Citrus Flavanones

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Rosa Tundis, Rosaria Acquaviva, Marco Bonesi,
Giuseppe Antonio Malfa, Barbara Tomasello, and Monica R. Loizzo

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Abstract

Plant foods represent a very rich source of phytochemicals, including flavonoids that play a prominent role as healthy compounds. Flavonoids are object of numerous studies for their antioxidant, anti-inflammatory, neuroprotective, anti-cancer, anti-obesity, and antidiabetic activities.

Whereas some classes of flavonoids are distributed in a wide range of plants, others, such as flavanones, are found only in specific species. Indeed, flavanones are present almost exclusively in *Citrus* species (Rutaceae). The aim of this

R. Tundis (✉) · M. Bonesi · M. R. Loizzo
Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy
e-mail: rosa.tundis@unical.it

R. Acquaviva · G. A. Malfa · B. Tomasello
Department of Drug Science – Biochemistry Section, University of Catania, Catania, Italy

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chapter is to report the most recent developments related to the bioactivities of *Citrus* flavanones, their bioavailability and metabolism, potential toxicity and side effects, application in foods, and critical analyses of their potential future application in food industries and for the formulation of pharmaceutical and nutraceutical products.

Keywords

Flavanones · Bioavailability · Bioactivities · Human studies · Application in food

8.1 Introduction

Plant foods represent a very rich source of bioactive compounds, among which are polyphenols. According to their chemical structures, polyphenols can be classified into phenolic acids, flavonoids, lignans, and stilbenes. The polyphenols that mainly characterize the human diet, representing about two-thirds and one-third of the total daily intake of these phytochemicals, are phenolic acids and flavonoids (Ovaskainen et al. 2008).

Several studies have reported an inverse relationship between flavonoids-rich foods and some degenerative diseases (Ma et al. 2018; Spagnuolo et al. 2015). According to their structures, dietary flavonoids are classified into six main groups, namely, anthocyanidins, flavanols, flavanones, flavones, flavonols, and isoflavones. Whereas some of these classes of flavonoids are distributed in a wide range of plant foods, others, such as flavanones, are found only in particular foods. In fact, this class of flavonoids is present almost exclusively in *Citrus* species. To a lesser extent, they are found also in some plants including tomatoes and mint. In *Citrus* fruits, flavanones account for approximately 95% of the total flavonoids (Peterson et al. 2006a, b). Flavanones occur mainly in flavedo, albedo, and segment membranes.

A total flavanones content in the range of 35–147 mg/100 g was found in *C. sinensis* (orange) (Peterson et al. 2006b). Naringin and narirutin were quantified in *C. paradise*. A content in the range of 44–106 mg/100 g was demonstrated (Peterson et al. 2006b). However, because generally in the preparation of juices, the albedo and membranous parts are discarded, flavanone levels in *Citrus* juices are lower. Indeed, levels of naringenin in the range of 17–76 mg/100 mL were found in the juice of *C. paradisi* (Ross et al. 2000) and levels of hesperidin and narirutin in the range 13–77 mg/100 mg were found in the juice of *C. sinensis* (Tomas-Barberan and Clifford 2000).

In Europe, *C. sinensis* represents the major source of *Citrus* flavanones (Zamora-Ros et al. 2010). Flavanones are present in all cultivars. However, red cultivars showed a higher number of flavanones (Grosso et al. 2013). *Citrus* fruits are largely consumed as fresh or as juices or are canned. Moreover, uses in cosmetic and pharmaceutical industries are reported. *Citrus* fruits have been used in traditional medicinal medicines of several countries including China, Korea, and Japan. In particular, Chinese Pharmacopoeia reports six *Citrus* species, namely, *C. aurantium* L., *C. medica* L., *C. sinensis* Osbeck, *C. reticulata* Blanco, and *C. wilsonii* Tanaka (Lv et al. 2015) to treat skin

inflammation, indigestion, cough, ringworm infections, muscle pain, and to lower blood pressure. Both in vitro works and evidences from epidemiological, clinical, and preclinical studies described the healthy promoting properties of *Citrus* species and flavanones (Kang et al. 2016; Loizzo et al. 2012, 2016; Rendeiro et al. 2016; Tundis et al. 2012; Zaidun et al. 2018; Zhou et al. 2018). The purpose of this chapter is to compile data related to the bioactivities of *Citrus* flavanones, their bioavailability and metabolism, application in foods, marketed products, potential toxicity and side effects, and critically analyze the future perspectives.

8.2 Bioactive Constituents of *Citrus* Species

In the *Citrus* genus, different types of flavonoids have been identified: flavanones, flavones, flavonols, and – uniquely to blood oranges – anthocyanins. The flavonoids contained in greater quantities in *Citrus* fruits are flavanones, representing approximately 95% of the total (Peterson et al. 2006b). This subclass of polyphenols is the intermediate structure for the biosynthesis of a wide range of flavonoids found throughout the plant kingdom. In the vegetal tissues, flavanones result from the condensation of two precursors: three molecules of malonyl-CoA with one molecule of *p*-coumaroyl-CoA by chalcone synthase (CHS), forming the 2′,4′,6′,4-tetrahydroxychalcone or naringenin chalcone (Fig. 1) (Forkmann and Heller 1999).

Eventually, chalcone isomerizes into (2S)-flavanone (5,7,4′-trihydroxyflavanone), otherwise known as naringenin. This reaction can occur spontaneously, due the instability of the chalcone structure or stereospecifically by chalcone–flavanone isomerase (CHI), which plays a significant role in the cyclization reaction of chalcones to form all flavonoid classes (Forkmann and Heller 1999; Aoki et al. 2000). The main *Citrus* flavanones aglycones are naringenin (5,7,4′-trihydroxyflavanone), hesperetin (4′-methoxy-3′,5,7-trihydroxyflavanone), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone), and isosakuranetin (4′-methoxy-5,7-dihydroxyflavanone) (Fig. 2).

In *Citrus* fruits, these four compounds are generally glycosylated at position 7 by either the disaccharide neohesperidose or rutinose (Fig. 3) producing four neohesperidose glycosides, namely, naringin, neoeriocitrin, neohesperidin, and poncirin, and four rutinose glycosides, namely, didymin, eriocitrin, hesperidin, and narirutin (Peterson et al. 2006b).

Neohesperidosides derivatives have a bitter taste, while rutinosides derivatives have no taste. Surprisingly, chemical hydrogenation of neohesperidin in alkaline solution produces the semisynthetic neohesperidin dihydrocalcone, a potent artificial sweetener (Kinghorn et al. 2010).

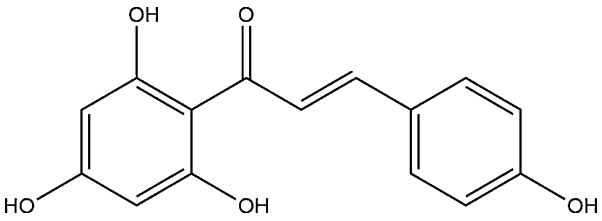

Fig. 1 Naringenin chalcone

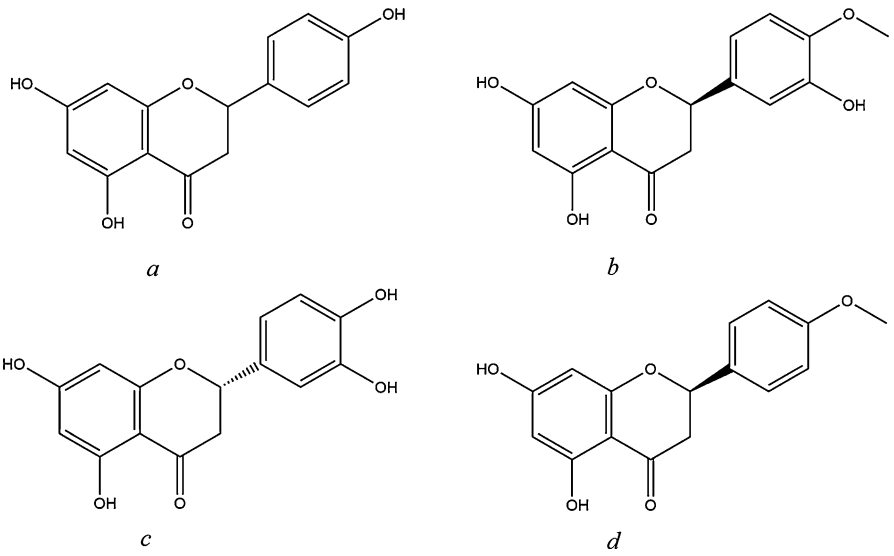

Fig. 2 *Citrus* flavanone aglycones: *a* naringenin, *b* hesperetin, *c* eriodictyol, *d* isosakuranetin

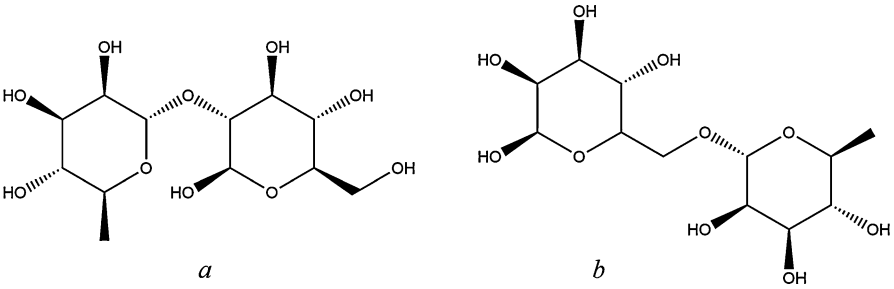

Fig. 3 Sugars present in *Citrus* flavanones glycosides: a neohesperidose and b rutinose

8.3 Bioavailability and Metabolism of Flavanones

To exert their beneficial properties, polyphenols should be released during digestion and absorbed (Parada and Aguilera 2007). Several works investigated how much of polyphenols can be absorbed after ingestion. Nevertheless, the poor bioavailability of these molecules have been highlighted.

Generally, the bioavailability of polyphenols is dependent on different factors including bioaccessibility, transport, and metabolism (Bouayed et al. 2012). Most studies that analyzed the bioavailability of *Citrus* flavanones involved hesperetin, naringenin, and their glycosides. After intake, flavanone glycosides are hydrolyzed in the small intestine and colon.

Subsequently, during the passage across small intestine and liver, aglycones are converted into their respective metabolites such as glucuronides, sulfoglucuronides, and sulfates, which are distributed at the various cell sites. A considerable quantity can be found in urine (Matsumoto et al. 2004). The deglycosylation of naringenin-7-*O*-β-D-glucoside happens early in the small intestine.

Naringenin-7-*O*-β-D-rhamnoglucosides occur in the colon (Choudhury et al. 1999). Indeed, Felgines et al. (2000) showed the presence of naringenin conjugates within 3 h in the plasma of rats fed with naringenin or its 7-*O*-β-D-glucoside. No naringenin metabolites neither naringenin-7-*O*-β-D-rhamnoglucoside were found. However, in the same work, similar naringenin concentrations after 10 h of ingestion were found regardless of the diet. This evidence demonstrated the delayed intestinal absorption of naringenin rhamnoglucosides.

