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Enemies of Citrus Fruit Juice: Formation Mechanism and State-of-the-Art Removal Techniques

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Abstract

Citrus fruits are among those commercially grown crops whose importance for immunoregulation has amplified recently in the post-COVID era. Unfortunately, bitterness and off-flavor development in citrus fruit juices is a key concern. Bitterness is predominantly caused by naringin and limonin, while nomilin, hesperidin, etc. are known to be the minor contributors. Although the paper describes the biological properties of these bittering compounds and their potential application, the presence of the same in juices is often not desired. Similarly, the presence of undesirable odors in stored juices is caused by compounds such as guaiacol, ethanol, acetaldehyde, 2-methyl-3-furanthiol, methional, terpinen-4-ol, and dimethyl trisulfide. This review primarily examines the bitter and undesirable flavor compounds found in citrus fruit juices that is created during the process of production and storage. It also explores the specific processes by which these compounds are formed. A range of debittering techniques has been proposed, that involves the addition of sugar, lye, β-cyclodextrin, hot water, adsorption using cellulose acetate and activated carbon, pre-treating the juice with

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sonication, supercritical fluid, enzymes, and microbial consortia. Similarly, strategies for averting the formation of off-flavor-related compounds can include treating the juice with electrical current, high pressure, microwave, ultrasound, and chemical inhibitors. This review is anticipated to guide the Citrus juice manufacturers in choosing one or more (amalgamated) technologies for achieving extended shelf-life.

Introduction

Almost 4,000 years ago, citrus fruits were cultivated[1] and are the world's largest fruit crop. A citrus fruit belongs to the Rutaceae family, whose leaves are usually transparent oil gland and whose flowers contain annular disks.[2] A citrus fruit is thought to have originated in southeastern Asia, and was later transported to the Middle East and Southern Europe, and then spread throughout the world with the assistance of missionaries and travelers who followed the path of civilization.[3] Because citrus fruit juice holds a prominent position, with remarkable demand among consumers around the world, a greater understanding of processing factors impacting the content of its nutritional quality parameter is needed, especially in light of the increasing need for citrus fruits, which is correlated with high vitamin C content as well as antioxidative phenolic compounds and flavanones. The citrus juice industry has several issues, one of which is delayed bitterness, that is defined by the juice gradually developing bitter compounds after it is extracted from the fruit.[4] The bitter taste is caused primarily by its limonoids and flavonoids, particularly limonin and naringin.[5] The application of novel sustainable strategies is critical for the reduction of post-harvest loss. Physical and quality losses resulting from fruits' metabolic changes, especially after harvest are the main causes of damage in citrus fruit.[6] Lye treatment, sugar addition, β-cyclodextrin, hot water method, enzymatic procedures utilizing microbial consortia, and sonication are being evaluated for their effectiveness in reducing citrus fruit bitterness. To mask the bitterness and enhance the flavor of citrus juice, artificial sweeteners, resins (adsorbents) and sequestrant have also been reported. Proposed methods for reducing bitterness involve i) removing bitter compounds, ii) removing obstacles in the physical world like pith, seeds, albedo, etc. iii) The methods employed include the use of scavengers and flavor enhancers like sugar, salt and florisil to mask the bitter aftertaste, enzymatic removal using naringinase and α-L-rhamnosidase, and the alteration of bitterness-producing circuits using genetic engineering techniques. The biology and mechanistic approaches of these postharvest technologies are briefly described herein.

(A) Biochemistry/Structure of Bittering Compound

Citrus fruits are rich in a diverse range of phytonutrients, each with its specific purpose and function.[7] These molecules vary in bitterness depending on the type of glycoside chain they include. The various types of phytonutrients include glucosinolates (organosulfur compounds), triterpenes, flavanones, flavones, flavonols, flavans, isoflavones, and isothiocyanates. Naringin (Flavanones) and limonin (Limonoid aglycones) are the main chemicals in lemon and kinnow that produce bitterness. (Figure 1) represent the chemical structure of important bittering compounds from the Citrus fruit.

The exact section, maturity stage, growth conditions, and species of plant can all affect the type and concentration of chemicals that cause bitterness. Table 1 lists the bitter substances that are present in citrus fruits.

Naringin

An essential disaccharide derivative is naringin (C27H32O14, M.W.: 580.5 g mol-1). The main bitter component found in fruit membranes and albedo, which is soluble in water, is Naringenin's 7-P-neohesperidoside. It is a kind of flavanone glucoside and is extracted into fruit juices.[16] Naringin is known as (2S)-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3 in IUPAC notation. Its concentration depends on how mature the fruit is, with ripened fruits having the lowest concentration. When the fruit ripens, α-L-rhamnosidase hydrolyzes naringin into prunin and L-rhamnose. Prunin has a bitterness that is 33% less than that of naringin, and is subsequently progressively hydrolyzed to create naringenin and D-glucose, as indicated below.

Naringin —α-L-Ramnosidase→ Prunin + Rhamnose

Purins —β-D-Glucosidase→ Naringenin + Glucose

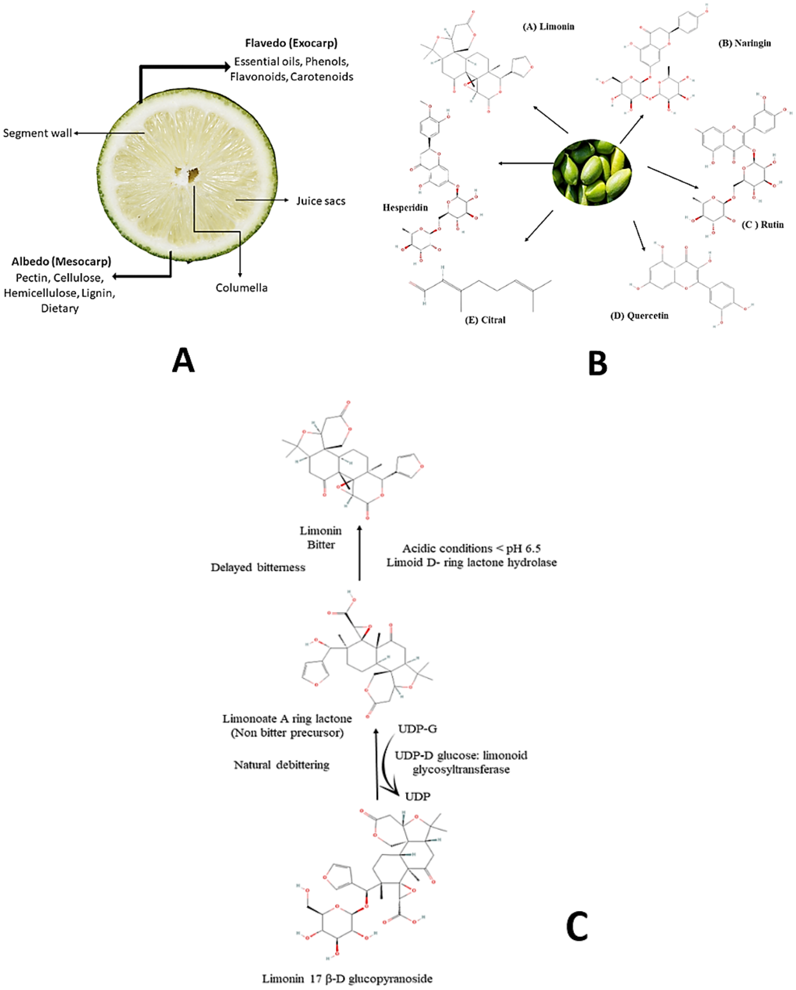


Fig.1: A) Structural anatomy of citrus fruit, B) Chemical structure of important bittering compounds from the Citrus fruit. A) limonin, B) Naringin, C) Rutin, D) Quercetin, E) Citral, F) Hesperidin, C) Mechanism of the Natural Debittering Process and Delayed Bitterness in Citrus Juice

Limonin

Citrus fruits contain limonin, which has a white hue and a molecular weight of $C_{26}H_{30}O_8$ (470.52 g mol-1). The highly oxygenated metabolite limonoid aglycones, which are connected to triterpene derivatives, are the source of limonin. Limonin is known by the IUPAC nomenclature 7,16-Dioxo-7,16-dideoxylimondiol. The Rutaceae and Meliaceae families of fruits contain them.[17] The aglycones and associated glucosides are two categories that make up the limonoids. Limonin, nomilin, limonin glucoside, and nomilinic acid are the components of limonoids.[18] Limonin is soluble in 100% ethanol and glacial acetic acid, but only slightly in water. The tetracyclic triterpenoids include limonin, also referred to as obaculactone and evodin, which is a secondary metabolite with significant biological activity in plants.

