Citrus R&T (e-ISSN 2236-3122)
Citrus Research & Technology, v. 38, n. 1, 2017
http://dx.doi.org/10.4322/crt.ICC063

Revisão/Review

Beyond vitamin C: the diverse, complex health-promoting properties of citrus fruits

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SUMMARY

Humans discovered citrus fruit centuries ago and today consider citrus among the most-healthy foods in the human diet. In addition to the carbohydrates, citrus fruit contains various health beneficial components such as carotenoids, vitamin C, and polyphenols, including flavonoids and anthocyanins. Additionally, citrus fruits contain biologically active compounds such as mono- and triterpenoids, coumarins, alkaloids, phytosterols, pectin, and polymethoxy flavones. Whole fruit contains these secondary metabolites at microgram to nanogram levels and processed juice has lower levels. However, despite their lower concentrations, these compounds can prevent several acute and chronic diseases, possibly via synergistic or cumulative effects with regular consumption. Accumulating evidence shows that citrus consumption reduces the risk of cardiovascular disease and obesity, and can promote weight loss and prevent infections. During the last three decades, further research has revealed additional benefits of citrus-derived compounds, such as inhibition of tumor growth due to their anti-inflammatory and anti-angiogenic properties. Interestingly, compounds found in non-edible parts of the citrus fruit, such as peels and pulp, have stronger activity against different cancers. Certain compounds, including obacunone, apigenin, synephrine, hesperidin, and pectin have shown activity parallel to existing natural cytotoxic compounds. Our group has conducted extensive research in chemical fingerprinting of different citrus fruits, isolating and separating active molecules, and exploring their health benefits using molecular, animal, and human studies. In the current article, we discuss characterization and isolation of key phytochemicals and explore the major biological benefits of using these molecules, ranging from cellular to systemic effects.

Index terms: *Citrus*, bioactive molecules, flavonoids, health benefits, limonoids.

Além da vitamina C: as propriedades diversas e complexas promotoras da saúde presentes nos citros

RESUMO

Os seres humanos descobriram os citros há séculos e hoje os consideram entre os alimentos mais saudáveis para sua dieta. Além dos carboidratos, os citros contêm vários componentes benéficos para a saúde, como carotenoides, vitamina C e polifenois, incluindo flavonoides e antocianinas. Além disso, as frutas cítricas contém compostos biologicamente ativos, tais como

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mono e triterpenoides, cumarinas, alcaloides, fitoesterois, pectina e polimetoxi-flavonas. A fruta inteira contém estes metabólitos secundários em microgramas ou nanograma e o suco processado tem níveis mais baixos. No entanto, apesar das suas concentrações mais baixas, estes compostos podem prevenir várias doenças agudas e crônicas, possivelmente através de efeitos sinérgicos ou cumulativos com o consumo regular. Diversas evidências mostram que o consumo de citros reduz o risco de doenças cardiovasculares e obesidade e pode promover a perda de peso e prevenir infecções. Durante as últimas três décadas, pesquisas adicionais revelaram benefícios adicionais de compostos derivados de citros, como a inibição do crescimento tumoral devido às propriedades antiinflamatórias e anti-angiogênicas. Curiosamente, os compostos encontrados em partes não comestíveis das frutas cítricas, como cascas e celulose, têm uma atividade mais forte contra diferentes tipos de câncer. Certos compostos, incluindo obacunona, apigenina, sinefrina, hesperidina e pectina mostraram atividade paralela aos compostos citotóxicos naturais existentes. Nosso grupo realizou extensas pesquisas em impressão química de diferentes frutas cítricas, isolando e separando moléculas ativas e explorando seus benefícios para a saúde usando estudos moleculares, animais e humanos. No artigo atual, discutimos a caracterização e isolamento de fitoquímicos chave e exploramos os principais benefícios biológicos do uso dessas moléculas, variando de efeitos celulares a sistêmicos.

Termos de indexação: *Citrus*, moléculas bioativas, flavonoides, benefícios para a saúde, limonoids.

HISTORICAL PERSPECTIVE OF CITRUS AND HEALTH

Some researchers believe that citrus is native to the subtropical and tropical areas of Asia, originating in certain parts of Southeast Asia including China, India, and the Malay Archipelago (Carr, 2017). The existing literature and ancient evidence indicates that the first wild ancestors of oranges and lemons evolved in Australia and New Guinea, where the first people probably began eating citrus around 3000 BCE (Carr, 2017). Citron fruit was consumed by people in China during the Stone Age and reached India and other Asian countries from China. Citron fruit may have reached China from other countries by floating in the ocean, or people on boats may have brought fruits to other places (Vikram et al., 2015).

According to ancient publications in China, the earliest reference to citrus was documented during the reign of Ta Yu (around 2205 to 2197 BCE) when citrus fruits (mandarins and pummelos) were highly prized and were only available for the imperial court. Lemon was originally grown in India whereas sweet oranges and mandarins are indigenous to China. Recent research suggests that some commercial species such as oranges, mandarins, and lemons originally came from Southeast Asia, but the true origins of citrus fruit are Australia, New Caledonia (off eastern Australia), and New Guinea.

