Chapter

PROCESSING OF CITRUS PEEL FOR THE EXTRACTION OF FLAVONOIDS FOR BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

Flavonoids are extra nutritional constituents that naturally occur in small quantities in plants. They are a family of polyphenolic compounds that are widespread in nature and are consumed as part of the human diet in significant amounts. The diversity in their structure and bioactivity of flavonoids make these compounds an interesting candidate for biotechnology based research. Extraction of flavonoids from citrus employing the use of various techniques such as chemical and physical methods is attempted in this write up. The biotechnological potential of flavonoids is not currently exploited to its maximum since extraction procedures are in developing phase. The current knowledge on the sources of citrus flavonoids and their potential activities in alleviating human health is also discussed.

Keywords: bioactives, flavonoids, supercritical, ultrasound, microwave, hypertension, antitumor.

1. INTRODUCTION

World citrus fruit production has been estimated to be 498 million metric ton (MMT) per annum with Brazil being the largest producer at 162 MMT followed by USA (FAO, 2010). Most of the citrus fruits are utilized for the production of juice and peel of the citrus fruit is remain unutilized (constituting about 50% of fresh fruit weight) which is becoming a major problem and demands urgent attention. Usually, citrus industries dry the residue and sell it either as raw material for pectin extraction or pelletize it for animal feeding (Khan et al., 2010). Recently, research has been focused upon increasing the capacity and sophistication of waste management systems, such as making value-added conversions and isolation of

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bioactive compounds or high value-chemicals from these agricultural wastes, in addition to their typical uses as raw materials for fertilizers, soil modifiers and animal feeds.

Bioactive compound like vitamin C, carotene (β-carotene), flavonoids, limonoids, essential oil, acridone alkaloids, fibers, minerals, vitamin B and other related nutrients like thiamine, riboflavin, nicotinic acid/niacin, pantothenic acid, pyridoxine, folic acid etc are present in citrus fruits (Hamdan et al., 2011; Benavente-Garcia et al., 1997). Out of all flavonoids, flavanone glycosides (polymethoxy flavones) present in citrus peel have a constructive influence on human health. In addition, these compounds seek scientific attention due to their physiological and pharmacological benefits.

Flavonoids are also of interest owing to their observed biological effects *in vitro* such as free-radical scavenging, modulation of enzyme activity, inhibition of cellular proliferation, their potential utility as antibiotic, anti-allergic, anti-ulcer, and anti-inflammatory agent (Hamdan et al., 2011; Yoshida et al., 2010; Ozcelik et al., 2008; Li et al 2006a; Havsteen 2002). Due to their high flavonoid content, citrus peels could be exploited by both pharmaceutical and food industries (Londoño-Londoño et al., 2010). Thus, extraction of flavonoids from citrus peel is attracting the attention of researchers to use them as natural food supplements to enhance the quality of life.

In recent years, citrus processing waste (CPW) has been used for the production of single cell protein (Kalra et al., 1989), bio-hydrogen (VenkataMohan et al., 2009), bio-ethanol (Boluda-Aguilar et al., 2010) and multiple enzymes (Puri et al., 2011; Puri et al., 2008). Functional compounds from CPW such as flavonoids and their further processing can be of great interest to the food and pharmaceutical industry as they retard oxidation of low-density protein, thereby reducing the risk of heart disease (Peluso et al., 2006). Flavonoids from citrus peel are comprised primarily of flavanone glycosides (naringin) (Mamma et al., 2008), polymethoxylated flavones aglycons (tangeritin) and flavone glycosides (rutin) (Sawalha et al., 2009). Thus recovery and enzymatic conversion of naringin, the abundant major flavonoid in citrus fruits, peel holds promises for byproduct utilization from citrus production. Naringin can be converted to naringenin, a free radical scavenger compound that reduces oxidative damage to DNA (Gao et al., 2006), and L-rhamnose. Rhamnose from naringin hydrolysis can be used as a precursor for the industrial production of aromatic compounds and flavors, as well as a chiral compound for chemical synthesis and as an inducer of recombinant protein expression in *Escherichia coli* (Zverlov et al., 2000).

A major reason for citrus peel unutilization is the unavailability of effective extraction procedures to obtain the flavonoids from the citrus peels (Ma et al., 2008a, b). This write up focuses on the flavonoid chemistry, methods of extraction and their potential applications.

