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Dietary Polyphenols as Functional Ingredients: Relevance to Citrus Fruits

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ABSTRACT

Numerous studies have demonstrated the biologically significant roles played by dietary polyphenols, such as antioxidants, antimicrobials, anticarcinogens or antimutagens, leading to their recognition as potential functional ingredients or food additives. This paper presents a revision of works published in recent years on the topic and looks at possible future trends in the sector. We also provide additional information on other biological effects of phenolics, with special attention to phenolics in citrus fruits, which could be of interest for the formulation of functional foods.

Keywords: flavonoids, fruits, functional compounds, healthy properties

CONTENTS

INTRODUCTION 120
BIOAVAILABILITY OF DIETARY POLYPHENOLS 121
TECHNOLOGICAL PROPERTIES OF FLAVONOIDS 121
Antioxidant activity 121
Antimicrobial activity 121
HEALTH EFFECTS OF PHENOLICS 122
Anticarcinogenic activity 122
Anti-inflammatory activity 123
Cardioprotective activity 123
Other health benefits associated with the activities of phenolics 123
PRINCIPAL PHENOLIC COMPOUNDS IN CITRUS FRUITS 123
Flavonoids 123
Cinnamic acid derivatives 124
Coumarins 124
Other phenolic compounds 124
CONCLUSIONS AND FUTURE TRENDS 124
ACKNOWLEDGEMENTS 124
REFERENCES 124

INTRODUCTION

Polyphenols are among the most common and widely distributed phytochemicals in fruits and vegetables, with 8000 phenolic structures currently known (Bravo 1998). These compounds are produced as the result of secondary plants metabolism and they are generally found bound to sugars, which means that they are water soluble (Guthrie and Kurawska 2001; Ross and Kasum 2002). The common structure of all polyphenols is an aromatic ring in the last hydroxyl group. Dietary polyphenols, commonly known as flavonoids, include several structurally heterogeneous subgroups and are known to contribute to color and taste in foods. Similarly, flavonoids have a common structure diphenylpropanes (C6-C3-C6), consisting of two aromatic rings binding across three carbons (**Fig. 1**) (Kris *et al.* 2002; Ross and Kasum 2002). One form of classification of flavonoids subdivides the phytochemicals into six types according to variations of the C ring: flavones, flavonols, flavanones, catechins, anthocyanidins and isoflavones (Ross and Kasum 2002; Rotelli *et al.* 2003). Recent interest in dietary polyphenols has concentrated on their likely health-giving benefits as reducing agents, more commonly termed antioxidants, which may play a role in the protection of body tis-

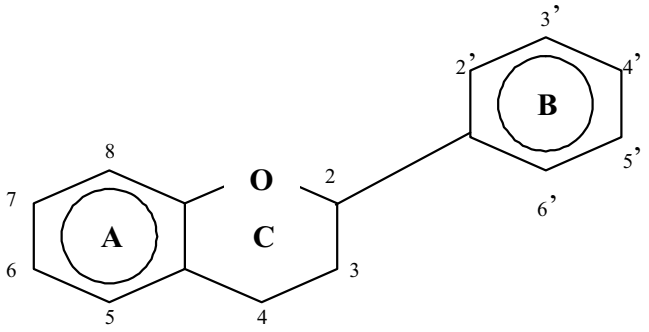

Fig. 1 Representative structure of flavonoids.

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sues against oxidative stress (Diplock *et al.* 2000; Duthie *et al.* 2000; Wollgast and Anklam 2000; Papadopoulou and Frazier 2004). There is strong evidence supporting the role of polyphenols in the prevention of age-related diseases, including cardiovascular disease and cancer. In this respect, it is of note that the nutrition community has recognized the importance of dietary polyphenols as health-promoting agents. The food industry, therefore, now has the opportunity of including polyphenols in products to provide health benefits, while offering a healthy image. The purpose of this work is to review recent information on the health effects and the technological properties of dietary polyphenols, with an emphasis on citrus polyphenols. This information could be of interest for the use of dietary polyphenols as functional ingredients in the food industry.

BIOAVAILABILITY OF DIETARY POLYPHENOLS

The absorption of flavonoids as a consequence of diet has been considered ineffective for a long time due to the presence of β-glycoside bonds (except for catechins) which can not to be hydrolysed by human enzymes. However, the absorption of these flavonoids is possible due to the existence of intestinal microbiota that are able to hydrolyse these bonds (Hollman and Katan 1999; Walle 2004). The nutritional flavonoids are absorbed from the gastro-intestinal tract after a preliminary degradation (Justesen *et al.* 2000; Heim *et al.* 2002; Ross and Kasum 2002). After the release of the aglycone form from glycosides by bacterial enzymes (Justesen *et al.* 2000; Guthrie and Kurawska 2001; Havsteen 2002), around 15% of the aglycone flavonoids are absorbed, along with biliary micelles, into the cells before being transferred to the lymph gland. The lymph allows the flavonoids to penetrate in the blood close to the liver and most (around 80%) is absorbed in the first step, part being linked to albumin serum. The other flavonoids are excreted through excrement or urine.

An important factor in the absorption of glycoside flavonoids from the gut is the associated sugar.

The average life of a typical flavonoid in the human body is estimated about 1 to 2 hours, although there is no research that provides specific data on the subject. It is important to highlight that the degradation of flavonoids by fecal microflora is almost complete after 48 hours *in vitro*, although this time differs for different flavonoids (Justesen *et al.* 2000). The uptake of 100 mg of polyphenols results in a concentration of about 300 μM in the gut. Normally the plasma levels will not exceed 1 μM. Urinary excretion varies from less than 1% to about 25% depending on their gut absorption, enterohepatic cycling, and metabolism (Murkovic 2003; Kanaze *et al.* 2007). In particular, the type of polyphenol and the glycosylation influence bioavailability. A higher degree of polymerization results in a sharp decrease in bioavailability (Murkovic 2003).

