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A Review of Phytochemical Composition, Conservation Strategies, and Food and Therapeutic Applications of Citrus Species

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Abstract

Citrus species, one of the most economically and therapeutically significant fruit groups, exhibits a diverse phytochemical composition that contributes to their extensive medicinal and industrial applications. This review synthesizes current knowledge on the phytochemistry, pharmacological properties, and functional roles of *Citrus* species in the food and nutraceutical industries. The presence of flavonoids, carotenoids, and essential oils underlies their antioxidant, antimicrobial, and anti-inflammatory effects. However, despite their wide utility, the genetic diversity of *Citrus* species is increasingly threatened by environmental changes, habitat loss, and emerging diseases. While numerous studies have explored their bioactive compounds, gaps remain in the experimental validation of their medicinal potential and the development of effective conservation strategies. This review underscores the necessity for integrative approaches that combine phytochemical research, sustainable agricultural practices, and genetic conservation efforts to ensure the long-term viability and utility of *Citrus* species.

Keywords: *Citrus*, Phytochemical, Conservation, Food Industry, Genetic Diversity

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Introduction

The fruits belonging to the genus *Citrus* are widely grown and considered as important fruit crops. According to the United States Department of Agriculture (USDA), the global orange production for 2021/2022 was estimated up to 1.8 million tons. As the climatic conditions have a direct impact on the harvest, *Citrus* is mostly cultivated in tropical and subtropical areas (Dinesh and Reddy, 2012). *Citrus* species belongs to the subfamily Aurantioideae of the plant family Rutaceae (Barsha et al., 2021). The subfamily is divided into subtribes Clauseneae (5 genera) and Citreae (28 genera). The Citreae tribe consists of three subtribes: Triphasiinae, Balsamocitrinae, and Citrinae. The Citrinae tribe is divided into 3 groups. The “true Citrus” is grouped into six genera (*Citrus*, *Clymenia*, *Eremocitrus*, *Fortunella*, *Microcitrus*, and *Poncirus*). But the recent findings by Bayer et al. (2009) suggested that even though subfamily Aurantioideae is a monophyletic group under the family Rutaceae, the two subtribes Clauseneae and Citrinae are not monophyletic indicating timely revisions are necessary for the traditional classification system.

The native range of the *Citrus* extends throughout the area of West Pakistan to north-central China, in the south across the East Indian Archipelago to New Guinea and the Bismarck Archipelago, northeastern Australia, New Caledonia, Melanesia and the western Polynesian islands. A study by Wu et al. (2018), which analyzed 60 diverse citrus species, revealed that Citrus underwent a rapid evolutionary radiation in Southeast Asia during the late Miocene epoch. This event was correlated with a noticeable decline in the monsoons. In the early Pliocene, the *Citrus* migrated across the Wallace line to Australia which gave rise to the Australian limes. This marks the second radiation of Citrus. There are three basic species of Citrus as *Citrus maxima* (pummelo), *Citrus reticulata* (mandarin), and *Citrus medica* (citron) according to the biochemical data presented by Scora (1975). The modern varieties of citrus (orange, lime, lemon, and grapefruit) are the results of recurrent hybridization among the ancestral species. Representative morphological features of the fruits and leaves of several citrus species are given in Figure 1.