Table 1: Principle Bitter compounds in various Citrus fruits

Fruit name	Botanical name	Fruit part	Bitter compound	Pharmacological activities	References
Grapefruit	*Citrus × paradisi*	Flavado, albeno, pith, juice, seeds	Naringin	Oxidative stress-induced diseases	(8)
Orange	*Citrus × sinensis*	Juice Whole fruit	Nobeletin	Positive effects in combating Alzheimer's disease (AD) and Parkinson's disease (PD)	(9) (10) (11)
Lemon	*Citrus limon*	Juice	Limonin	Cancer (pancreatic), Increase	(8)
Tangerine	*Citrus tangerine*	Juice	Limonin	mitochondrial membrane potential, decrease cell proliferation	
Sweet orange	*Citrus × sinensis*	Juice	Narirutin	Antihypertensive, choles terol-lowering, and insulin -stimulating properties	(12)
Sour orange	*Citrus × aurantium*	Juice	Neohesperidin Naringin Narirutin	Antioxidant, anti-infla-mmatory and antia-poptotic effects	(13)
Kinnow	*Citrus nobilis × Citrus deliciosa*	Pulp, juice, peels, residue, seeds	Naringin Limonin	Anti-dyslipidemia, anti-obesity and anti-diabetic and antifibrotic, asthma	(14)
			Limonene Fortunellon and nomilin	Alzheimer's disease (AD) Increase Adriamycin-dependent cell death	(15)

Materials and Methods

Researchers employed a variety of debittering techniques to stop the accumulation of bitter chemicals in citrus fruits as they grew and matured. On the basis of physical, chemical, and biological processes, numerous debittering methods have been created.

Physical Methods

Resins

Resins consist of resin acid ($C_{20}H_{30}O_2$), waxes, and other fatty acids. Commercially, resins are mostly derived from trees belonging to the Pinaceae and Dipterocarpaceae groups. Both non-woody and woody plants have unique surface glands and interior ducts where resin is produced. They are plant metabolic waste products that can be removed through infection or incision.[19] The minerals, acids, and amino acid composition of navel orange juice was unaffected by the processing of resins. Dowex Optipore L285 application causes a fall in titrable acidity in oranges. Amberlite XAD 7HP is used to lessen the bitterness of orange juice.[20] Their findings confirmed the efficiency of Amberlite XAD-7HP treatment as the utilized approach lowers limonin from orange juice by 90–96%. The used physical and chemical treatments for debittering citrus juices are listed in Table 2.

Table 2: Use of Physical and chemical treatments for debittering different Citrus juices

Source	Chemical used	Conc. Used	Temp.	pH	Outcome	References
Pummelo juice	Sodium hydroxide Citric acid	1.25-1.75% 1%	82°C Room temp.	4.05-4.10	An increase in pH reduces the bitterness of juice.	(21)
Pummelo juice	Sodium bicarbonate	-	Room temp.	4.25-4.75	Juice extracted from treated fruit contained considerably less ascorbic acid than juice from untreated samples.	
Tangerine Citrus Reticulata Blanco Juice	β-cyclode -xtrin	5%	30°C	-	Limonin reduced by 80.71%.	(22)
Lime Juice	β- cyclo -dextrin	2%	Ambient temp.	-	Limonene reduced from 23.24 to 19.93 g ml^{-1}.	(23)
Orange Juice	Amberlite XAD-16HP and Dowex Optipore L285	-	(20-50) °C	-	A significant reduction in limonin content (ppm) was seen, resulting in a drop in limonin content (ppm) from 10.67 to 0.06.	(24)
Orange juice	Amberlite XAD-7HP	-	30°C 40°C 50°C 60°C	-	Limonin content (ppm) reduced from 11.4-5.4 ppm, especially at 40°C.	(20)
Grapefruit juice	Naringin -ase or by physical adsorption with Amberlite IRA-400.	-	20°C	-	Physical adsorption with Amberlite_IRA-400 maintain the antioxidant and biomolecule protection capability of freshly squeezed grapefruit juice.	(25)

Adsorptive Debittering

Since limonin and naringin must be removed from citrus juice during debittering, many adsorptive materials have been researched since 1960. Polyamides are effective in selectively adsorbing significant levels of limonin from Washington navel orange juice. With distinct matrices, their combinations, and cellulose acetate, different degrees of debittering have been accomplished, and two porous polymers have demonstrated a strong affinity for limonin. Citrus fruit juices are packaged in cellulose acetate films because they have a decrease potential for naringin. Enzyme immobilization is currently popular since it prolongs the duration of enzymatic activity and makes it easier for enzymes to be used repeatedly. There are numerous approaches to immobilize enzymes, including a) using inert materials for immobilization; b) trapping enzymes inside polymerized gel lattices; c) using versatile compounds to cross-link functional proteins; and d) covalently connecting enzymes to insoluble materials.[8]

Fruit Juice Extraction Methods for Averting Bitterness

The flavorless non-bitter precursor (limonoate A-ring lactone) can be found in the membranes and cytoplasm of fruit juice cells. This precursor is exposed to the acidic environment of the juice during fruit extraction and slowly transforms into bitter limonin.[26] It has become a vital instrument for citrus cultivators globally as the process of extracting juice from its fruit has advanced significantly from the previous laborious process of squeezing to an automated juice-extracting machine. Although modern juice extractors result in a faster extraction of juice from fruits, crushing the seeds and juicy sac during the extraction process may contribute to bitterness in the juice. Bitterness in juice is decreased by manually removing seeds from fruits before juice extraction. There are various types of citrus juicers, including vertical hand presses, hydraulic or pneumatic presses, press bowl type and spinning bowl type, centrifugal beach juicer machines, motorized juice extractor machines, cup hand-held citrus Juicer, dome shape juicers, and screw type. The citrus fruits must first be cut in half down the middle before being placed on the juicer. Some manual citrus juicers include handles that are used to press the fruit and squeeze out the juice, while others have cone-shaped cups that are used to press the fruit until the juice is extracted. It was discovered that the juice obtained by squeezing or peeling fruit and then extracting juice with a soft press in a way that seeds are not crushed is superior to other ways, with the exception of the fact that there is less juice recovery.[27] Sweet orange (Mosambi) and pummelo juice can be extracted using an electrical juicer.