TECHNOLOGICAL PROPERTIES OF FLAVONOIDS

Antibacterial and antioxidant activities are two of the most important technological properties of dietary polyphenols. The antioxidant and antimicrobial mechanisms of the principal phenolic compounds are shown in **Table 1**.

Table 1 Technological properties of phenolic compounds.

Property	Mechanism
Antioxidant	➢ Direct electron location
	➢ Enzymic reduction
	➢ ROS[a] quenching
Antimicrobial	➢ Membrane permeability modification
	➢ Interferes with metabolic synthesis of macromolecules and nucleic acids
	➢ Decreasing electron transport

[a]ROS: reactive oxygen species.

Antioxidant activity

Oxygen-reactive species are formed *in vivo* during aerobic metabolism and may cause damage to DNA, protein and lipids despite the natural antioxidant defense system. The accumulation of irreparably damaged metabolites can result in cancer, atheroesclerosis, diabetes and chronic inflamemations (Ross and Kasum 2002; Baghurst 2003; Lule and Xia 2005).

The antioxidant activity of phenolic compounds is well-known. Such activity has been attributed both to the ability to scavenge free radicals and to the chelating capacity of scavenging metallic ions.

Several authors have demonstrated that some flavonoids show an antioxidant capacity in a large range of chemical-oxidative systems, because of their ability to scavenge practically all known oxidant structures, including the superoxide anion, hydrogen peroxide, hydroxyl radical, singlet oxygen, alkolxyl and peroxyl radicals (alkyl, aryl and nitrogen derivatives), both in aqueous and organic systems (Benavente-García *et al.* 1997; Ross and Kasum 2002; Edenharder and Grünhage 2003).

This hydrophylicity of flavonoids leads to the initial radical chains binding to the membrane interface, a characteristic which prevents the reactions of the radical chains from progressing (Havsteen 2002). These antioxidant capacities of flavonoids are closely related with their special structure. Three structural groups are important in determining their capacity to scavenge: 1) the O-dihydroxy (catechol) structure in the B ring, which is the obvious radical target site; 2) the 2,3- double bond in conjugation to the 4-keto function, which is responsible for electron delocalization; and 3) the presence of both hydroxyl groups (the 3-OH and the 5-OH) for maximal radical-scavenging potentials (Benavente-Garcia *et al.* 1997; Lien *et al.* 1999; Murkovic 2003). In addition to this radical scavenging capacity, the relatively high stability of the "antioxidant radical" intermediate formed is of great importance (Benavente-Garcia *et al.* 1997; Cetkovic *et al.* 2004).

In addition to their radical-scavenging activity, some flavonoids can chelate metals ions. This increases their antioxidant activity, because they eliminate and neutralize the metallic ions from the hepatocytes. The chelation of metallic cations does not necessarily inactivate the free radical scavenging capacity of flavonoids. Iron is sensitive to oxygen and therefore ferric iron and superoxide are formed, which can induce the generation of hydrogen peroxide. The reaction of iron with hydrogen peroxide generates hydroxyl radicals, which oxidize the nearby biomolecules. In this process, known as the Fenton reaction, the production of hydroxyl radicals is related to the concentration of iron and copper. The different structures of the flavonoids, 3',4'-catechol, 4-oxo and 5-OH, largely inhibit the Fenton oxidation reaction. These polyphenols are more effective inhibitors of the oxidation induced by the metal, than the non-metallic components (Heim *et al.* 2002).

Candidate phenolic antioxidants in foods include flavonoids, anthocyanins, catechins, chalcones, hydroxybenzoic, and hydroxycinnamic acids, many of which are present in fruit juices (Lule and Xia 2005).

Antimicrobial activity

Although less potent than antibiotics, phenolics are interesting antimicrobial agents when used alone or in combination with synthetic additives (Pszczola 2002; Lule and Xia 2005). Their mode of action is concentration-dependent, and comparatively high concentrations (up to 2000 ppm) are possible.

Since flavonoids are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found to be effective *in vitro* antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex

with bacterial cell walls (Cowan 1999).

Phenols seem to affect membrane permeability, decreesing electron transport and nutrient uptake, and possibly interfering with the metabolic synthesis of macromolecules and nucleic acids. The small sizes of the attached groups facilitate their passage through the cell membrane. Even though synthetic preservatives do not usually kill spores, their growth can be inhibited by phenols because of their ability to permeate the spore coat (Nichas 1995). Gallic, *p*-hydroxybenzoic acid, and related phenolics have been found to retard or partially inhibit the growth and toxin production of *Clostridium botulinum* types A and B, and the inhibitory activity increases with a decrease in the bacterial cell concentration (Pierson and Reddy 1982).

Both the dissociation of the acid moiety and the presence of one or more reactive double bonds contribute to the antimicrobial effect. A linear relationship between the number of carbon atoms in an alkali chain and the antimicrobial activity has been observed, whereas an increased number of hydroxyl groups in the phenol group and greater oxidation level seems to increase toxicity (Cowan 1999).

The inhibitory potential of the functional groups attached to the benzene ring has been estimated to increase from COOH, p-OH, CHO to CH=CH, whereas m-OH has no effect and OCH_3 has a stimulating effect (Tran and Chamber 1985, 1986). In the case of aromatic acids, they are less toxic than the corresponding aldehydes. Synergistic inhibitory effects have also been reported (Klinke *et al.* 2001).

Phenolic acids, such as ferulic, coumaric, sinapic or caffeic acid, show antimicrobial activity comparable or superior to that of mixtures of benzoic acid and sorbic acid (Cirigliano *et al.* 2000). Mixtures of chlorogenic acid, caffeic acid, gallic acid and protocatechuic acid have shown antimicrobial action against *Escherichia coli*, *Salmonella typhimurium* and *Bacillus cereus* (Rodríguez de Sotillo *et al.* 1998). Several authors have described the antimicrobial activity of different phenolic compounds against the microbes usually associated with food spoilage (Fernández-López *et al.* 2005; Viuda-Martos *et al.* 2007, 2008c).

