersen, S.B. Protein engineering: design and engineering on the nano scale. In Applied Biocatalysis, second ed., Amsterdam: Harwood Academic Publications, **2005**; 263-310.
27. Izabel, S.; Zacarias, T.v.; Rodrigo Patera, B.; Suzymeire, B. Microorganism-Produced Enzymes in the Food Industry. In Scientific, Health and Social Aspects of the Food Industry, Benjamin, V., Ed.; IntechOpen, Rijeka, **2012**; 83-94, https://doi.org/10.5772/31256.
28. Hasan, F.; Shah, A.A.; Hameed, A. Industrial applications of microbial lipases. *Enzyme Microb. Technol.* **2006**, *39*, 235-251, https://doi.org/10.1016/j.enzmictec.2005.10.016.
29. Pan, L.; Zhang, Y.; Zhang, F.; Wang, Z.; Zheng, J. α-L-rhamnosidase: production, properties, and applications. *World J. Microbiol. Biotechnol.* **2023**, *39*, 191, https://doi.org/10.1007/s11274-023-03638-9.
30. Lou, H.; Liu, X.; Liu, S.; Chen, Q. Purification and Characterization of a Novel α-L-Rhamnosidase from *Papiliotrema laurentii* ZJU-L07 and Its Application in Production of Icariin from Epimedin C. *J. Fungi* **2022**, *8*, 644, https://doi.org/10.3390/jof8060644.
31. Sehrawat, N.; Devi, S.; Devi, A.; Yadav, M. Anti-diabetic and anti-cancerous potential of citrus flavonoid naringin: A review. *Med. Plants - Int. J. Phytomed.* **2018**, *10*, 171-175, https://doi.org/10.5958/0975-6892.2018.00028.X.
32. Yadav, M.; Sehrawat, N.; Kumar, S.; Sharma, A.K.; Kaur, N.; Singh, A.; Nara, R.; Kumar, S. Therapeutic Potential of Citrus Flavonoid Naringin in Management of Osteoporosis: Current Status and Future Perspectives. *Asian J. Biol. Sci.* **2021**, *10*, 527-532, https://doi.org/10.5530/ajbls.2021.10.70.
33. Sehrawat, N.; Upadhyay, S.K.; Sharma, A.K.; Kumar, S.; Yadav, M. Emerging Renoprotective Role of Citrus Flavonoid Naringin: Current Pharmaceutical Status and Future Perspectives. *Curr. Pharmacol. Rep.* **2021**, *7*, 96-101, https://doi.org/10.1007/s40495-021-00256-7.
34. Yadav, M.; Sehrawat, N.; Singh, M.; Upadhyay, S.K.; Aggarwal, D.; Sharma, A.K. Cardioprotective and Hepatoprotective Potential of Citrus Flavonoid Naringin: Current Status and Future Perspectives for Health Benefits. *Asian J. Biol. Life Sci* **2020**, *9*, 1-5, http://dx.doi.org/10.5530/ajbls.2020.9.1.
35. Trpathi, A.; Nara, R.; Sehrawat, N.; Singh, M.; Sehrawat, A.; Preet, K.; Yadav, M. Molecular characterization of naringinase producing bactreria *Pseudomonas resinovorans* RMCSJ-28. *Indian J. Nat. Sci.* **2024**, 15, 74375-74380.

36. Cavia-Saiz, M.; Muñiz, P.; Ortega, N.; Busto, M.D. Effect of enzymatic debittering on antioxidant capacity and protective role against oxidative stress of grapefruit juice in comparison with adsorption on exchange resin. *Food Chem.* **2011**, *125*, 158-163, https://doi.org/10.1016/j.foodchem.2010.08.054.
37. Ribeiro, I.A.; Rocha, J.; Sepodes, B.; Mota-Filipe, H.; Ribeiro, M.H. Effect of naringin enzymatic hydrolysis towards naringenin on the anti-inflammatory activity of both compounds. *J. Mol. Catal. B Enzym.* **2008**, *52*, 13-18, https://doi.org/10.1016/j.molcatb.2007.10.011.