2. THE CHEMISTRY OF FLAVONOIDS

In 1936, Rusznyak and Szent-Gyorgyi studied the biological activities of plant pigments and proposed name flavonoids (Bohm, 2006). Flavonoids are aromatic secondary plant metabolites, belong to a group of polyphenolic compound and are widely distributed in plants. They are frequently found in fruits (e.g. orange, grapefruit, apple, and strawberry etc.), vegetables (i.e. onion, broccoli, green pepper and tomato), cereals (soybeans and different herbs), common foodstuffs as well as in herbal remedies and dairy products. However, red

wine, tea, dark chocolate, grapes and apples are the common and regularly consume source of flavonoids. Over 5000 naturally occurring flavonoids have been characterized from various sources including plant and dairy products. Flavonoids play a vital role in plant physiology as they inhibit exocytosis of auxin indolyl acetic acid (IAA) which play major role in plant growth regulation (Havsteen, 2002). In the field of food technology, flavonoids are well known for their ability to contribute towards bitterness (Puri et al. 2010; Puri et al. 2000).

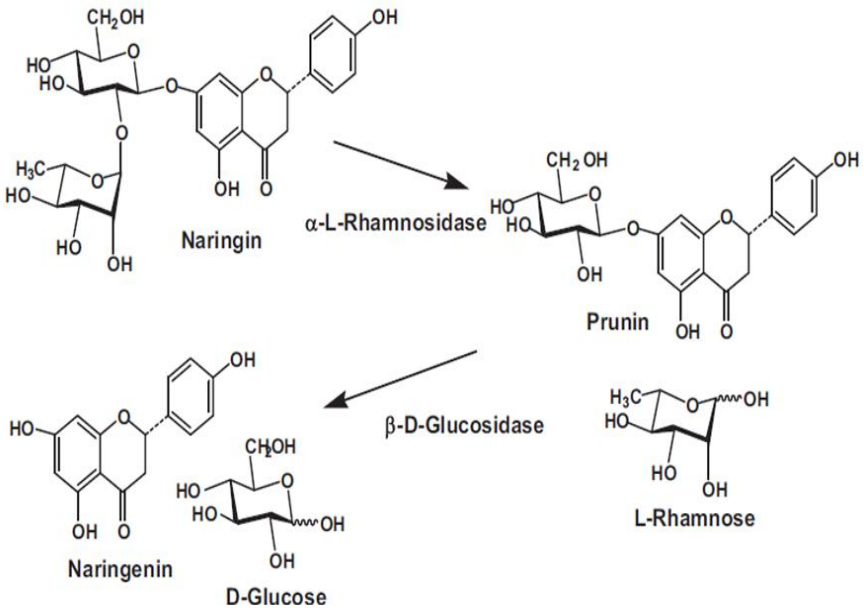

Figure 1. The structure of naringin and its degradation product.

The chemical structure of flavonoids shows that it is a water soluble polyphenolic molecule containing 15 carbon (C6-C3-C6) atoms. The skeleton consists of two phenyl rings connected to three carbon bridges. Two of the phenyl rings connected by a short chain of three-carbon bridge (Figure 1). One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-member.

They are categorized into various subclasses including flavones, flavonols, flavanone, isoflavanones, isoflavanoids, anthocyanidins, and catechins (Havsteen, 2002).

2.1. Structure

The principal flavonoids isolated in citrus (Benavente-Garcia et al., 1997) are listed in Table 1.

Table 1. Principal flavonoids isolated in *citrus*, with structure and substitution pattern

Flavonoid	*Citrus* sp.	Molecular weight/ Chemical	C-ring Structure [a]	substitution pattern