Antifungal activities for phenolic compounds presents in different fruits, vegetables and spices have also been described. Nychas (1995) indicated that phenolic compounds could denature the enzymes responsible for spore germination or interfere with the amino acids involved in germination. Once the phenolic compounds have crossed the cellular membrane, interactions with membrane enzymes and proteins cause an opposite flow of protons, affecting cellular activity. Davidson (2001) reported that the exact cause-effect relation for the mode of action of phenolic compounds, such as thymol, eugenol and carvacrol, has not been determined, although it seems that they may inactivate essential enzymes, react with the cell membrane, or disturb genetic material functionality. Different phenolic compounds present in extracts from several spices and citrus fruits show antifungal activity against *Aspergillus niger* and *Aspergillus flavus* (Viuda-Martos *et al*. 2008a, 2008b).

A number of flavonoids present in foods of plant origin possess antiviral activity (Lule and Xia 2005). For example, tannins from strawberries have the ability to inactivate polio, enteric and herpes viruses (Konowalchuk and Speirs 1976). Quercetin, found in a number of fruits such as apple, apricot, fig, plum, strawberry and tomato has shown antiviral activities against the herpes virus, influenza virus and polio virus *in vivo* and *in vitro* studies (Middleton 1986; Musci 1986).

HEALTH EFFECTS OF PHENOLICS

Phenolic compounds contribute to the sensory properties of foods, particularly their color and astringency. The effect of polyphenols on health includes protection against environmental stresses. This health-promoting effect has aroused the interest of scientists, food manufactures and consumers alike, since a wide range of pharmacological and biochemical activities (anticarcinogenic, antiinflamatory and antiatherogenic) have been attributed to these compounds (Garrote *et al.* 2004).

Anticarcinogenic activity

Because of their antioxidant properties and their ability to absorb ultraviolet light, flavonoids can act at all levels of the carcinogenic process: in the damage to DNA (or in the initial steps), during tumoral growth (or in the previous steps) and during invasion (or in the proliferative phases) (Benavente-Garcia *et al.* 1997).

Flavonoids are involved in a great number of regulatory routes, such as cellular growth, metabolic energy, cell division, transcription, gene reparation, cellular transmission, inflammation, among others. These processes are related with a great number of different types of cancer. Flavonoids can act as antioxidants, free radical scavengers, enzymes hormones (including neurotrasmittor) and antihormones inhibitors, or inducers of gene expression. These effects can be explained by the high mobility of their electrons in the benzene nucleus and by the similarity between flavonoids and the different substances inherent in the biochemistry of biological cells.

For all these reasons, flavonoids act as chemical-protectors in three principal ways: first, they prevent the metabolism of cancer; secondly, they prevent the proliferation of tumorigenic cells by inactivating them or by regulating the activity of the pro-oxidant enzymes and, finally, they induce the death of carcinogenic cells (apoptosis) (Marín *et al.* 2002).

Of the biochemical routes that are influenced by flavonoids, the routes that often act upon cytoplasmic/nuclear hormonal receptors are more sensitive than the routes that act upon the enzymatic inhibitor mechanisms and similar processes, which need a high concentration of flavonoids (Havsteen 2002).

Therefore, flavonoids can influence the development of several cancers in different ways through interacting with hormonal receptors andt through their influence on enzymes and on the energy resources of cells (Benavente-Garcia *et al.* 1997; Havsteen 2002).

Flavonoids affect cellular metabolism in different ways, either by affecting the level of cellular membranes or the level of intracellular enzymes. The effects of flavonoids often include the inhibition of glycolysis, which is important since this metabolic route is often very active in cellular tumors. Flavonoids can affect the activity of several enzymes involved in the transduction signs of mithogenesis (kinase, phospholipases, phosphodiesterases) and other regular enzymes, which are critical for growth and cellular proliferation (Benavente-Garcia *et al.* 1997).

Guthrie and Kurawska (2001) and So *et al.* (1996) have reported antiproliferative effects for hesperetin, tangeritin and the nobilentin on carcinogenic breast cells (MDA-MB-435). A synergic effect has been observed for combinations of flavonoids containing tocotrienoles with tamoxifen, which is a drug that is used against breast cancer (Carroll *et al.* 1996; Guthrie and Kurawska 2001). The experiments of Guthrie *et al.* (2000) demonstrated that flavonoids inhibit the proliferation of both positive and negative estrogen receptor breast cancer cells. Many vegetables and fruits possess ellagic and cholrogenic acids, which serve as potential preventers against several carcinogens (Huang *et al.* 1992; Lule and Xia 2005). Recent studies have shown that some phenolic antioxidants present in extra virgin olive oil are involved in the etiology of fat-related neoplasms, such as cancer of the breast and colorectum (Owen *et al.* 2000).

It has been shown that some environmental toxins with oncogenic activity (aromatic hydrocarbons and free radicals) are detoxified by the oxidation properties of flavonoids. Flavonoids can facilitate the breaking off of aromatic rings and the breaking down to relatively safe smaller products, like aromatic carbonic acids that can be excreted in urine (Havsteen 2002).

Several authors have suggested that some food phenolics may have potential use as anticancer agents in humans

(Manthey and Guthrie 2002).

Anti-inflammatory activity

The anti-inflammatory effect of some flavonoids (diosmin, hesperidin and others) depends on how they influence the metabolism of arachidonic acid and histamine. These flavonoids inhibit the secretion of lysosomal enzyme and arachidonic acid from the membrane due to the inhibition of lipoxygenase, cycloxygenase and phospholipase A_2 (Benavente-Garcia *et al.* 1997; Manthey and Guthrie 2002; Rotelli *et al.* 2003).

The inhibition of arachidonic acid production in the inflamed cells results in a lower quantity of arachidonic matter being provided to the lipoxygenase and cycloxygenase pathways. On one hand, this involves a decrease in endoperoxide, prostaglandin and thromboxane levels and, on the other, a decrease in hydroperoxy and hydroxyeicosatrienoic acids, a fall that underlines the lower production of histamine, which acts during the first steps of the inflammation process (Benavente-Garcia *et al.* 1997).