An Egyptian tomb painting from 1000 BCE depicts citron fruit, suggesting a possible migration from Asian countries. These citrons were not fleshy; people consumed the rind and used the fruit mainly for perfumes. Indian medical doctors reported that citrus could cure scurvy (vitamin C deficiency) and other sickness. Citrons reached ancient Greece and Rome not much later; Theophrastus described the fruit in 310 BCE and the Roman writers Virgil and Pliny called citrons mala Medica, “Persian apples” (Carr, 2017).

Interestingly, citron is not the ancestor of the oranges and lemons we eat today. Current citrus fruits came from pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*). Chinese or Indian gardeners bred the pomelo and the mandarin sometime before 314 BCE to obtain new fruits such as the bitter orange (*Citrus aurantium*) and the sweet orange (*Citrus sinensis*). Indians used bitter orange, which they named naranga trees, (Sanskrit for ‘an orange tree’), to prepare pickles. These oranges spread west along the Silk Road and reached West Asia by the time of Ibn Sina (who used it in a recipe), reaching Europe in the Middle Ages, where people used citrus to make marmalade, and North Africa, where Albertus Magnus mentioned bitter oranges around 1250 ACE. Table 1 depicts major milestones in our discovery of citrus and its health benefits.

Saladin’s medical doctor, Maimonides, wrote about medical uses of lemon in the 1100s, and after that people began to eat lemons in southern Europe and North Africa. Jewish people in Egypt combined lemons with another new food, sugar, to make lemonade. By 1450 CE, farmers began to grow lemons around Genoa, in Italy. Sweet oranges probably grew only in India and China until near the end of the Middle Ages. Islamic traders brought Indian sweet oranges to East Africa, and they eventually brought sweet oranges to Genoa. People were growing oranges as well as sugar in the Canary Islands in the Atlantic Ocean before the end of the 1400s. When Portuguese explorers

Table 1. Major milestones in citrus research from discovery to super food

Year/ period of discovery	Major finding	References
3000 BCE	Report on discovery of orange and lime by mankind.	
1000 BCE	Painting of citron in an Egypt tomb.	
310 BCE	Citron use mentioned in Greece.	
314 BCE	Pumelo and mandarin were bred in India and China.	
1250	Bitter orange mentioned by the medieval writer Albertus Magnus.	(Carr, 2017)
1100	First mention of medicinal use of citrus.	
1450	People started cultivating lemon in Italy.	(De Candolle, 1885)
1493	Citrus introduced into Caribbean islands.	(McClure, 1982)
1500–1600	Citrus introduced into South America.	
1700	Citrus introduced into South Africa and Australia.	(Scora, 1975)
1753	First report on application of citrus fruits in health (prevention of scurvy).	(Bartholomew, 2002)
1932	Dr. Szent-Gyorgyi discovers ascorbic acid (vitamin C).	(Naidu, 2003)
1938	Limonin first identified in oranges by Higby.	(Higby, 1938)
1828	Hesperidin first isolated by Leberton from citrus.	(Higby, 1941)
1849	Limonene first described by Deville.	(Croteau, 1998)
1857	Naringin discovered by De Vry in all the tissues of *Citrus decumana*.	(Poore, 1934)
2000	Effect of limonoids on prevention of colon cancer demonstrated in animal models.	(Tanaka et al., 2001)

began sailing to India and China, they brought back better varieties, and oranges became very fashionable in Europe in the late 1500s. In northern Europe, where it was too cold for oranges, very rich people in the 1600s built special glass greenhouses to grow oranges.

Spanish and Portuguese explorers introduced citrus to the Americas, and orchards first appeared in Florida and California around 1655 and 1769, respectively. In the 18th century, lime became part of the first clinical study when James Lind, a naval surgeon, used it to cure scurvy (Lind, 1757). He provided a group of sailors who had scurvy (12 British sailors, 2 in each group) with different food ingredients such as cider, diluted vitriol (sulfuric acid), vinegar, seawater, lime/orange, and spicy paste. The supplement was given with acidic food for a month, but due to a shortage of supply, oranges and lemons were given only for 6 days. The sailors receiving citrus got better, but the other sailors continued to have scurvy. This observation gave the impetus for further study on the role of citrus in prevention of scurvy (Stewart & Guthrie apud Draper, 1953). Since then, lime and orange juice have become a part of the daily diet for sailors and a part of diet in the western world.

Citrus plants generally are evergreen shrubs or small trees, bearing flowers, which yield a strong scent. The fruits can have different forms (for example, round, oblong, or elongated) and various sizes from 3.8 to 14.5 cm in diameter. Citrus fruits generally consist of an outer skin or rind made up of an epidermis (a leathery and waxy layer), the flavedo (a sub-epidermal layer that contains color and oil sacks producing aromatic oils), the albedo (a spongy layer below the flavedo, a source of flavanones), and vascular bundles. As citrus fruits ripen the total sugar increases, the acidity decreases, the ascorbic acid content increases, the peel color changes, and the fruit gets larger (Liu et al., 2012).