Naringenin and its glucuronides were found in plasma and brain of rats 10 min after administration of a concentration of 20 mg/kg (Peng et al. 1998). Higher concentrations of naringin were detected in the bile and liver (Tsai 2002). Manach et al. (2003) confirmed that in humans, both naringin and hesperidin are absorbed in the distal part of the intestine, and once deglycosylated, during their transfer from the luminal side of the gut to the portal vein, aglycones are sulfated and/or glucuronated by uridine diphosphate (UDP)-glucuronosyltransferase and sulfotransferase.

Erlund et al. (2001) evaluated the concentration in human plasma of naringenin and hesperetin aglycones after ingestion of grapefruit or orange juice (8 ml/kg body weight). A range of 0.6–6.0 mmol/l with a peak concentration of 6.0 mmol/l for naringenin (from grapefruit juice) and 2.2 mmol/l for hesperetin (from orange juice) was found. A considerable distribution to tissues for both flavanones was suggested (Erlund et al. 2001). Successively, the ingestion of 135 mg of both hesperetin and naringenin under fasting conditions in 6 volunteers resulted in their appearance as metabolites in blood plasma 20 min later. Peak plasma concentration of 2.7 and 7.4 mmol/l for hesperetin and naringenin was reached 4.0 and 3.5 h after ingestion, respectively (Kanaze et al. 2007).

After ingestion of 1 l of orange juice (characterized by a content of 444 mg of hesperidin and 96.4 mg of narirutin), analysis over 24 h led to C(max) values at 1.28 and 0.20 mmol/L for hesperetin and naringenin (after deconjugation), respectively. The levels in urine of flavanones, expressed as percentage of their intake, amounted to 7.9% and 6.4% for naringenin and hesperetin, respectively. The dose not considerably affected the urinary excretion of flavanones (Manach et al. 2003). Generally, naringenin is more bioavailable than hesperetin as resulted by both plasma and urine analyses (Gardana et al. 2007; Kanaze et al. 2007).

The study of Cao et al. (2010) aimed to explain the different efficacy of two Zhi Zhu Wan (ZZW) varieties on the basis on the results of pharmacokinetics of hesperetin and naringenin. ZZW is a Chinese formulation that contains *Atractylodes Rhizome* and *Fructus Citrus Immaturus*, which derived from *C. aurantium* and *C. sinensis*. Although the immature *C. aurantium* and *C. sinensis* fruits showed many common constituents, there are significant differences in their quantity and in the presence of other characteristics compounds (Liu et al. 2008; Wang et al. 2008). These chemical differences reflect the different clinical uses. After oral

administration of *C. aurantium*, both naringenin and hesperetin were detected in plasma and demonstrated similar pharmacokinetic parameters. After oral administration of *C. aurantium*, both hesperetin and naringenin were detected in plasma and showed comparable pharmacokinetic parameters. After oral administration of *C. sinensis*, only hesperetin was detected. Moreover, it was found that the pharmacokinetic properties for hesperetin in *C. sinensis* was different from hesperetin in *C. aurantium*, and it was slowly eliminated. Based on all these results, it can be concluded that hesperetin can be considered the effective compound for both ZZW varieties.

The main plasma and urinary metabolites of aglycones are obtained by glucuronidation and sulfation pathways. Glucuronide metabolites dominate with a percentage of about 87%. However, the importance of the other metabolites should not be neglected (Manach et al. 2003). Hesperetin-7-*O*-β-D-glucuronide, hesperetin-3′-*O*-β-D-glucuronide, hesperetin sulfoglucuronide, and hesperetin diglucuronide are the main metabolites of hesperetin (Matsumoto et al. 2004; Mullen et al. 2008). Naringenin-7-*O*-β-D-glucuronide, naringenin-4′-*O*-β-D-glucuronide, naringenin-4′-*O*-sulfate-7-*O*-β-D-glucuronide, naringenin-4′,7-*O*-disulfate, and naringenin-4′-*O*-β-D-glucuronide-7-*O*-sulfate are the most abundant metabolites of naringenin (Brett et al. 2009; Tripoli et al. 2007).

A good determinant of flavanones intestinal absorption is their ability to permeate of epithelial cells. The faster absorption of aglycones in comparison to glycosides was demonstrated in humans by Miyake et al. (2006) that investigated the mechanism of absorption of eriocitrin and eriodictyol.

The transport of flavonoids across the cell membranes in general imply the presence of ATP-binding cassette (ABC) transporters, present in the enterocytes apical or basolateral membrane, and allow the excretion back into the intestinal lumen or uptake into the blood, respectively. Intestinal ABC transporters related to the flavonoids include breast cancer resistance protein (BCRP), P-glycoprotein, and multidrug resistance proteins. Kobayashi and Konishi (2008) confirmed that the aglycone hesperetin was better absorbed across Caco-2 cell monolayers in comparison to its glycoside hesperidin. The absorption occurs through transcellular passive diffusion and a proton-coupled active transport (Kobayashi et al. 2008). In the same year, Brand et al. (2008) revealed that hesperetin-7-*O*-β-D-glucuronide and hesperetin-7-*O*-sulfate are mainly transported to the apical side in Caco-2 monolayers. Instead, it was demonstrated that hesperetin aglycone is able to permeate the basolateral side of the monolayers of Caco-2 cell line. The results from this work showed that BCRP-mediated transport could be one of the key steps that could limit the bioavailability of hesperetin (Brand et al. 2008). Successively, Brand et al. (2010) analyzed the differences in metabolism and transport, and the activity of separated hesperetin enantiomers in in vitro models by using (i) human intestinal fractions that contain UDP-glucuronosyl transferases or sulfotransferases, (ii) Caco-2 cell monolayers, and (iii) mouse Hepa-1c1c7 cells transfected with human EpRE-controlled luciferase.

These results showed relatively small differences in the metabolism, transport, and activity between (S)- and (R)-hesperetin. This is important for the consideration

that experiments that are performed with commercially available racemic hesperetin may adequately reflect what can be expected for the naturally occurring (S)-hesperetin.

Taking onto account that the bioavailability of healthy compounds may be influenced by the food matrix in which they are to be found, the bioavailability and metabolism of *C. sinensis* juice flavanones, namely, naringenin-7-*O*-rutinoside and hesperetin-7-*O*-rutinoside, and the impact of the ingestion of the juice with a yogurt were studied (Mullen et al. 2008). Human plasma and urine were collected over a 24 h after the consumption of juice (250 mL) with and without of yogurt (150 mL). Juice contains 168 and 12 μmol of hesperetin-7-*O*-rutinoside and naringenin-7-*O*-rutinoside, respectively, paracetamol (1 g) and lactulose (5 g). It was demonstrated that the investigated dairy food matrix may delay the intestinal absorption of both naringenin-7-*O*-rutinoside and hesperetin-7-*O*-rutinoside with no impact on their bioavailability. The role of the dietary matrix (yogurt) on the bioavailability of flavanones was studied in a successive work in which it was demonstrated that the quantity of flavanone metabolites excreted in urine after 24 h of *C. sinensis* juice consumption has reduced about 7 times when the orange juice is ingested with yoghurt (Roowi et al. 2009). Probably, this reduction is due to the alteration of flavanones metabolism by the action of the microflora in the large intestine.

Flavanones demonstrated to be resistant to oxidative reactions, while their isomerization into chalcones was observed mainly for the orange juice. The processing of pasteurization for 30 s at 95 °C, freezing, and concentration of juice showed no effect on the in vitro flavanones bioaccessibility (Gil-Izquierdo et al. 2002). Chalcones were found in higher quantities in industrially pressed juice in comparison to the manually pressed juice.

Spigoni et al. (2017) recently investigated the bioavailability in humans of different metabolites of *Citrus bergamia* (bergamot) phenolics (including flavanones) after juice intake. In particular, the potential use of bergamot in the system of lipotoxicity-induced myeloid angiogenic cells (MACs) impairment was analyzed. MACs play a crucial role in endothelial functionality and repairing processes. However, the lipotoxic effects of some compounds, such as palmitic acid and stearic acid, may decrease these activities. After consumption of bergamot juice, the circulating flavanone metabolites in plasma and urine were assessed. Twelve flavanone phase II conjugates were identified and quantified. Then, the effects at physiological concentrations of hesperetin-7-*O*-glucuronide, naringenin-7-*O*-glucuronide, hesperetin-3′-*O*-glucuronide, and naringenin-4′-*O*-glucuronide were investigated on gene expression of apoptosis and inflammation markers in MACs after exposition to stearic acid. Hesperetin-7-*O*-glucuronide and naringenin-4′-*O*-glucuronide demonstrated to be able to mitigate stearate-induced inflammation in MACs.

Previously, Aschoff et al. (2016) analyzed the bioavailability and the colonic catabolism of flavanones from orange juice by using a dose 2.4-fold higher in comparison to the flavanones from fruits. This choice is due to the consideration that the juice contains flavanones at lower concentrations as compared to the fruits. In this randomized two-way cross-over project, twelve healthy subjects are invited to

consume a meal including either pasteurized juice or fresh fruits, providing 751 and 1774 μmol as total *Citrus* flavanones content, respectively.

Naringenin, deglucuronidated and desulfated hesperetin, and four catabolites, namely, hippuric acid, 4-hydroxyhippuric acid, 3-(3′-hydroxy-4′-methoxyphenyl) propionic acid, and 3-(3′-hydroxyphenyl)hydracrylic acid, were quantitated in 24-h urine.

The urinary excretion of hesperetin as well as postprandial catabolites excretion showed nonsignificant differences after consumption of both orange juice and fruits. In conclusion, the excretion of flavanones after ingestion of orange fruits did not differ from that succeeding at juice consumption, although the use of a 2.4-fold higher dose. Probably, this is due to a saturation of absorption or flavanones entrapment in the matrix of the fruit that is rich in fibers.

At the end of this paragraph, some general considerations are necessary. Numerous studies show marked individual differences in the bioavailability of this class of compounds, due to both molecular factors, including the activity and/or synthesis of enzymes responsible of the biotransformation and transporters, and physiological factors, including gastric motility, body composition, and body weight. Variations have been described for biotransformation enzymes and secretory transporters associated with flavonoids, including uridine diphosphate glucuronosyltransferases, CYP3A4, and P-glycoprotein (Dai et al. 2001; Fisher et al. 2000; Hall et al. 1999; Lown et al. 1995; van der Kolk et al. 2000). Determinant for the bioavailability of flavonoids are also the composition and activity of the gastrointestinal microflora.

8.4 Bioactivities of *Citrus* Flavanones: *Animal Experiments*

Flavanones extracted from *Citrus* fruits are involved in many essential physiological functions: as an antioxidant, anti-inflammatory, and antitumor activity (Table 1). They are able to counteract the endogenous and exogenous biological stimuli. They can also reduce cholesterol and triglyceride levels in experimental animals (Jeon et al. 2007; Habauzit et al. 2011; Horcajada et al. 2008).

Citrus flavanones also possess antioxidant activities, although these activities are poorer in respect to many other polyphenols (Jeon et al. 2002). Their anti-inflammatory, antitumor, anti-atherogenic properties cannot be explained solely based on their antioxidant properties. Investigations of their mechanism of action suggested that they can act as free radical scavengers and, also, modulate cellular signalling processes or may themselves serve as signalling molecules.

Several animal studies have been published during the past few years (Acquaviva and Iauk 2010; Nijveldt et al. 2001; Ross and Kasum 2012). A lot of attention has been paid to the anticarcinogenic properties of flavanones. In particular, hesperidin has been shown to inhibit mammary, urinary bladder, and colon carcinogenesis in laboratory animals (So et al. 1996; Yang et al. 1997; Tanaka et al. 1997; Miyagi et al. 2000).