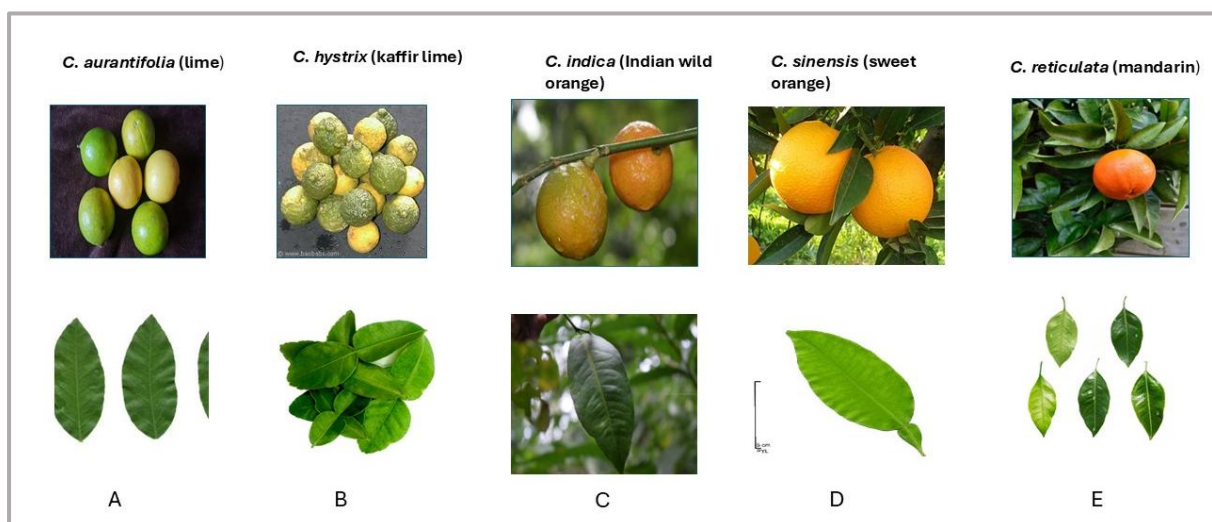


Figure 1. Morphological features of selected Citrus species (A) *C. aurantifolia* (lime), (B) *C. hystrix* (kaffir lime), (C) *C. indica* (Indian wild orange), (D) *C. sinensis* (sweet orange), and (E) *C. reticulata* (mandarin). Each panel shows representative ripe fruits (top) and leaves (bottom).

Citrus is produced globally in more than 140 countries. The world's leading citrus producers include China, Brazil, the U.S.A., India, Mexico, and Spain, which together account for two-thirds of global production (United States Department of Agriculture [USDA], 2020). The FAO reported that in 2019, mainland China was the highest global producer of citrus, with about 37 million tons (FAO, 2019). Citrus fruits contain many secondary metabolites such as alkaloids, flavonoids, limonoids, coumarins, carotenoids, phenolic compounds together with essential oils (Lv et al., 2015). When the nutritional value of Citrus fruits is considered, they are a valuable source of vitamin C and other macronutrients. There are also many micronutrients available including potassium, Vitamin B complex, phosphorus, magnesium, copper, and Vitamin E (Abu and Maruf, 2021).

Current research on citrus mainly focuses on understanding citrus genomics. More specifically, how the evolution and domestication of citrus can be explained using genomics (Gonzalez-Ibeas et al., 2021). This review will focus on identifying the chemical compositions and the applications of Citrus species in medicinal uses, and food industry.

Polyphenol profiles of selected Citrus species

The chemical composition of the genus *Citrus* includes several major classes of phytochemicals such as polyphenols, alkaloids, coumarins, and carotenoids. Among these, polyphenols represent the most abundant and widely studied class. To provide a comprehensive overview, **Table 1** presents different subclasses of polyphenols found in various *Citrus* species, alongside examples of the species in which they are most abundant. It also outlines their respective phytochemical categories. **Figure 2** complements this table by illustrating the general chemical structures of the main polyphenolic subclasses found in *Citrus* species.

The Table 1 summarizes reported concentrations of key flavonoids in citrus fruits (per 100 g) and the analytical methods used. Both fresh weight (FW) and dry weight (DW) are given when available; the tissue (e.g. whole fruit, peel, juice) is noted.

Table 1: Polyphenolic compounds found in Citrus species

No.	Class	Compound	Citrus Species (common name)	Quantity mg/100 g (FW/DW; tissue/part)	Method	References
1	Flavanone aglycone	Hesperetin	<i>C. sinensis</i> (orange)	11.95 (FW; juice)	HPLC–DAD	Chen et al. (2020); USDA (2013)
			<i>C. latifolia</i> (lime)	43.00 (FW; juice)		
		Naringenin	<i>C. sinensis</i> (orange)	2.14 (FW; juice)	HPLC–DAD	USDA (2013)
			<i>C. latifolia</i> (lime)	0.38 (FW; juice)		
		Eriodictyol	<i>C. sinensis</i> (orange)	0.17 (juice)	HPLC–DAD	USDA (2013)

