Health Benefits and Bioactive Components of the Fruits from *Opuntia ficus-indica* [L.] Mill.♦

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ABSTRACT

The health-promoting properties of edible fruits from *Opuntia ficus-indica* have been the object of recent interest. Scientific evidence has been provided about benefits from the consumption of the fruits in humans, with special attention to the non-nutritive components as potentially active antioxidant phytochemicals. Information about bioavailability and bioactivity of betalains, mode of action as antioxidants in cells, and other biological models are now available. The use of cactus pear components as nutraceuticals and functional food is discussed.

Keywords: *Opuntia ficus-indica*, edible cactus, betanin, indicaxanthin, betalains, health benefits, in vivo, in vitro, natural oxidants, free-radical scavengers

1. INTRODUCTION

An equilibrated life-style, a balanced diet, no smoking, and moderate physical activity are fundamental for maintaining a healthy status. Importantly, epidemiological evidence has been provided that various age-related pathologies, including cardiovascular diseases, cancer and neurodegenerative disorders have a minor incidence among people usually consuming a traditional Mediterranean-style diet, rich in fruit and vegetables (Ames et al., 1993; Lampe, 1999; Lee et al., 2004; Rice-Evans and Miller, 1985). Since these diseases originate in or are aggravated by oxidative stress there has been substantial agreement in considering the antioxidant food components essential to long-term positive health outcomes (Trichopoulou and Vasilopolou, 2000). In this context, apart from antioxidant vitamins, polyphenol compounds (mainly flavonoids), the most represented pigments in the plant kingdom, have actively been researched to find association between benefits from certain foods and content of these substances in those foods (Peterson and Dwyer, 1998; Ross and Kasum, 2002).

Cactus pear (*Opuntia ficus-indica* [L] Mill.) originating from Mexico is now spread all over the Mediterranean basin, including Sicily. Therapeutic properties of the green parts of the plant, the cladodes, have very long been known in the traditional medicine (Cornett, 2000; Knishinsky, 1971), however potential activities of the fruit, beyond nutritional benefits, have been explored only recently. This review summarises the most recent acknowledgements about the positive health effects arising from consuming cactus-pear fruits, and reports findings on the protective effects from the administration of fruit extracts to the whole animal. A body of experimental evidence has recently been provided that beside conferring them appealing and pleasant appearance, indicaxanthin and betanin, the characteristic betalain pigments of the cactus fruits, are to be considered among the natural colors with potential health-promoting properties. Data on bioavailability, antioxidant activity in biological environments, from membranes to

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intact cells and lipoproteins, and the influence of these pigments on redox-regulated pathways involved in cell growth and inflammation are also reviewed.

2. BENEFITS FROM FRUIT INGESTION *in vivo*

2.1. *Decrease of body oxidative stress in humans*

Pro-oxidant factors that bring about an increased formation of free radicals or other reactive oxygen species continuously attack our body systems. Both cellular mechanisms (defects in mitochondrial respiration, specific enzymes, low-grade inflammation) and exogenous factors (smoking, pollution, drugs) may contribute to this (Sies, 1991). The main aim in achieving oxidative balance is to reduce the pro-oxidant factors, especially smoking, and to maintain endogenous protective systems, specific enzymes and thiols such as glutathione (GSH), by introducing radical-scavenging molecules, i.e., antioxidant vitamins (vitamins A, C, E), polyphenol compounds, and other dietary constituents, that minimize the effects of pro-oxidant factors and maintain the appropriate endocellular redox milieu. Unfortunately, the balance between pro-oxidation and anti-oxidation is never perfect, so that a certain degree of oxidative damage even occurs in healthy persons, while serious oxidative stress is present in case of impaired and/or reduced endogenous defence mechanisms due to pathological events or exposure to excessive pro-oxidant conditions.

The body's global antioxidant status (TEAC test, Miller and Rice-Evans, 1996), as well as individual antioxidant vitamins, and a number of markers of oxidative stress have been measured in plasma and cells of healthy volunteers before (baseline) and after fifteen-days during which they ingested fresh fruit pulp of Sicilian *Opuntia ficus-indica* (250 g twice daily), in addition to their usual diet (Tesoriere et al., 2004a). With respect to the baseline, a remarkable increase of plasma vitamin E and vitamin C was observed, whereas vitamin A and TEAC did not vary significantly.

Free-radical-driven oxidation of lipids is a central feature of oxidant stress, which is why quantification of end-products of lipid peroxidation such as F_2-isoprostanes and malondialdehyde (MDA) is considered to be a measure of whole-body oxidative damage (Janero, 1990; Roberts and Morrow, 2000). After supplementation with cactus-pear fruit, the plasma level of 8-epi-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and MDA decreased by about 30% and 75%, respectively (Tesoriere et al., 2004a). In addition, the ratio between GSH, the most powerful intracellular antioxidant, and its oxidized form (GSSG), measured in red blood cells, shifted towards a higher value, indicating reduction of oxidative damage and enhancement of the reducing potential of the cells. As a comparison, after a six-weeks washout, the same individuals received a supplementation of vitamin C, at a dosage comparable to the amount ingested with the fruit, twice a day for fifteen days. Supplementation with vitamin C did not affect any oxidative stress marker. It was concluded that consumption of cactus-pear fruits may positively affect the body redox balance, would decrease the oxidative damage to lipids, and improve the antioxidant status in healthy humans. Components of the fruit other than antioxidant vitamins may play a role in the observed effects.