Table 1 Major effects of flavanones

Flavanones	Effects	Mechanisms of action	References
Hesperidin Naringenin Naringenin	Antioxidant	ROS ⇓ Antioxidant enzymes ⇑ Lipid peroxidation production ⇓ Nonenzymic antioxidants ⇑	Acquaviva and Iauk 2010 Akiyama et al. 2010 Nandakumar and Balasubramanian 2011
Hesperidin Naringenin Naringenin	Anticancer	Apoptosis ⇑ DNA binding of NFkB ⇓ DNA binding of tumor initiation ⇓ iNOS ⇓ COX-2 ⇓ Regulation of both phase I and phase II metabolizing enzymes	Acquaviva and Iauk 2010 Leonardi et al. 2010 Nandakumar and Balasubramanian 2011
Hesperidin Naringenin Naringenin	Anti-lipidemic	Cholesterol ester synthesis ⇓ 3-Hydroxy-3-methylglutaryl-coenzyme A reductase ⇓ Acyl coenzyme A: cholesterol O-acyltransferase ⇓ LDL⇓ Cholesterol levels⇓	Lee et al. 1999a,b Kurowska et al. 2000
Hesperidin Naringenin Naringenin	Antidiabetic	ALT⇓ AST⇓ GLUT-4 antagonism Modification of insulin sensitivity Improve glycolytic and gluconeogenetic enzymes Impeded TNF-α expression Induces AMPK	Akiyama et al. 2010 Priscilla et al. 2015 Vinayagam and Xu 2015
Hesperidin Naringenin Naringenin	Anti-neurodegenerative	ROS ⇓ Antioxidant enzymes ⇑ Counteract the degeneration of the nigrostriatal dopaminergic projection Anti-inflammatory activity ⇑ TNF-α ⇓ Aβ deposition ⇓ APP expression⇓	Leem et al. 2014 Li et al. 2015

Flavanones may act with different mechanisms of action, such as inhibiting carcinogen activation, stimulating carcinogen detoxification, scavenging free radical species, controlling cell cycles, inhibiting cell proliferation, inducing cellular apoptosis, and inhibiting angiogenesis, metastasis, and growth factors activity.

Nandakumar and Balasubramanian (2011) have investigated the antigenotoxic activity of hesperidin and have demonstrated that the daily administration of this compounds (30 mg/kg BW) for 45 days prevented 7,12-dimethylbenz(α)anthracene (DMBA)-induced experimental breast cancer formation. Probably this effect is due to the regulation of both phase I and phase II metabolizing enzymes and due to its strong antioxidant activity.

In addition, in vivo studies have shown that naringenin could suppress the early stage of colon cancer by attenuating inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) levels in carcinogen-injected rats (Leonardi et al. 2010). It is known that inducible iNOS is another enzyme that plays a pivotal role in mediating inflammation. Therefore, the anti-inflammatory effect of flavanones may be due both to their antioxidant activity and to their effect on enzymes involved in the inflammatory cascade.

It was demonstrated that phenolic compounds present in the juice of blood oranges might prevent lipid peroxidation and the formation of atherosclerotic plaques (Acquaviva and Iauk 2010; Sorrenti et al. 2004). Hesperidin and naringenin also act on lipid metabolism; in fact, they are able to regulate the apolipoprotein B secretion by HepG2 cells, probably through the inhibition of cholesterol ester synthesis, and to inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase and acyl coenzyme A cholesterol O-acyltransferase in rats (Lee et al. 1999a, b).

Moreover, a decrease in serum LDL and hepatic cholesterol levels was observed in rabbits fed with a cholesterol-rich diet and supplemented with orange juice (Kurowska et al. 2000).

An increase in high-density lipoprotein levels has been shown in hypercholesterolemic patients who consumed orange juice (Erlund 2004). Other biological activities attributed to naringenin and hesperidin include antidiabetic properties.

It is known that peroxisome proliferator-activated receptor gamma (PPARγ) plays a critical role in peripheral glucose homeostasis and in modulating insulin sensitivity. PPARγ induces glucose uptake by directly or indirectly increasing the transcription of genes encoding proteins such as GLUT4. The partial antagonism, exerted by flavanones, may be useful in improving insulin sensitivity (Vinayagam and Xu 2015). Recently, it was demonstrated that a 4-week diet-based administration of hesperidin (10 g/kg), in streptozotocin (STZ)-induced diabetic type 1 rats, induced a decrease of blood glucose compared to nondiabetic rats. In addition, hesperidin did not influence both bone metabolism and body weight in diabetic rats. Moreover, the antidiabetic effect of hesperidin may be due to its ability to inhibit pancreatic damage, thanks to its antioxidant activity, increasing insulin secretion and consequently decreasing glucose levels, and altering glycogen contents in the diabetic tissues by the improvement of glycolytic and gluconeogenetic enzymes (Akiyama et al. 2010). In addition, several studies showed that also naringenin possesses an insulin-mimetic effect; naringenin, in fact, is able to decrease blood glucose levels in healthy male Wistar rats (Vinayagam and Xu 2015). In particular, the oral administration of naringenin (25 mg/kg bw) for 45 days exerts a significant inhibition of intestinal α-glucosidase activity in diabetic rats induced by streptozotocin (STZ). This effect delays the absorption of carbohydrates with a consequent significant decrease in postprandial glycemic levels. Moreover, naringenin treatment, in diabetic rats, improved GLUT-4 and induced 5′ adenosine monophosphate-activated protein kinase (AMPK) activation, that increases glucose tolerance and insulin sensitivity, while at the same time decreasing TNF-α expression and ALT, AST levels in the serum, preventing STZ-induced liver damage in rats (Priscilla et al. 2014, 2015).

Scientific research has shown that flavanones can provide benefits in the prevention and treatment of metabolic diseases due to their antioxidant activity and their ability to modulate the expression of some proteins involved in hyperglycemia and hyperlipidemia.

It is reported that flavanones possess neuroprotective effects against various types of insult associated with neurodegenerative diseases, including Parkinson and Alzheimer diseases.

The neuroprotective activity of the *Citrus* flavanones may be due to their capacity to cross the blood-brain barrier and to arrest free radical-induced oxidative damage, which is known to play a pivotal role in many degenerative diseases. Antunes et al. (2014) have demonstrated that hesperidin mitigated both the increased levels of ROS and the activity of glutathione reductase induced by 6-hydroxydopamine.

Moreover, their neuroprotective action is mediated by the interaction with specific intracellular targets that are implicated in several signalling pathways important for maintaining the cellular homeostasis. Naringin is able to counteract the degeneration of the nigrostriatal dopaminergic (DA) projection by increasing the level of glia-derived neurotrophic factor (GDNF) in nigral DA neurons, with a concomitant activation of mammalian target of rapamycin complex 1 (mTORC1) (Leem et al. 2014). Moreover, naringin exerts its anti-inflammatory activity in CNS mitigating the increase of TNF-α induced by 1-methyl-4-phenylpyridinium (MPP+) in microglia (Leem et al. 2014).

Li et al. (2015) demonstrated that the pre-treatment with hesperidin (100 mg/kg body weight) for 10 days recovered deficits in non-cognitive nesting capability and social interaction and attenuated Aβ deposition, plaque-associated amyloid precursor protein (APP) expression, and microglial activation and TGF-β1 immuno-reactivity in both cerebral cortex and hippocampus of transgenic APP/PS1 APP/PS1 mice. Certainly further clinical studies must be performed to validate the neuroprotective activities of the flavanones.

8.5 Benefits (Human Studies)

Citrus flavanones have shown many interesting effects in vivo and in vitro models, including antioxidant properties, cholesterol and triglycerides reduction, cell proliferation and angiogenesis inhibition, estrogenic activity, nitric oxide (NO) level modulation, and decrease of lymphocyte immobilization and platelet aggregation (Bellocco et al. 2009; Barreca et al. 2009; Codoner-Franch and Valls-Belles 2010). Instead, their effectiveness in humans remains controversial because of both the intersubject variability in flavanones pharmacokinetics and the chemical transformation carried out by gut microbiota that in turn influences their absorption and biological activity as well.

Citrus healthy properties are mostly related to their antioxidant capacity. However, in healthy subjects, *Citrus* flavanones do not have significant antioxidant effects, suggesting that their antioxidant potency is negligible in normal conditions (Testai and Calderone 2017). Conversely, they can improve endogenous antioxidant

defense in non-healthy individuals, allowing a reduced risk of certain chronic diseases and the prevention of some cardiovascular disorders as well as certain kinds of cancer (Tomás-Navarro et al. 2014).

Rangel-Huerta et al. (2017) demonstrated that the consumption of an orange juice with a high content of flavanones improves oxidative stress and inflammatory biomarkers by decreasing the serum levels of hydroxyoctadecadienoic acid (9-HODE+13-HODE) and dihydroxyoctadecanoic acid (12,13-DiHOME and 9,10-DiHOME), as well as increasing the levels of 12-hydroxyeicosatetraenoic acid (12-HETE) compared to normal flavanones juice in 30 subjects from the BIONAOS study (Biomarkers In Overweight And Obese Adults).

Moreover, *Citrus* flavanones also have anti-inflammatory properties, mainly hesperetin, naringenin, and their glycosylated derivatives (Barreca et al. 2017). These molecules are capable of regulating cellular inflammation process, due to their antioxidant properties, but also by interacting with key enzymes (such as protein kinase, phosphodiesterase, lipoxygenase, cyclooxygenase, and phospholipase), inhibiting arachidonic synthesis, downregulating of NFkB activation and consequently of pro-inflammatory cytokines (TNF-a, IL-6, and IL-1), chemokines, COX-2, and iNOS (Tomás-Navarro et al. 2014; Bodduluru et al. 2016). Results of in vivo research and clinical trials support their use as compounds readily available at a low cost without any side effects or intolerance.

8.5.1 Cardiovascular Disease and *Citrus* Flavanones

Cardiovascular diseases (CVD), including myocardial infarction, coronary heart disease, strokes, cardiomyopathy, and other heart disease, are one of the leading causes of morbidity and mortality in worldwide. Epidemiological, clinical, and preclinical studies have deeply analyzed the relationship between *Citrus* flavanone intake and the risk of cardiovascular disease (Hollman et al. 2010; Testai and Calderone 2017). Their results suggest that *Citrus* flavanones prevent cardiovascular disease positively influencing some cardio-metabolic parameters (He et al. 2006; He et al. 2007; Gan et al. 2015) that are the main risk factors of CVD, such as hypertension, dyslipidemia, overweight, and hyperglycemia.

A number of sources suggest the cardioprotective activity of hesperidin and its aglycone form, hesperetin in particular (Barreca et al. 2017). A prospective study carried out on approximately 10,000 Finnish men and women reveals a 20% reduction in cerebrovascular diseases in those who consumed the highest levels of flavanones (4.7–26.8 mg aglycone/day) (Knekt et al. 2002).

A significant inverse correlation between flavanone consumption and cerebral ischemia has been found in women who consume high levels of flavanones (>63 mg/day) *versus* low levels (<13.7 mg/day) (Cassidy et al. 2012). Moreover, a systematic review and meta-analysis of prospective cohort studies reported that flavonoids intake, especially of flavanones, was associated with a decreased risk of cardiovascular disease ($p = 0.002$) (Wang et al. 2014).