			<i>C. limon</i> (lemon)	21.36 (juice)		
		Neohesperidin	<i>C. bergamia</i>	≈36.1– 82.4 (Juice)	–	–
		Didymin (isosakuranetin-7-O-rutinoside)	<i>C. aurantium</i> (bitter orange)	85.25 (DW;pulp)	UPLC–PDA	Chen et al. (2020)
2	Flavanone O-glycoside	Hesperidin (hesperetin-7-O-rutinoside)	<i>C. sinensis</i> (orange)	205.20 (FW; peel)	Ultrasound extraction + HPLC (UAE-HPLC)	Khan et al. (2010)
		Naringin (naringenin-7-O-neohesperidoside)	<i>C. sinensis</i> (orange)	70.30 (FW; peel)	Ultrasound extraction + HPLC (UAE-HPLC)	Khan et al. (2010)
3	Flavone - C-glycosides	Vicenin-2	<i>C. bergamia</i>	44–55 mg/L (Juice)	HPLC-DAD / LC-MS (reported as juice concentrations)	Barreca et al. (2020)
4	Flavone aglycone	Luteolin	<i>C. limon</i> (lemon)	1.90 (FW; juice)	HPLC–DAD	USDA (2013)
		Kaempferol	<i>C. limon</i> (lemon)	0.03 (FW; juice)	HPLC–DAD	USDA (2013)
5	Flavonol aglycone	Quercetin	<i>C. limon</i> (lemon)	1.14 (FW; juice)	HPLC–DAD	USDA (2013)
5	Flavone glycoside	Diosmin (diosmetin-7-O-rutinoside)	<i>C. aurantium</i> (bitter orange)	. ~189 (DW; pulp)	UPLC–PDA (Chen et al.)	Chen et al. (2020)
		Nobiletin	<i>C. reticulata</i> (mandarin)	1137.0 (DW; peel)	UPLC–PDA	Chen et al. (2020)
		Tangeretin	<i>C. reticulata</i> (mandarin)	536.75 (DW; peel)	UPLC–PDA	Chen et al. (2020)
6	Polymethoxyflavone	5-Hydroxy-6,7,8,3',4'-pentamethoxyflavone	<i>C. reticulata</i> (mandarin)	211.38 (DW; peel)	UPLC–PDA	Chen et al. (2020)
		Sinensetin	<i>C. reticulata</i> (mandarin)	297.99 (DW; peel)	UPLC–PDA	Chen et al. (2020)

Data reported as fresh weight (FW) and/ or dry weight (DW).

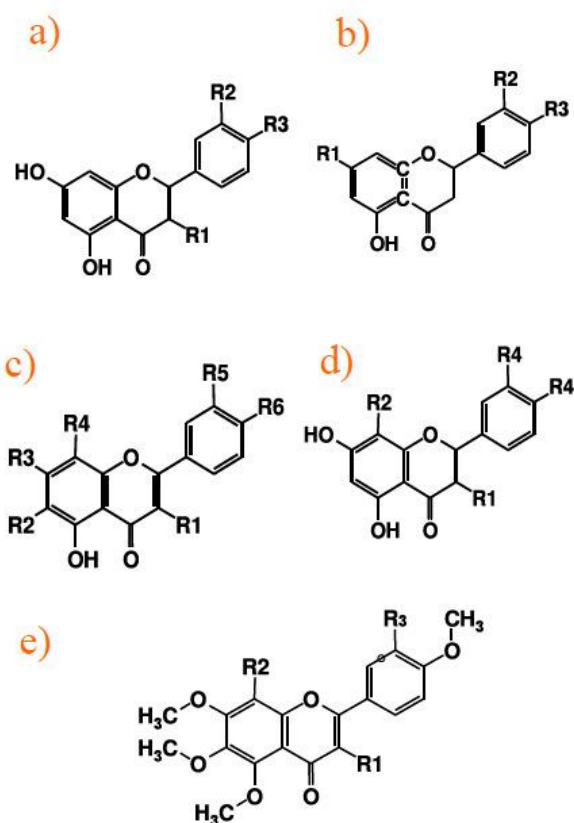


Figure 2. General chemical structures of the polyphenols found in Citrus species. (a) Flavanone aglycones, (b) Flavanone-O-glycosides, (c) Flavone aglycones, (d) Flavone-C-glycosides and Flavone-O-glycosides, (e) Polymethoxyflavones.