		structure		
Naringin	*C. paradise* *C. aurantium*	604 Da/ $C_{29}O_{14}H_{32}$	FLA	5,4¢-OH 7-*O*-Neo[b]
Neoeriocitrin	*C. aurantium*	596.53 Da/$C_{27}H_{32}O_{15}$	FLA	5,3¢,4¢-OH 7-*O*-Neo
Hesperidin	*C. sinensis*	583 Da/ $C_{30}O_{12}$ H_{31}	FLA	5,3¢-OH, 4¢- OMe 7-*O*-Rut[b]
Diosmin	*C. sinensis* *C. limonia*	583 Da/ $C_{30}O_{12}$ H_{31}	FLO	5,3¢-OH 4¢-OMe 7-*O*-Rut[b]
Rutin	*C. limonia*	664.57 Da/ $C_{27}H_{30}O_{16}{\cdot}3(H_2O)$	FOL	5,7,3¢,4¢-OH 3-*O*-Rut
Naringenin	*C. paradisi*	271 Da/ $C_{15}O_5H_{11}$	FLA	5,7,4¢-OH
Eriodictyol	*C. aurantium*	290.27 Da/ $C_{15}H_{14}O_6$	FLA	5,7,3¢,4¢-OH
Hesperetin	*C. sinensis*	288 Da/ $C_{15}O_6H_{13}$	FLA	5,7,3¢-OH 4¢-OMe
Apigenin	*C. paradisi*	270 Da /$C_{15}O_5H_{10}$	FLO	5,7,4¢-OH
Luteolin	*C. limonia* *C. aurantium*	286 Da /$C_{15}O_6H_{10}$	FLO	5,7,3¢,4¢-OH
Diosmetin	*C. sinensis*	288 Da/ $C_{15}O_6H_{12}$	FLO	5,7,3¢-OH 4¢-OMe
Kaempferol	*C. paradisi*	286.24 Da/ $C_{15}H_{10}O_6$	FOL	5,7,3,4¢-OH
Quercetin	*C. limonia*	302 Da/ $C_{15}O_7H_{10}$	FOL	5,7,3,3¢,4¢-OH
Tangeretin	*C. aurantium* *C. paradise* *C. limonia*	372.37 Da/ $C_{20}H_{20}O_7$	FOL FOL FLO	5,7,3,4¢-OH 5,7,3,3¢,4¢-OH 5,6,7,8,4¢-OMe

[a] FLA, flavanone; FLO, flavone; FOL, flavonol. [b]Neo, neohesperidoside; Rut, rutinoside.

i) *Flavonoids*

They are derived from 2-phenylchromen-4-one2-phenyl-1, 4-benzopyrone structure based on the oxidation saturation present in heterocyclic C-ring. Flavonoids are again divided into subclasses (e.g. quercetin, rutin).

ii) *Iso-Flavonoids*

They are a subclass of flavonoids with 3-phenylchromen-4-one backbone with no hydroxyl group substitution at 2 positions. This subclass of flavonoids is not found in plants or plant product in large amount.

iii) *Neo-flavonoids*

It is a class of flavonoids with 4-phenylchromen backbone with no hydroxyl group substitution at 2 positions. Neoflavonoids are biogenetically and structurally close to flavonoids and isoflavonoids.

iv) *Minor-flavonoids*

Some of the natural product like aurones and chalcones also have a common backbone structure of flavonoids C_6-C_3-C_6 and are consider as minor flavonoids.

v) *Flavonoids in citrus peel*

Citrus peel contains number of polyphenolic compound having the chemical backbone of naringin, rutin, hesperidin, quercetin which are available in good quantity. However, other flavonoids like narirutin, diosmin, didymin, sinensetin, tangeretin and kaempferol etc are available in trace quantities. Naringin is a flavone glycosidase having a chemical back bone of C6-C3-C6 carbon framework.

The chemical structure of the naringin shows that two sugar molecules (glucose and rhamnose) are attached to try-hydroxyl flavonols structure (Figure 1). Naringin has an anti-oxidant, anti-proliferative, cholesterol lowering activity. The in vitro studies of flavonoids have shown that naringin is beneficial in some of the chronic diseases such as cerebrovascular disease and asthma (Yoshida et al., 2010).

Hesperidin is another flavone glycoside having chemical formula C28H34O16, found in large quantity in citrus peel. It is also known as Vitamin P and a predominant flavonoid in lemons and oranges. The peel and membranous parts of citrus peel has highest concentration of flavonoid. The structure of flavonoids naringin and hesperidin are similar to each other. Rutin, having a chemical formula C27H30O16, is a flavone glycosidase, found abundantly in citrus fruits.

3. METHODS OF FLAVONOID EXTRACTION

Medical interest in citrus plants derived bioactives or drugs has led to an increased need for ideal extraction methods, which could obtain the maximum of the bioactive constituents in a shortest processing time with a low cost.