Many clinical trials have focused their attention on modulation of CDVs risk factor by *Citrus*.

A meta-analysis of three randomized clinical trials, including 233 patients, demonstrated a correlation between grapefruit intake and a reduction in systolic blood pressure and waist circumference in overweight and obese adults (Onakpoya et al. 2017). In general, these effects of flavanones on hypertension, particularly naringenin and hesperetin, are due to improvement of vasodilation and endothelial function (Salehi et al. 2019) through endothelial production of NO and activation of voltage-operated calcium channels and potassium currents (Liu et al. 2014).

Metabolic syndrome, characterized by altered glucose metabolism, elevated blood pressure, dyslipidemia, and obesity is one of the main cardiovascular risk factor. A cohort clinical trial performed on 10,000 Polish subjects reveals that habitual consumption of flavonoids, among which flavanones, reduces the incidence of metabolic syndrome (Grosso et al. 2017).

Rizza et al. (2011) carried out a clinical trial of 3 weeks on 28 subjects with metabolic syndrome demonstrating that hesperidin (500 mg/day) reduces E-selectin expression, cholesterol, and ApoB level and induces enhancement of NO levels.

Intake of 300 mL of fruit juice (containing 95% of *Citrus* flavonoids) produces no variations of glucidic parameters but improvement of lipidic panel, with decrease in the cholesterol, LDL-C and C-reactive peptide levels, in subjects with metabolic syndrome ($n = 33$) compared with healthy subjects ($n = 20$) in a clinical trial of 4 or 6 months (Mulero et al. 2012).

High level of lipoprotein and cholesterol in plasma as well as hyperinsulinemia and hyperlipidemia led to development of atherosclerosis with the plaque formation in arteries, which is a gateway for cardiovascular diseases. Among flavanones, naringenin is the most effective to decrease triglycerides and LDL-C and inhibit glucose uptake as well. On the contrary, it promotes the improvement of high-density lipoprotein (HDL-C) level and antioxidant defenses, decreasing atherosclerosis-related genes expression (Orhan et al. 2015). These effects can be due to molecular structures characterized by the presence of a hydroxyl mevalonate moiety resembling that of statins, a drug able to inhibit cholesterol biosynthesis (Barreca et al. 2017).

Clinical evidences show that 400 mg/day of naringenin administrated for 2 months causes a reduction of the LDL-C, cholesterol and ApoB levels and an increase in HDL-C levels and detoxifying enzyme only in hypercholesterolemic subjects ($n = 30$) (Jung et al. 2003).

In addition, bergamot fruit shows anti-cholesterolemic properties both in animal and human studies (Cappello et al. 2016; Constans et al. 2015). A *C. bergamia* extract (Bergavit® containing 150 mg of flavonoids with 16% neoeriocitrin, 47% neohesperidin and 37% naringin) reduced cholesterol levels and improved lipidic and lipoproteic panel in mild hypercholesterolemic patients ($n = 88$) (Toth et al. 2016).

The results from clinical trial are controversial. In fact, Morand et al. (2011) observed no difference in fasting glucose, insulinemia, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in 24 overweight subjects after

drinking juice contained 292 mg of hesperidin and 47.5 mg of narirutin for 4 weeks. This flavanone combination, instead, reduced pressure parameter (4 mmHg), ameliorated postprandial microvascular reactivity, and some biomarkers of oxidative stress. Similar results have been obtained with a combination of naringin and hesperidin (500 mg plus 800 mg) in a randomized controlled study that saw 4 weeks of treatment involving moderately hypercholesterolemic patients. These findings, according to some authors, could be related to the difference in individual pharmacokinetic parameters.

Several clinical trials have demonstrated the positive effect of *Citrus* flavonoids in the reduction of cardiovascular risk factors. Although some of the mechanisms responsible for the beneficial effects of *Citrus* flavanones on the cardiovascular system are unclear, the nutraceutical value of these fruits in cardiovascular disease therapy should be considered as valid approach.

8.5.2 Diabetes and *Citrus* Flavanones

Type 2 diabetes mellitus (T2DM) represents a worldwide health problem and is a chronic, progressive disease characterized by elevated blood glucose levels and insulin resistance.

Chronic hyperglycemia causes complications, such as cardiovascular disease, diabetic retinopathy, renal dysfunction, and leg ulcers (Marathe et al. 2017). A healthier diet plays a key role in the T2DM prevention as suggested by various epidemiological studies (Schwingshackl et al. 2017). Nutraceutical products from plants have been reported to reduce hyperglycemia and lipid disorders in individuals with T2DM or with a predisposition to T2DM (Kaleem and Ahmad 2018).

Among *Citrus* flavanones, naringin and naringenin have been shown to control diabetes and its related complications in animal and cell studies by improving glucose tolerance and insulin sensitivity but also reducing plasma and/or hepatic cholesterol and triglyceride levels (Assini et al. 2013; Sharma et al. 2015).

A nutraceutical product rich in *Citrus* flavanones, limonoids, and tocotrienols (Diabetinol®) showed a significantly reduced peak hyperglycemic response, TC, and LDL-C levels and decreased HbA1c levels after 3 months supplementation in subjects with mild impaired fasting glucose (Judy et al. 2010). Similarly results have been achieved in subjects with greater severity of impaired fasting glucose ($\leq$15.4 mmol/L) in a 24-week, randomized, double-blind, placebo-controlled, parallel study carried out by Evans (Evans et al. 2015).

Ethanol extracts of *C. junos* (yuja) peel (YE) contain naringin, hesperidin, rutin, quercetin, and tangeretin, showing antidiabetic properties in animal models (Kim et al. 2013), and have been assayed by individuals with impaired fasting glucose (IFG) in an 8-week, randomized, double-blinded, crossover, placebo-controlled clinical trial. The authors reported that YE significantly reduced fasting plasma glucose levels, fasting plasma insulin, postprandial glucose, and c-peptide and homeostatic model assessment-insulin resistance compared to the placebo group (Hwang et al. 2015).

A body of evidence suggests that oxidative stress is a main mechanism in pathogenesis of diabetes. Bonina et al. (2002) evaluated some serum oxidative stress biomarkers in T2DM patients ($n = 33$) who took ROC supplement (50 mg/d, orally) for 2 months. They observed an oxidative stress improvement characterized by high levels of free thiol groups and reduced concentration of serum free radical. In addition, the glycemic profile remained stable in all subjects for the whole period, and the total antioxidant status was unmodified by ROC administration.

Despite the numerous preclinical studies demonstrated the useful of *Citrus* flavanones in diabetes, further human studies focused on dosage, bioavailability, efficacy, and safety are required.

8.5.3 Cancer and *Citrus* Flavanones

A growing amount of literature data evidences that the risk of cancer can be reduced with a diet rich in vegetables and fruits (Boffetta et al. 2010). Some epidemiological studies reported that *Citrus* fruit consumption is protective against a variety of human cancers. *Citrus* fruit exerts this chemopreventive action through the protection of DNA from injury and the inhibition of the early phase of carcinogenesis by promotion of xenobiotic detoxification process.

A recent review of Italian and Swiss case-control studies (10,000 cases of 14 different cancers and about 17,000 controls) has shown that the high consumption of *Citrus* fruit is associated with a reduced risk of cancers of the digestive tract and larynx (Turati et al. 2015). Previously the analysis data from a series of case-control studies regarding *Citrus* fruits intake and risk of several types of cancer has found that the ORs for the highest versus lowest category of *Citrus* fruit consumption varied from 0.42 to 0.82 for esophageal, oral cavity, pharyngeal, laryngeal, stomach, and colorectal cancer. Instead, the authors have found no consistent association with breast, endometrial, ovarian, prostate, and renal cell cancer. This observed protective effect against cancers of digestive and upper respiratory tract is linked to the content on flavanones, vitamin C, and other compounds with antioxidant, antimutagenic, and antiproliferative properties contented in *Citrus* fruit (Foschi et al. 2010). In addition, the meta-analysis done by Bae and Kim confirm the association between intake of *Citrus* fruit and gastric cancer risk evidencing a 13% reduction of gastric cancer. In particular, the authors reported that 100 g of *Citrus* fruit intake per day inhibits cardia gastric cancer by 40% (Bae and Kim 2016). *Citrus* flavanones such as naringin, hesperidin, and 20-hydroxyflavanone (2HF) also have antitumor activities. They inhibit tumor growth and promote cancer cell apoptosis through cell cycle arrest and caspase-activation induced by either death receptors or mitochondrial pathways, as demonstrated by both in vitro and in vivo studies. All these studies show the potential anticancer activity of *Citrus* flavanones even if presently no clinical studies support these findings and the current intake of *Citrus* fruits is insufficient to induce apoptosis of cancer cells in humans.

8.5.4 Neurodegenerative Disease and *Citrus* Flavanones

Neurodegenerative disorders including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and Huntington's disease are debilitating, incurable diseases that are becoming increasingly prevalent (Wyss-Coray 2016). Much attention is paid to dietary supplementation for maintaining cognitive function in old age and delaying the onset of neurodegenerative diseases. Epidemiologic studies reveals that higher intakes of flavonoids over 10–15 year are associated with a reduced rate of cognitive decline in old age (Kesse-Guyot et al. 2012; Letenneur et al. 2007).

Most of the human studies focused on flavanols and anthocyanins (Krikorian et al. 2012), while few researches have been performed with flavanones, even though they are the main components of the most commonly consumed juices and also are easily absorbed and cross the blood-brain barrier (Manach et al. 2005). A cross-sectional epidemiologic survey of 1091 men and women born in 1936 reported a positive association between flavanone consumption and crystallized intelligence (Butchart et al. 2011). Kean et al. (2015) carried out a controlled, placebo-matched, crossover, randomized, double-blind human-intervention trial on healthy older adults ($n = 37$) that drunk daily a flavanone-rich orange juice (305 mg) for 8 weeks. They reported an improvement of global cognitive function compared to low-flavanone control, while no difference in mood and blood pressure was observed. These findings underlined that constant daily flavonoid intake provides cognitive benefits not only to adults with mild cognitive impairment or neurodegenerative disease but also to healthy older adults. Up to date, no human studies provide data to support possible mechanisms that underlie positive cognitive effects of flavanones. Future research should be conducted to clarify the potential role of flavanone-based dietary supplementation to maintain and increase cognitive function in healthy young adults and mitigate cognitive impairment in older adults with neurodegenerative disease.

8.6 Application in Food

Many *Citrus* fruits, including particularly orange, clementine, tangerine, and grapefruit, are eaten fresh, unlike more acidic fruits such as limes and lemons that are generally not eaten fresh. In fact, generally, lemonade or limeade are beverages that are prepared by diluting derived juices and by adding sugar. *Citrus* juices and rinds are used in different drinks. The colorful peel of some *Citrus* fruits is used in cooking as a flavoring.

Although the consumption of *Citrus* fruits is reported since ancient times, *Citrus* processing was not possible until the development of both thermal and concentration processes. Since then, the *Citrus* industry has quickly developed, becoming soon prominent among food industries. Now, the *Citrus* industry is the second largest fruits processing industry, after the grape industry that principally produces wine. Approximately 30% of *Citrus* fruits is processed to obtain juice.

The variation of *Citrus* flavanones content in beverages and foods is dependent on different factors including *Citrus* species and cultivar, growth conditions, fruits' ripeness, postharvest processing, cooking process, and storage conditions.