Hot Water Method and Filtration Technique

It is an efficient physical method to reduce the bitterness of citrus fruits. During this treatment fruits are incubated in 50°C water for 20 to 30 minutes, then removed from the water and manually peeled and juiced. By using this treatment pummelo juice reduces TSS, acidity, and ascorbic acid. To prevent bitter components from being introduced, certain machinery and membranes are used, including a filter press and hollow fiber membranes/ultra-filtrations (UF).[21] To improve juice clarity, hollow fiber membranes and ultra-filtrations are used. Hollow fiber membranes block pulp entry and suspended particles. Juice that has been clarified will be further treated chemically and/or physically. Ultra filtration membrane modules have the ability to extend the shelf life of kinnow juice for a period of 60 days without any chemicals.[28]

Ultrasonication Treatment

One of the non-destructive, quick, adaptable, and promising green technologies being applied in the food business is ultrasonication.[29] The process of ultrasound involves compressing and expanding sound waves as they move through a liquid medium. In the liquid or close to the surface of the target material, this results in hydrodynamic cavitation, which includes the formation, expansion, and collapse of countless small vapor bubbles. This process is the foundation for Ultrasound-Assisted Extraction, which causes the solvent to penetrate the cell wall and facilitates mass transfer. Ultrasound in the range of 20–100 kHz is used for extraction process.[30] Ultrasonication has emerged as one such technique to meet consumer demands for fruit juices that not only maintain but also enhance their nutritional value. It is asserted that fruit juice will retain its nutritional value, freshness, and microbiological safety.[29]

The naringin concentration of pomelo juice is significantly impacted by sonication treatment under optimal conditions when combined with debittering agents. The sonication process efficiently increased the efficiency of enzyme hydrolysis and resin's adsorption effectiveness. Additionally, the processing time was cut by 30 minutes. The sonication process significantly increased the hydrolytic activity of rhamnosidase and glucosidase, resulting in decreased levels of bitterness and enhanced activity in pomelo juice. The treatment also had an impact on the bioactive components of juice; resin treatment caused the greatest harm to phytochemicals, whereas the enzymatic method enhanced some activities. Additionally, increased C-O stretch increases the likelihood that the carbon-oxygen bonds in sonicated naringin would shatter, which prompts naringinase to attack the bond. Sugars and organic acids were found to be efficiently lost during ultrasonic-assisted resin debittering. In comparison to resin-treated juice, the volatile components were maintained relatively more in enzyme-treated juice.[31]

Supercritical CO_2 Extraction

Supercritical water is a liquid that maintains itself at a critical the pressure of 1.0-22.1 MPa and critical temperature of 374ºC (above boiling point) without going through any phase changes. Under carefully controlled conditions of temperature and pressure, an average of 25% of the limonin content was reduced in nighty one-hour runs. Vitamin C, acids (such as citric acid) and total amino acid levels in the juice were not affected by supercritical CO_2 extraction. There was, however, a slight reduction in the oil content (e.g.- d-limonene) of the juice. Supercritical carbon dioxide is utilized as a solvent in place of organic solvents because of its fluid-like behavior and gas-like features.

Chemical Method

Lye Method

Citrus juices are subjected to a lye treatment, where they are exposed to sodium hydroxide at a temperature of 82–83°C for a duration of 40–60 seconds, in order to remove bitterness from the fruits. The fruits are thereafter immersed in a solution of citric acid with a specified concentration and subsequently cleansed with flowing water to eliminate any excess sodium hydroxide.[21] A variety of fruits of varying maturities can be treated with lye treatment to prevent debittering. The fruit's exterior, creamy-white portion reacts with hydroxyl and carboxylic acids during the lye treatment. The effects of various methods to thicken kinnow juice. Lye treatment was the best approach for debittering juice among many techniques (florisil, and their combinations).

Florisil

Activated magnesium silicates are the chemical name for the white, odorless substance known as florisil. The concentration of limonin in grapefruit juice is decreased from 8.8 to 1.7 ppm and naringin from 326 ppm to 159 ppm by employing florisil at a 20% concentration.

The percentage of total acid of grapefruit juice was decreased from 0.81 to 0.33%. Florisil influenced the ascorbic acid and lowered the sugar of kinnow juice. It was discovered that the use of florisil decreased the levels of ascorbic acid (18.14 mg/100 g) and lowering sugar (3.53%).[32]

β-Cyclodextrin

According to research investigations, β -cyclodextrin can take the bitterness away from fruit juices. Bitter gourd juice can be thickened using β -cyclodextrin. They use β-cyclodextrin at concentrations of 0.25 to 2%, 1.5% of which were effective in reducing bitterness in bitter gourd. Five percent β-cyclodextrin at 30°C for 60 minutes causes a reduction of 80.71% in the limonin level of tangerine juice.[22] Adding 2% β-cyclodextrin to lime juice33 for 10 minutes reduced the amount of limonin from 23 to 20 g/ml.[29] Orange and grapefruit juice aqueous solutions were used to extract limonin and naringin using a β-cyclodextrin polymer in a batch continuous column mode. Interestingly, the amount of ascorbic acid, total dissolved solids (Brix), and acidity remained unchanged despite the elimination of components such as naringenin, 7-P-rutinoside, coumarins, and flavonoids. Its suitability for scale-up study was enhanced by revitalizing the β-cyclodextrin polymer through extraction with an organic solvent. Consumer priorities for this debittered juice (which, incidentally, had a superior flavor) over the control were indicated by a panel of analysts.[34]

Limitations of Physiochemical Methods

The physiochemical method has several limitations that prevent its implementation on an industrial scale. Juice undergoes some changes in taste, turbidity, aroma, and flavor during physiochemical processing that are not favorable to the consumer's acceptance. Processing results in the loss of some important ingredients when bittering chemicals are removed. Because these techniques are not practical for use on a big scale, researchers are working to create more environmentally friendly and sustainable alternatives for the debittering of fruit juices.

Biological methods

Enzyme Production by Microbial Consortia: Microbial Strains are Continuously
utilized to produce a variety of specialized enzymes, such naringinase and α-L-rhamnosidase for debittering applications. For the production of naringinase the strains specifically used are *A. oryzae, A. foetidus, A. niger, A. flavus, Bacillus sp., Pseudomonas sp., Streptomyces sp, Fusarium solani, Escherichia coli, Aspergillus brasiliensis, Rhizophus Stolonifer, Bacillus cereus, ungal* and bacterial strains frequently utilized for the making of *α-L-rhamnosidase* are *Clavispora lusitaniae, A. niger, Aspergillus ochraceous, A. wentii, A. sydowii, A. foetidus.*[35]

The Substrate for the Synthesis of Enzymes

Around the world, various substrates are being used to screen microbial strains for the synthesis of α-L-rhamnosidase and naringinase. Rice bran, wheat bran, maize cob, orange peel, sugarcane bagasse, soybean hull and rice straw[36] are all regularly utilized substrates for the synthesis of α-L-rhamnosidase. Substrates that were used for the production of naringinase during fermentation were grapefruit rind; grapefruit pericarp powder; and rice bran, wheat bran, sugarcane bagasse, citrus peel, press mud, orange rind, citrus fruit and peel and lemon peel.[35] The enzyme-based method is more cost-effective because enzymes can be immobilized on suitable surfaces, allowing them to be used repeatedly over long periods. The purpose of this screening is to determine whether microorganisms have the capacity to produce particular enzymes for industrial use. According to the substrate, microbial strains, and incubation conditions, different types and numbers of enzymes may be produced. Citrus peel, rice bran, sugarcane bagasse, press mud, wheat bran was only a few of the substrates that Aspergillus niger (MTCC-1344) was able to use to manufacture the naringinase enzyme.

In a study simultaneously debittered and clarified mixture of citrus juice using tannase and poly-galacturonase to increase nutrient levels.[37] It was investigated that maximum enzyme-mediated debittering and clarity were anticipated when Assam lemon, Kachai, and pomelo were combined in the ratio of 45:30:25 based on response surface methodology-based optimization. Various bitter substances in combined citrus fruit juice can also be degraded by an enzyme mixture. Thus, the mixed citrus fruit juice was simultaneously debittered and clarified by tannase and polygalacturonase.

