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Innovative Physical and Chemical Processing Techniques for the Debittering of the Juices of Citrus Fruits, A Short Comprehensive Overview

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Abstract An intense bitterness associated with citrus industry is specifically originated due to the presence of chemically bitter metabolites in citrus fruits, the conditions and the duration of storage along with the actual process of juice extraction. Incessant research efforts are being made by scientists worldwide to discover new technological pathways to remove unwanted bitterness. Out of such numerous physical, chemical and biological methodologies being universally made available while competing towards becoming cost effective at the industrial production level; a few of the prevalent and prominent ones are mentioned herein.

Keywords Limonoid, Flavonoid, Delayed Bitterness, Debittering, Citrus Fruit Juice Industry

1. Introduction

Citriculture is recognized as an important sector of world's food production and nutrition. Citrus fruits can easily be included among the most renowned categorical fruits liked by the billions all over the globe. They are valued highly due to their richness in specific minerals, vitamins and a few bioactive compounds. Their consumption throughout the world is preferred either directly or in the form of fresh juice and/or processed fruit products [1-3]. Oranges consumed, primarily in the form of juice, comprise 67% of total worldwide citrus production. The citrus juice and its products procured from certain varieties of oranges, mandarins, grapefruits, and lemons are inclined towards introducing bitterness within a few hours after extraction from its parent fruit [5-8]. As per studies, the small particles of the albedo containing certain enzymes along with fragments of the central bundle and segments of carpella in the fruit which get incorporated during the juice extraction process are primarily responsible for the introduction and development of excessive and undesirable bitterness. The freshly extracted fruit juice is devoid of any bitterness in taste but interestingly, the longer storage times involved due to the packing and transport of the citrus juice and its products are also observed to substantially contribute to introducing unwanted bitterness. The phenomenon of 'delayed bitterness' became a great topic of interest for the researchers due to the post extraction processing and storage duration of fruit juice and subsequently, plenty of efforts have been invested so far in the research specifically in developing methods to counter the effects of bitterness. Even though to name a few, naringin, tangeretin, nobiletin, sinensetin, quercetin, limonin, nomilin and neo hesperidin are identified to be some of the chemically bitter metabolites present in citrus fruits; particularly, limonin and nomilin (limonoid) and naringin (flavonoid) along with their respective acidic forms, specifically recognized as the primary components of the group of compounds called limonoids are to be blamed for causing an intense bitterness in oranges [9-11]. Accordingly, discovering new pathways to remove limonoids after harvesting and processing along with preventing the formation of its precursors prior to harvest are the fundamental research approaches prevalently focused upon. In citrus fruits under acidic conditions, limonoate A-ring lactone (a non-bitter compound) is observed to convert into limonin (bitter compound) and the rate of conversion is studied to be much higher under low pH conditions [12-15]. As per another study the two different ways through which the bitterness in juice extracted from bitter and non-bitter fruits are, i) Presence on flavonoids and their derivatives in fruits, ii) Conversion of tasteless form of compounds into the bitter ones [16,17]. Furthermore, the type of fruit, fruit parts, cultivars and conditions under which they are growing also actively influence the concentration of the bitterness causing components in citrus fruits [18-21]. Overall, the introduction of bitterness attributed to these various factors, in turn results in the deterioration in the overall quality of citrus fruits products, a subsequent reduction in consumer acceptability, followed by a decline in economic value. In general, the citrus industry worldwide has acknowledged bitterness as a

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major obstacle accountable for yearly loss of approximately USD 6-8 million [22,23]. Inventing methodologies to efficiently effectuate the debittering of citrus fruit juices along with enhancing their shelf-life has been a focus of the research of scientists around the globe and subsequently, several physical, chemical and biological procedures are being screened. The previously existing three categorical methods exposed for their technical limitations were unfortunately found to be endangering stability and quality of the juice by affecting components other than limonoids, in turn, were equally responsible for severe economic damage [24-26]. In the light of the only back-then available and accepted commercial method involving the blending of excessive bitter juice with a non-bitter juice to reduce the levels of bitter citrus components, the imminent invention and subsequent implementation of an inciteful and financially profitable methods at industrial level is quintessential [27].