Additionally, some flavonoids show antiinflammatory action through the inhibition of prostaglandins E_2, which plays a very important role in the inflammation process. The flavonoids in question inhibit the enzyme, cycloxygenase (COX), which plays a key part in the formation of prostaglandins (El-Shafae and El-Domiaty 2001).

Cardioprotective activity

Cardiovascular diseases are the most frequent cause of death in industrialized countries. However, various epidemiological studies have demonstrated that a diet rich in fruits and vegetables decreases such risks (Kurawska *et al.* 2000; Havsteen 2002; Fernández-Lopez *et al.* 2004).

Recent evidence has shown that polyphenols have an important effect on the intricate regulatory process involved in these diseases. A common factor of these diseases is an inflammation of the vascular wall, which is denominated atherosclerosis, although the tendency to develop the disease depends strongly on the genetic disposition of the individual and environmental factors, such as the composition of the diet, the intake of alcohol and tobacco and psychological stress. Polyphenols can act at different stages of disease (Havsteen 2002; Kris *et al.* 2002; Ross and Kasum 2002).

The key to the biosynthesis of cholesterol is an enzyme, HMG-CoA reductase (3-hydroxy-3methyl-glutaryl-coenzyme A reductase), which is regulated by phosphorylation/desphosphorylation reactions. This is regulated by the action of dependent protein phosphoquinase cAMP (cyclical AMP), which phosphorylizes the residuals of serine and threonine using ATP. The coenzyme cAMP is attached to the enzyme PDE cAMP (AMP cyclical phosphodiesterase), which is inhibited by flavonoids (Benavente-Garcia *et al.* 1997; Havsteen 2002). The consequence is that the concentration of cAMP increases, as does the phosphorylation of the HMG-CoA, but, despite this increase, the production of endogenous choresterol decreases. Additionally, flavonoids can interact with protein-phosphatase, which liberates aliphatic phosphoesters from reductase HMG-CoA, and thus restore the activity of this enzyme. This process results from the fact that metalloenzyme phosphatases add a highly divalent metal, ion (Zn^{2+}), which is essential for their action. As the metallic ions have great affinity for flavonoids, they produce a change in the position of the metallic ion, which has a negative effect on the catalytic action. Consequently, flavonoids inhibit the HMG-CoA through a double mechanism (Benavente-Garcia *et al.* 1997; Havsteen 2002; Choi *et al.* 2003).

Polyphenols also exert their influence on steroid metabolism. The hydrophobicity of the aglycone group of flavonoids suggests that these are introduced into hepatocytes by the lipoprotein released from the lysosomes and they are linked to the receptors of the cytoplasmatic steroids that are introduced inside the cell nucleus (Havsteen 2002). There, either the receptor section of the complex reacts with the elements regulating the steroids, or flavonoids act between the base of this segment (So *et al.* 1996; Havsteen 2002; Zern and Fernández 2005).

Flavonoids show an apparent regulatory action on the erythrocyte aggregation and inhibit the ratio of erythrocyte sedimentation (RSE) (Benavente-Garcia *et al.* 1997).

Orallo *et al.* (2005) reported the potential vasorelaxant effect of some citrus flavonoids related to the inhibition of cyclic nucleotide phosphodiesterase.

Other health benefits associated with the activities of phenolics

Trans-cinnamic acid has been proposed for preventing and/or treating several diabetic complications because of its ability to inhibit aldose reductase and prevent the conversion of glucose into sorbitol (Lee 2002).

Some authors have reported that certain polyphenols can provoke the inhibition of prostaglandins COX (cycloxygenase) and prevent the development of the mechanisms of pain (El-Shafae and El-Domiaty 2001; Havsteen 2002).

One of the ways flavonoids affect asthma and allergies is through the inhibition of the biosynthesis of cicosanoids. This plays an important role in the regulating the contraction of bronchial smooth muscle since nasal epithelium cells have superficial receptors for leucotrienes. Flavonoids are reported to inhibit the secretion of histamine and serotonin by the mast cells activated by the allergen (Benavente-Garcia *et al.* 1997; Havsteen 2002). Nevertheless, the mechanism used by flavonoids is still unknown.

PRINCIPAL PHENOLIC COMPOUNDS IN CITRUS FRUITS

Cinnamic acid derivatives, coumarins and flavonoids are the major phenolic compound groups occurring in citrus fruits, where they are found in the free form and/or as glycosides (**Table 2**) (Horowitz and Gentili 1977; Manthey and Grohmann 2001; Wilfried *et al.* 1994a, 1994b). Their concentration differs according to the section of the fruits.

Flavonoids

The four main types of flavonoids present in citrics are flavanones, flavones, flavonols and the anthocyans (the last one only in orange juice) (**Fig. 2**) (Tripoli *et al.* 2007). Flavonones are the most abundant, while only some species of red orange (blood) have anthocyans (Benavente Garcia *et al.* 1997; Mazza 1998).

Table 2 Principal phenolic compounds in citrus fruits.