Use of Limonin

It was found that limonate A-ring lactone is converted into tasteless limonin glucosides, such as limonin 17-L-D glucopyranoside. An enzyme called UDP-D-glucose-limonoid glucosyltransferase is responsible for converting glucose to limonoid. This enzyme extracts glucose units from uridine di-phosphoglucose (UDPG) and transfers them to limonoids to produce non-bitter glucosides, which reduce citrus limonoid bitterness.[38]

Limonin D-ring Hydrolase

The hydrolase has catalyzed the reversible lactonization-hydrolysis of limonin's open and closed D-ring lactones and limonoate's A-ring lactone. Hydrolysis activity of both sources of the enzyme occurs at pH 8.0 using both limonin and limonoate A-ring lactone as substrates. The reverse reaction occurred at pH 6.0, i.e., the closing of the D-ring to form the lactone; lactonization. The enzyme did not catalyze the reversible opening and closing of the A-ring lactones of limonoids. The type of reaction catalyzed, i.e., hydrolysis or lactonization, appeared to be pH-dependent. The enzyme also catalyzed the hydrolysis of other limonoid-type compounds such as nomilin, deacetyl- nomilin and ichangin, obacunone, which differ from limonin structurally only in the vicinity of the A- and A^1-rings. The enzyme, however, was inactive towards deoxylimonin and deoxylimonic acid indicating that the double bond at carbons 14 and 15, or removal of the epoxide group, interfered with catalysis.[39]

Limonin D-ring lactone hydrolase had no influence on the equilibrium of the reaction, which was determined largely by the pH of the juice. At citrus juice pH, the equilibrium was towards formation of the lactone, i.e., limonin. This enzyme, therefore, has little significance as a de-bittering enzyme. (Figure 1 C) illustrates mechanism of the natural debittering process and delayed bitterness in Citrus Juice.

Limonoate dehydrogenases -Freshly made juice contained limonoate A-ring lactone, which the dehydrogenase converted to 17-dehydrolimonoate

A-ring lactone, preventing it from turning bitter. However, demonstrating juice debittering using isolated enzymes proved a significant step towards creating a biological method for limonoid bitterness reduction in citrus juices.

Naringinase

Naringinase (EC 3.2.1.40) is a debittering enzyme that is widely used. It is a complex mixture of the enzymes -L-rhamnosidase (EC 3.2.1.40) and -D-glucosidase (EC 3.2.1.21), which, through a two-step process, hydrolyzes the bitter chemical naringin into naringenin (a non-bitter compound)[40] naringinase has also been detected in various bacteria, fungi, and yeasts in addition to plants. Naringinase is used in the fruit juice industry to debitter various citrus fruit liquids and also aids in enhancing the stability and organoleptic qualities of the citrus juices[41] (Figure 2) illustrates the functional principle of naringinase.

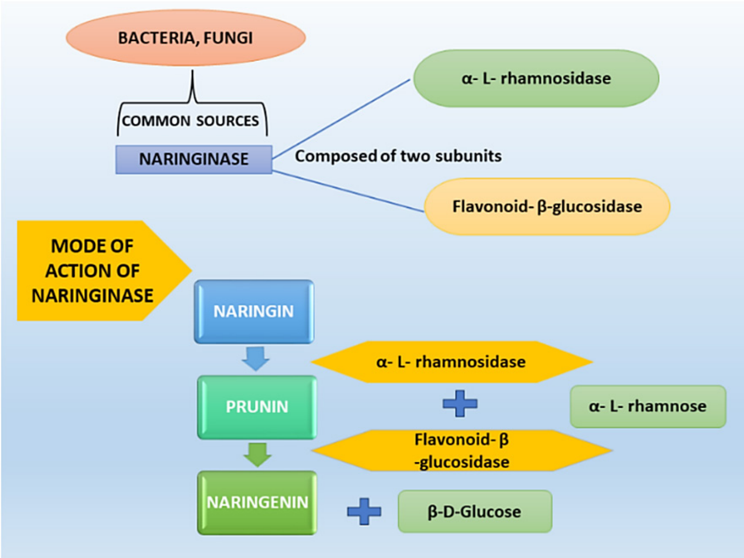


Fig. 2: Mode of action of naringinase

Immobilization of Naringinase

Enzyme immobilization has been demonstrated to be a successful strategy for sustaining the long-term continuous use of enzymes. Trapping enzymes in polymerized gel is one of the numerous methods for immobilizing enzymes that have been reported. Some of the elements that significantly affect enzyme immobilization include temperature, pH, the presence or absence of prosthetic groups, functional groups, etc., as well as the employment of multifunctional reagents to produce cross-linking, covalent bonding, and the utilization of inert material.[35]

In a tubular-type bioreactor, the naringinase enzyme, which was isolated from Penicillium and immobilized on chitin, was discovered to be a reliable way to hydrolyze naringin in fruit juices. Conflicting results were reported for naringinase made from A. niger. As a result, fruit juices' carbohydrates are crucial in serving as the naringinase enzyme's competitive or non-competitive inhibitors. The study reported the optimum values of pH and temperature was value 3.7 and 55°C respectively for the fiber method of enzyme entrapment which was the same as that of its natural form. However, the heat stability of the enzyme was increased.

Immobilized the Penicillium decumbens naringinase enzyme, which was then used to debitter grapefruit juice after being immobilized on chitosan microspheres.[42] The immobilization of naringinase onto chitosan microspheres was shown to be a straightforward and effective process. It may also be reused as a biocatalyst and simplifies the handling of the enzyme. Immobilized enzyme that exhibits peak activity at acidic pH and low reaction temperature is ideal for use in acidic environments, such as citric juice, and there have been no reports of the juice's nutritional content or other qualities degrading as a result. 75% of the naringin was eliminated by naringinase immobilized on chitosan microspheres, bringing the level below the threshold.

On a magnetic polysaccharide framework, Bodakowska-Boczniewicz[42] and Garncarek immobilized the naringinase enzyme in 2020. A. niger was used to isolate the enzyme. Dextran aldehyde was used to cross-link the locust bean gum-based immobilization. According to the study, the immobilized enzyme is very stable at both high temperatures and acidic environments. After being immobilized, the pH optimum for the enzyme (naringinase) activity changed from 4 to 3.5. In addition to heat stability, the immobilized enzyme had a longer half-life than free enzyme. The same enzyme was discovered to have operational stability when immobilized and cross linked with dextran aldehyde, making it preferred for practical applications such as naringin hydrolysis.

Other Methods of Debittering

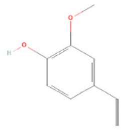
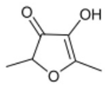
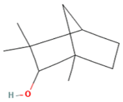
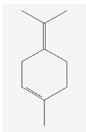
Adding Syrup

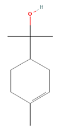
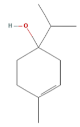
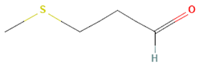
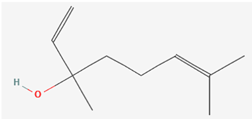
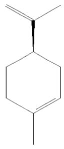
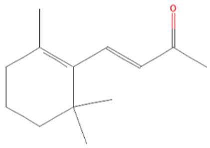
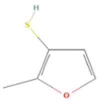
The degree of bitterness in kinnow juice is determined by the acidity and sugar content of the juice sacs. The amount of sugar in the juice sacs of the kinnow fruit is strongly impacted by the temperature, amount of light, and methods of cultivation. As the kinnow fruit ripened throughout the higher temperature led in a decline in acid content and a rise in sugar content.[43] The total soluble solids (TSS) concentration of kinnow pulp can range from 9.5-16%. Sugar syrups are applied to alleviate the bitterness. The amount of bitterness producing chemicals in kagzi lime juice has decreased by 60.38% and 39.76%, respectively.[33]

Off-flavor Development in Citrus Juice after various Processing and Storage

Mandarin juice, grapefruit juice, and orange juice are among the most popular citrus juices due to their alluring aroma. When citrus juice is processed and stored, off-flavor compounds are formed and aroma-active components are reduced. More than 300 volatile fragrance molecules originate in citrus fruit have been found to exhibit substantial aroma-active properties.[44] The odor active value (OAV) is an essential metric for evaluating both flavor dilute (FD) components and aroma-active compounds. Seventy aroma-active components of citrus juice were statistically evaluated and found, with odor active value ≥1 suggesting that a single odorant contributed to the overall aroma. These components included aldehydes, ketones, alcohols, esters, terpenes, and others. According to the screening criteria, aroma-active compounds were evaluated based on their FD factor or OAV greater than or equal to 1.[45] Table 3 listed the off-flavor markers identified in Citrus juice.