38. Liu, S.; Grierson, D.; Xi, W. Biosynthesis, distribution, nutritional and organoleptic properties of bitter compounds in fruit and vegetables. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 1934-1953, https://doi.org/10.1080/10408398.2022.2119930.
39. Nara, R.; Tripathi, A.; Sehrawat, N.; Sehrawat, A.; Upadhyay, SK.; Malik, K. *et al*. Molecular Characterization of a New Bacterial Source of Naringinase as *Paenibacillus stellifer* RAMCM-44 and Fermentative Production of Naringinase at Shake- Flask Level. *Asian J Biol Life Sci*. **2024,** 13, 168-175. https;//doi.org/10.5530/ajbls.2023.13.22.
40. Sehrawat, N.; Yadav, M.; Singh, M.; Kumar, V.; Sharma, V.R.; Sharma, A.K. Probiotics in microbiome ecological balance providing a therapeutic window against cancer. *Semin. Cancer Biol.* **2021**, *70*, 24-36, https://doi.org/10.1016/j.semcancer.2020.06.009.
41. Sharma, V.R.; Singh, M.; Kumar, V.; Yadav, M.; Sehrawat, N.; Sharma, D.K.; Sharma, A.K. Microbiome dysbiosis in cancer: Exploring therapeutic strategies to counter the disease. *Semin. Cancer Biol.* **2021**, *70*, 61-70, https://doi.org/10.1016/j.semcancer.2020.07.006.
42. Sharma, V.; Sharma, N.; Sheikh, I.; Kumar, V.; Sehrawat, N.; Yadav, M.; Ram, G.; Sankhyan, A.; Sharma, A.K. Probiotics and Prebiotics Having Broad Spectrum Anticancer Therapeutic Potential: Recent Trends and Future Perspectives. *Curr. Pharmacol. Rep.* **2021**, *7*, 67-79, https://doi.org/10.1007/s40495-021-00252-x.
43. Sharma, V.; Sharma, A.K.; Punj, V.; Priya, P. Recent nanotechnological interventions targeting PI3K/Akt/mTOR pathway: A focus on breast cancer. *Semin. Cancer Biol.* **2019**, *59*, 133-146, https://doi.org/10.1016/j.semcancer.2019.08.005.
44. Quintero, M.-B.M.; Paz, A.-V.C.; Holguín, A.L.V.; Galán, J.-P.M. Naringinase immobilized on modified banana peel with potential application in the citrus industry. *Ingeniería Investigación y Desarrollo* **2023**, *23*, 33-42, https://doi.org/10.19053/1900771X.v23.n1.2023.15844.
45. Olson, A.C.; Gray, G.M.; Guadagni, D.G. Naringin bitterness of grapefruit juice debittered with naringinase immobilized in a hollow fiber. *J. Food Sci.* **1979**, *44*, 1358-1361, https://doi.org/10.1111/j.1365-2621.1979.tb06438.x.
46. RoitNer, M.; Schalkhammer, T.; Pittner, F. Preparation of prunin with the help of immobilized naringinase pretreated with alkaline buffer. *Appl. Biochem. Biotechnol.* **1984**, *9*, 483-488, https://doi.org/10.1007/BF02798402.
47. Jimeno, A.; Manjon, A.; Canovas, M.; Iborra, J.L. Use of naringinase immobilized on glycophase-coated porous glass for fruit juice debittering. *Process Biochem.* **1987**, *22*, 13-16.
48. Tsen, H.-Y.; Tsai, S.-Y. Comparison of the kinetics and factors affecting the stabilities of chitin-immobilized naringinases from two fungal sources. *J. Ferment. Technol.* **1988**, *66*, 193-198, https://doi.org/10.1016/0385-6380(88)90047-7.