Phenolic group	Subgroup	Compound
Flavonoids	Flavanones	naringenin, eriodictyol, hesperetin, isosakuranetin
	Flavanone glycosides	naringin, neoeriocitrin, hesperidin
	Polymethoxylated flavones	nobiletin, sinensetin
	Glycosylated flavones	diosmin, neodiosmin
Cinnamic acid derivatives	Hydroxycinnamic acid	
	Glycosides of cinnamic acid	phlorin, coniferin
Coumarins		Xanthyletin, seselin, scoparone, auraptene

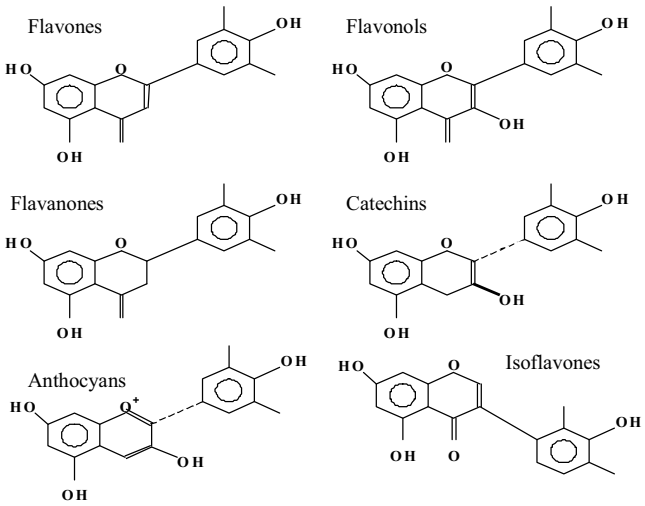

Fig. 2 Chemical structure of the principal flavonoids found in citrus fruits.

In the mature fruit, the flavonoid concentration is highest in the tissue (95-99%) and lowest in the juice (1-5%). Of the tissues, the highest content is in the peel (albedo 30-50% and flavedo 10-20%) and the lowest is in the pulp (30-50%) (Braddock 1999).

The peel is the primary waste product from processing citrus fruits (orange peels represent approximately 45% of the fruit mass). The highest concentration of flavanones and flavanone glycosides occur in the peel (Kannes *et al.* 1993; Bocco *et al.* 1998). For example, orange and grapefruit peels contain between 13.5 and 22.3 g flavonoids/kg of dry weight (Sinclair 1972; Bocco *et al.* 1998). The presence of four flavanones, namely, naringenin, eriodictyol, hesperetin and isosakuranetin has been reported. According to Kannes *et al.* (1992), flavanone glycosides, such as naringin, neoeriocitrin and hesperidin comprise 50 to 80% of total flavonoids in citrus fruits. In orange peels, hesperidin is the predominant glycoside (Manthey and Grohmann 1996) while in lemon peels neoeriocitrin and naringin are predominant (Bocco *et al.* 1998; Schieber *et al.* 2001).

Polymethoxylated flavones, a group of phenolic compounds unique to citrus species, mostly accumulate in the peel (Ortuño *et al.* 2002) and their profile in citrus fruit is a characteristic of each species (Gaydou *et al.* 1987). The total content of polymethoxylated flavones in the peel oil of citrus fruits ranges from 1.88 g/L in oranges to 6.49 g/L in common mandarin (Gaydou *et al.* 1987). Polymethoxylated flavones exert potent anticarcinogenic, anti-inflammatory and cardioprotective activities (Benavente-García *et al.* 1997; Kawaii *et al.* 1999a, 1999b; Manthey *et al.* 1999, 2001).

Several glycosylated flavones have been detected in citrus fruits. Of these, diosmin and neodiosmin have been found in higher concentrations in the flavedo than in albedo, while only small quantities of these flavones are present in pulp (Marín and Del Río 2001). Flavanone glycosides contribute to the taste and bitterness of citrus juice. Although their fungistatic and fungitoxic properties are well-established (Kefford and Chandler 1970), these compounds exert only a weak antiproliferative activity toward cancer cell lines (Kawaii *et al.* 1999a).

Cinnamic acid derivatives

The total hydroxycinnamic acid content in the juices of different orange varieties ranges from 53.1 to 158.8 mg/L (Rapisarda *et al.* 1998), while in citrus peels and seeds the range is from 144 to 2956 mg/kg dry matter (Bocco *et al.* 1998). Other glycosides of this acid, namely, phlorin and coniferin, have been detected in citrus fruit juices, citrus peels and/or their by-products (Louche *et al.* 1998; Braddock and Bryan 2001). According to Louche *et al.* (1998), phlorin mainly accumulates in the albedo of the peel (0.8 to 1.1 mg/kg).

Coumarins

The accumulation of certain coumarins, namely, xanthyletin, seselin and scoparone, has been associated with the infection of citrus fruits by pathogens (Ben-Yehoshua *et al.* 1988; Stange *et al.* 1993). Treating citrus fruits with UV radiation also stimulates the biosynthesis of coumarins (Riov *et al.* 1971, 1972). Masuda *et al.* (1992) found another, coumarin, namely auraptene, in a number of citrus species where its concentration ranged from 10.32 g/kg dry weight in juice sacs to 16.6 in peels. Auraptene exerts an inhibitory effect against chemical cancerogenesis in some rodent models (Murakami *et al.* 1997; Tanaka *et al.* 1997, 1998).

Other phenolic compounds

Other compounds found in citrus fruits include phenolic acids, which are predominantly located in the flavedo (Peleg *et al.* 1991; Manthey and Grohmann 2001), such as caffeic, p-coumaric, ferulic and sinapic acids in the form of esters, amides and glycosides (Mouly *et al.* 1997; Bocco *et al.* 1998). In addition, very small amounts of free phenolic acids are present.

CONCLUSIONS AND FUTURE TRENDS

Considering that real scientific interest in the role of dietary polyphenols in maintaining human health only started about 10 years ago, there has been a remarkable progress in the number of published studies which provide convincing data on the effect of these compounds on some aspects of health. Consequently, the dietary supplement industry has introduced a substantial number of supplements containing polyphenols into the market. Furthermore, the food industry has also taken the opportunity to include polyphenols in their products, which in addition of its technological effects, could add important healthy benefits (functional foods). As long as these compounds are obtained from foods that are highly consumed in the Mediterranean diet (citrus fruits), consumer acceptance can be assured.

However, the use of these compounds as food ingredients may induce reactions with food constituents and so provoke changes in the physico-chemical properties and activities of both, which is an aspect that must be investigated.