Several methods like solvent extraction (Xu et al., 2007; Zia-ur-Rehman, 2006; Anagnostopoulou et al., 2006; Li et al., 2006a; Jeong et al., 2004; Manthey and Grohmann, 1996), hot water extraction (Xu et al., 2007), alkaline extraction (Bocco et al., 1998; Curto et al., 1992), resin-based extraction (Kim et al., 2007; Calvarano et al., 1996), electron beam- and γ-irradiation-based extractions (Kim et al., 2008), supercritical fluid extraction (Giannuzzo et al., 2003) and enzyme-assisted extraction (Puri et al., 2011; Li et al., 2006b) have been reported in the literature for the extraction of flavonoids from citrus peel. Few of these approaches are discussed below:

3.1. Chemical Methods

Many of the industries particularly the pharmaceutical and food industries use solvents for the extraction of bioactive compounds from citrus materials. Chemical methods use organic solvents, such as hexane, methanol, ethanol, petroleum ether, benzene, toluene, ethyal acetate, isopropanaol, acetone, etc., to extract flavonoids from the plant materials. The extraction occurring by diffusion transfers from the solids to the surrounding solvents is known as leaching. The operating temperature and time of extraction are specific to the nature of the plant materials.

Five citrus peels (Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange) were used for the extraction of phenolic substances with ethanol. The highest amount of polyphenolics (162 mg per 100 g fresh peel) were obtained when grapefruit peel was extracted with 72% ethanol at 80 ^{o}C, followed by Yen Ben lemon (118 mg), orange (74 mg) and Meyer lemon (60 mg). Recovery increased with the increase in ethanol concentration up to 85 % ethanol (Li et al., 2006a).

A finger lime (*Citrus australasica*) fruit is one of the five native citrus species endemic to Australia, which grow mainly in the rainforests of Queensland and New South Wales. An extract was prepared from the peel with dichloromethane for the extraction of limonene. The peel was covered again with dichloromethane (SDS, Atrasol, 900 ml) and left at room temperature for 12 h. The combined extracts were dried over magnesium sulphate and concentrated in a column at atmospheric pressure to extract new terpenyl compounds (Delort and Jaquier, 2009).

Dried peel powder of *Citrus decumana* was extracted by maceration process using solvents of increasing polarity (Sood et al., 2009). Maceration was first carried out with hexane followed by chloroform, ethyl acetate and methanol, respectively. The powdered material was extracted with each solvent three times at room temperature over a period of 24 h. The material was kept for 24 h between each successive solvent for proper drying. The extracts were filtered and concentrated under vacuum on a rotary evaporator at 40°C and stored in a refrigerator for further analysis.

Naringin isolation from the albedo of Khao Taeng-gwa (KY) peel was carried by methanol extraction followed by its crystallisation in water. Direct water extraction from the peel was followed and each experiment was repeated three times using methanol extract. The process with highest naringin yield was used for isolation of naringin from the albedo and flavedo portions of peel from the other Khao Nam Pheung (KN) and Tong Dee (TD) cultivars. In large scale naringin isolation, powdered KT cultivar of pomelo peel was macerated in methanol for 3 days (Sudto et al., 2009). The extracted slurry with methanol was dried with a rotary evaporator under reduced pressure at 45 °C. Water was added to the dry methanolic extract. After stirring at 70°C for 2 h, dichloromethane was added and the mixture left for 4 days at 25°C. The naringin crystals formed in the aqueous layer were filtered and harvested (Sudto et al., 2009).

In one of the studies, dry powder of plant tissues was homogenized in dimethyl sulfoxide: methanol (1:1, v/v) in an ultraturrax blender (Bermejo et al., 2011). The supernatant after centrifugation (12,000 rpm; 15 minute; 4°C) was filtered (0.45 µm) and analysed by HPLC-DAD and HPLC-MS for the presence of flavonoids using a reverse-phase column (C18). A gradient mobile phase consisting of acetonitrile (solvent A) and 0.6% acetic acid (solvent B) was used at a flow rate (1 mL/min). Compounds were indentified on the basis of comparison of their retention times, UV-Visible and mass spectrum data with corresponding standards (Mata Bilbao et al., 2007; Weber et al., 2006).

These techniques are associated with below mentioned limitation: i) require several hours to obtain flavonoid extracts, ii) large volumes of solvent are spent for the extraction of flavonoids, iii) solvents needs to be evaporated which adds extra cost and loss of quality.