49. Tsen, H.-Y.; Tsai, S.-Y.; Yu, G.-K. Fiber entrapment of naringinase from *Penicillium* sp. and application to fruit juice debittering. *J. Ferment. Bioeng* **1989**, *67*, 186-189, https://doi.org/10.1016/0922-338X(89)90120-7.
50. Puri, M.; Marwaha, S.S.; Kothari, R.M. Studies on the applicability of alginate-entrapped naringiase for the debittering of kinnow juice. *Enzyme Microb. Technol.* **1996**, *18*, 281-285, https://doi.org/10536/DRO/DU:30095405.
51. Mishra, P.; Kar, R. Treatment of Grapefruit Juice for Bitterness Removal by Amberlite IR 120 and Amberlite IR 400 and Alginate Entrapped Naringinase Enzyme. *J. Food Sci.* **2003**, *68*, 1229-1233, https://doi.org/10.1111/j.1365-2621.2003.tb09630.x.
52. Pedro, H.A.L.; Alfaia, A.J.; Marques, J.; Vila-Real, H.J.; Calado, A.; Ribeiro, M.H.L. Design of an immobilized enzyme system for naringin hydrolysis at high-pressure. *Enzyme Microb. Technol.* **2007**, *40*, 442-446, https://doi.org/10.1016/j.enzmictec.2006.07.018.
53. Ribeiro, I.A.C.; Ribeiro, M.H.L. Kinetic modelling of naringin hydrolysis using a bitter swe*et al*fa-rhamnopyranosidase immobilized in k-carrageenan. *J. Mol. Catal. B Enzym.* **2008**, *51*, 10-18, https://doi.org/10.1016/j.molcatb.2007.09.023.

54. Puri, M.; Kaur, H.; Kennedy, J.F. Covalent immobilization of naringinase for the transformation of a flavonoid. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1160-1165, https://doi.org/10.1002/jctb.1303.
55. Şekeroğlu, G.; Fadıloğlu, S.; Göğüş, F. Immobilization and characterization of naringinase for the hydrolysis of naringin. *Eur. Food Res. Technol.* **2006**, *224*, 55-60, https://doi.org/10.1007/s00217-006-0288-y.
56. Busto, M.D.; Meza, V.; Ortega, N.; Perez-Mateos, M. Immobilization of naringinase from *Aspergillus niger* CECT 2088 in poly (vinyl alcohol) cryogels for the debittering of juices. *Food Chem.* **2007**, *104*, 1177-1182, https://doi.org/10.1016/j.foodchem.2007.01.033.
57. Magario, I.; Ma, X.; Neumann, A.; Syldatk, C.; Hausmann, R. Non-porous magnetic micro-particles: Comparison to porous enzyme carriers for a diffusion rate-controlled enzymatic conversion. *J. Biotechnol.* **2008**, *134*, 72-78, https://doi.org/10.1016/j.jbiotec.2007.12.001.
58. Shilpa, V.S.; Sreerag, N.K.; Gopalakrishnan, K.; Shahada, C.; Farha, R. Debittering citrus fruit juices, advances in immobilized enzyme technology: A review. *Pharma. Innov. J.* **2023**, *12*, 3096-3101.
59. Ribeiro, M.H.L.; Rabaça, M. Cross-linked enzyme aggregates of naringinase: novel biocatalysts for naringin hydrolysis. *Enzyme Res.* **2011**, *2011*, 851272.
60. Cao, L.; van Langen, L.M.; van Rantwijk, F.; Sheldon, R.A. Cross-linked aggregates of penicillin acylase: robust catalysts for the synthesis of β-lactam antibiotics. *J. Mol. Catal. B Enzym.* **2001**, *11*, 665-670, https://doi.org/10.1016/S1381-1177(00)00078-3.
61. Wilson, L.; Illanes, A.; Soler, L.; Henríquez, M.J. Effect of the degree of cross-linking on the properties of different CLEAs of penicillin acylase. *Process Biochem.* **2009**, *44*, 322-326, https://doi.org/10.1016/j.procbio.2008.11.010.