Table 3: Off-Flavor Markers found in Citrus juice

Off flavor marker compound	Odor	Structure
p-vinyl guaiacol	smokey, burnt	
4-Hydroxy-2,5-dimethyl-3(2H) -furanone (DMHF)	heated sugar, caramel	
β-fenchyl alcohol	cleaner, soil	
α-terpinolene	old orange	

α-terpineol	musty, spicy, woody, citrusy, herbaceous	
terpinen-4-ol	sweaty, citrus	
Methional	bad, green, potato	
Linalool	cleaner, fruity, candy, floral, citrusy, woody, green, sweet	
Limonene	cleaner, lemon, mint, citrusy, lemony, orange, fresh, sweet	
β-ionone	floral, woody	
2-methyl-3-furanthiol	unpleasant odor	

Citrus juice loses most of its aroma-active ingredients and forms off-flavor compounds, which leads to the development of off-flavor in the juice.[46] The most essential concern during citrus juice preparation and storage are the change in aroma. Off-flavor compounds were also present in citrus juices that were impacted by temperature, light, oxygen, and physiological factors (fruit maturity, packaging, diseases, and centrifugal preparation for the hazy orange juice debittering process).

Light-Induced Off-Flavor

The "sunlight"-flavored chemical methanethiol is produced when Strecker degradation of methionine is triggered by light. The principal off-flavor compounds present in citrus fruits are four volatile Sulphur Compound VSCs (methanethiol, dimethyl sulphide, dimethyl trisulfide, and methional).[47]

Physiological Factors causing Off-Flavor

The aroma quality of citrus juices will also be strongly impacted by citrus fruit ripeness, fruit disease and other processing techniques (such as centrifugation, filtering, and the debittering process). The ripening stage of the mandarin fruit had an impact on the volatiles that were glycosidic bound.[48] Eleven fragrance components dramatically varied in citrus juice especially orange during maturity, with practically all detected aldehydes peaking at complete ripening as opposed to the majority of terpenes in juice extracts, which peaked at earlier ripening stages.[49]

Off-Flavor Causing Compound of Citrus Fruit Juice

Fruit juice that has been aged has a markedly different aroma than juice that has freshly been squeezed.

A juice exposed to processing and unrefrigerated storage for long periods of time tends to have cooked and heated type notes. The volatiles that contribute to "fresh" and "green" notes are greatly reduced in processed and aged juices.[50] The process of processing and storing citrus juice causes flavor changes, which weaken the aroma and produce off-flavors. Different degradation mechanisms have been implicated in the off-flavor formation. A variety of precursors (aldehydes, ketones, alcohols, and esters) are involved in degradation of carbohydrates, vitamins, and carotenoids, aromatic terpenes, sulfur-containing amino acids.[51]

Results and Discussion

Degradation of Carotenoids and Apocarotenoids

Degradation of carotenoids may be the cause of the undesirable flavor in citrus juice. Geranial and neral, two isomeric monoterpene aldehydes that combine to form citral, were found in higher concentrations in frozen concentrate orange juices. Processed orange juice contains higher quantities of citral.[52] Citral can be produced by oxidising the unsaturated double bond in β-cryptoxanthin, as was further established by researchers when they explored the link between fragrance compounds and carotenoids.53 Additionally, prior studies have shown that the handling and storing of citrus juice results in the degradation of carotenoids, which is linked to the fact that carotenoids contain several unsaturated conjugated double bonds that are liable to break.[50] Orange juice at full maturity contained significantly fewer odor-active volatiles, namely nurosoprenoids degraded from carotenoids. According to these results, these odor-active volatiles significantly affect the juice aroma profile, which is strongly affected by the mutation responsible for color differences.[53]

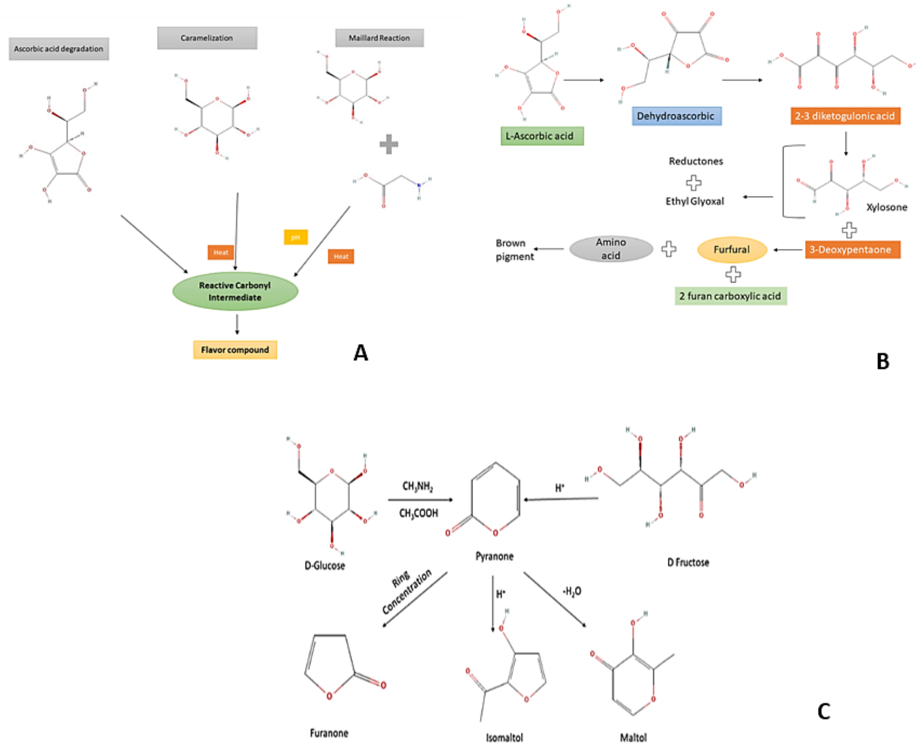


Fig. 3: A) The three nonenzymatic browning routes. Ascorbic acid degradation, caramelization, and the Maillard reaction, B) The mechanism of ascorbic acid degradation, C) Carbohydrate degradation to form furaneol

Degradation of Carbohydrates

One of the most significant chemical reaction pathways that leads to the deterioration of juice products' quality during storage is non-enzymatic browning. This process can be broadly described as the breakdown of sugars, ascorbic acid, and other carbonyl compounds that react with nitrogen-containing molecules to give off-colors and tastes. The following are the three non-enzymatic browning pathways: 1) Ascorbic acid degradation, 2) The Maillard reaction, which occurs when an amine component (such an amino acid) combines with a reducing sugar during heat processing and storage 3) Only reducing sugars are utilized in caramelization, which is recommended at high temperatures (above 150 °C). (Figure 3A) provides a quick schematic of the three non-enzymatic browning routes and their interaction.