This table presents a comprehensive list of polyphenolic compounds identified across various Citrus species, categorized into major flavonoid classes including flavanone aglycones, flavanone O-glycosides, flavone aglycones, flavone-C/O-glycosides and polymethoxyflavones. The data are compiled from multiple analytical studies employing HPLC-DAD or HPLC-MS, following hydroalcoholic extraction from citrus peel, juice, or whole fruit. Flavanones such as hesperetin, naringenin, and their glycosylated derivatives (e.g., hesperidin, naringin, narirutin) are predominant in *C. sinensis*, *C. reticulata*, and *C. paradisi*. Species-specific markers such as naringin in grapefruit (*C. paradisi*) and nobiletin in sweet orange (*C. sinensis*) are highlighted. Unique compounds like vicenin-2 and fortunellin are especially characteristic of *C. japonica* and other less common citrus varieties. Glycosylation and methoxylation patterns contribute to the structural diversity observed. The consistent use of analytical methodologies across studies allows for reliable interspecies comparisons. Overall, the data underscore the rich polyphenolic composition of citrus fruits and highlight notable species-level differences relevant to nutritional and taxonomical evaluations.

Ethnobotanical, medicinal and historical uses of Citrus

The citrus fruits have been a valuable component of herbal medicine systems globally, and their uses abound in Nigeria, Indonesia, China, and elsewhere. The fruits are both nutritional and medicinal, exploiting their abundant phytochemical constituents to combat a wide range of diseases. In the Benue, Kaduna, and Cross River states of Nigeria, sweet orange (*C. sinensis*) is

used in the control of hypertension, diabetes, malaria, and typhoid fever. The dominant therapeutic components are bark and leaves, which are given through decoctions or infusions (Esan et al., 2024). The Agatu community utilizes some of the citrus species, bitter and sour—to treat cancer, inflammation, skin ailments, and gastrointestinal disorders. Special preparations are derived from fruit peels or pulp extracts, which may be used topically or ingested (Olotu et al., 2022). Citrus species remain central to global ethnomedicine, with current research verifying conventional uses through phytochemical and clinical studies. Their varied roles—from staples as foods to disease management tools, affirm the continued continuity between cultural tradition and natural resource use. Aside from being a popular fruit, the most prized use of Citrus species is their medicinal applications. Table 2 describes the recorded ethnobotanical, traditional and medicinal uses of Citrus species from around the world.

Table 2: Comparative Phytochemical Profiles and Bioactivities of Major Citrus Species

Citrus species	Tissue/Part	Therapeutic Uses (Mechanistic Explanation)	Key References
<i>C. aurantifolia</i> (lime)	Juice / whole fruit	antioxidant (vitamin C and flavonoids scavenge ROS)	(Liu et al., 2022)
		anti-inflammatory (flavonoids inhibit TNF- α /NF- κ B signaling)	
		antibacterial (limonene/citral in oil disrupt microbial membranes)	
		cardiovascular support (vitamin C and citrus flavonoids may enhance NO bioavailability).	
<i>C. aurantium</i> (bitter orange)	Peel (selected cultivars)	anti-obesity (protoalkaloid p-synephrine stimulates β -adrenergic thermogenesis/lipolysis)	(Chen et al., 2020)
		antimicrobial and antioxidative (peel limonene and flavonoids damage microbes and neutralize free radicals)	
		digestive aid (essential oils aid digestion)	
		anxiolytic (volatile oils like linalool act on CNS GABA receptors; hypothesized sedative effect).	
<i>C. hystrix</i> (kaffir lime)	Peel	antioxidant (phenolics scavenge radicals)	(Lubinska-Szczygeł et al., 2023)
		antimicrobial/anti-inflammatory (leaf/peel oils contain citronellal, limonene – damage bacterial membranes and inhibit COX enzymes)	
		skin/oral health (antimicrobial, wound-healing via vitamin C and volatile oils)	