62. Xiao, A.; You, H.; Wu, C.; Ni, H.; Yang, Q.; Cai, H. Immobilization and characterization of naringinase from *Aspergillus aculeatus* onto magnetic Fe_3O_4 nanoparticles. *Nanosci. Nanotechnol. Lett.* **2015**, *7*, 770-778, https://doi.org/10.1166/nnl.2015.2029.
63. Nunes, M.A.P.; Vila-Real, H.; Fernandes, P.C.B.; Ribeiro, M.H.L. Immobilization of Naringinase in PVA–Alginate Matrix Using an Innovative Technique. *Appl. Biochem. Biotechnol.* **2010**, *160*, 2129-2147, https://doi.org/10.1007/s12010-009-8733-6.
64. Puri, M.; Kaur, A.; Singh, R.S.; Schwarz, W.H.; Kaur, A. One-step purification and immobilization of His-tagged rhamnosidase for naringin hydrolysis. *Process Biochem.* **2010**, *45*, 451-456, https://doi.org/10.1016/j.procbio.2009.11.001.
65. Luo, J.; Li, Q.; Sun, X.; Tian, J.; Fei, X.; Shi, F.; Zhang, N.; Liu, X. The study of the characteristics and hydrolysis properties of naringinase immobilized by porous silica material. *RSC Adv.* **2019**, *9*, 4514-4520, https://doi.org/10.1039/c9ra00075e.
66. Kumar, S.; Joshi, J.; Kumar, V.; Gautam, P.; Singh, S.; Kohli, D. Effect of Immobilized Enzymes Naringinase and Tannase Produced from *Aspergillus* Sp. Isolate Mk156394 Isolated from Rotten Pomelo on Quality Characteristics of *Citrus Limetta* Juice and Process Optimization by Using Response Surface Methodology. *Biointerface Res. Appl. Chem.* **2020**, *11*, 9646-9657, https://doi.org/10.33263/BRIAC112.96469657.
67. Ferreira, L.; Afonso, C.; Vila-Real, H.; Alfaia, A.; Ribeiro, M.H.L. Evaluation of the Effect of High Pressure on Naringin Hydrolysis in Grapefruit Juice with Naringinase Immobilised in Calcium Alginate Beads. *Food Technol. Biotechnol.* **2008**, *46*, 146-150.
68. Ribeiro, M.H.L.; Afonso, C.; Vila-Real, H.J.; Alfaia, A.J.; Ferreira, L. Contribution of response surface methodology to the modeling of naringin hydrolysis by naringinase Ca-alginate beads under high pressure. *LWT-Food Sci. Technol.* **2010**, *43*, 482-487, https://doi.org/10.1016/j.lwt.2009.09.015.
69. Yu, C.; Li, Q.; Tian, J.; Zhan, H.; Zheng, X.; Wang, S.; Sun, X.; Sun, X. A facile preparation of immobilized naringinase on polyethyleneimine-modified Fe_3O_4 magnetic nanomaterials with high activity. *RSC Adv.* **2021**, *11*, 14568-14577, https://doi.org/10.1039/d1ra01449h.
70. Zheng, X.; Li, Q.; Tian, J.; Zhan, H.; Yu, C.; Wang, S.; Sun, X. Novel Strategy of Mussel-Inspired Immobilization of Naringinase with High Activity Using a Polyethylenimine/Dopamine Co-deposition Method. *ACS Omega* **2021**, *6*, 3267-3277, https://doi.org/10.1021/acsomega.0c05756.
71. Rathee, P.; Chaudhary, H.; Rathee, S.; Rathee, D.; Kumar, V.; Kohli, K. Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. *Inflamm. Allergy-Drug Targets* **2009**, *8*, 229-235, https://doi.org/10.2174/187152809788681029.

72. Sharma, A.K.; Sharma, V.R.; Gupta, G.K.; Ashraf, G.M.; Kamal, M.A. Advanced Glycation End Products (AGEs), Glutathione and Breast Cancer: Factors, Mechanism and Therapeutic Interventions. *Curr. Drug Metab.* **2019**, *20*, 65-71, https://doi.org/10.2174/1389200219666180912104342.