Cooking processes, including boiling, baking, frying or microwave, are responsible of numerous changes in physical characteristics and chemical composition of food matrix (Zhang and Hamauzu 2004). Ismail et al. (2004) found that thermal treatment decreased the total phenolic content in food matrix. Previously, Gil-Izquierdo et al. (2002) analyzed some processes used for the production of orange juice at industrial scale, namely, mild and standard pasteurization, concentration, freezing, and squeezing. Moreover, commercial squeezing was compared with domestic squeezing in order to evaluate the influences of these processes on orange juice composition. Taking into consideration flavanones, mild and standard pasteurization processes did not influence the flavanones content. A slight decrease of flavanones content in the soluble fraction was proved. Only didymin decreased with a percentage of 52%.

The freezing process produced a considerable decrease of flavanones content in the soluble fraction. Comparing commercially and domestically squeezed orange juice, commercial squeezing gives a major content of flavanones than domestic squeezing. In agreement with this study, Moura and de Sylos (2009) analyzed samples of tangor murcott and orange juice (a mixture of Valencia and Pera varieties) and evidenced that pasteurization and concentration processes of juice did not affect significantly the amount of flavones hesperidin and narirutin.

The flavanone hesperetin is used as food enhancer and sweetener in a wide variety of dessert and alcoholic beverage (Ley et al. 2005).

Naringin is listed in Commission Decision 1999/217/EC establishing a register of flavoring substances in application of Regulation EC 2232/96, allowing its use in food without restriction. It has been subsequently evaluated by EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in the Flavouring Group Evaluation 32 (EFSA 2011) with an interim finding that it is considered safe for use in food but that more data on human intake is desirable. A further possible application of naringenin in food matrix regards the inhibition of genotoxic compounds produced at 100–180 °C in particular acrylamide, which is dangerous for human health (Cheng et al. 2009). More than 30 years ago, Horowitz and Gentili (1971) reported that the peels of oranges and lemons contained a number of compounds, which could be converted into sweeteners.

8.7 Safety: Toxicity and Side Effects

Numerous studies demonstrated as *Citrus* flavanones are safe and well tolerated. Among these, particularly naringenin and hesperidin are investigated.

EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has assessed naringin in 2010 as a food flavoring. Data from chronic oral feeding studies in rats showed that naringenin up to 5 mg/kg complete feed is safe with an appreciable margin of safety.

Based on mammalian studies, only transient amounts of naringin and its metabolites residues in animal tissues are to be expected. This would represent an unimportant contribution to human exposure. Therefore, the use of the flavanone in animal's nutrition is evaluated safe for the consumer. However, the EFSA's panel on additives and products or substances used in animal feed (FEEDAP) could not conclude on the safety and efficacy of the product when delivered in water for drinking because related data are not available (EFSA 2011).

Li et al. (2014) investigated the potential chronic toxicity in Sprague-Dawley (SD) rats of naringin by oral gavage for 6 months followed by a recovery period of 1 month at doses of 0, 50, 250, and 1250 mg/kg. During both these periods, no mortality and/or toxicologically substantial variations in hematology, biochemical analyses, organ weights, ophthalmology, serum sex hormone, and histopathological and macroscopic examinations were reported. A slight, reversible, and non-pathological hair loss was observed during the treatment. However, it was not considered to be of toxicological importance. Overall, the obtained results demonstrated that the levels with no adverse effects of naringin, when orally administered in rats for 6 months, are greater than 1250 mg/kg/day.

Several animal and human studies have been reported that hesperidin is safe and well tolerated (Meyer 1994). The hesperidin safety was demonstrated on patients with rheumatoid arthritis administered 3 g of glucosyl hesperidin (G-Hsd) every morning for a 3-month treatment (Kometani et al. 2008). In another work, 94 menopausal women had a daily intake of 0.9 g of hesperidin with 0.3 g of hesperidin methyl chalcone and 1.2 g of vitamin C for 1 month (Smith 1964).

One of the most popular formulation containing hesperidin is Daflon 500 mg. This formulation has been analyzed in animals and humans trials (Meyer 1994). In animal studies, the safety of Daflon 500 mg is demonstrated by (i) a LD_{50} (lethal dose 50) of 3 g/kg, that is, 180 times the daily therapeutic dose, and (ii) by the absence of toxic effects after repeated oral dosing for 13 and 26 weeks, using a dose that represents 35 times the daily dosage in the rat and primate, respectively. The passage into breast milk and the transplacental passage are negligible. Clinical trials have collected more than 2850 patients treated with Daflon 500 mg at the dosage of 2 tablets/day for the period 6 weeks–1 year and satisfied the international scientific requirements. Side effects are essentially of a gastrointestinal or autonomic nature. Hemodynamic parameters as well as laboratory parameters were uninfluenced even by prolonged treatment for 1 year. Satisfactory clinical acceptability already confirmed in the short term was equally found in long-term treatment.

In animal studies, the concomitant administration of hesperidin with β-adrenergic blocking agents, calcium channel blockers, or statins significantly changed the maximal plasma concentration and the absorption of these drugs (Cho et al. 2009; Piao and Choi 2008; Uesawa and Mohri 2008).

In the study of Cho et al. (2009) the effects of hesperidin on the bioavailability and pharmacokinetic parameters of diltiazem and its desacetyldiltiazem (its main active metabolite) in rats, were investigated. Diltiazem (15 mg/kg) was orally administered in the presence or absence of hesperidin (1, 5 or 15 mg/kg), administered 30 min before the calcium channel blocker. Compared with the control group,

hesperidin (at doses of 5 or 15 mg/kg but not at 1 mg/kg) considerably modified the pharmacokinetics of diltiazem. Hesperidin (15 mg/kg) significantly increased the C (max) of desacetyldiltiazem but not significantly modified the desacetyldiltiazem metabolite-parent *ratio*. In conclusion, data from this work demonstrated the ability of hesperidin to enhance in rats the bioavailability of diltiazem probably by increasing its absorption and by reducing its first-pass metabolism in the intestine and in the liver via inhibition of cytochrome P450 3A or P-glycoprotein.

8.8 Marketed Products

Greater attention to health and social changes has led to the growth of the natural products market not only in the pharmaceutical and nutraceutical sectors but also in the food sector in Europe and in United States. This growth trend has been facilitated by the updated Novel Food legislation that makes easier for exporters to introduce new products. Sustainability in terms of reutilization of plant and food by-products is still a strong trend, demanded by companies as well as consumers (https://www.cbi.eu/market-information/natural-ingredients-health-products/trends/).

Citrus fruits represent a rich source of nutrition and vitamins and are important additions to any diet lacking in this components. *Citrus* are one of the key products in both food and beverage. Due its high nutritional value, *Citrus*-derived compounds could be used in innovative health functional food and beverage. Among *Citrus* flavanones, hesperetin, naringenin, eriodictyol, isosakuranetin, and their glycoside are the main abundant compounds. The *Citrus* flavanones are marketed individually or in the form of a mix, as nutraceuticals, since they act synergistically with the vitamin C to neutralize free radicals and counteract oxidative stress. The biological activities of *Citrus* flavanones are thought to be particularly beneficial for capillary strength, probably due an action on collagen (Kawaguchi et al. 2006). For this reason, these supplements are used in treatment of venous diseases such as chronic venous insufficiency, varicose veins, spider veins, and hemorrhoids (Mastantuono et al. 2015).

Moreover, hesperidin and naringenin are natural aromatase inhibitors. This activity is linked to a reduction in estrogen circulation that inhibited platelet aggregation and positively affect blood-clotting mechanisms which reduces the risk of cardiovascular disease. Bioflavonoids from *Citrus* possess also powerful anti-inflammatory properties (Benavente-García et al. 1997).

Recently, a researcher demonstrated the effect of naringenin on metabolic syndrome and obesity (Alam et al. 2014). Consequently, food supplements based on these bioactive compounds have been developed for this specific indication. Although the optimum daily doses of *Citrus* flavanones have not been determined, the label of nutraceutical products recommend 2000–6000 mg of these compounds for adults each day. This dose take into account the low toxicity of flavanones and the rare occurrence of side effects following their intake (Panche et al. 2016).

In food industry, hesperetin was used to fortified foods. A recent study proposed the nano-incapsulation of this flavanone in nanostructure lipid carriers coated with

different biopolymers such as alginate, chitosan, and low methoxypectin. Physico-chemical and sensorial analysis evidenced that developed nanoparticles could be applied for milk fortification to mask flavanone bitterness, inhibit color change, and enhance its solubility (Fathi and Varshosaz 2013). Further studies are necessary to identify other possible applications in food and nutraceutical industries.

8.9 Patents

The available information on patents was collected from some databases such as Espacenet (European Patent Office), Google Patents, and SciFinder. The research focuses on *Citrus* flavanones alone or as active ingredient in formulations used for the treatment of several pathological conditions. The search was performed in January 2019. The used keywords were: *Citrus* flavanones, naringenin, hesperetin, hesperidin, didymin, eriodictyol, neoeriocitrin, neohesperidin, poncirin, didymin, eriocitrin, and narirutin. A very high number of patents was found. For this reason, the research was limited to the last year (2018). After the removal of duplicated patents, the documents found were processed individually in an attempt to classify them based on the date and purpose for which they were registered (Table 2). Most of the patents found for the year 2018 do not concern single flavanones but are related to extracts or to mixtures of compounds (including flavanones) or to formulations in which are present flavanones.

Most of the patents have been designed and registered with the purpose of creating new products and formulations with specific biological activities. In particular, products with anti-inflammatory, and neuroprotective activity, products useful for the prevention and/or treatment of dermatitis, and skin damage caused by radiation and for repelling mosquitoes were prepared. Instead, fewer patents are present with regard to extraction and identification techniques. Among these, the patent CN108490094A reported the method for the determination of 22 flavonoids and phenolic acids in *Citrus* fruits.

8.10 Future Perspectives

Being *Citrus* flavanones daily present in the diet, their impact on human health is of relevance.

Extensive studies were carried out in order to investigate their ability to promote health and to provide protection against chronic diseases with high social impact including cardiovascular diseases, diabetes, and obesity. Among these compounds, particularly hesperetin, hesperidin, naringenin, naringin, and eriodictyol are mainly investigated.

In this chapter, we summarized and analyzed data related to the bioactivity of *Citrus* flavanones, their bioavailability and metabolism, their application in foods, marketed products, and safety.