Genetic analysis confirmed that most citrus varieties are hybrids derived from a few ancestral varieties. Approximately 25 true-breeding species of citrus are commonly available for consumption. Several studies have classified citrus species for identification and chemical characteristics of citrus fruits including enzymes, fatty acids, hydrocarbon profiles, flavonoid patterns, and carotenoid composition (Vikram & Uckoo et al., 2015). Figure 1 depicts the structures of reported citrus limonoids that are responsible for its anticancer and therapeutic effects. Table 2 presents the health-promoting compounds

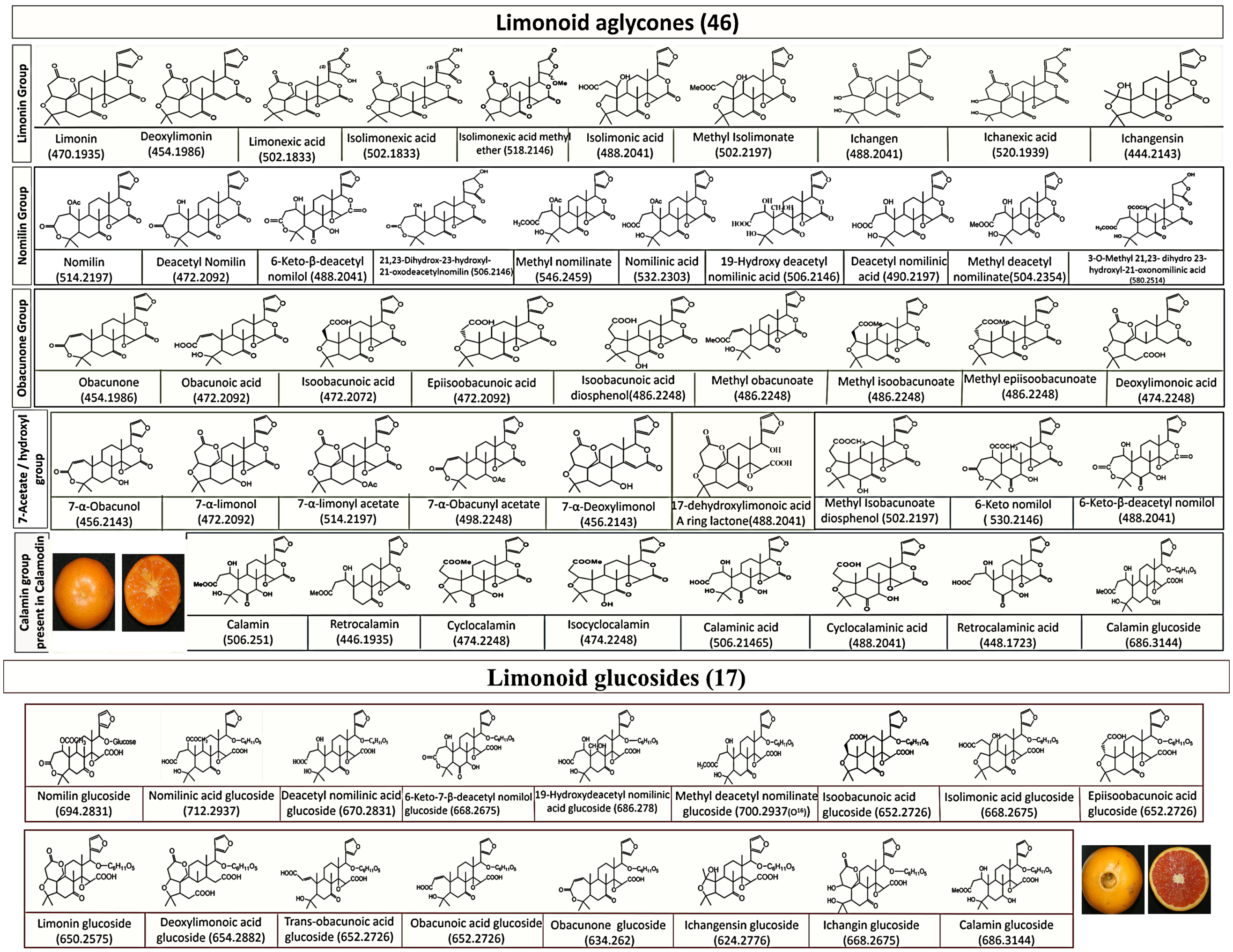

Figure 1. Biologically active limonoids reported from different varieties of citrus fruits.

such as limonoids, flavonoids, volatiles, carotenoids, coumarins, and sterols.

MAJOR HEALTH-BENEFITS OF CITRUS

A diet rich in fruits and vegetables can help in maintenance of health and prevention of chronic diseases. Several epidemiological studies suggest that a diet containing lots of fruits and vegetables can help prevent chronic diseases such as cardiovascular disease, stroke, and different forms of cancers. Based on the literature, epidemiological observations, and clinical evidence, certain foods are classified as functional foods or health foods, because of their established health-protective or health-promoting effects. However, recent clinical research suggests that, in citrus, these health benefits may be not limited to anti-oxidant activities. Below, we discuss the benefits and potential mechanisms of action of phytochemicals found in citrus and provide a broad classification of the different types of evidence (i.e. *in vitro* or *in silico* studies, animal or *in vivo* studies, and clinical studies).