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REFERENCES

Baghurst K (2003) The Health Benefits of Citrus Fruits. Report to Horticulture Australia Ltd. *CSIRO Health Science Nutrition*, 1-95

Benavente-García O, Castillo J, Marin FR, Del Río JA (1997) Uses and properties of citrus flavonoids. *Journal of Agricultural and Food Chemistry* **45**, 4505-4515

Ben-Yehoshua S, Shapiro B, Kim JJ, Sharoni J, Carmeli S, Kashman Y (1988) Resistance of citrus fruit to pathogens and its enhancement by curing. In: Goren R, Mendel K (Eds) *Proceedings of the 6th International Citrus Congress*, Balanban Publishing, Rehovot, Israel, pp 1371-1374

Blank CB, Poli C, Marder M, Roguin LP (2004) Antiproliferative activity of various flavonoids and related compounds: additive effect of Interferon-α2b. *Biorganic Medical and Chemical Letters* **14**, 133-136

Bocco A, Cuvelier ME, Richard H, Berset C (1998) Antioxidant activity and phenolic composition of peel and seed extracts. *Journal of Agricultural and Food Chemistry* **46**, 2123-2129

Braddock RJ, Bryan CR (2001) Extraction parameters and capillary electrophoresis analysis of limonin glucoside and florin in citrus by-products. *Journal of Agricultural and Food Chemistry* **49**, 5982-5988

Braddock RJ (1999) *Handbook of Citrus and Processing Technology*, Wiley-Interscience Publication, Canada, 596 pp

Bravo L (1998) Polyphenols: chemistry, dietary, sources, metabolism and nutri-

tional significance. *Nutrition Review* **56**, 317-333

Carroll KK, Guthrie N, So FV, Chambers AF (1996) Anticancer properties of flavonoids with emphasis on citrus flavonoids. In: Rice-Evans CA, Packer L (Eds) *Flavonoids in Health and Disease*, CRC Press, Boca Raton, Florida, pp 437-446

Cetkovic GS, Djilas S.M, Canadanovic-Brunet JM, Tumbas VT (2004) Antioxidant properties of marigold extracts. *Food Research International* **22**, 643-650

Choi M, Lee JS, Park EM, Lee MK (2003) Cholesterol-lowering properties of citrus flavonoides and polyphenolic compounds and their relevance to antioxidative activity. *Nutritional Sciences* **6**, 31-41

Cirigliano MC, Farrel FJ, Mckenna RT, Rothenberg PJ (2000) Flavoring materials for use in tea containing beverages. US patent, US 6,022,576.

Cowan MM (1999) Plant products as antimicrobial agents. *Clinical Microbiology Review* **27**, 379-383

Davidson PM (2001) Chemical preservatives and naturally antimicrobials. In: Beuchat MP, Montville LR (Eds) *Food Microbiology, Fundamentals and Frontiers*, ASM Press, Washington DC, pp 593-628

Diplock AT, Charleux JL, Croizer-Willi G, Kok FJ, Rice-Evans C, Robertfroid M, Stalh W, Vina-Ribes J (2000) Functional foods and defence against reactive oxidative species. *British Journal of Nutrition* **1**, S77-S112

Duthie GG, Duthie SJ, Kyle JAM (2000) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutritional Research Review* **13**, 79-106

Edenharder R, Grünhage D (2003) Free radical scavenging abilities of flavonoids as mechanism of protection against mutagenicity induced by tert-butyl hydroperoxide or cumene hydroperoxide in *Salmonella typhimurium* TA102. *Mutation Research* **2**, 1-18

El-Shafae AM, EL-Domiaty MM (2001) Improved LC methods for determination of diosmin and/or hesperidin in plant extracts and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* **26**, 539-545

Fernández-López J, Fernández-Gines JM, Alesón-Carbonell L, Sendra E, Sayas-Barberá ME, Pérez-Álvarez JA (2004) Application of functional citrus by-products to meat products. *Trends in Food Science and Technology* **15**, 176-185

Fernández-López J, Zhi N, Alesón-Carbonell L, Pérez-Alvarez JA, Kuri V (2005) Antioxidant and antibacterial activities of natural extracts: Application in beef meatball. *Meat Science* **69**, 371-380

Garrote G, Cruz JM, Moure A, Domínguez H, Parajó JC (2004) Antioxidant activity of byproducts from the hydrolytic processing of selected lignocellulosic materials. *Trends in Food Science and Technology* **15**, 191-200

Gaydou EM, Bianchini JP, Ramananarivo RP (1987) Orange and mandarin oils differentiation using polymetoxylate flavone composition. *Journal of Agricultural and Food Chemistry* **35**, 525-529

Guthrie N, Morley K, Hasegawa S, Manners GD, Vandergberg T (2000) Inhibition of human breast cancer cells by citrus limonoids. In: Berhow MA, Hasegawa S, Manners GD (Eds) *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*, American Chemical Society, USA, pp 164-174

Guthrie N, Kurawska EM (2001) Anticancer and cholesterol lowering activities of citrus flavonoids. In: Wildman REC (Ed) *Handbook of Nutraceuticals and Functional Foods,* CRC Press, Boca Raton, Florida, pp 113-126

Havsteen BH (2002) The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics* **96**, 67-202

Heim KE, Tagliferro AR, Bobiliya DJ (2002) Flavonoid antioxidant: chemistry and structure-activity relationships. *Journal of Nutritional Biochemistry* **13**, 572-584

Hollman PCH, Katan MB (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food and Chemical Toxicology* **37**, 937-942

Horowitz RM, Gentili B (1977) Flavonoids constituents of citrus. In: Nagy S, Shaw PE, Veldhuis MK (Eds) *Citrus Science and Technology*, AVI Plublising, Westport CT, pp 397-426

Huang MT, Ho CT, Lee CY (1992) *Phenolic Compounds in Foods and their Effects on Health II*, American Chemical Symposium Series 507, American Chemical Society, Washington, DC, 320 pp

Justesen U, Arrigoni E, Larsen BR, Amado R (2000) Degradation of flavonoid glycosides and aglycones during in vitro fermentation with human faecal flora. *Lebensmittel-Wissenschaft und-Technologie* **33**, 424-430

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. *European Journal of Clinical Nutrition* **61**, 472-477