With the development of the "Green Chemistry" concept during the last few years, environment-friendly techniques are becoming more and more attractive.

3.2. Physical Methods of Extraction

3.2.1. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) has been documented as an effective method for preparing bioactive products from plant materials (Modey et al., 1996). The combined liquid like solvating capabilities and gas-like transport properties of supercritical fluids make them particularly suitable for the extraction of diffusion-controlled matrices such as plant tissues. Moreover, the solvent strength of supercritical fluid can be manipulated by changing pressure (*P*) and/or temperature (*T*); therefore, it may achieve a remarkably high selectivity. This tunable solvation power of supercritical fluid is particularly useful for the extraction of complex samples such as plant materials (Reverchon et al., 2006).

High-pressure flavonoids extraction from *Citrus sulcata* was performed. The dried *C. sulcata* peel and edible fruit powder (1 g) was mixed with 50 ml of 40% ethanol and extracted for 30 min at 100 atmospheres, respectively. After depressurization, the extracted solution was collected, filtered through a 0.45-μm membrane, and concentrated by freeze-drying; the collected extract was stored at -20 °C in the refrigerator (Wang et al., 2011).

3.2.2. Microwave-Assisted Extraction (MAE)

MAE has received a great attention mainly due to considerable savings in processing time, solvent consumption and energy (Spigno and Faveri, 2009). A laboratory scale microwave extraction apparatus operated at atmospheric pressure with microwave frequency of 2450 MHz was used for the extraction purpose. The process parameters like microwave power 10 to 800 W, temperature 1-120 °C and time 1 to 999 s were linearly adjusted. The extracts were filtered through nylon filter (0.45 μm) and analyzed for free phenolic acid content by HPLC. Yields were better when compared with the traditional methods. MAE showed many advantages, such as shorter time, less solvent requirement, higher extraction rate, savings of energy and better products with lower cost. The performance of the method was confirmed by different antioxidant assay systems. The results were promising and demonstrated the practical feasibility of MAE to substitute the traditional time-consuming techniques for efficient extraction of phenolic compounds from citrus mandarin peels (Hayat et al., 2009).

3.3.3. Ultrasound-Assisted Extraction (UAE) Method

The extraction of bioactive compounds under ultrasound irradiation (20-100 kHz) is one of the upcoming extraction techniques that can offer high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and temperature and lower energy input *(Chemat et al., 2008)*.

Ultrasonic-assisted extraction has proven to significantly decrease extraction time and increase extraction yields in many vegetable materials. During sonication, the cavitation process causes the swelling of cells or the breakdown of cell walls, which allow high diffusion rates across the cell wall or a simple washing-out of the cell contents (Vinatoru, 2001). Besides the solvent, temperature and pressure, better recoveries of cell contents can be obtained by optimising ultrasound application factors including frequency, sonication power and time, as well as ultrasonic wave distribution (Londoño-Londoño et al., 2010; Wang and Weller, 2006). Optimisation of ultrasound-assisted extraction (UAE) has been described recently to extract hesperidin from Penggan (Citrus reticulata) peel (Ma et al., 2008a), and phenolic acids and flavanone glycosides from Satsuma Mandarin (Citrus *unshiu* Marc) peel (Ma et al., 2009) (Table 2). Workers further refined there process for extracting hesperidin from *C. reticulate* peel by increasing frequency 60 kHz for 60 min at 40°C in the presence of methanol (Ma et al., 2008c). The highest yield was obtained with dry material in 30 min versus wet material. Dry material has more porosity and the solvent diffusion rate which resulted in higher yield.

The citrus peels from three species; Tahiti lime (Citrus *latifolia*), sweet orange (Citrus sinensis) and oneco tangerine (Citrus reticulata) were obtained for extraction of hesperidin and other related citrus flavonoids such as naringin.