<i>C. indica</i> (Indian wild orange)	Fruit / peel	antioxidant and antimicrobial (rich in limonene and other terpenes disrupting microbes)	(Benedetto et al., 2023)
		digestive/respiratory remedy (volatile oils are carminative and expectorant. Mechanistically, flavonoids would reduce gut inflammation and support mucosal immunity reducing IL-6/TNF- α)	
<i>C. limon</i> (lemon)	Peel	strong antioxidant (high vitamin C and eriocitrin/hesperidin scavenge ROS)	(Magalhães et al., 2023)
		anti-inflammatory (inhibits NF- κ B and COX-2)	
		antimicrobial (limonene/citral oil disrupts pathogen membranes)	
		cardioprotective (flavanones boost NO and endothelial function)	
		anti-diabetic (flavonoids increase insulin secretion and AMPK activity);	
<i>C. medica</i> (citron)	Peel / pulp	skin benefits (vitamin C is cofactor for collagen synthesis, flavonoids protect from UV)	(Dadwal et al., 2022)
		anti-inflammatory (flavonoids reduce prostaglandin and cytokine production)	
		antioxidant (high vitamin C, limonene, suppress free radicals)	
		antiviral (essential oils may disrupt viral envelopes)	
		wound healing (vitamin C enhances collagen crosslinking and NO signaling for repair)	
<i>C. sinensis</i> (sweet orange)	Peel / pulp / juice	antioxidant and anti-inflammatory (flavanones hesperidin/naringin scavenge ROS and inhibit TNF- α /NF- κ B)	(Chen et al., 2020)
		antibacterial (juice and peel oils kill pathogens via membrane damage)	
		neuroprotective (reducing oxidative stress, modulating cholinesterase)	
		antidiabetic (enhances insulin release, GLUT4 expression)	
		cardiovascular benefits (hesperidin boosts NO, lowers cholesterol via CETP inhibition)	

<i>C. reticulata</i> (mandarin)	Peel / pulp / juice	Antioxidant and anti-inflammatory (rich in nobiletin/tangeretin, which inhibit COX-2 and NF-κB)	(Chen et al., 2020)
		antimicrobial (peel limonene kills bacteria)	
		improves lipid metabolism (flavonoids downregulate ACAT2/MTP and upregulate LDL receptors, lowering LDL)	
		skin protective (flavonoids suppress UV-induced damage).	
<i>C. japonica</i> (kumquat)	Whole fruit / peel	antioxidant (flavonoids and citric acid scavenge ROS)	(Lou et al., 2015; Lou, 2016)
		antibacterial (peel oils similar to other citrus)	
		anti-inflammatory (reduces cytokines)	
		anti-cancer (polymethoxylated flavones like tangeretin induce cancer cell apoptosis via p53-dependent mitochondrial and Fas/FasL pathways)	
<i>C. junos</i> (yuzu)	Whole fruit / juice	antioxidant and anti-inflammatory (rich in hesperidin, naringenin – boost NO and inhibit proinflammatory cytokines)	(Yoo et al., 2004)
		cardiovascular protection (improves endothelial function via NO)	
		antibacterial (peel limonene oil disrupts microbes)	
		improves circulation.	
<i>C. australasica</i> , <i>C. garrawayi</i> , <i>C. mitis</i> (Australian, calamondin)	Peel / whole fruit	antioxidant (high polyphenol content scavenges free radicals)	(Lou & Ho., 2017)
		antibacterial and anti-inflammatory (peel oils contain compounds like limonene that disrupt pathogens and flavonoids that reduce IL-6/TNF-α)	
		antidiabetic (polymethoxyflavones modulate glucose uptake)	
		immune support (reduce oxidative stress in immune cells)	

Food Industry applications of *Citrus* species

Health benefits associated with bioactive compounds found in *Citrus* species are among the main reasons the citrus-based food industry is expanding. *Citrus* fruits are well-known rich sources of natural bioactive compounds which promote health. As discussed, the main citrus phytochemicals include carotenoids, vitamins, organic acids, dietary fiber, and essential oils,

which have promising biological activities due to their free radical-scavenging, anti-inflammatory, and anti-cancer properties (Liu *et al.*, 2022). This biochemical richness positions citrus fruits as valuable ingredients in developing functional foods and natural additives.