Kannes K, Tisserat B, Berthow M, Vandercook C (1992) Phenolic composition of various tissues in *Rutaceae* species. *Phytochemistry* **31**, 967-974

Kannes K, Tisserat B, Berthow M, Vandercook C (1993) Phenolic composition of various tissues in Rutaceae species. *Phytochemistry* **32**, 1529-1536

Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M (1999a) Antiproliferative activity of flavonoids on several cancer lines. *Bioscience, Biotechnology and Biochemistry* **63**, 896-899

Kawaii S, Tomono Y, Katase E, Ogawa K, Yano M (1999b). Antiproliferative effects of the readily extractable fractions prepared from various citrus juices on several cancer lines. *Journal of Agricultural and Food Chemistry* **47**, 2509-2521

Kefford JB, Chandler BV (1970) *The Chemical Constituents of Citrus Fruits*, Academic Press, New York, 420 pp

Klinke HB, Thomesen AB, Ahring BK (2001) Potential inhibitors from wet oxidation of wheat straw and their effect on grown and ethanol production by *Thermoanaerobacter mathranii*. *Applied Microbiological Biotechnology* **57**, 631-638

Konowalchuk J, Speirs J (1976) Antiviral activity of fruit extracts. *Journal of Food Science* **41**, 1013-1019

Kris PM, Hecker KD, Bonanome A, Coval SM, Binkoski EB, Hilpert K, Griel AE, Etherton TD (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine* **113**, 71S-88S

Kurawska EM, Borradaile NM, Spence JD, Carroll KK (2000) Hypocholesterolemic effects of dietary citrus juices in rabbits. *Nutrition Research* **20**, 121-129

Lee HS (2002) Characterization of major anthocyanins and the color of red-flesh Budd blood orange (*Citrus sinensis*). *Journal of Agricultural and Food Chemistry* **50**, 1243-1246

Lien EJ, Ren S, Bui HH, Wang R (1999) Quantitative structure-activity relationship analysis of phenolic Antioxidants. *Free Radical from Biological Medicine* **26**, 285-294

Louche LMM, Gaydou EM, Lesage JL (1998) Determination of phlorin as peel marker in orange (*Citrus sinensis*) fruit and juices. *Journal of Agricultural and Food Chemistry* **46**, 4191-4197

Lule SU, Xia W (2005) Food phenolics, pros and cons: A review. *Foods Reviews International* **21**, 367-388

Manthey JA, Grohmann K (1996) Concentrations of hesperidin and other orange peel flavonoids in citrus processing by-products. *Journal of Agricultural and Food Chemistry* **44**, 811-814

Manthey JA, Grohmann K (2001) Phenols in citrus peel byproducts: concentration of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agricultural and Food Chemistry* **49**, 3268-3273

Manthey JA, Grohmann K, Mantanari A, Ash K, Manthey CL (1999) Polymethoxylated flavones derived from citrus suppress tumor necrosis factor-α expression by human monocytes. *Journal of Natural Products* **62**, 441-444

Manthey JA, Guthrie N (2002) Antiproliferative activities of citrus flavonoids against six human cancer cell lines. *Journal of Agricultural and Food Chemistry* **50**, 5837-5843

Manthey JA, Guthrie N, Grohmann K (2001) Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Current in Medical Chemistry* **8**, 135-153

Marín FR, Frutos MJ, Pérez-Álvarez JA, Martínez-Sánchez F, Del Río JA (2002) Flavonoids as nutraceuticals; structural related antioxidant properties and their role on ascorbic acid preservation. In: Atta-Ur-Rahman (Ed) *Studies in Natural Products Chemistry* (Vol XXVI), Elsevier, London, UK, pp 741-778

Marín FR, Del Río JA (2001) Selection of hybrids and edible *Citrus* species with a high content in the diosmin functional compound. Modulating effect of plant growth regulators on contents. *Journal of Agricultural and Food Chemistry* **49**, 3356-3362

Masuda T, Muroya Y, Nakatani N (1992) Coumarin constituents of the juice oil form citrus hassaku and their sposmolytic activity. *Bioscience, Biotechnology and Biochemistry* **56**, 1257-1260

Mazza G (1998) Anthocyanins in grapes and grape products. *CRC Critical Reviews in Food Science and Nutrition* **35**, 341-371

Middleton EJ (1986) Some effects of flavonoides on mammalian cell systems. In: Farkas L, Gabor M, Kallay F (Eds) *Flavonoids and Bioflavonoids*, Elsevier, Amsterdam, The Netherlands, pp 381-425

Mouly PP, Gaydou EM, Faure R, Estiene JM (1997) Blood orange juice authentication using cinnamic acid derivatives. Variety differentiations with flavanone glycoside content. *Journal of Agricultural and Food Chemistry* **45**, 373-377

Murakami A, Kuki W, Takahashi Y, Yonei H, Nakaramura Y, Otho Y, Oigashi H, Koshmizu K (1997) Auraptene, a citrus coumarin, inhibits 12-tetradecanoylphorbol-13-acetat-induced tumor promotion in ICR mouse skins, possibly through suppression of superoxide generation in leukocytes. *Japan Journal of Cancer Research* **88**, 4443-4452

Murkovic M (2003) Phenolic compounds. In: Caballero B, Trugo L, Finglas P (Eds) *Encyclopedia of Food Science and Nutrition*, Academic Press, London, UK, pp 4507-4514

Musci I (1986) Combined antiviral effect of quercetin and interferon on the multiplication of herpes simplex virus in cell cultures. In: Farkas L, Gabor M, Kallay F (Eds) *Flavonoids and Bioflavonoids*, Elsevier, Amsterdam, The Netherlands, pp 333-398

Nychas GJE (1995) Natural antimicrobials from plants. In: Gould GW (Ed) *New Methods of Foods Preservation,* Blackie Academic and Professional/ Chapman and Hall, Glasgow, UK, pp 25-68