Table 2. Extraction of polyphenols under ultrasound irradiation

Plant material	Analytes	Comments	References
Satsuma Mandarin Citrus *unshiu* Marc	Phenolic acids (PA)	UAE time = 10-40 min; maceration for 8 h for similar yields of PA	Ma et al., 2009
Folium eucommiae	Flavonoids	UAE was found more efficient than heating, microwave- and enzyme-assisted extractions	Huang et al., 2009
Wheat bran *Triticum aestivum*	Phenolics-rich heteroxylans	Extraction time reduced	Hromádková et al., 2008
Penggan *C. reticulata*	Hesperidin	Comparable yields and less degradation of hesperidin compared with soxhlet extraction	Ma et al., 2008c
Satsuma mandarin *C. unshiu* Marc	Phenolic acids and flavanone glycosides	Increase in polyphenol content and antioxidant activity of extracts	Ma et al., 2008b
Penggan *C. reticulata*	Total phenolic content (TPC)	TPC increased on increasing irradiation time and temperature	Ma et al., 2008a
Winged burning bush *Euonymus alatus*	Flavonols rutin and quercetin	UAE efficiency monitored by microscopy	Yang and Zhang, 2008

The extraction was carried out in ultrasonic cleaning bath, operating at 60 kHz (Ma et al., 2008a).

A factorial design (2^2) was used to optimize the extraction process from tangerine peel by identifying the effect of two active factors in improving yield percentage and total phenolic

compounds. The effect of water content in the citrus peel material (0% and 75%), extraction time (30 and 90 min), peel /water ratio (g/mL) were studied. The hesperidin extraction was accomplished by successive acid/base precipitations from flavonoids fraction. The hesperetin was obtained by atmospheric pressure acid-catalysed hydrolysis of hesperidin and used as model to establish differences between glycoside and aglycone. Structural identification of glycoside and aglycone was made by HPLC/MS comparing fragmentation profile with reference standards (Grohmann et al., 2000).

The UAE of phenolic antioxidants from orange peels with ethanol-water mixtures appeared effective in comparison to conventional procedure. The results indicate that the sonication power as the most influential factor in the UAE process followed by temperature and ethanol to water ratio. Although the same volumes of solvent were used in both extraction processes, the duration of the ultrasound-assisted process and consequently the energy input were drastically reduced without affecting the overall yield. Hence, UAE can be called an 'environment-friendly' or 'green' technique. Overall, ultrasound-assisted extraction of polyphenols from abundant food by-products such as orange peels and by using food grade solvents has a strong potential of industrial development as an efficient and environment-friendly process for the preparation of extracts rich in natural antioxidants aimed at replacing synthetic antioxidants.

4. HEALTH BENEFITS OF FLAVONOIDS

Flavonoids are attracting more attention of scientific world due to health related properties, which are based in their antioxidant activity. Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. Studies have shown that flavonoids prevent the oxidation of low-density lipoprotein thereby reducing the risk for the development of atherosclerosis.

4.1. Antitumor Effects

The antitumor activity of flavonoids is still a point of discussion. Antioxidant systems are frequently inadequate, and damage from reactive oxygen species is proposed to be involved in carcinogenesis. Reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens.

Flavonoids can inhibit carcinogenesis (Fotsis et al., 1997). Some flavonoids-such as fisetin, apigenin, and luteolin are stated to be potent inhibitors of cell proliferation (Caltagirone et al., 2000). A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequent incidence of lung cancer. This effect was mainly ascribed to quercetin, which provided > 95% of the total flavonoid intake in that particular study. Quercetin and apigenin inhibited melanoma growth and influenced the

invasive and metastatic potential in mice. This finding may offer new insights about possible therapies for metastatic disease. Furthermore, it has been speculated that flavonoids can inhibit angiogenesis. Angiogenesis is normally a strictly controlled process in the human body. The process of angiogenesis is regulated by a variety of endogenous angiogenic and angiostatic factors. It is switched on, during wound healing. Pathologic, unregulated angiogenesis occurs in cancer. Angiogenesis inhibitors can interfere with various steps in angiogenesis, such as the proliferation and migration of endothelial cells and lumen formation. Among the known angiogenesis inhibitors, flavonoids seem to play an important role. However, the mechanism behind the antiangiogenetic effect of flavonoids needs elucidation. A possible mechanism could be inhibition of protein kinases. These enzymes are implicated to play an important role in signal transduction and are known for their effects on angiogenesis (Androutsopoulos et al., 2010).

4.2 Anti-Atherosclerotic Effects

Flavonoids are likely to have a major influence on the vascular system because of anti-oxidative properties. Oxygen radicals can oxidize low density lipoproteins (LDL), which injures the endothelial wall and thereby promotes atherosclerotic changes. A few clinical studies have pointed out that flavonoid intake protect against coronary heart disease. Also flavonoids if consumed regularly might reduce the risk of death from coronary heart disease in elderly men (Hertog et al., 1993) stated that the. Furthermore, a Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations (Arai et al., 2000). Oxidative stress and vascular damage are postulated to play a key role in dementia, and the intake of red wine is reported to prevent the development of dementia (Orgogozo et al., 1997). The intake of flavonoids was reported to be inversely related to the risk of incident dementia (Commenges et al., 2000).