Table 2 Patents overview of *Citrus* flavanones in 2018

Title	Date	Patent no.	Application no.	Use
Co-crystal of isoniazid and naringenin and preparation method of co-crystal	2018-09-28	CN 108586332A	CN 201810359523	Improvement of naringenin solubility and isoniazid activity
Application of naringenin and derivatives in the prevention and treatment of Alzheimer's disease	2018-11-13	CN 108785301A	CN 2018-11042921	Neuroprotective
Application of naringenin and naringin in tumor radiotherapy	2015-09-30	CN 104940932A	CN 2015-10404716	Tumor radiotherapy
Flavonoids pharmaceutical compositions and preparations and applications	2018-10-26	CN 108704037A	CN 2018-10626248	Prevention and treatment of dermatitis and skin damage caused by radiation
Mosquito repellent liquid	2018-04-21	CN108391665A	CN 201810363142	Repelling mosquitoes
Antibacterial coating and preparation method of green	2018-09-21	CN108559343A	CN 201810445336	Antimicrobial
Method for the determination of 22 *Citrus* fruit flavonoids and phenolic acids species	2018-09-04	CN108490094A	CN 201810274708	Compounds determination
Naringin microsphere silk fibroin/ hydroxyapatite composite scaffold and preparation method	2018-06-15	CN108159502A	CN 201810184370	Application to bone defect areas of different shapes
Application of naringin to preparation of medicine for treating and delaying intervertebral disc degeneration	2018-07-13	CN108272812A	CN 201810122205	Treating and delaying intervertebral disc degeneration
Such compositions and their use in the preparation of anti-inflammatory drugs	2018-09-04	CN108478549A	CN 201810497584	Anti-inflammatory

(continued)

Table 2 (continued)

Title	Date	Patent no.	Application no.	Use
Application of hesperetin, tangeretin and glycyrrhetinic acid in the inhibition of chloride channel	2018-11-16	CN108815154A	CN 201810866735	Treatment of diarrhea, heart, lung, gastric, brain, psychotic and ocular disorders, and rhinitis
Narirutin with effect of restraining allergic asthma	2018-05-25	CN108066353A	CN 201810138317	Effect of restraining allergic asthma
Whitening moisturizing skin care product	2018-06-22	CN108186496A	CN 201810199635	Whitening and moisturizing effects

The available data from animal models and clinical trials together with epidemiological studies suggest that this class of phytochemicals may beneficially affect both aetiology and physiopathology of several diseases. However, despite the ever-increasing number of studies on this flavonoids class, their health effects are still unknown or partially known. This is due to various reasons: (a) often interpretation of results from performed studies is problematic, because several different mechanisms and pathways may be involved; (b) serum biomarkers together with data from dietary intake could be used when studying the biological properties of *Citrus* flavanones; (c) clinical studies that investigate both high and low doses of *Citrus* flavanones are warranted; (d) bioavailability of each compound of a mix should be monitored, because marked interindividual variation could confound results; and (e) information about daily intake and bioavailability needs to be more fully developed.

8.11 Cross-References

- ▶ Dietary Flavonols and O-Glycosides
- ▶ Prenylated Flavonoids in Food
- ▶ Soy Isoflavones

References

Acquaviva R, Iauk L (2010) Natural polyphenols as anti-inflammatory agents. Front Biosci S2:318–331

Akiyama S, Katsumata S, Suzuki K, Ishimi Y, Wu J, Uehara M (2010) Dietary hesperidin exerts hypoglycemic and hypolipidemic effects in streptozotocin-induced marginal type 1 diabetic rats. J Clin Biochem Nutr 46:87–92

Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD (2014) Effect of *Citrus* flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr 5:404–417

Antunes MS, Goes ATR, Boeira SP, Prigol M, Jesse CR (2014) Protective effect of hesperidin in a model of Parkinson's disease induced by 6-hydroxydopamine in aged mice. Nutrition 30:1415–1422

Aoki T, Akashi T, Ayabe SJ (2000) Molecular and biochemical characterization of 2-hydroxyisoflavanone dehydratase. Involvement of carboxylesterase-like proteins in leguminous isoflavone biosynthesis. Plant Res 113:475–488

Aschoff JK, Riedl KM, Cooperstone JL, Högel J, Bosy-Westphal A, Schwartz SJ, Carle R, Schweiggert RM (2016) Urinary excretion of *Citrus* flavanones and their major catabolites after consumption of fresh oranges and pasteurized orange juice: a randomized cross-over study. Mol Nutr Food Res 60:2602–2610

Assini JM, Mulvihill EE, Huff MW (2013) *Citrus* flavonoids and lipid metabolism. Curr Opin Lipidol 24:34–40

Bae JM, Kim EH (2016) Dietary intakes of *Citrus* fruit and risk of gastric cancer incidence: an adaptive meta-analysis of cohort studies. Epidemiol Health 38:e2016034

Barreca D, Laganà G, Tellone E, Ficarra S, Leuzzi U, Galtieri A, Bellocco E (2009) Influences of flavonoids on erythrocyte membrane and metabolic implication through anionic exchange modulation. J Membr Biol 230:163–171

Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Laganà G, Daglia M, Meneghini S, Nabavi SM (2017) Flavanones: *Citrus* phytochemical with health-promoting properties. Biofactors 43:495–506

Bellocco E, Barreca D, Laganà G, Leuzzi U, Tellone E, Ficarra S, Kotyk A, Galtieri A (2009) Influence of L-rhamnosyl-D-glucosyl derivatives on properties and biological interaction of flavonoids. Mol Cell Biochem 321:165–171

Benavente-García O, Castillo J, Marin FR, Ortuño A, Del Río JA (1997) Uses and properties of *Citrus* flavonoids. J Agric Food Chem 45:4505–4515

Bodduluru LN, Kasala ER, Madhana RM, Barua CC, Hussain MI, Haloi P, Borah P (2016) Naringenin ameliorates inflammation and cell proliferation in benzo(a)pyrene induced pulmonary carcinogenesis by modulating CYP1A1, NFκB and PCNA expression. Int Immunopharmacol 30:102–110

Boffetta P, Couto E, Wichmann J, Ferrari P, Trichopoulos D, Bueno-de-Mesquita HB, van Duijnhoven FJ, Büchner FL, Key T, Boeing H, Nöthlings U, Linseisen J, Gonzalez CA, Overvad K, Nielsen MR, Tjønneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Lagiou P, Naska A, Benetou V, Kaaks R, Rohrmann S, Panico S, Sieri S, Vineis P, Palli D, van Gils CH, Peeters PH, Lund E, Brustad M, Engeset D, Huerta JM, Rodríguez L, Sánchez MJ, Dorronsoro M, Barricarte A, Hallmans G, Johansson I, Manjer J, Sonestedt E, Allen NE, Bingham S, Khaw KT, Slimani N, Jenab M, Mouw T, Norat T, Riboli E, Trichopoulou A (2010) Fruit and vegetable intake and overall cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). J Natl Cancer Inst 102:529–537

Bonina FP, Leotta C, Scalia G, Puglia C, Trombetta D, Tringali G, Roccazzello AM, Rapisarda P, Saija A (2002) Evaluation of oxidative stress in diabetic patients after supplementation with a standardised red orange extract. Diabetes Nutr Metab 15:14–19

Bouayed J, Deußer H, Hoffmann L, Bohn T (2012) Bioaccessible and dialyzable polyphenols in selected apple varieties following in vitro digestion vs. their native patterns. Food Chem 131:1466–1472

Brand W, Van der Wel PAI, Rein MJ, Barron D, Williamson G, van Bladeren PJ, Rietjens IMCM (2008) Metabolism and transport of the *Citrus* flavonoid hesperetin in Caco-2 cell monolayers. Drug Metab Dispos 36:1794–1802

Brand W, Shao J, Hoek-vanden Hil EF, Van Elk KN, Spenkelink B, Haan LHJD, Rein MJ, Dionisi F, Williamson G, Van Bladeren PJ, Rietjens IMCM (2010) Stereoselective conjugation, transport and bioactivity of S- and R-hesperetin enantiomers in vitro. J Agric Food Chem 58:6119–6125

Brett GM, Hollands W, Needs PW, Teucher B, Dainty JR, Davis BD, Brodbelt JS, Kroon PA (2009) Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. Br J Nutr 101:664–675

Butchart C, Kyle J, McNeill G, Corley J, Gow AJ, Starr JM, Deary IJ (2011) Flavonoid intake in relation to cognitive function in later life in the Lothian Birth Cohort 1936. Br J Nutr 106:141–148

Cao H, Chen X, Sun H, Tetsuro S, Zhou J, Sun W, Lv H, Wang X (2010) Pharmacokinetics-based elucidation on disparity in clinical effectiveness between varieties of Zhi Zhu Wan, a Traditional Chinese Medical formula. J Ethnopharmacol 128:606–610

Cappello AR, Dolce V, Iacopetta D, Martello M, Fiorillo M, Curcio R, Muto L, Dhanyalayam D (2016) Bergamot (*Citrus bergamia* Risso) flavonoids and their potential benefits in human hyperlipidemia and atherosclerosis: an overview. Mini-Rev Med Chem 16:619–629

Cassidy A, Rimm EB, O'Reilly EJ, Logroscino G, Kay C, Chiuve SE, Rexrode KM (2012) Dietary flavonoids and risk of stroke in women. Stroke 43:946–951

Cheng KW, Zeng X, Tang YS, Wu JJ, Liu Z, Sze KH, Chu IK, Chen F, Wang M (2009) Inhibitory mechanism of naringenin against carcinogenic acrylamide formation and non enzymatic browning in Maillard model reactions. Chem Res Toxicol 22:1483–1489

Cho YA, Choi DH, Choi JS (2009) Effect of hesperidin on the oral pharmacokinetics of diltiazem and its main metabolite, desacetyldiltiazem, in rats. J Pharm Pharmacol 61:825–829

Choudhury R, Chowrimootoo G, Srai K, Debnam E, Rice-Evans CA (1999) Interaction of the flavonoid naringenin in the gastrointestinal tract and the influence of glycosylation. Biochem Biophys Res Commun 265:410–415

Codoner-Franch P, Valls-Belles V (2010) *Citrus* as functional foods. Curr Top Nutraceut Res 8:173–183

Constans J, Bennetau-Pelissero C, Martin JF, Rock E, Mazur A, Bedel A, Morand C, Bérard AM (2015) Marked antioxidant effect of orange juice intake and its phytomicronutrients in a preliminary randomized cross-over trial on mild hypercholesterolemic men. Clin Nutr 34:1093–1100

Dai D, Tang J, Rose S, Hodgson E, Bienstock RJ, Mohrenweiser HW, Goldstein JA (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. J Pharmacol Exp Ther 299:825–831

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (2011) Scientific Opinion on the safety and efficacy of naringin when used as a sensory additive for all animal species. EFSA J 9:2416. [12 pp]. https://doi.org/10.2903/j.efsa.2011.2416

Erlund I (2004) Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 24:851–874

Erlund I, Meririnne E, Alfthan G, Aro A (2001) Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr 131:235–241

Evans M, Judy WV, Wilson D, Rumberger JA, Guthrie N (2015) Randomized, double-blind, placebo-controlled, clinical study on the effect of Diabetinol(®) on glycemic control of subjects with impaired fasting glucose. Diabetes Metab Syndr Obes 8:275–286

Fathi M, Varshosaz J (2013) Novel hesperetin loaded nanocarriers for food fortification: production and characterization. J Funct Foods 5:1382–1391

Felgines C, Texier O, Morand C, Manach C, Scalbert A, Régerat F, Rémésy C (2000) Bioavailability of the flavanone naringenin and its glycosides in rats. Am J Physiol Gastrointest Liver Physiol 279:G1148–54

Fisher MB, VandenBraden M, Findlay K, Burchell B, Thummel K, Hall SD (2000) Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. Pharmacogenetics 10:727–739

Forkmann G, Heller W (1999) Biosynthesis of flavonoids. In: Sankawa U (ed) Comprehensive natural products chemistry, Vol. 1: polyketides and other secondary metabolites including fatty acids and their derivatives. Elsevier, Oxford, pp 713–748

Foschi R, Pelucchi C, Dal Maso L, Rossi M, Levi F, Talamini R, Bosetti C, Negri E, Serraino D, Giacosa A, Franceschi S, La Vecchia C (2010) *Citrus* fruit and cancer risk in a network of case-control studies. Cancer Causes Control 21:237–242