Functional food ingredient

The use of citrus as a functional food ingredient primarily stems from its high antioxidant and dietary fiber content. Powdered citrus fibres are increasingly incorporated in various food matrices such as bakery, meat, confectionery, and dairy products to improve their nutritional and textural qualities. As an example, according to research the addition of normal or shear-activated citrus fiber to gluten-free bread formulations enhances hydration and improves breadcrumb texture. Citrus fiber increases the size of breadcrumb alveolus and reduces staling by making the bread more cohesive, elastic, and resilient, particularly in formulations based on starches like corn and tapioca (Bugarín and Gómez, 2023).

Similarly, fat replacement studies reveal that fortification with 50% debittered orange fiber achieves a 55% fat reduction (from 10% to 4.5%) in bakery products while improving stability and nutritional properties (Caggia et al., 2020).

Apart from their utilization in bakery products, citrus fibers demonstrate substantial functional capabilities throughout diverse segments of the food industry. When integrated into meat products, these fibers have been found to augment water retention, enhance textural qualities, and aid in fat reduction, thus facilitating the creation of healthier formulations. In dairy applications, including yogurt and cheese, the incorporation of citrus fibers results in increased viscosity, improved product stability, and a favorable impact on the overall nutritional profile. Similarly, in confectionery foods, citrus fibers function as good fat replacers in addition to their roles as texture and shelf-life extenders. The multifunctionality of these properties highlights the versatility of citrus fibers as valuable ingredients to produce nutritionally enhanced and texture-modified foods across a range of food categories. Regarding the application of citrus extracts as food additives, the research explored the use of citrus peel extracts to maintain the oxidative stability of meatballs during frozen storage. The study revealed that natural antioxidant extracts derived from citrus peels effectively control lipid oxidation in meat products by inhibiting the enzymatic reactions that cause oxidative damage (Nishad et al., 2019).

Another study by Romero-Lopez et al, developed muffins incorporating different proportions of dietary-fiber-rich orange bagasse. It is found that prepared muffins (with 15% extract) had a high dietary fiber (15.3%) and low fat (15%) content compared to the control muffins.

Food colorant

There is a trend in the food industry to replace synthetic food colorants with natural colorants due to concerns over the harmful effects to consumers, such as allergenicity, hyperactivity in children, and carcinogenicity. Citrus fruit peel is an excellent source of carotenoids which not only impart orange-yellow hues, but these compounds also promote health benefits.

Barman et al. (2023) extracted β -carotene from orange peel waste and developed a stable nano emulsion to be used as a natural colorant in food products. Their study showed that adding this nano emulsion to fruit juice significantly enhances its color, providing a viable alternative to synthetic orange-yellow colorants. Nano emulsions have been reported to reduce the limitations of natural colorants, such as low water solubility, low stability during processing and storage. Similarly, another study has optimized the ultrasound-assisted extraction process of total carotenoids from mandarin peel and demonstrated its potential to reduce the use of tartrazine which is a dye that is widely used in bakery products like cakes and bread, suggesting its further application as a natural coloring agent. (Ordóñez-Santos et al., 2021). Despite such positive developments, some obstacles remain ahead of the widespread industrial application of colorants from citrus peels. The extraction processes, especially those involving emerging technologies like ultrasound or nanoemulsification, tend to necessitate considerable investment and energy consumption, resulting in elevated production costs in relation to synthetic dyes. Furthermore, natural pigments may also exhibit variation in pigment content based on fruit variety, production season, and extraction efficiency, affecting batch-to-batch consistency and color uniformity in final food products. Stability to different food processing and storage conditions such as pH fluctuations, heat and lights conditions are another critical area that requires optimization of formulation.