Degradation of Ascorbic Acid

(Figure 3 B) illustrates a representative ascorbic acid breakdown reaction process. In an acidic environment, native ascorbic acid easily undergoes oxidation to produce dehydroascorbic acid (DHA) i.e., extremely unstable and responsive in aqueous solutions. Furfural and its derivatives are important markers of fruit juice quality that are produced when dehydroascorbic acid (DHA) interacts with sugars in anaerobic conditions.[46] It should be noted that ascorbic acid is also used commercially to prevent browning since it can lower pH and function as a radical scavenger. Ascorbic acid is well known for avoiding enzymatic browning in fruits and other items because it inhibits polyphenol oxidase. Ascorbic acid is converted to DHA when utilised to prevent the synthesis of possible degradative chemicals, which encourages the pathways that lead to browning and flavor development. Numerous storage experiments have shown that ascorbic acid increases the color development of the juice during long-term storage.

Research on furfural as an indicator of both off-flavor and off-color development is considerable. Furfural is a byproduct of the acid-catalyzed breakdown of carbohydrates; it can also combine with other ingredients in juice to form compounds such as 5-methyl furfural, furfuryl alcohol, and 5-hydroxymethyl furfural. Koca claims that sugars have a very minor role in the production of furfural and 5-HMF, with ascorbic acid degradation being the only chemical mechanism responsible for their formation.

Maillard Reaction

There are multiple pathways involved in the development of off-flavors in any food system. The bitter taste of furaneol causes it to be used in aged orange juice as an unpleasant flavoring agent. Furaneol is known as 2,5-dimethyl-4-hydroxy-3-(2H)-furanone. The caramel odor found in many processed foods is also caused by it. Reducing carbohydrates is an essential component of the Maillard reaction. By weight, the simple sugars fructose, sucrose, and glucose make up the majority of the sugar content in orange and apple juice, with traces of rhamnose.[54] However, under acidic conditions, sucrose hydrolyzes into glucose and fructose, which is indirectly related to Maillard browning. Rhamnose level of orange juice is steady when compared to fresh juice, indicating that other, more common reducing sugars are a major factor in the deterioration of off flavors.

The Strecker degradation and Maillard reaction in citrus juice result in the production of potent undesirable compounds known as furans and furanone. Recent research suggests that the chemical reactions that took place during the preparation and preservation of citrus juice produce these furans and furanone. Furaneol is mostly created when reducing sugars are broken down by the caramelization and Maillard processes, which serve as a marker for the off flavors in orange juice. On the other hand, furanone was produced when carbohydrates were broken down.[50] (Figure 3C) illustrates carbohydrate degradation to form furaneol.

Terpene Degradation

In citrus juice, terpenes commonly make up more than 90% of all odor substances.[55] These are the primary aroma compound in citrus juice. α -terpineol, which is currently employed as a marker for fruit juice quality, is one of the main off-flavor chemicals produced via terpene breakdown processes. It is understood that this substance is created through the non-oxidative terpene breakdown process, often from the precursor limonene and linalool. In order to create α-terpineol, limonene, the most prevalent terpene in orange juice, is degraded. α-terpineol can also be produced by the degradation of linalool, a significant terpene alcohol and character-impacting component of fresh orange juice. Due to the synthesis of α-terpineol and the loss of linalool, the sensory perception of the substance is altered. (Figure 4A) depicts a reaction mechanism showing the synthesis of α-terpineol from limonene and linalool.

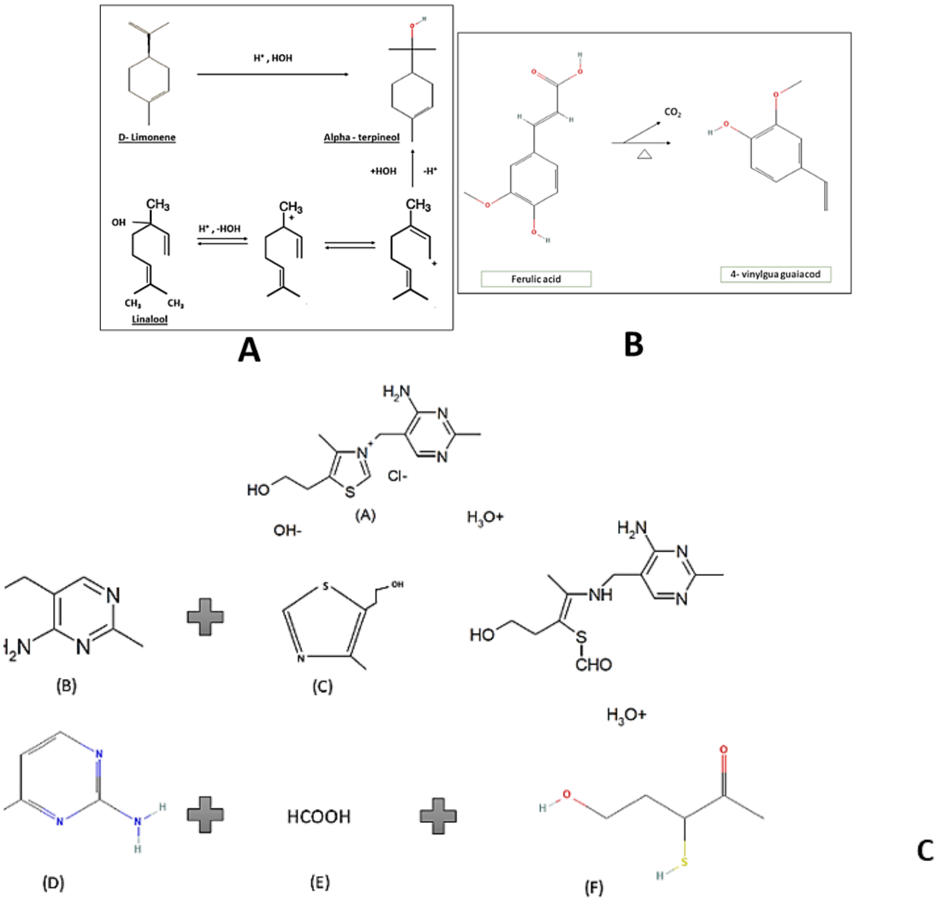


Fig.4: A) Mechanism of reaction demonstrating how limonene and linalool combine to generate α-terpineol, (B) Degradation of ferulic acid to p-vinylguaiacol, C) The mechanism of thiamin (Vitamin B1) degradation pathways. A=thiamin hydrochloride, B = pyrimidine moiety, C = thiazoles moiety, D = diaminopyrimidine, E = formic acid, and F = 5-hydroxy-3-mercapto-2-pentanone

Degradation of Sulfur-Containing Amino Acids

Citrus juice processing and storage resulted in the production of a few volatile sulfur compounds (VSCs), including mercaptans, thioethers, disulfides, polysulfides, and other sulphides. These compounds may significantly contribute to the cooked, sulfurous, meaty smell of juice. VSCs are typically present in the juice, peel, and oil of tropical citrus fruits, which contribute to the pleasant aroma.[56]

However, the off-flavor in grapefruit juice, orange juice, and mandarin juice that was created during processing and storage was caused by a number of volatile sulfurous compounds, including the dimethyl sulphide, 2-methyl-3-furanthiol, methional, methanethiol, and the dimethyl trisulfide. The precursor of mesityl oxide or the cysteine or glutathione conjugate are two potential mechanisms for 4-mercapto-4-methylpentan-2-one synthesis. Several researchers have recently investigated how VSCs contribute to off-flavors in other thermally processed juices.[57]