IN VITRO STUDIES: RADICAL-SCAVENGING ASSAYS SHOW THE IMPORTANCE OF CAROTENOIDS

The biological activities of most compounds are initially screened using *in vitro* studies, which are directly or indirectly linked to the pharmacological effect. One widely used assay is screening for radical scavenging or antioxidant activity. This activity can be linked to prevention of chronic diseases including cancer

Table 2. Distribution of phytochemicals in different parts of citrus plants

Part of plant		Class of compounds	Major compounds	References
Leaf		Flavonoids Essential oil Phenolics	Sabinene, γ-terpinene, *(E)*-β-ocimene, p-cymene Paoli et al., 2016	(Kawaii et al., 2000)
Root		Flavonoids	5, 8-Dihydroxy-6, 7, 4′-trimethoxyflavone	(Nagy, 1980; Intekhab & Aslam, 2009)
		Lipids	β-sitosterol, campesterol	
Fruit	Seed	Limonoids	Limomin, nomilin, obacunone, obacunone glucosides	(Jayaprakasha et al., 2007; Sandoval-Montemayor et al., 2012)
		Coumarins	5,7-Dimethoxycoumarin, bergamotene	
		Essential oil	Fatty acids and fixed oil, phytosterols	
	Peel	Flavonoids Polymethoxy flavone Volatile Oil Carotenoids Pectin	Hesperidin Nobiletin d-Limonene β-Carotene, lutein Pectin	(Manthey & Grohmann, 2001) (Viuda-Martos et al., 2008)
	Pulp	Polyphenols Polymethoxy flavone Pectin	Tannin, phytate, flavonoids Nobiletin and tangeretin Pectin	(Raman et al., 2005; Viuda-Martos et al., 2008)

and cardiovascular disease. Citrus fruits contain many molecules known for their radical scavenging activity such as polyphenols, which include flavanones, glycosylated flavanones, phenolic acids, and flavonols, anthocyanins etc. Additionally, potent radical scavenging ability was also demonstrated in peels and seeds of certain citrus varieties. Researchers have reported hydrophilic, hydrophobic, and biological sample-based (LDL peroxidation, human blood cells, animal organ-based) radical-scavenging assays (Jayaprakasha et al., 2008a, b; Lagha-Benamrouche & Madani 2013; Escobedo-Avellaneda et al., 2014).

Carotenoids, potent antioxidants, occur in the plant kingdom and are found in all citrus fruits. These colored compounds can efficiently quench free radicals, minimizing the damage caused by oxidative stress. Consumption of carotenoids at higher levels was speculated to have pro-oxidative properties in smokers and individuals with high levels of free radicals, and to reduce harmful effects. Another major health benefit associated with carotenoids is their provitamin-A activity. Among the carotenoids, β-carotene and β-cryptoxanthin contain a β-type non-substituted ring, which is one of the key structural components required for functioning as a vitamin A precursor. β-carotene has the highest pro-vitamin A activity among all the carotenoids, with each molecule producing 2 units of retinal which is then reduced to vitamin A (retinol).

In addition to carotenoids, anthocyanins, present in blood orange and other citrus species, also known have radical scavenging activity by citrus juice/fruits (Barreca et al., 2014; Fallico et al., 2017).

CELL-BASED STUDIES SHOW THE IMPORTANCE OF LIMONOIDS

In cell-based studies, cancer-related studies predominate, followed by studies of antimicrobial activity. Most of the studies conducted preliminary screenings of large numbers of samples or examined individual components for their possible mechanisms of inhibition of cancer cells. Citrus fruit juice, solvent extracts from juice, peel, pulp, seed, whole fruit, purified compounds have been subjected to cell line studies. Limonoids, major triterpenoids found in citrus, demonstrated anticancer activities in both *in vitro* and animal studies (Table 3). Our lab demonstrated that limonoids have the potential to inhibit colon cancer (Vanamala et al., 2006; Jayaprakasha et al., 2010), ovarian cancer, and neuroblastoma (Tian et al., 2001; Poulose et al., 2007) and inhibit the growth of estrogen receptor-negative