Furthermore, regulatory routes to market for novel natural colorants can be complex and lengthy, with the risk of delayed market entry. Consumer acceptance, otherwise favorable to natural ingredients, can also be influenced by changes in sensory characteristics such as taste, texture and color.

Briefly, although citrus-peel-derived carotenoids are promising multifunctional natural colorants with health-promoting added value, research in the future should emphasize the development of cost-effective and scalable extraction techniques, pigment stability improvement, and extensive safety and sensory testing. The inclusion of these aspects will be paramount to overcome

challenges such as the high cost of using natural colorants in industrial food applications.

Flavoring agent

Citrus essential oils can be used as natural flavoring agents, which have become a better alternative to carcinogenic synthetic flavors used in the food industry. Essential oils are extracted from the peel of citrus fruit which is a rich source of terpenoids. Bergamot is a citrus fruit scientifically known as *Citrus bergamia*, and it is a hybrid of bitter orange (*Citrus aurantium*) and lemon. It is well known for its aromatic oil, which is extracted from the rind of the fruit and widely used in flavoring (such as in Earl Grey tea). It is also a rich source of linalool and linalyl acetate, both of which have promising flavor. It is used as a flavoring agent in some flour-based confectionery in recipes to replace bergamot peels. The main challenge involved in natural citrus flavor is the flavor degradation that leads to reduced intensity and the development of undesirable off-flavors, which reduce their market potential (Wedamulla et al., 2022).

Thickening agent

A thickening agent is a food additive that increases a liquid's viscosity without significantly altering its other properties. Edible thickeners are mostly used in sauces, soups, and puddings without altering their taste. Citrus peel is an excellent source of pectin which is a commonly used thickening agent in the food industry (Cui et al., 2021; Gavahian et al., 2021). A study extracted pectin from both white and red grapefruit peels and found that the gel-forming properties of the extracted pectin were comparable to those of commercial pectin (Mohamed.,2016). Correspondingly, another research group developed ice cream using frozen Kinnow peel, both blanched and unblanched, at concentrations of 1%, 3%, and 5%. The inclusion of Kinnow peel improved the appearance, flavor, and overall acceptability of the ice cream samples. Based on sensory evaluation, the optimal levels of frozen Kinnow peel were identified as 3% for unblanched and 5% for blanched.

Zhang et al. (2018) extracted pectin enriched with rhamnogalacturonan-I from citrus peel and found that it had superior thickening properties compared to commercial pectin.

Food packaging

Recently, citrus processing waste has been utilized in creating food packaging films because of its antioxidant and antimicrobial properties. Food packaging films can be made using citrus processing waste in various forms, such as directly utilizing citrus peel powder or indirectly using active compounds extracted from the waste, including pectin, essential oils, and seed extracts. This packaging film is used as a preservation technique in different industries such as aquatic products, baked food, fruits, vegetables and meats (Yun and Liu.,2022). Few studies have explored the use of films containing citrus peel powder. A study has developed an active packaging film using orange peel and pomegranate peel powders, which were then used as the primary packaging of white bread samples. It is found that the bread with packaging film exhibited lower weight loss, and a reduced total viable bacterial count compared to unwrapped samples, due to the water vapor barrier and antimicrobial properties of the film (Venkatesh and Sutariya., 2022). Another study developed a film with antioxidant and antimicrobial properties by incorporating pomelo peel powder, sodium alginate, and tea polyphenol. When applied to soybean oil stored at 50°C for 30 days, the films effectively slowed the increase in peroxide value, attributed to their strong oxygen barrier and antioxidant capabilities (Wu et al.,2019).

As described earlier, citrus pectin is a structural polysaccharide constituting 20–30% of citrus peel dry weight and is commonly extracted from citrus peels. Due to its excellent gelling and film-forming capacities, it has been widely used in food packaging films. These films are often developed using solvent-casting methods (Yun and Liu, 2022). To enhance the properties of these films, they are sometimes combined with plasticizers such as glycerol, sorbitol, and polyglycerol. Additionally, more versatile citrus-based composite active packaging has been developed by blending citrus pectin with other polymers, such as agar, alginate, chitosan, gelatin, and starch. According to recent developments, composite packaging films made from citrus pectin and sodium alginate, enhanced with varying concentrations of pterostilbene (PTE), demonstrated good mechanical properties. However, the addition of PTE reduced the antioxidant properties but improved moisture resistance due to changes in the microstructure of the film. The films also

exhibited reduced water solubility and increased thermal stability after calcium chloride cross-linking.