Phenolic Acids and Tannin Deterioration

A free form of ferulic acid is transformed into guaiacol, vanillin, 4-ethyl guaiacol, and 4-vinyl guaiacol either thermally or enzymatically. In citrus juice, guaiacol and 4-vinyl guaiacol have often been found as off-flavors. Alicyclobacillus species create the majority of guaiacol; nevertheless, the precise method by which microorganisms produce guaiacol is still unknown. The fresh orange juice's 4-vinyl guaiacol concentration increased after four days at 37°C.[58] The strongest fragrance component produced during storage period of heat induced orange juice is said to be p-vinyl guaiacol (cooked, nutty). (Figure 4B) showed degradation of ferulic acid to p-vinyl guaiacol.[58]

Thiamin Degradation Pathway

Thiamine undergoes heat degradation when a hydroxyl ion breaks the C-N link between the pyrimidine and thiazole molecules. After degradation, the thiazole moiety (III) generates other highly aromatic thiazoles such as 4,5-dimethylthiazole (roasted pork) and 4-methylthiazole (green hazelnut). When thiazole ring in thiamin hydrochloride is hydrolyzed, 5-hydroxy-3-mercapto-2-pentanone (VI), an aroma intermediate, is produced. (Figure 4C) demonstrates the method of thiamin (Vitamin B1) degradation routes.[46]

Changes in other Aroma Compounds

Different studies have discovered seven odor-active esters, such as methyl butanoate, ethyl acetate, and ethyl butanoate. The addition of fruity and flowery overtones by ethyl-2-methylpropanoate, thyl-2-methylbutanoate, ethyl octanoate, and ethyl hexanoate. After concentrated processing, the concentration of essential fragrance molecules decreases, which causes a significant loss of aroma. One penten-3-one, a crucial fragrance component, was necessary for the NFC orange juice's aroma but it was decreased after storage.[59]

Degradation of Essential Oil (Terpenes and Limonoids)

Citral, α and β-pinene, limonene, and γ-terpinene are the five main components of lemon oil. It has been demonstrated that these reactions, which take place in the presence of copper catalysts and air, cause substantial oxidation of α- and β-pinene and γ-terpinene. Lemon oil has a high concentration of unsaturated and oxygen-functionalized terpenes, making it very susceptible to oxidation. Temperature, ultraviolet radiation, and metal traces acting as catalysts have all been proven to have an impact on oxidation. Citral, together with D-limonene, c-terpinene, and α - and β -pinenes, are the primary contributors to the flavor and odor of lemon oil and lemon essence. P-cymene can be created by the oxidation of limonene and γ-terpinene, as well as by the acid-catalyzed cyclization and dehydration of citral. P-cymene is a key component of off-flavors.[46]

Monoterpenes including limonene, linalool, and 4-terpineol are major contributors to the volatile components of orange peel oil and juice. These monoterpenes may go through hydration-dehydration processes via carbocation intermediates when exposed to an acidic pH and high temperatures.[60] Linalool and limonene, two compounds found in citrus juices, have previously been found to be thermally unstable.60 Ten damaged molecules in canned orange juice were discovered after being stored at 35°C for 12 weeks; three of these molecules, α-terpineol, 4-vinyl guaiacol, and furaneol, were found to have foul odors. When 2.5 ppm (the off-flavor threshold level) of α-terpineol is added to freshly squeezed orange juice, a stale, musty, or piney aroma may be perceived. Despite the fact that linalool degradation was seen under all circumstances, the production of α-terpineol was only seen between 35 and 45°C. Additionally, the model juice containing 1 g/L limonene demonstrated higher α-terpineol accumulation for at least 4 weeks of storage at all three temperatures. Additionally, it was found that at 35 °C, the production of α-terpineol from linalool was quicker than the formation of limonene. The pH of buffer solutions at 4 and 35ºC for 4 weeks had a significant impact on its production from both substrates. The buildup of α-terpineol was greatest at an acidic pH (exactly 2.8), supporting an acid-catalyzed hydration process.

Non-Conventional and Non-Thermal Technologies to Remove off Flavor of Citrus Juice

In comparison to traditional debittering methods non-conventional technologies and non-thermal processing techniques such as microwave heating, ohmic heating, pulsed electric field, ultrasound and supercritical water extraction have a great impact on inactivating enzymes, microorganisms and reducing off-flavor.[61]

Microwave processing - Electrical treatments such as microwave heating involve emitting electromagnetic waves through a magnetron and guiding them through space to a target. A microwave is an electromagnetic wave with a frequency ranging from 300 MHz to 300 GHz. Microwaves in industrial settings are typically operated at frequencies ranging between 915 MHz and 2.45 GHz, (Figure 10A) represent a schematic diagram of microwave processing.[62] The MWH technology has several advantages, such as reducing processing time, ensuring good process control, and saving space. Electromagnetic energy maximizes and retains food quality rather than conventional heating. As microwaves can achieve high processing temperatures in shorter periods of time, they can be used to process fruit and vegetable juices more efficiently. It retains nutritional properties as well as sensory properties.

Ohmic Heating

The aroma compounds in ohmic heat-treated juice are higher than in traditional pasteurized orange juice, which has a shorter sensory shelf life. This technology has a number of advantages: it reduces surface fouling, which occurs when the product is overheated, it has low maintenance costs and high energy conversion efficiency and it maintains a higher nutritional value in food products as well as reducing off flavor. Ohmic heating works well in fruit and vegetable juices that contain a lot of water and ionic salts.[63] This type of product can be processed using OH for uniform and rapid heating, which is highly effective for the reduction of microbial organisms and for the inactivation of enzymes, with a useful effect on the nutritional and sensory properties of the product. Furthermore, Ohmic heating is more energy-efficient, less expensive, and quicker to use than conventional thermal methods. A further benefit is the fact that 97 percent of the electricity provided is converted into heat.[64] (Figure 10B) represent the schematic diagram of ohmic heating.

High-Pressure Processing

High-pressure processing (HPP) minimizes the loss of nutrients and fragrances while inactivating microbes and enzymes.[65] Several studies have demonstrated that HPP is superior to other methods in preserving the nutritional value and aroma of fruit juice and purée. Because of its low processing temperature, high pressure processing not only has limited effects on covalent bonds but also on tiny molecules such as volatile chemicals, pigments, vitamins, and antioxidants.[66] This resulted in the commercial acceptance of this procedure as a technique to increase the shelf life of juices and make products of excellent quality. Sensory tests revealed that the mango juice treated with HPP at 600 MPa for 5 minutes at 25°C was more similar to fresh than the pasteurized sample,[67] even though the content of B myrcene, d-limonene, and 4-carene in the HPP-treated juice was much lower than that in the fresh juice. A schematic illustration of high-pressure processing is presented in (Figure 10C).

Pulsed Electric Field (PEF)

Pulsed electric field (PEF) is one of the non-thermal technologies that has been investigated the most. It is used to inactivate microorganisms and preserve the organoleptic and nutritional properties of fruit and vegetable juices. The Pulsed electric field method is shown in Figure 10D. In this procedure, a product is sandwiched between two electrodes and exposed to brief bursts of high-intensity electrical field (generally between 10 and 40 kV/cm) (normally 5–30 μs). Thermally treated orange juice contained a larger amount of undesirable volatile chemicals than PEF-treated orange juice, including decanal. The PEF-treated orange juice had a less prominent unpleasant cooked orange aroma and a stronger fresh orange aroma as compared to autoclaved orange juice.[68]

Ultrasound Processing

The aroma concentration of pasteurized orange juice (43.97 μg/g) was lower than that of mandarin juice processed with ultrasonication (19 kHz for 36 min at 50 °C).[69] Additionally, following ultrasound-activated enzyme treatment, limonene and α-pinene dramatically increased and notes that were green, citrus, flowery, and woody like decreased by 21%, 13%, 11%, and 25%. Supercritical CO_2 with ultrasonic assistance allows for the production of high-quality orange juice while preserving its fresh-tasting organoleptic properties.[71] The orange liquids with ultrasonication had as close to fresh juice-like sensory characteristics as feasible.[72] (Figure 10E) represents the process of ultrasonication.