Table 3. Cancer prevention studies reported for citrus and its constituents

Model used	Active constituents	Mechanism/mode of action	Reference
Colon cancer cells (SW480, HT-29 and CaCo2)	Limonin/obacunone and its glucosides, limonexic acid, nomilin, volatile oil isolimonic acid, ichanexic acid	Induction of apoptosis, inhibition of inflammation, cell signal and cell cycle	(Chidambara Murthy et al., 2011a, b) (Patil et al., 2009; Kim et al., 2011) (Jayaprakasha et al., 2008a, b)
AOM-induced rat colon and bowel cancer model (F344)	Auraptene Obacunone & limonin Nobiletin Orange juice Diosmin and Hesperidin	Induction of phase II drug-metabolizing enzymes Induction of apoptosis and inhibition of inflammation Reduced cell-proliferation activity, increased the apoptotic index, and decreased prostaglandin E, Tumor reduction, decreased labeling index and proliferation zone in the colonic mucosa Inhibition of inflammation and proliferation index	(Tanaka et al., 1998) (Tanaka et al., 2001, Suzuki et al., 2004) (Miyagi et al., 2000) (Tanaka et al., 1997)
Breast cancer cell lines (hormone-dependent and independent)	All flavonoids of citrus juice Genistein Limonin, nomilin, obacunone, limonexic acid, isolimonexic acid, nomilinic acid glucoside, deacetyl nomilinic acid glucoside, limonin glucoside, and obacunone glucoside	Proliferation inhibition Proliferation inhibition Apoptosis and anti-aromatase	(So et al., 1996) (Guthri et al., 2000; Kim, Jayaprakasha et al., 2013)
Breast cancer Animal model (DMBA induced)	All flavonoids of citrus juice d-Limonene and perillyl alcohol	Inhibition of tumor size Induction of proliferation inhibition and Phase II Drug-metabolizing Enzymes	(So et al., 1996) (Crowell, 1997)
Prostate cell lines (LNCaP and PC-3 cells)	Modified citrus pectin	Apoptosis and anti-angiogenesis	(Nangia-Makker et al., 2002)

Table 3. Continued...

Model used	Active constituents	Mechanism/mode of action	Reference
Prostate cancer animal model	Modified citrus pectin	Tumor inhibition and metastasis inhibition	(Nangia-Makker et al., 2002)
Prostate cancer clinical trial	Modified citrus pectin	Increased PSA doubling time	(Guess et al., 2003)

and positive human breast cancer cells (Tian & Miller et al., 2001; Kim et al., 2013). Additionally flavonoids, such as hesperidin, lutein, naringin, apigenin, and quercetagetin, are abundant in citrus fruits; flavonoids also have demonstrated significant inhibition of proliferation and anti-inflammatory activity *in vitro* and *in vivo*. One of the studies suggested that among most abundant flavonoids in citrus, apigenin and quercetagetin are the two major flavonoids which are effective against colon adenocarcinoma (SW480) cells (Chidambara Murthy et al., 2012). Lycopene and β-carotene are prominent in red grapefruits, which are known for several other health benefits such as protection of DNA from peroxidation, anti-cancer activity, suppression of tumors, and immune-modulation.

The monoterpenes present in citrus fruits, such as limonene and perillyl alcohol, are effective in cancer prevention and therapy (Crowell, 1999; Jayaprakasha et al., 2012; Jayaprakasha et al., 2013). Monoterpenes decrease the incidence of chemically induced tumors in the skin, liver, lung, breast, and forestomach of rats (Gould, 1995). We have demonstrated the ability of d-limonene-rich volatile oil to inhibit angiogenesis and inflammation, and induce apoptosis in colon cancer cells (Murthy et al., 2011). Limonene and other monoterpenes exert their chemopreventive effect by blocking the initiation of carcinogenesis by inducing phase I and phase II carcinogen-metabolizing enzymes thereby inducing detoxification of carcinogens. Limonene induces expression of several enzymes, such as CYB 2B1, CYP2C (specifically, subfamily CYP2C9, CYP2D6) epoxide hydratase, glutathione-S-transferase, and UDP-glucuronyl transferase (Rabi & Bishayee, 2009).

Citrus fruits demonstrated antimicrobial activity, including antibacterial and antifungal properties against pathogenic and nonpathogenic strains (Mandalari et al., 2007). Among the different compounds responsible for such activity are D-limonene, D-dihydrocarvone, m-Mentha-6, 8-diene and α-terpineol. Additionally, flavonoids, limonoids, and coumarin found in citrus also demonstrated antimicrobial activity. *Salmonella* Typhimurium and *E. coli* O157:H7 are major foodborne pathogens and cause significant mortality and morbidity, especially in children and immuno-compromised individuals. Studies conducted in our laboratory suggest that certain limonoids function as negative effectors of *Salmonella* Typhimurium and *Escherichia* coli O157:H7 virulence (Vikram et al., 2012). In addition to insect deterrent activity, certain limonoids also possess potent quorum sensing and antivirulence activities against Gram-negative pathogens. Therefore, in bacterial infections, the limonoid aglycones are likely to be synthesized in much the same fashion as in insect damage response and will help protect the fruits. Additionally, nomilin, which is the precursor of limonoids and distributed in various tissues such as leaf, stem, immature and mature fruits, was an effective virulence inhibitor, suggesting an important role in defense against bacterial pathogens.

In addition to their anti-cancer and antimicrobial activities, limonoids also seem to protect against HIV infection by inhibiting p-24 antigen activity, protease activity, and replication of HIV-1 (Battinelli et al., 2003). Monoterpenes of most citrus fruits have antifungal activity against common fungi associated with food contamination such as *Aspergillus niger*, *Aspergillus flavus, Penicillium chrysogenum*, and *Penicillium verrucosum* (Viuda-Martos et al., 2008). Similarly, flavonoids found in citrus i.e. quercetin, apigenin rutin, robinetin, myricetin, and galangin, also have anti-fungal activity against pathogenic and nonpathogenic fungi (Cushnie & Lamb, 2005).