Gan Y, Tong X, Li L, Cao S, Yin X, Gao C, Herath C, Li W, Jin Z, Chen Y, Lu Z (2015) Consumption of fruit and vegetable and risk of coronary heart disease: a meta-analysis of prospective cohort studies. Int J Cardiol 183:129–137

Gardana C, Guarnieri S, Riso P, Simonetti P, Porrini M (2007) Flavanone plasma pharmacokinetics from blood orange juice in human subjects. Br J Nutr 98:165–172

Gil-Izquierdo A, Gil MI, Ferreres F (2002) Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. J Agric Food Chem 50:5107–5114

Grosso G, Galvano F, Mistretta A, Marventano S, Nolfo F, Calabrese G, Buscemi S, Drago F, Veronesi U, Scuderi A (2013) Red orange: experimental models and epidemiological evidence of its benefits on human health. Oxidative Med Cell Longev 2013:157240

Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A (2017) Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. Eur J Nutr 56:1409–1420

Habauzit V, Sacco SM, Gil-Izquierdo A, Trzeciakiewicz A, Morand C, Barron D, Pinaud S, Offord E, Horcajada MN (2011) Differential effects of two *Citrus* flavanones on bone quality in senescent male rats in relation to their bioavailability and metabolism. Bone 49:1108–1116

Hall SD, Thummel K, Watkins PB, Lown KS, Benet LZ, Paine MF, Mayo RR, Turgeon DK, Bailey DG, Fontana RJ, Wrighton SA (1999) Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 27:161–166

He FJ, Nowson CA, MacGregor GA (2006) Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. Lancet 367:320–326

He FJ, Nowson CA, Lucas M, MacGregor GA (2007) Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Hum Hypertens 21:717–728

Hollman PC, Geelen A, Kromhout D (2010) Dietary flavonol intake may lower stroke risk in men and women. J Nutr 140:600–604

Horcajada MN, Habauzit V, Trzeciakiewicz A, Morand C, Gil-Izquierdo A, Mardon J, Lebecque P, Davicco MJ, Chee WS, Coxam V, Offord E (2008) Hesperidin inhibits ovariectomized-induced osteopenia and shows differential effects on bone mass and strength in young and adult intact rats. J Appl Physiol 104:648–654

Horowitz RM, Gentili B (1971) In: Birch GG, Green LF, Coulson CB (eds) Sweetness and sweeteners. Applied Science, London, pp 69–80

https://www.cbi.eu/market-information/natural-ingredients-health-products/trends/. Accessed 18 Oct 2018

Hwang JT, Yang HJ, Ha KC, So BO, Choi EK, Chae SW (2015) A randomized, double-blind, placebo-controlled clinical trial to investigate the anti-diabetic effect of *Citrus junos* Tanaka peel. J Funct Foods 18:532–537

Ismail A, Marjan ZM, Foong CW (2004) Total antioxidant activity and phenolic content in selected vegetables. Food Chem 87:581–586

Jeon SM, Bok SH, Jang MK, Kim YH, Nam KT, Jeong TS, Park YB, Choi MS (2002) Comparison of antioxidant effects of naringin and probucol in cholesterol-fed rabbits. Clin Chim Acta 317:181–190

Jeon SM, Kim HK, Kim HJ, Do GM, Jeong TS, Park YB, Choi MS (2007) Hypocholesterolemic and antioxidative effects of naringenin and its two metabolites in high-cholesterol fed rats. Transl Res 149:15–21

Judy W, Stogsdill W, Judy D, Judy J (2010) Efficacy of Diabetinol™ on glycemic control in insulin resistant hamsters and subjects with impaired fasting glucose-a pilot study. J Funct Foods 2:171–178

Jung UJ, Kim HJ, Lee JS, Lee MK, Kim HO, Park EJ, Kim HK, Jeong TS, Choi MS (2003) Naringin supplementation lowers plasma lipids and enhances erythrocyte antioxidant enzyme activities in hypercholesterolemic subjects. Clin Nutr 22:561–568

Kaleem M, Ahmad A (2018) Flavonoids as nutraceuticals. In: Therapeutic, probiotic, and unconventional Foods. Academic, London, pp 137–155

Kanaze FI, Bounartzi MI, Georgarakis M, Niopas I (2007) Pharmacokinetics of the *Citrus* flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. Eur J Clin Nutr 61:472–477

Kang P, Ryu KH, Lee JM, Kim HK, Seol GH (2016) Endothelium- and smooth muscle-dependent vasodilator effects of *Citrus aurantium* L. var. amara: Focus on $Ca^{(2+)}$ modulation. Biomed Pharmacother 82:467–471

Kawaguchi K, Maruyama H, Kometani T, Kumazawa Y (2006) Suppression of collagen-induced arthritis by oral administration of the *Citrus* flavonoid hesperidin. Planta Med 72:477–479

Kean RJ, Lamport DJ, Dodd GF, Freeman JE, Williams CM, Ellis JA, Butler LT, Spencer JP (2015) Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults. Am J Clin Nutr 101:506–514

Kesse-Guyot E, Fezeu L, Andreeva VA, Touvier M, Scalbert A, Hercberg S, Galan P (2012) Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. J Nutr 142:76–83

Kim SH, Hur HJ, Yang HJ, Kim HJ, Kim MJ, Park JH, Sung MJ, Kim MS, Kwon DY, Hwang JT (2013) *Citrus junos* tanaka peel extract exerts antidiabetic effects via AMPK and PPAR-γ both in vitro and in vivo in mice fed a high-fat diet. Evid Based Complement Alternat Med 2013:921012

Kinghorn AD, Chin YW, Pan L, Jia Z (2010) Natural products as sweeteners and sweetness modifiers. In: Comprehensive natural products II. Chemistry and biology, vol 3. Elsevier, Oxford, pp 269–315

Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A (2002) Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76:560–568

Kobayashi S, Konishi Y (2008) Transepithelial transport of flavanone in intestinal Caco-2 cell monolayers. Biochem Biophys Res Commun 368:23–29

Kobayashi S, Tanabe S, Sugiyama M, Konishi Y (2008) Transepithelial transport of hesperetin and hesperidin in intestinal Caco-2 cell monolayers. Biochim Biophys Acta 1778:33–41

Kometani T, Fukuda T, Kakuma T, Kawaguchi K, Tamura W, Kumazawa Y, Nagata K (2008) Effects of alpha-glucosylhesperidin, a bioactive food material, on collagen-induced arthritis in mice and rheumatoid arthritis in humans. Immunopharmacol Immunotoxicol 30:117–134

Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD, Sadat-Hossieny S (2012) Concord grape juice supplementation and neurocognitive function in human aging. J Agric Food Chem 60:5736–5742

Kurowska EM, Borradaile NM, Spence JD, Carrol CC (2000) Hypocholesterolemic effects of dietary *Citrus* juices in rabbits. Nutr Res 20:121–129

Lee SH, Jeong TS, Park YB, Kwon YK, Choi MS, Bok SH (1999a) Hypocholesterolemic effect of hesperetin mediated by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A cholesterol acyltransferase in rats fed high-cholesterol diet. Nutr Res 19:1245–1258

Lee SH, Park YB, Bae KH, Kwon YK, Lee ES, Choi MS (1999b) Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyltransferase in rats. Ann Nutr Metab 43:173–180

Leem E, Nam JH, Jeon MT, Shin WH, Won SY, Park SJ, Choi MS, Jin BK, Jung UJ, Kim SR (2014) Naringin protects the nigrostriatal dopaminergic projection through induction of gdnf in a neurotoxin model of Parkinson's disease. J Nutr Biochem 25:801–806

Leonardi T, Vanamala J, Taddeo SS, Davidson LA, Murphy ME, Patil BS, Wang N, Carroll RJ, Chapkin RS, Lupton JR, Turner ND (2010) Apigenin and naringenin suppress colon carcinogenesis through the aberrant crypt stage in azoxymethane-treated rats. Exp Biol Med (Maywood) 235:710–717

Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P (2007) Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 165:1364–1371

Ley J, Kindel G, Paetz S, Riess T, Haug M, Schmidtmann R, Krammer G (2005) Use of hesperetin for enhancing the sweet taste. Patent no. WO2007014879A1

Li P, Wang S, Guan X, Cen X, Hu C, Peng W, Wang Y, Su W (2014) Six months chronic toxicological evaluation of naringin in Sprague-Dawley rats. Food Chem Toxicol 66:65–75

Li CY, Zug C, Qu HC, Schluesener H, Zhang ZY (2015) Hesperidin ameliorates behavioral impairments and neuropathology of transgenic APP/PS1 mice. Behav Brain Res 281:32–42

Liu L, Shan S, Zhang K, Ning Z, Lu X, Cheng Y (2008) Naringenin and hesperetin, two flavonoids derived from *Citrus aurantium* up-regulate transcription of adiponectin. Phytother Res 22:1400–1403

Liu Y, Niu L, Cui L, Hou X, Li J, Zhang X, Zhang M (2014) Hesperetin inhibits rat coronary constriction by inhibiting Ca(2+) influx and enhancing voltage-gated K(+) channel currents of the myocytes. Eur J Pharmacol 735:193–201

Loizzo MR, Tundis R, Bonesi M, Menichini F, De Luca D, Colica C, Menichini F (2012) Evaluation of *Citrus aurantifolia* peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. J Sci Food Agric 92:2960–2967

Loizzo MR, Tundis R, Bonesi M, Sanzo GD, Verardi A, Lopresto CG, Pugliese A, Menichini F, Balducchi R, Calabrò V (2016) Chemical profile and antioxidant properties of extracts and essential oils orom *Citrus* × *limon* (L.) Burm. cv. Femminello Comune. Chem Biodivers 13:571–581

Lown KS, Fontana RJ, Schmiedlin-Ren P, Turgeon DK, Watkins PB (1995) Interindividual variation in intestinal mdr1: lack of short term diet effects. Gastroenterology 108:A737

Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, Cheng Xiao C, Lu C, Yuanyan Liu Y (2015) *Citrus* fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chem Cent J 9:68–82

Ma L, Sun Z, Zeng Y, Luo M, Yang J (2018) Molecular mechanism and health role of functional ingredients in blueberry for chronic disease in human beings. Int J Mol Sci 19:2785–2804

Manach C, Morand C, Gil-Izquierdo A, Bouteloup-Demange C, Rémésy C (2003) Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. Eur J Clin Nutr 57:235–242

Manach C, Williamson G, Morand C, Scalbert A, Rémésy C (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S

Marathe PH, Gao HX, Close KL (2017) American diabetes association standards of medical care in diabetes. J Diabetes 9:320–324

Mastantuono T, Battiloro L, Sabatino L, Chiurazzi M, Di Maro M, Muscariello E, Colantuoni A, Lapi D (2015) Effects of *Citrus* flavonoids against microvascular damage induced by hypoperfusion and reperfusion in rat pial circulation. Microcirculation 22:378–390

Matsumoto H, Ikoma Y, Sugiura M, Yano M, Hasegawa Y (2004) Identification and quantification of the conjugated metabolites derived from orally administered hesperidin in rat plasma. J Agric Food Chem 52:6653–6659

Meyer OC (1994) Safety and security of Daflon 500 mg in venous insufficiency and in hemorrhoidal disease. Angiology 45:579–584