2.2. *Cardiovascular protective effects in humans*

There is substantial evidence that oxidized low-density lipoproteins (LDLs) are central to early events leading to atherosclerosis (Navab et al., 2004; Steinberger et al., 1989). Minimally oxidized LDLs induce the expression of proteins acting as monocyte chemoattractants in the vascular endothelial cell, thereby promoting monocyte infiltration and formation of foam cells in the coronary artery wall (Ross, 1999). It has been shown that supplementing healthy humans with fruits from Sicilian *Opuntia ficus-indica* (two 250 g fruit-pulp servings per day for fifteen days) ameliorated the oxidative status of LDL, as evident from the decrease of the resident LDL hydroperoxides (Tesoriere et al., 2004a). In addition, LDLs purified from plasma of healthy volunteers 3 h after a single ingestion of 500 g of Sicilian *Opuntia ficus-*

indica fruit pulp were more resistant to oxidation induced by copper ions than did LDL isolated before the fruit meal from the same volunteers (Tesoriere et al., 2004b).

Though long-term studies and epidemiological evidence are still required, these studies suggest beneficial effects from dietary cactus-pear fruits to reduce the risk of cardiovascular disease.

2.3. *Antiulcer effects in rats*
Both macroscopic and microscopic observations showed that a 9 days pre-treatment of rats with a juice obtained from whole fruits (including peel) of Sicilian *Opuntia ficus-indica*, had a protective action on ethanol-induced gastric ulcer (Galati et al., 2003).

2.4. *Hepatoprotective effects in rats*
It has been shown that juice from whole fruits of Sicilian *Opuntia ficus-indica* reduced the carbon tetrachloride-induced liver damage, when orally administered 2 h after the toxic agent (Galati et al., 2005). Preventive effects were also assessed by applying the juice for 9 consecutive days before inducing liver toxicity. Histology evaluation, and measurement of the plasma level of hepatic enzymes provided evidence of the improvement of hepatocytes.

3. PROTECTIVE EFFECTS OF FRUIT EXTRACTS IN ANIMALS

3.1. *Cancer chemoprevention*
The suppression of ovarian tumor growth by aqueous extracts from whole fruits of *Opuntia ficus-indica* from Arizona was studied in nude mice, and compared with that of the chemopreventive agent N-(4-hydroxyphenyl) retinamide (4-HPR) (Zou et al., 2005). Immunohistochemistry staining was performed to examine the gene expression. The fruit extracts, injected i.p. (intraperitoneal) one day prior to tumor cells injection, and then during the following 6 weeks, significantly suppressed tumor growth, and modulated expression of tumor-related genes, with effects comparable with those caused by 4-HPR.

3.2. *Cerebral ischemia*
Methanol extracts from whole dried fruits of *Opuntia ficus-indica* from South Korea, preventively administered to gerbils, protected against global ischemic injury surgically induced (Kim et al., 2006). Histological examination showed that the neuronal cell damage in the hippocampal CA1 region, evaluated at 5 days after ischemia, was reduced by more than 30%. These findings may suggest that preventive administration of *Opuntia ficus-indica* extracts may be helpful in alleviating the excitotoxic neuronal damage induced by global ischemia.

4. ACTIVITY OF FRUIT EXTRACTS IN BIOLOGICAL MODELS

Methanolic extracts from the red, yellow, and white fruit from Sicilian cultivars of *Opuntia ficus-indica* (1 to 5 mg edible pulp), dose-dependently inhibited lipid oxidation induced by organic hydroperoxide in isolated human red blood cells, and by either azo-compound-derived free radicals, or copper ions, in isolated human LDLs (Butera et al., 2002). Using α-tocopherol as a reference, the extracts from the white cultivar showed higher protective effects than the red and yellow ones in all models examined.

Pre- and co-treatment of cultured mouse neurons with methanol extracts of *Opuntia ficus-indica* fruits from South Korea, attenuated the *N*-methyl-D-aspartate-, kainate-, and oxygen-glucose deprivation-induced neurotoxicity (Kim et al., 2006).

The methanol extract of *Opuntia ficus-indica* also produced dose-dependent neuroprotective effects on hydroxyl- and superoxide radical-mediated neuronal damage to mouse primary cortical cultures (Ha et al., 2003; Wie, 2000).