ANIMAL STUDIES SHOW THE IMPORTANCE OF LIMONOIDS

Limonoids act as feeding deterrents for insects, thus protecting plants from damage by these foliage eaters and many animal experiments have examined this activity.

Investigation of insect antifeedant activity of citrus limonoids identified limonin, nomilin, and obacunone as primary antifeedant limonoids (Ruberto et al., 2002). Structure-activity studies revealed that the epoxide and furan functional groups of limonoids are essential for their antifeedant activity. Although much debated, increasing oxidation and skeletal rearrangement appears to be associated with increased deterrent activity.

A few *in vivo* studies have tested the hypolipidemic and antiobesity effects of limonoids. Feeding of citrus fruit and juice has shown a hypocholesterolemic effect in rats and rabbits. In a study feeding orange juice or grapefruit juice reduced serum LDL cholesterol by 43% and 32%, respectively. This was associated with a reduction of total liver cholesterol in the orange juice group (18%) and with hepatic cholesterol ester reduction in both groups (42%). The juices were not acting as intestinal sequestrants, since neither increased fecal cholesterol nor bile acid excretion. In fact, cholesterol excretion decreased in the orange juice and grapefruit juice group by 44% and 48%, respectively (Kurowska et al., 2000a). One of the limonoids, nomilin, demonstrated antiobesity and antihyperglycemic effects in mice fed a high-fat diet. Synephrine from citrus is known for its effect in prevention of becoming overweight and developing obesity. It acts by increasing lipolysis and fatty acid oxidation to reduce levels of cholesterol and LDL (Kurowska et al., 2000c).

Many animal experiments have examined the cardio-protection and cancer-prevention activities of citrus compounds. Limonin and nomilin inhibit forestomach neoplasia in a mouse model (Lam & Hasegawa 1989). Three limonoids (ichangensin, deoxylimonin, and obacunone) have been implicated in the inhibition of oral tumors in a hamster cheek pouch model (Miller et al., 2004). The protective effects of limonoids appears to emanate from their function as inducers of phase I and phase II metabolism, possibly by inducing glutathione S-transferase enzyme in the small intestine mucosa (Lam et al., 1989). *In vivo* studies using animal models have also demonstrated the ability of limonoids to induce phase-II enzymes. In addition, suppression of tumors in the cheek, intestine, and oral cavity was demonstrated in animal models. Research from our group has shown inhibition of azoxymethane-induced colon carcinoma by grapefruit phytochemicals (Vanamala et al., 2006) and induction of phase-II enzymes (in lung, small intestine, whole stomach, and liver) by nomilin, deacetyl nomilin, and isoobacunoic acid and mixture of limonoids in mice (Perez et al., 2010).

Citrus components have also been shown to benefit bone health, specifically by preventing osteoporosis. Feeding of citrus juice for 2 months to orchidectomized male rats prevented symptoms of osteoporosis. Total antioxidant capacity, femoral density, alkaline phosphatase, acid phosphatase, and urinary excretion of hydroxyproline are the key markers measured to understand the prevention of osteoporosis (Deyhim et al., 2006). Similarly, feeding of orange pulp at 2.5, 5.0, and 10% of diet significantly improved osteoporosis-associated symptoms. Hesperidin, another flavone abundant in citrus fruits, prevented ovariectomy-induced bone loss in 6-month-old rats when fed at 0.5% of diet for three months (Horcajada et al., 2008). Similar studies have shown that hesperidin can help in prevention of osteoporosis in males and females. Other constituents of citrus known to support bone health include carotenoids (β-cryptoxanthin), volatile oil, phytosterols, and other phenolics.

HUMAN STUDIES AND CLINICAL TRIALS SHOW THE IMPORTANCE OF WHOLE CITRUS

James Lind's 1747 study on the effect of lemon and orange consumption on scurvy is considered the first reported case-control clinical study (Lind, 1757; Morand et al., 2011). Results from Lind's study demonstrated that citrus fruits, specifically limes and oranges, can reverse the effects of scurvy. The study also mentioned the loss in vitamin C content during storage of fruits. Apart from prevention of scurvy, several retrospective studies have examined diabetes, hypertension, and obesity (Stewart & Guthrie apud Draper, 1953; Ono et al., 2011; Kengne et al., 2012). Additionally, small prospective case control and randomized studies have examined these conditions (Morand & Dubray et al., 2011). There have not been many studies on tumors and associated symptoms.

Cardiovascular disease is the leading cause of morbidity and mortality in the modern world. The increased production of reactive oxygen species, oxidative stress, and inflammation are associated with increased cardiovascular risk in patients with diabetes, hypertension, and obesity. There is convincing evidence on prevention of cardiovascular diseases by regular consumption of fruits and vegetables, specifically citrus fruits. Bioactive constituents such as flavonoids (including polymethoxyflavones), carotenoids, ascorbic acid, and other polyphenols are speculated to be responsible for the cardioprotective activities of citrus fruits. The mechanisms by which these compounds exhibit cardioprotective activity include radical scavenging/antioxidant, hypolipidemic, vasodilator (antihypertensive), anti-inflammatory, and antiatherogenic effects. They also help prevent and reduce the risk of atherosclerosis. Certain limonoids also function in reduction of LDL cholesterol in a rabbit model and apo-B production in HepG-2 cells (Kurowska et al., 2000b) suggesting beneficial effects in cardiovascular diseases.