73. Sheikh, I.; Sharma, V.; Tuli, H.S.; Aggarwal, D.; Sankhyan, A.; Vyas, P.; Sharma, A.K.; Bishayee, A. Cancer Chemoprevention by Flavonoids, Dietary Polyphenols and Terpenoids. *Biointerface Res. Appl. Chem.* **2021**, *11*, 8502-8537, https://doi.org/10.33263/BRIAC111.85028537.
74. Amudha, K.; Pari, L. Beneficial role of naringin, a flavanoid on nickel induced nephrotoxicity in rats. *Chem.-Biol. Interact.* **2011**, *193*, 57-64, https://doi.org/10.1016/j.cbi.2011.05.003.
75. Sheldon, R.A.; Basso, A.; Brady, D. New frontiers in enzyme immobilisation: robust biocatalysts for a circular bio-based economy. *Chem. Soc. Rev.* **2021**, *50*, 5850-5862, https://doi.org/10.1039/d1cs00015b.
76. Gao, X.; Feng, T.; Liu, E.; Shan, P.; Zhang, Z.; Liao, L.; Ma, H. Ougan juice debittering using ultrasound-aided enzymatic hydrolysis: Impacts on aroma and taste. *Food Chem.* **2021**, *345*, 128767, https://doi.org/10.1016/j.foodchem.2020.128767.
77. Ram, G.; Sharma, V.R.; Sheikh, I.; Sankhyan, A.; Aggarwal, D.; Sharma, A.K. Anti-cancer potential of natural products: recent trends, scope and relevance. *Lett. Appl. NanoBioSci.* **2020**, *9*, 902-907, https://doi.org/10.33263/LIANBS91.902907.
78. Singh, M.; Kumar, V.; Sehrawat, N.; Yadav, M.; Chaudhary, M.; Upadhyay, S.K.; Kumar, S.; Sharma, V.; Kumar, S.; Dilbaghi, N.; Sharma, A.K. Current paradigms in epigenetic anticancer therapeutics and future challenges. *Semin. Cancer Biol.* **2022**, *83*, 422-440, https://doi.org/10.1016/j.semcancer.2021.03.013.
79. Pinho-Ribeiro, F.A.; Zarpelon, A.C.; Fattori, V.; Manchope, M.F.; Mizokami, S.S.; Casagrande, R.; Verri, W.A. Naringenin reduces inflammatory pain in mice. *Neuropharmacology* **2016**, *105*, 508-519, https://doi.org/10.1016/j.neuropharm.2016.02.019.
80. Yin, J.; Liang, Y.; Wang, D.; Yan, Z.; Yin, H.; Wu, D.; Su, Q. Naringenin induces laxative effects by upregulating the expression levels of c-Kit and SCF, as well as those of aquaporin 3 in mice with loperamide-induced constipation. *Int. J. Mol. Med.* **2018**, *41*, 649-658, https://doi.org/10.3892/ijmm.2017.3301.
81. Ke, J.-Y.; Banh, T.; Hsiao, Y.-H.; Cole, R.M.; Straka, S.R.; Yee, L.D.; Belury, M.A. Citrus flavonoid naringenin reduces mammary tumor cell viability, adipose mass, and adipose inflammation in obese ovariectomized mice. *Mol. Nutr. Food Res.* **2017**, *61*, 1600934, https://doi.org/10.1002/mnfr.201600934.
82. Karim, N.; Jia, Z.; Zheng, X.; Cui, S.; Chen, W. A recent review of citrus flavanone naringenin on metabolic diseases and its potential sources for high yield-production. *Trends Food Sci. Technol.* **2018**, *79*, 35-54, https://doi.org/10.1016/j.tifs.2018.06.012.