2. A Quick Compilation of the Most Widely Implemented Debittering Methods

Citrus fruits contain numerous types of phytonutrients with specific functionality and usefulness. Depending upon the type of glycoside chain, phytonutrients are classified as flavanones, flavones, flavonols, flavans, isoflavones, triterpenes, limonoid aglycones, glucosinolates viz. organosulphur compounds and isothiocyanates [28-32]. Researchers have broadly categorized the basic mechanism behind various debittering methods which are briefly summarized herein. These include, i) Removal of bitter compounds, ii) Removal of physical barriers such as pith, iii) Utilization of flavor enhancers and bitter compounds scavengers such as salt, sugar and florisil, iv) Utilization of enzymes such as naringinase and α L-rhamnosidase, v) Utilization of genetical engineering techniques for modulating the synthetic pathways of bitter compounds [22]. Food scientists and researchers all over the world have contributed immense efforts to reduce the accumulation of bitter compounds during the development and maturing of citrus fruits viz. chemical sprays, agronomic practices and post-harvest treatment of fruit to name a few. Many such prevalently practiced physical and chemical procedures are addressed here in brief. In addition, there exist numerous types of genetical engineering techniques along with biological methods incorporating technologies associated with enzymes, bacteria/microbial strains and substrates which when implemented either individually or in tandem with ultrasound sonication [33] have been observed to assist greatly in debittering the juices of citrus fruits. Nevertheless, it is to be noted that considering the vastness of these fascinating techniques the detailed explanation of their utilization is deliberately excluded from this short communication.

2.1. Physical Methods

i) Resins: Resins being water insoluble and soluble in organic solvents, basically are mixture of heterogeneous fatty acids, waxes, resenes and resin acid ($C_{20}H_{30}O_2$). They are synthesized in specific surface glands and internal ducts of both non-woody and woody plants of family Pinaceae and Dipterocarpaceae and are extracted from the same [34]. Methods have been developed and published [35-38] where various types of resin materials viz. strong and weak, cationic and anionic etc. were utilized to successfully eliminate bitterness by reducing more than 90% of limonin from fruit juice without compromising the nutritional value.

ii) Membrane Assisted Processing: To eliminate the unwanted flavors from the fruit juices, a fascinating route has been adopted by deploying a synthetic solution-diffusion membrane which selectively allows four types of limonoids viz. limonin, limonoic acid, nomilin, and nomiloic acid to pass through while retaining desired flavor and nutritional components. The drastically reduced performance of conventional porous filtration membrane can be attributed to its selectivity being dictated by the molecular size along with a phenomenon of concentration polarization resulting in fouling [39]. In contrast to the principle of convective flow, the selectivity of solution-diffusion membrane possessing minimal concentration polarization is dependent on the molecular solubility where the transport of solute occurs by permeating through membrane via molecular diffusion resembling liquid-liquid extraction rather than filtration. A thin layer of a hydrophobic liquid stabilized by the microporous, polymer film contained within the membrane, even though separates two aqueous solutions whose acid concentrations differ by pH units of 10 or more is observed to validate an outstandingly yearlong chemical stability. A classic practice involves separation of limonoids by running in the directions opposite each other the flows of a feed solution containing orange juice (pH 3.2) and a stripping solution consisting of Sodium $Hydroxide_{(aq.)}$ (pH 12-13) on the left and right sides of the membrane respectively, thus, effecting the rapid and irreversible diffusion of limonoids from the acidic to the basic side by dissolving in membrane's hydrophobic liquid. Most importantly this method successfully demonstrated to be uncompromised on retaining the nutritional quality and flavor of the juice along with being substantially economical. Furthermore, the successful research and development of a membrane system would not only offer a practical and commercially viable procedure for the removal of limonoids from all types of citrus juices but the foundation for fabricating other supported-liquid membranes for the selective removal of off-flavors and off-colors in fruit-juice concentrates would also be made available. It is noteworthy to mention that membrane assisted methodology also extends its advantages with proven potential to deliver a means for the removal of polyphenol off colors in natural sweeteners produced from apple or pear concentrates.