Orallo F, Camina M, Alvarez E, Basaran H, Lugnier C (2005) Implication of cyclic nucleotide phosphodiesterase inhibition in the vasorelaxant activity of the citrus-fruits flavonoid naringenin. *Planta Medica* **71**, 99-107

Ortuño AM, Arcas MC, Botia JM, Fuster MD, Del Río JA (2002) Increasing resistance against *Phytophora citrophthora* in tangelo: Nova fruits by modulating polymethoxyflavones levels. *Journal of Agricultural and Food Chemistry* **66**, 217-220

Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartscha H (2000) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer* **36**, 1235-1247

Papadopoulou A, Fraizier RA (2004) Characterization of protein-polyphenol interactions. *Trends in Food Science and Technology* **15**, 186-190

Peleg H, Naim M, Rouseff RL, Zehavi U (1991) Distribution of bound and free phenolics acids in orange (*Citrus sinensis*) and grape fruit (*Citrus paradisi*). *Journal of the Science of Food and Agriculture* **60**, 417-426

Pierson MD, Reddy NR (1982) Inhibition of *Clostridium botulinum* by antioxidants and related phenolic compounds in comminuted pork. *Journal of Food Science* **47**, 1926-1933

Pszczola DE (2002) Antimicrobials: setting up additional hurdles to ensure food safety. *Food Technology* **56**, 99-107

Rapisarda P, Carollo G, Fallico B, Tomaselli F, Maccarone E (1998) Hydroxycinnamic acids as markers of Italian blood orange juices. *Journal of Agricultural and Food Chemistry* **46**, 464-470

Riov J, Goren R, Monselise SP, Kahan RS (1971) Effect of gamma radiation on the synthesis of scopoletin and scopolin in grape fruit peel in relation to radiation damage. *Radiation Research* **45**, 326-334

Riov J, Monselise SP, Goren R (1972) Simulation of phenolic biosynthesis in citrus peel by gamma radiation. *Radiation Research Review* **3**, 417-427

Rodriguez de Sotillo D, Hadley M, Hola RT (1998) Phenolics in aqueous potato peel extracts a nonmutagenic antioxidant with potential antimicrobial activity. *Journal of Food Science* **63**, 907-910

Ross JA, Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects and safety. *Annual Review in Nutrition* **22**, 19-34

Rotelli AE, Guardia T, Osvalda-Járez A, De La Rocha NE, Pelzer LE (2003) Comparative study of flavonoids in experimental models of inflamemation. *Pharmacology Research* **48**, 601-606

Schieber A, Stintzing FC, Carle R (2001) By-products of plan food processing as a source of functional compounds-recent developments. *Trends in Food Science and Technology* **12**, 401-413

Sinclair WB (1972) *The Grape Fruit its Composition, Physiology and Products*, University of California, Los Angeles, USA, 600 pp

So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK (1996) Inhibition of human breast cancer cell proliferation and delay of mamary tumorigenesis by flavonoids and citrus juices. *Nutrition and Cancer* **26**, 167-181

Stange JrRR, Midland SL, Eckerd JW, Sims JJ (1993) An antifungal compound produced by grapefruit and Valencia orange after wounding of the peel. *Journal of Natural Products* **56**, 1627-1627

Tanaka T, Kawabat K, Kakumota M, Hara A, Murakami A, Kuki W, Takasaki Y, Yonei H, Maeda M, Ota T, Odasima S, Yamane T, Koshimizu K, Ohigashi H (1998) Citrus auraptene exerts dose-dependent chemoprotective activity in large bowel tumorgenesis: the inhibition correlates with suppression of cell proenzymes. *Cancer Research* **58**, 2550-2556

Tanaka T, Kawabat K, Kakumota M, Makita H, Hara A, Murakami A, Kuki W, Takasaki Y, Yonei H, Koshimizu K, Ohigashi H (1997) Citrus auraptene inhibits chemically induced colonic aberrant crypt foci in male F344 rats. *Carcinogenesis* **18**, 2151-2161

Tran AV, Chambeer RP (1985) Red oak wood derived inhibitors in the ethanol fermentation of xylose by *Pichia stipitis* CBS 5776. *Biotechnology Letters* **7**, 841-846

Tran AV, Chambeer RP (1986) Ethanol fermentation of red oak acid prehydrolysate by the yeast *Pichia stipitis* CBS 5776. *Enzimology, Microbiology and Technology* **11**, 387-393

Tripoli E, La Guardia M, Giammanco S, Di Majo D, Giammanco M (2007) *Citrus* flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry* **104**, 466-479

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2007) Antifungal activities of clove, thyme and oregano essential oils. *Journal of Food Safety* **27**, 91-101

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2008a) Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *International Journal of Food Science* **43**, 526-531

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2008b) Antifungal activity of lemon, mandarin, grapefruit and orange essential oils. *Food Control* **19**, 1130-1138

Viuda-Martos M, Ruíz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2008c) Antibacterial activity of lemon, mandarin, grapefruit and orange essential oils. *Journal of Food Safety* **28**, 567-576

Walle T (2004) Serial review: flavonoids and isoflanoides (Phytoestrogens): absorption, metabolism and bioactivity. *Free Radical Biology Medicine* **36**, 829-837

Wilfried CO, Sigrid JO, Detavernier CM, Huyghebaert A (1994a) Characterization of orange juice (*Citrus sinensis*) by flavone glycoside. *Journal of Agricultural and Food Chemistry* **45**, 2183-2190

Wilfried CO, Sigrid JO, Detavernier CM, Huyghebaert A (1994b) Characterization of orange juice (*Citrus sinensis*) by polymethoxylated flavanone glycoside. *Journal of Agricultural and Food Chemistry* **45**, 2191-2195

Wollgast J, Anklam E (2000) Polyphenols in chocolate: is there a contribution to human health? *Food Research International* **33**, 449-459

Zern TL, Fernández ML (2005) Cardioprotective effects of dietary polyphenols. *Recent Advances in Nutritional Sciences* **22**, 2291-2294