83. Salehi, B.; Fokou, P.V.T.; Sharifi-Rad, M.; Zucca, P.; Pezzani, R.; Martins, N.; Sharifi-Rad, J. The Therapeutic Potential of Naringenin: A Review of Clinical Trials. *Pharmaceuticals* **2019**, *12*, 11, https://doi.org/10.3390/ph12010011.
84. Zobeiri, M.; Belwal, T.; Parvizi, F.; Naseri, R.; Farzaei, M.H.; Nabavi, S.F.; Sureda, A.; Nabavi, S.M. Naringenin and its Nano-formulations for Fatty Liver: Cellular Modes of Action and Clinical Perspective. *Curr. Pharm. Biotechnol.* **2018**, *19*, 196-205, https://doi.org/10.2174/1389201019666180514170122.
85. Cataneo, A.H.D.; Kuczera, D.; Koishi, A.C.; Zanluca, C.; Silveira, G.F.; Arruda, T.B.d.; Suzukawa, A.A.; Bortot, L.O.; Dias-Baruffi, M.; Verri, W.A.; Robert, A.W.; Stimamiglio, M.A.; Duarte dos Santos, C.N.; Wowk, P.F.; Bordignon, J. The citrus flavonoid naringenin impairs the *in vitro* infection of human cells by Zika virus. *Sci. Rep.* **2019**, *9*, 16348, https://doi.org/10.1038/s41598-019-52626-3.
86. Sharma, V.; Sehrawat, N.; Sharma, A.; Yadav, M.; Verma, P.; Sharma, A.K. Multifaceted antiviral therapeutic potential of dietary flavonoids: Emerging trends and future perspectives. *Biotechnol. Appl. Biochem.* **2022**, *69*, 2028-2045, https://doi.org/10.1002/bab.2265.
87. Alberca, R.W.; Teixeira, F.M.E.; Beserra, D.R.; de Oliveira, E.A.; Andrade, M.M.d.S.; Pietrobon, A.J.; Sato, M.N. Perspective: The Potential Effects of Naringenin in COVID-19. *Front. Immunol.* **2020**, *11*, 570919, https://doi.org/10.3389/fimmu.2020.570919.
88. Sharma, V.; Sharma, A.K.; Yadav, M.; Sehrawat, N.; Kumar, V.; Kumar, S.; Gupta, A.; Sharma, P.; Chakrabarti, S. Prediction Models based on miRNA-disease Relationship: Diagnostic Relevance to Multiple Diseases Including COVID-19. *Curr. Pharm. Biotechnol.* **2023**, *24*, 1213-1227, https://doi.org/10.2174/1389201024666221025114500.
89. Sharma, A.K.; Sharma, V.; Sharma, A.; Pallikkuth, S.; Sharma, A.K. Current Paradigms in COVID-19 Research: Proposed Treatment Strategies, Recent Trends and Future Directions. *Curr. Med. Chem.* **2021**, *28*, 3173-3192, https://doi.org/10.2174/0929867327666200711153829.

90. Sharma, V.; Aggarwal, D.; Sharma, A.K.; Chandran, D.; Sharma, A.; Chopra, H.; Emran, T.B.; Dey, A.; Dhama, K. An overview on monkeypox, current paradigms and advances in its vaccination, treatment and clinical management: Trends, scope, promise and challenges. *J. Pure Appl. Microbiol.* **2022**, *16*, 3000-3012, https://doi.org/10.22207/JPAM.16.SPL1.21.
91. Tutunchi, H.; Naeini, F.; Ostadrahimi, A.; Hosseinzadeh-Attar, M.J. Naringenin, a flavanone with antiviral and anti-inflammatory effects: A promising treatment strategy against COVID-19. *Phytother. Res.* **2020**, *34*, 3137-3147, https://doi.org/10.1002/ptr.6781.
92. Chen, H.; Du, Q. Potential Natural Compounds for Preventing SARS-CoV-2 (2019-nCoV) Infection. *Preprints* **2020**, 2020010358, https://doi.org/10.20944/preprints202001.0358.v3.