Miyagi Y, Om AS, Chee KM, Bennink MR (2000) Inhibition of azoxymethane-induced colon cancer by orange juice. Nutr Cancer 36:224–229

Miyake Y, Sakurai C, Usuda M, Fukumoto S, Hiramitsu M, Sakaida K, Osawa T, Kondo K (2006) Difference in plasma metabolite concentration after ingestion of lemon flavonoids and their aglycones in human. J Nutr Sci Vitaminol 52:54–60

Morand C, Dubray C, Milenkovic D, Lioger D, Martin JF, Scalbert A, Mazur A (2011) Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J Clin Nutr 93:73–80

Moura LM, de Sylos CM (2009) The effect of the manufacturing process on the *Citrus* juice on the concentrations of flavanones. Aliment Nutr 19:379–384

Mulero J, Bernabé J, Cerdá B, García-Viguera C, Moreno DA, Albaladejo MD, Avilés F, Parra S, Abellán J, Zafrilla P (2012) Variations on cardiovascular risk factors in metabolic syndrome after consume of a *Citrus*-based juice. Clin Nutr 31:372–377

Mullen W, Archeveque MA, Edwards CA, Matsumoto H, Crozier A (2008) Bioavailability and metabolism of orange juice flavanones in humans: impact of a full-fat yogurt. J Agric Food Chem 56:11157–11164

Nandakumar N, Balasubramanian MP (2011) Hesperidin protects renal and hepatic tissues against free radical-mediated oxidative stress during DMBA-induced experimental breast cancer. J Environ Pathol Toxicol Oncol 30:283–300

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425

Onakpoya I, O'Sullivan J, Heneghan C, Thompson M (2017) The effect of grapefruits (*Citrus* paradisi) on body weight and cardiovascular risk factors: a systematic review and meta-analysis of randomized clinical trials. Crit Rev Food Sci Nutr 57:602–612

Orhan IE, Nabavi SF, Daglia M, Tenore GC, Mansouri K, Nabavi SM (2015) Naringenin and atherosclerosis: a review of literature. Curr Pharm Biotechnol 16:245–251

Ovaskainen ML, Torronen R, Koponen JM, Sinkko H, Hellstrom J, Reinivuo H, Mattila P (2008) Dietary intake and major food sources of polyphenols in Finnish adults. J Nutr 138:562–566

Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:1–15

Parada J, Aguilera JM (2007) Food microstructure affects the bioavailability of several nutrients. J Food Sci 72:R21–R32

Peng H, Cheng F, Huang Y, Chen C, Tsai T (1998) Determination of naringenin and its glucuronide conjugate in rat plasma and brain tissue by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 714:369–374

Peterson JJ, Beecher GR, Bhagwat SA, Dwyer JT, Gebhardt SE, Haytowitz DB, Holden JM (2006a) Flavanones in grapefruit, lemons, and limes: a compilation and review of the data from the analytical literature. J Food Compos Anal 19:S74–S80

Peterson JJ, Dwyer JT, Beecher GR, Bhagwat SA, Gabhardt SE, Haytowitz DB, Holden JM (2006b) Flavanones in oranges, tangerines (mandarins), tangors and tangelos: a compilation and review of the data from the analytical literature. J Food Compos Anal 19:S66–S73

Piao YJ, Choi JS (2008) Enhanced bioavailability of verapamil after oral administration with hesperidin in rats. Arch Pharm Res 31:518–522

Priscilla DH, Roy D, Suresh A, Kumar V, Thirumurugan K (2014) Naringenin inhibits α-glucosidase activity: a promising strategy for the regulation of postprandial hyperglycemia in high fat diet fed streptozotocin induced diabetic rats. Chem Biol Interact 210:77–85

Priscilla DH, Jayakumar M, Thirumurugan K (2015) Flavanone naringenin: an effective anti-hyperglycaemic and antihyperlipidemic nutraceutical agent on high fat diet fed streptozotociin induced type 2 diabetic rats. J Funct Foods 14:363–373

Rangel-Huerta OD, Aguilera CM, Perez-de-la-Cruz A, Vallejo F, Tomas-Barberan F, Gil A, Mesa MD (2017) A serum metabolomics-driven approach predicts orange juice consumption and its impact on oxidative stress and inflammation in subjects from the BIONAOS study. Mol Nutr Food Res 61:1600120

Rendeiro C, Dong H, Saunders C, Harkness L, Blaze M, Hou Y, Belanger RL, Corona G, Lovegrove JA, Spencer JP (2016) Flavanone-rich *Citrus* beverages counteract the transient decline in postprandial endothelial function in humans: a randomised, controlled, double-masked, cross-over intervention study. Br J Nutr 116:1999–2010

Rizza S, Muniyappa R, Iantorno M, Kim JA, Chen H, Pullikotil P, Senese N, Tesauro M, Lauro D, Cardillo C, Quon MJ (2011) *Citrus* polyphenol hesperidin stimulates production of nitric oxide in endothelial cells while improving endothelial function and reducing inflammatory markers in patients with metabolic syndrome. J Clin Endocrinol Metab 96:E782–E792

Roowi S, Mullen W, Edwards CA, Crozier A (2009) Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans. Mol Nutr Food Res 53:S68–S75

Ross JA, Kasum CM (2012) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 22:19–34

Ross SA, Ziska DS, Zhao K, ElSohly MA (2000) Variance of common flavonoids by brand of grapefruit juice. Fitoterapia 71:154–161

Salehi B, Fokou PVT, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, Sharifi-Rad J (2019) The therapeutic potential of naringenin: a review of clinical trials. Pharmaceuticals 12:E11

Schwingshackl L, Hoffmann G, Lampousi AM, Knüppel S, Iqbal K, Schwedhelm C, Bechthold A, Schlesinger S, Boeing H (2017) Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. Eur J Epidemiol 32:363–375

Sharma M, Akhtar N, Sambhav K, Shete G, Bansal AK, Sharma SS (2015) Emerging potential of *Citrus* flavanones as an antioxidant in diabetes and its complications. Curr Top Med Chem 15:187–195

Smith CJ (1964) Non-hormonal control of vaso-motor flushing in menopausal patients. Chic Med 67:193–195

So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK (1996) Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and *Citrus* juices. Nutr Cancer 262:167–181

Sorrenti V, Di Giacomo C, Russo A, Acquaviva R, Barcellona ML, Vanella A (2004) Inhibition of LDL oxidation by red orange (*Citrus sinensis*) extract and its active components. Cell Biol Toxicol 69:480–484

Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, Nabavi SF, Devi KP, Loizzo MR, Tundis R, Nabavi SM (2015) Genistein and cancer: current status, challenges, and future directions. Adv Nutr 6:408–419

Spigoni V, Mena P, Fantuzzi F, Tassotti M, Brighenti F, Bonadonna RC, Del Rio D, Dei Cas A (2017) Bioavailability of bergamot (*Citrus bergamia*) flavanones and biological activity of their circulating metabolites in human pro-angiogenic cells. Nutrients 9:E1328–E1343

Tanaka T, Makita H, Kawabata K, Mori H, Kakumoto M, Satoh K, Hara A, Sumida T, Tanaka T, Ogawa H (1997) Chemoprevention of azoxymethane induced rat colon carcinogenesis by the naturally occurring flavonoids, diosmin and hesperidin. Carcinogenesis 18:957–965

Testai L, Calderone V (2017) Nutraceutical value of *Citrus* flavanones and their implications in cardiovascular disease. Nutrients 9:502–515

Tomas-Barberan FA, Clifford MN (2000) Flavanones, chalcones and dihydrochalcones. Nature, occurrence and dietary burden. J Sci Food Agric 80:1073–1080

Tomás-Navarro M, Vallejo F, Tomás-Barberán FA (2014) Bioavailability and metabolism of *Citrus* fruit beverage flavanones in humans (chapter 40). In: Watson RR, Preedy VR, Zibadi S (eds) Polyphenols in human health and disease, vol. 1. Academic Press is an imprint of Elsevier, Amsterdam, pp 537–551

Toth PP, Patti AM, Nikolic D, Giglio RV, Castellino G, Biancucci T, Geraci F, David S, Montalto G, Rizvi A, Rizzo M (2016) Bergamot reduces plasma lipids, atherogenic small dense LDL, and subclinical atherosclerosis in subjects with moderate hypercholesterolemia: a 6 months prospective study. Front Pharmacol 6:299

Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M (2007) *Citrus* flavonoids: molecular structure, biological activity and nutritional properties: a review. Food Chem 104:466–479

Tsai TH (2002) Determination of naringin in rat blood, brain, liver, and bile using microdialysis and its interaction with cyclosporin A, a P-glycoprotein modulator. J Agric Food Chem 50:6669–6674

Tundis R, Loizzo MR, Bonesi M, Menichini F, Mastellone V, Colica C, Menichini F (2012) Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of *Citrus aurantifolia* Swingle, *C. aurantium* L., and *C. bergamia* Risso and Poit. peel essential oils. J Food Sci 77:H40–H46

Turati F, Rossi M, Pelucchi C, Levi F, La Vecchia C (2015) Fruit and vegetables and cancer risk: a review of southern European studies. Br J Nutr 113(Suppl 2):S102–S110

Uesawa Y, Mohri K (2008) Hesperidin in orange juice reduces the absorption of celiprolol in rats. Biopharm Drug Dispos 29:185–188

van der Kolk DM, de Vries EG, van Putten WJ, Verdonck LF, Ossenkoppele GJ, Verhoef GE, Vellenga E (2000) P-Glycoprotein and multidrug resistance protein activities in relation to treatment outcome in acute myeloid leukemia. Clin Cancer Res 6:3205–3214

Vinayagam R, Xu B (2015) Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab 12:60

Wang X, Sakurai T, Chen X, Sun H, Wang Z, Sun Q, Sun W, Cao H (2008) Hydrolysis of flavanone glycosides and degradation of the corresponding aglycones from dried immature *Citrus* fruit by human fecal flora in vitro. Planta Med 74:1751–1755

Wang X, Ouyang YY, Liu J, Zhao G (2014) Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. Br J Nutr 111:1–11

Wyss-Coray T (2016) Ageing, neurodegeneration and brain rejuvenation. Nature 539:180–186

Yang M, Tanaka T, Hirose Y, Deguchi T, Mori H, Kawada Y (1997) Chemopreventive effects of diosmin and hesperidin on *N*-butyl-*N*-(4 hydroxybutyl)nitrosamine-induced urinary-bladder carcinogenesis in male ICR mice. Int J Cancer 73:719–724

Zaidun NH, Thent ZC, Latiff AA (2018) Combating oxidative stress disorders with *Citrus* flavonoid: Naringenin. Life Sci 208:111–122

Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventós RM, Berenguer T, Jakszyn P, Barricarte A, Ardanaz E, Amiano P, Dorronsoro M, Larrañaga N, Martínez C, Sánchez MJ, Navarro C, Chirlaque MD, Tormo MJ, Quirós JR, González CA (2010) Estimation of dietary sources and flavonoid intake in a Spanish adult population (EPIC-Spain). J Am Diet Assoc 110:390–398

Zhang D, Hamauzu Y (2004) Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem 88:503–509

Zhou L, Cui M, Zhao L, Wang D, Tang T, Wang W, Wang S, Huang H, Qiu X (2018) Potential metabolic drug-drug interaction of *Citrus aurantium* L. (Rutaceae) evaluating by its effect on 3 CYP450. Front Pharmacol 9:1–11