Supercritical Carbon Dioxide

Supercritical carbon dioxide (SC-CO_2) or dense phase carbon dioxide (DPCD) is one of the non-thermal methods for inactivating microorganisms and enzymes in liquid food, such as fresh juices. Food is treated for five to thirty minutes at low temperature (20 to 50°C) and moderate pressure (below 50 MPa) with either sub-critical or supercritical (i.e., pressurized) CO_2.[73] All of the following are some of the many benefits of CO_2: it is safe, non-flammable, non-corrosive, and has a low critical temperature that makes it possible to create non-thermal processes. It also lessens the impact on the nutritional and sensory qualities of food. Numerous studies have been conducted to determine how well SC-CO_2 processing preserves juices, including mango, tomato, orange, apple, guava, and melon. The technique for producing supercritical carbon dioxide is shown in (Figure 10F)

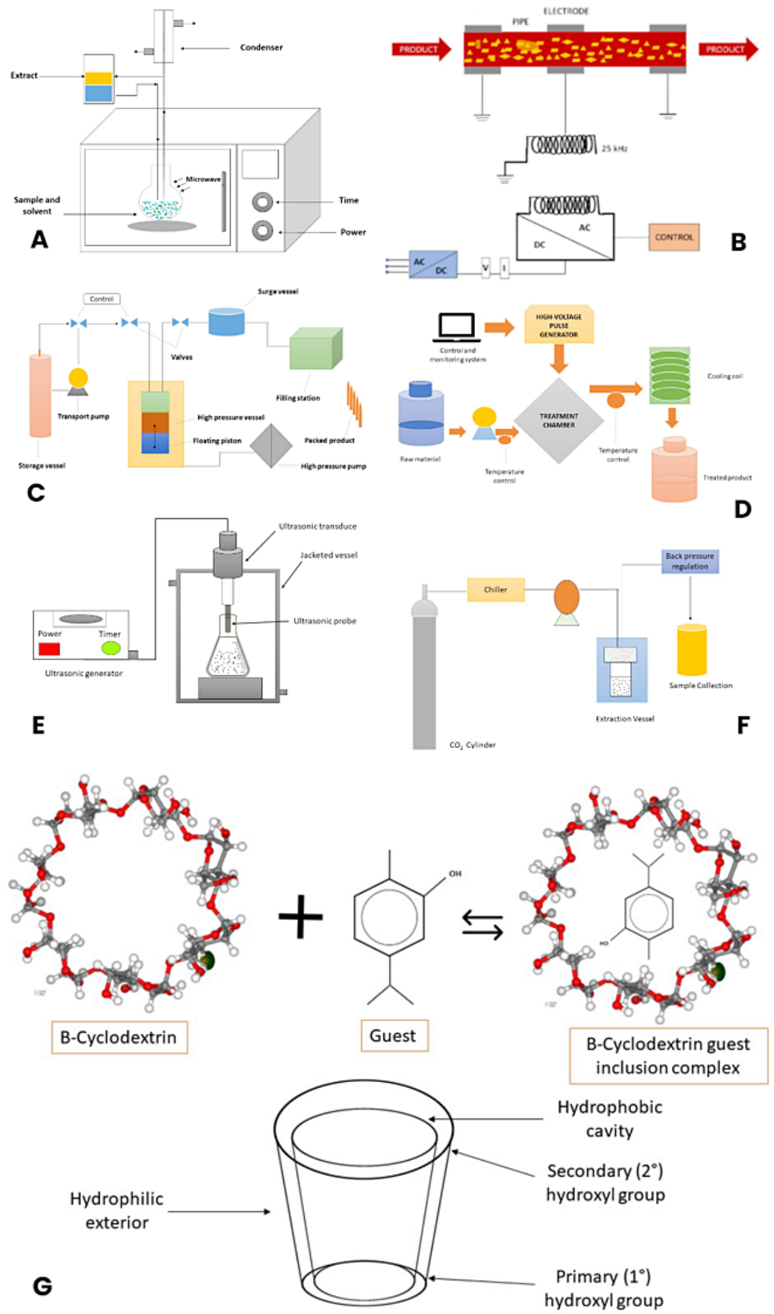


Fig. 5: Schematic representation of A) Microwave processing B) Ohmic heating C) High pressure processing D) Pulse Electric Field E) Ultrasonication equipment F) Supercritical water extraction G) β-Cyclodextrin (the association of β-CD and guest molecule to form β- cyclodextrin inclusion complex

Chemical Treatment Techniques

A hydrophobic cavity and a hydrophilic outer surface make up the distinctive molecular structure of cyclodextrins. Because of the structural flexibility of cyclodextrin, it can create inclusion complexes with a variety of molecules, including bitter substances, (Figure 10G) represent the association of β-CD and guest molecule to form β- cyclodextrin inclusion complex. Cyclodextrins can combine with the bitter components, including limonin and naringin, in citrus juice to cause debittering. Cyclodextrins have a hydrophobic chamber that can encapsulate bitter chemicals, effectively limiting their interaction with taste receptors on the tongue and lowering the feeling of bitterness.[74]

Conclusion

The findings of this study indicate that the use of advanced techniques such as ultrasonication, microwave, supercritical water extraction, pulse electric field, etc under optimum conditions and in combination with debittering agents, significantly impacts the naringin concentration of citrus juice. Green technologies enhance the adsorption efficiency of resin or other adsorptive materials and significantly boost the hydrolysis efficiency of the enzyme and reduced the processing time.

"Green consumerism," or the preservation of food using "friendly compounds" is the newest trend in food technology. Studies in the literature have demonstrated that flavonoids and other bioactive substances are crucial for giving citrus extracts their biological properties. Citrus extracts are powerful antibacterial, anticancer, anti-inflammatory, antidiabetic, cardioprotective, and neuroprotective agents, as shown by the research reviewed. Citrus extracts have been utilized to minimize contamination and stop yeast deterioration in various food products. Citrus extract has been demonstrated to be a natural food additive for extending the shelf life of several fruits. Based on scientific studies on the removal of the bitter compounds from juice, it has been shown that screw-type juice extractors extract juice with less bitter components more effectively than other methods. Cost-effective, simple, dependable, and user-friendly approaches are becoming increasingly popular these days.

Industrial Significance

Citrus pulp and seeds are mostly to blame for the bitterness of juice, which reduces the shelf life and market acceptance of juice.

Enhancing the effectiveness of removing bitterness and decreasing the duration of treatment using novel technology has the potential to enhance the cost-effectiveness of the process. Using modern technology for debittering citrus juice can effectively and quickly eliminate the unpleasant bitter taste caused by naringin, hence enhancing the overall quality and customer acceptance of the juice. An intriguing and challenging method of reducing bitterness in fruit juice, apart from chemical treatments that are costly to implement on an industrial scale, is to harness the potential of genetic engineering techniques. The bitterness in juice issue may be resolved via transgenic plants. The synthesis of limonin and naringin in citrus fruits may be stopped by making the appropriate alterations to the genome at particular sites.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval

Informed Consent Statement

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

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

- **Jyotismita Konwar:** collected literature, compiled information and drafted original manuscript.
- **Mamoni Das:** surveyed literature and revised the Tables.
- **Moloya Gogoi:** surveyed literature and revised the references and manuscript.
- **Pranjal Kumar Kaman:** revised the manuscript and corrected the language.
- **Soumitra Goswami:** supervised the draft and surveyed literature. J.S. (Jadav Sarma) and
- **Purnima Pathak:** surveyed literature.
- **Manashi Das Purkayastha:** conceived the idea, wrote and revised/edited the manuscript and received the research grant. All authors read and approved the final manuscript.

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