One large clinical study indicated that flavonoids may reduce mortality from coronary heart disease (Gorinstein et al., 2006; Hertog et al., 1993). Various cohort studies indicated an inverse association between flavonoid intakes and coronary heart disease mortality (Skibola et al., 2001). These studies are promising and indicate that flavonoids may be useful food compounds.

4.3 Antidiabetic Effects

Flavonoids, especially quercetin, has been reported to possess antidiabetic activity. Vessal et al., (2003) reported that quercetin brings about the regeneration of pancreatic islets and proprably increases insulin release in streptozotocin-induced diabetic rats. Also in another study, quercetin stimulated insulin release and enhanced Ca^{2+} uptake from isolated islets cell which suggest a place for flavonoids in noninsulin- dependent diabetes (Hif and Howell. 1985; Hif and Howell 1984). The citrus bioflavonoids namely hesperidin and naringin both play crucial roles in controlling the progression of hyperglycemia. This may be done partly by increasing hepatic glycolysis and glycogen concentration and/or by lowering hepatic gluconeogenesis (June et al., 2004).

4.5. Free Radical Scavenging

Many *in vitro* studies have demonstrated the potent peroxyl radical scavenging abilities of flavonoids in inhibiting lipid peroxidation and oxidation of low density lipoproteins (LDL) (Castelluccio et al., 1995). There have been few studies on the ability of flavonoids and phenolic acids to scavenge reactive nitrogen species. Nitrous oxide (NO) is such a species produced by the action of nitric oxide synthase in endothelial cells and neurones. At sites of inflammation, iNOS is also induced and further NO synthesis activated. Concomitant production of nitric oxide and superoxide radical at such sites of chronic inflammation induces the production of peroxynitrite. Peroxynitrite is a toxic oxidising and nitrating species which is produced by rapid interaction of superoxide radical and nitric oxide (Beckman et al., 1994). Nitrated proteins are immunogenic and nitration can alter their function and stability, thus interfering with cell signalling pathways, cytoskeletal structures and repair mechanisms, and nitrosation of tyrosine has been suggested to be responsible of the onset of the apoptotosis. Thus the ability to inhibit peroxynitrite-dependent nitration of proteins has a potentially significant contributory role in inhibiting damage to biomolecules mediated by reactive nitrogen species. Flavonoids are ideal candidates for this role.

The activity of flavonoids to inhibit peroxynitrite-dependent nitration of tyrosine is structure-dependent. Thus, catechol-containing phenolics inhibit by scavenging peroxynitrite through electron donation whereas monohydroxycinnamates and flavonoids with monophenolic B rings intercept the reaction between tyrosine and peroxynitrite via the anticipated mechanism of competitive nitration. Several flavonoids and phenolic compounds are powerful inhibitors of nitrous acid dependent nitration and DNA deamination *in vitro* (Oldreive et al., 1998). Thus flavonoids from plant materials might provide a gastro-protective effect under corditois in which high levels of reactive nitrogen species are produced. Flavonoids and phenolic compounds with hydroxyl groups can also interact with transition metal ions to form chelates. These chelates might be stable, or redox cycling might take place leading to the reduction of iron or copper to a more pro-oxidant form and the oxidised quinone.

CONCLUSION AND FUTURE DIRECTIONS

There is requirement of simple and economically valuable method for the extraction of citrus flavonoids since plant based bioactives is considered safe for food processing application. Although techniques like physical extraction methods have shown promise in recent years, however environment friendly methods are desirable. In this regard, enzyme assisted extraction of flavonoids combined with aqueous solvents for extracting flavonoid is an attractive proposal. In enzyme-assisted aqueous extraction, the enzymes can rupture the polysaccharide-protein colloid in the cell wall creating an emulation that interferes with extraction.

Enzymes normally function at an optimal temperature, they can still be used over a range of temperatures, providing flexibility for both cost and product quality. Thus, a market exists for eco-friendly extraction methods for the production of a variety of flavonoids.

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