Citrus fruits such as oranges, grapefruits, limes, and lemons are of specific interest for their cardioprotective effects, such as hypolipidemic and antihypertensive activities. Edible parts, juice, extracts of different parts of fruits. and major phytochemicals found in citrus have demonstrated cardioprotective ability *in vitro* and *in vivo*. These properties are mainly attributed to flavonoids, ascorbic acid, fiber, polymethoxyflavones, and other polyphenols. In a cross-over study involving 24 healthy overweight men, both orange juice and hesperidin reduced diastolic blood pressure over 4 weeks, suggesting that hesperidin may be responsible for this beneficial effect of citrus fruit. However, multiple cardiovascular biomarkers were evaluated as an outcome, which increases the probability of chance findings.

Apart from flavonoids, citrus fruits also contain phytosterols. In a randomized, placebo-controlled clinical study, orange juice with plant sterols or a reduced calorie orange juice beverage with sterols significantly lowered LDL-C levels in mildly hypercholesterolemic subjects when consumed twice a day with meals (Devaraj et al., 2006). Although consumption of orange juice alone failed to affect plasma inflammatory markers, addition of plant sterols to orange juice or orange juice supplemented with sterols (2g/d) resulted in a significant attenuation of plasma biomarkers (IL-1b and IL-6 levels). These cytokines are potent inducers of synthesis of C-reactive protein and this could be the mechanism by which orange juice or orange juice with sterol supplementation may lower C-reactive protein levels.

Incorporation of dietary options that may lower inflammatory biomarkers would be an important strategy to reduce risk from cardiovascular diseases and cancer. Overall, studies have provided some support for the potential benefits of flavanone intake for cardiovascular health, but further large-scale randomized clinical studies are required to elucidate the potential benefits of citrus fruits against chronic diseases such as cardiovascular disease. A large clinical study (805 men) indicated that flavonoids may reduce mortality from coronary heart disease (Hertog et al., 1993). These promising studies indicate that flavonoids may be useful dietary compounds. Orange juice can antagonize oxidative stress and inflammatory processes. This effect is attributed to the presence of hesperidin and naringenin, which are known to suppress generation of reactive oxygen species *in vitro*. Blood orange have an enhanced antioxidant activity and cardiovascular protective effects, which is attributed to the anthocyanins found in these fruits. Thus, regular intake of blood orange juice may have beneficial effects on the endothelial function of subjects with increased cardiovascular risk. This is mainly due to reduction of oxidative stress or anti-inflammatory properties.

Among different clinical investigations on citrus, prevention of obesity and associated symptoms are the most promising. Oral administration of orange juice reduced serum LDL cholesterol and hepatic cholesterol esters; it also increased HDL, triglycerides, and folate concentration (Kurowska et al., 2000b). Similar effects were reported for consumption of extracts rich in synephrine and flavonoids such as quercetin, rutin, hesperetin, and naringenin, which are predominant in citrus fruits. Among citrus fruits, grapefruits have high levels of naringin and sweet oranges, mandarins, and lemons have high levels of hesperidin. In a study designed to examine the effects of orange juice on vascular function, moderately overweight subjects who consumed 500 mL juice containing 292 mg of hesperidin and 47.5 mg of narirutin, daily for 4 weeks had significant improvement in postprandial microvascular endothelial reactivity and decrease in diastolic blood pressure compared to the subjects receiving a control drink (Morand et al., 2011). Similar results were reported upon consumption of red grapefruit, blond grapefruit, and pommelo fruits daily for 30 days. Consumption of these fruit juices improved the lipid profile of hypercholesterolemic patients diagnosed with coronary artery disease. Their total cholesterol was reduced by 15.5 and 7.9% compared to control in case of groups fed red and blond grapefruits juice, respectively. Additionally, LDL (reduction was 20.3% for red and 10.7 for blond grapefruits, compared to control) and triglyceride levels (17.2% and 5.6% for red and blond grapefruits, respectively, compared to control) decreased with consumption of fruit juice, which clearly indicate the benefit to human health.

Although the observed effect was not attributed to any specific compound, synergistic activity of several compounds may account for the observed protections. Vitamin C and flavanones are well known for scavenging the free radicals and reactive oxygen molecules, protecting against oxidative damage, inhibiting the formation of carcinogens, and protecting DNA. Hesperidin (250 mg) given twice-a-day to hyper-triglyceridemic patients for 24 weeks resulted in significant reduction of plasma triglyceride and apolipoprotein B levels (Craig, 1997), whereas 400 mg of naringin for 8 weeks produced a 17% decrease in LDL cholesterol and a 14% decrease in triglyceride levels in hypercholesterolemic subjects (Jung et al., 2003).