The annual global industrial citrus waste production is estimated at 120 million tons where the peels constitute 40-50% of the total fruit mass. Therefore, application of pectin and powder from citrus peels as thickening agents in food preparation and in food packaging presents attractive solutions to the global environmental impact of citrus peel waste as well.

Conservation of wild Citrus

The use of natural products such as medicine or green medicine is slightly increasing in the world due to their safety, cultural value, and lesser side effects. Wild and endemic citrus plants are at the top of the list of these frequently used medicinal plants due to their remarkable medicinal value and availability of many chemical compounds which are already the basis of certain medicines (Sharma et al., 2020). The over usage of wild and endemic citrus plants for medicinal or any other anthropogenic activity can harm their existence as usually these plants are found only with small population sizes distributed within a limited area (Borah et al., 2018). Other threats to the citrus populations are illegal human interactions like logging, extensive firewood collection, and encroachment. Some Citrus species are included in the endangered category, enabling their conservation an urgent requirement that can protect the existing genetic diversity and promote the further cultivation of the species (Sharma et al., 2020). The conservation of citrus species can be carried out using either *in situ* or *ex situ* methods, with *in situ* conservation being the preferred approach. As wild Citrus are found in very small population sizes, loss of population at some localities can cause the immediate loss of genetic diversity. A study on *C. hongheensis*, a wild citrus found in the Yunnan region of China, found a deficiency in heterozygotes among the populations, indicating inbreeding. Therefore, when discussing conservation, *in situ* conservation to maintain high outcrossing and other historically significant processes are important. The preservation of populations *in situ* would be beneficial, as it may help maintain the genetic structure of the species (Yang et al., 2010). Habitat fragmentation, combined with small population size, is a major factor affecting genetic diversity and should be considered when discussing conservation strategies for a species. Interpopulation gene flow can be accelerated by the growth of isolated populations, which may result from human activities that facilitate dispersal. Transplantation also can be a good option, but it can have some risks like altering the genetic composition which results in decreased fitness. Therefore, it is not recommended to use it as the first option. In small and fragmented populations, the genetic variations may be insufficient to match certain environmental adaptations and to avoid outcrossing depression. So that the artificially propagated plants from local seed sources can be a good alternative with increased fitness to reintroduce into existing populations to increase the effective population size (Yang et al., 2010).

Through biotechnological approaches, many modern conservation methods are available for Citrus. The tissue culture of Citrus species can be used to mass propagate. It can also overcome the infertility of Citrus due to many fungal infections such as *Fusarium* and *Sclerotium*. The usual methods for Citrus propagation are layering, budding, or cutting which is limited to the time period when buds are available but micropropagation can be done regardless of the season. As Citrus is considered a difficult-to-root crop, micropropagation enables to obtain of a mass number of plants in a limited space under controlled conditions throughout the year (Usman, Muhammad,

& Fatima, 2005). Biotechnological approaches like genetic engineering, haploid induction, and somaclonal variations can improve the traits *in vitro* conditions. All these methods have paved the way to obtain high-quality and disease-free planting stock for breeders and farmers. Plant cell culture can be used to obtain transgenic plants in the future. Cryopreservation and the slow growth *in vitro* storage can be used as alternatives to the field gene banks to provide secure germplasm collection for future generations (Sharma et al., 2020).

Conclusion

The Citrus species contain many valuable phytochemicals with medicinal and other uses in addition to being a delicious and popular fruit. The medicinal uses should be investigated more to have a proper understanding and to be applied in pharmaceutical development. The population size of wild Citrus is relatively low when compared with the cultivated ones implying a potential lowering of genetic diversity. Therefore, urgent measures should be taken to conserve the endangered species of the genus Citrus.

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Conflict of interest statement

The authors declare no conflict of interest.

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