iii) Fruit Juice Extraction: The process by which the juice is extracted from the fruit plays a crucial role while determining the overall bitterness. A study [40,18,19] reported lowest bitterness in juice due to a gentle pressing of fruits as compared to untreated counterparts. Also, crushing

seeds along with juicy sac is observed to be contributing in bitterness to juice. A study [41] successfully demonstrated the use of screw type juice extractor to be effective in decreasing the bitterness in juice. Another study [42] supported the amount of bitterness causing components to be minimum in juice when extracted from seedless fruits.

iv) Hot Water Filtration: Many researchers [22,43-45] showed that a variety of fruits when treated with hot water at a certain temperature for a certain time, followed by filtration, are recognized to produce the juice with reduced bitterness. The fruits are then subjected to juice extraction after manual peeling. Immediately post-extraction, the juice is clarified by ultra-filtration through a specific filter press equipped with hollow fiber membranes possessing the specific permeability are capable to retain large sized molecules whereas smaller ones could pass through, thus, retaining the suspended particles and pulp.

2.2. Chemical Methods

i) Lye: Lye treatment has been proven to achieve debittering of juices and hence is widely applied to a variety of fruits. Fruits are treated with a certain concentration of NaOH solution, certain temperature for a certain time span, followed by rinsing with citric acid solution further followed by immediate washing under tap water to remove excess sodium hydroxide [22]. The outer creamy-white part of peeled fruit is observed to react with hydroxyl and carboxylic groups during the lye treatment and as a result the hydrophilic derivatives are removed while washing with water. The concentration of sodium hydroxide contributes as a deciding factor for debittering [46] as it is observed to act efficiently only up to a specific threshold. Another study [47] on kinnow juice ranked lye treatment to be the best among a few other techniques practiced for debittering. Additionally, the removal of white papery segment from kinnow fruits during lye treatment is found to be effective in maximal debittering of juice [48,41].

ii) Florisil: This white colored, odorless compound, chemically known as activated Mg-silicates, is used as an important debittering agent. Florisil with increasing concentration was observed to significantly affect the amount of bitterness causing compounds, (specifically limonin and naringin) in grapefruit juice as per a study [49]. A study [50] on kinnow juice reportedly reduced the amounts of ascorbic acid along with its sugar content.

iii) β-cyclodextrin: The use of β-cyclodextrin is proven to reduce the bitterness from gourd [51]. As per another study [23,52] the limonin content from the tangerine and lime juices were observed to have considerably reduced when they were treated with a certain concentration of β-cyclodextrin solutions at certain temperature for a certain time span.

2.3. Other Methods

Syrup Treatment: Being a well-established fact that bitterness is dependent on the sugar to acid ratio in the juice; it is now known that specific environmental conditions such as temperature, light irradiance along with agricultural practices also significantly affect the sugar content in juice. For example, an increase in temperature during ripening phase is observed to result in decreasing acid level while increasing sugar content and hence, sugar syrups are utilized to balance the bitterness of juice [53-55].

3. Conclusions

A universal plan is being made available to the citrus industry with all these multiple technological approaches to increase the acceptance of citrus products in turn, resulting in not only an improved organoleptic quality but also providing a longer shelf life. The fact that oranges along with various other citrus fruits being consumed are comprised of 2/3rd of total global citrus production itself testifies the reputation of its juice in the worldwide populous. In general, a personal proclivity for the juice based on 'with or without pulp' could pose a marginal nuisance, which being a critical aspect of these various commercially focused scientific projects would soon be preferentially rectified. Nevertheless, an individual not favoring orange juice would be a rare site, adequately proclaiming the monetary triumph of all these technical endeavors. In conclusion, the research, development and implementation of these numerous debittering processes are crucially important considering citrus's ever-expanding demand in the market worldwide.

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