Citrus sudachi Hort. ex Shirai (Rutaceae), called "sudachi", is a small, round, green citrus fruit that is mainly cultivated in Japan. A randomized, double-blind, placebo-controlled trial was performed with 40 participants with abdominal obesity and metabolic risk factors including hypertension, impaired glucose tolerance, and elevated triglyceride levels. Test subjects were fed 1.3 g dried *Citrus sudachi* peel powder for 5 weeks. There were no side effects observed with treatment. In the treated group, serum triglyceride, body weight, waist circumference, and serum triglyceride levels significantly decreased compared to the control group (Akaike et al., 2014). Similarly, supplementation of Moro orange (*Citrus sinensis* (L.) Osbeck, also known as blood orange) juice extract (400 mg/day) for 12 weeks produced a significant reduction in body weight, BMI, and waist and hip circumference compared with the placebo-treated group. There are several clinical studies explaining the benefit of bitter orange (*Citrus aurantium*) extract and its primary constituent *p*-synephrine. Over 20 studies have been reported involving more than 350 subjects who consumed synephrine alone or in combination with other ingredients. Supplementing *p*-synephrine for 6–12 weeks increased resting metabolic rate and energy expenditure, and modestly increased weight loss. In these studies, over 30% of studies have shown that caffeine was also consumed along with *p*-synephrine (the range was 10–80 mg/day) (Stohs et al., 2012).

Calcium is a key ingredient responsible for bone health, and citrus juice is a good source of calcium; therefore, regular consumption is known to support bone health. Citrus has shown significant benefits in protection and promotion of bone health as evident from both animal and human studies. Certain flavanones also promote bone health. Consumption of citrus juice directly influences serum antioxidant status associated with bone quality and bone health as measured by bone density, strength, and other physicochemical properties. These protective properties of citrus fruits are attributed to the components such as ascorbic acid, calcium, flavonoids, and carotenoids present in the citrus fruits (Mandadi et al., 2009).

POSSIBLE WAYS TO ENHANCE THE BIOACTIVITY OF CITRUS COMPOUNDS

One important pre-requisite for the health benefits of citrus compounds is their bioavailability. For example, carotenoids are lipophilic compounds and therefore co-intake of fat significantly increases their solubilization followed by micellization (van het Hof et al., 2000; Ho et al., 2016). Processing citrus juice using high thermal processing, homogenization, and addition of fat significantly increases micellization, which may in turn lead to greater bioavailability of carotenoids (Uckoo et al., 2013a, 2013b).

Another major concern with optimum utilization is the concentration of some of the bioactive molecules in the edible portion of the fruits (Shanmugam et al., 2017). Most efficient molecules like obacunone, apigenin, synephrine and other molecules known for efficient health benefits are found in very low concentrations in the edible part (less than ppm). Some of the proposed methods to enhance the consumption of these ingredients in diet include:

- Enhance the content of these compounds in whole fruit using biotechnological (elicitation, gene manipulation, silencing, etc.) and breeding routes.
- Extract these compounds from seed or peel and add them to juice or food.
- Formulation of food recipes or supplements using parts of the fruit rich in active compounds (many recipes use citrus peel).
- Consuming fruit is more likely to be beneficial compared to juice, as many components will be removed while filtering the juice.
- Use of a food base that can easily solubilize the key ingredient by nanoencapsulation (Jayaprakasha et al., 2016; Hu et al., 2017). For example, use of oil or fat base to enhance the bioavailability of hydrophobic compounds (aglycones, carotenoids, volatiles, and phytosterols).
- Increase the popularity of citrus fruits and its products, specifically in children and young adults.
- Certain limonoids and other phytochemicals impart a bitter taste and hydrophobic in nature. These compounds can be masked or encapsulated with protein or formulating in to nanoparticles, or by additional ingredients or processing/cooking techniques (Shanmugam & Acharya et al., 2017).

FUTURE RESEARCH DIRECTIONS

Considering the popularity of citrus fruit and juice across the globe, research and promotion is essential for its optimum utilization and consumption. Areas in which there is a need for research include: enhancing the levels of biologically active phytochemicals in the edible part of fruits and enhancing bioavailability of active phytochemicals. There is a need for development of formulations or recipes from non-edible parts such as peel, seed, and lamella, as these parts contain good amounts of active phytochemicals. More than 50% of the citrus byproducts go to waste during processing (Uckoo et al., 2013a, b). Several industrial processes use this waste as a source of nutraceuticals (Rudra et al., 2015). Citrus juice industrial waste and byproducts can be effectively used for extraction/formulation of vitamins, minerals, and phytochemicals. For citrus, synephrine, hesperidin, polymethoxyflavones, limonoids, hesperidin, and dietary fiber supplements can be formulated from waste generated by the citrus juice industry. Additionally, research to provide clinical evidence for benefits of citrus in cancer, allergy, and other chronic disease will be of great benefit.

ACKNOWLEDGMENTS

KNC Murthy would like to thank the management of Ramaiah Medical College & Hospitals for their support and encouragement for research. This project is based upon work supported by the USDA-NIFA 2010-34402-20875, “Designing Foods for Health”, through the Vegetable & Fruit Improvement Center and Texas State funding 2013- 121277 VFIC-TX State appropriation.

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Received: February 06, 2017
Accepted: October 18, 2017