Aqueous extracts from the whole fruit of cactus pear from Arizona were used to treat immortalized ovarian and cervical epithelial cells, as well as ovarian, cervical, and bladder cancer cells (Zou et al., 2005). The treatment of the cells with varied extract amounts, for 1, 3, or 5 days, caused a dose- and time-dependent increase in apoptosis and growth inhibition, and affected cell cycle of cancer cells by increasing G1 and decreasing G2 and S phases.

5. BIOACTIVE COMPONENTS OF EDIBLE FRUITS FROM CACTUS PEAR

Cactus pear has recently received considerable attention in the scientific community for its bioactive components, which may provide health benefits beyond basic nutrition. Apart from antioxidant vitamins such as vitamin C, E, and carotenoids, with vitamin C being the most important quantitatively (Livrea and Tesoriere, 2004), the fruit contains peculiar phytochemicals, such as the betalain pigments (Fernandez-Lopez and Almela, 2001; Forni et al., 1992), and small amounts of polyphenol compounds that are present in the peel (Galati et al., 2003; Kuti, 2004). In addition, taurine, a cell-protective β-amino acid with antioxidative effects (Devamanoharan et al., 1998; Weiss et al., 1982), has been assessed in fruits of *Opuntia ficus-indica* from Mexico, South Africa and Italy, and thiol compounds (GSH) have been demonstrated in the fruit pulp of the Sicilian cultivars (Stintzing et al., 1999; Tesoriere et al., 2005b).

5.1. *Betalains*

The betalain pigments are condensation products of betalamic acid with various amino acids to form betaxanthins, or with *cyclo*-Dopa or glycosyl-derivatives of *cyclo*-Dopa to form betacyanins (Piattelli, 1981; Steglich and Strack, 1990). These molecules are synthesized and stored in the vacuolar compartment of plants of the order of the Caryophyllales (Stintzing et al., 2002; Castellar et al., 2003), where they are usually dissolved as bis-anions (Wyler, 1969). Two of these compounds, betanin and indicaxanthin (Figure 1), that characterize the fruits of cactus species such as the *Opuntia ficus-indica*, received recent attention for their antioxidant activity in a number of biological lipid environments *in vitro*, from human low-density lipoproteins to cell membranes (Kanner et al., 2001; Tesoriere et al., 2003; Tesoriere et al., 2005a). The interaction of the molecules with the lipid structures has been considered as the basis of such an activity.

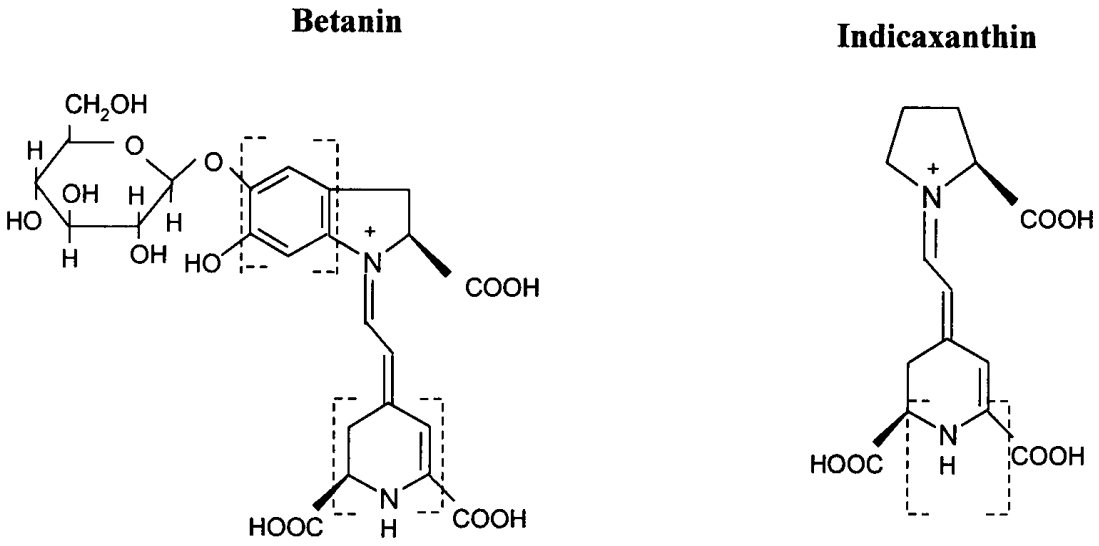

Figure 1. Molecular structure of betanin and indicaxanthin. The behaviour in lipid environments (Kanner et al., 2001; Tesoriere et al., 2006b) suggests an amphiphilic nature of the molecules (the parts with lipophilic character are shown in brackets).

Dialysis experiments provided evidence that betanin and indicaxanthin can bind to biological membranes, and to either DPPC or large unilamellar soybean-PC liposomes (Kanner et al., 2001, Tesoriere et al., 2006b). Charge-related interactions with polar head groups of membrane constituents, or even inclusion or adsorption reaction with the lipid aggregates could be accounted for by the chemistry of these molecules, that indeed may be considered as amphiphilic-like compounds. A number of findings on the redox activity of these substances have been reported in the latest years (Butera et al., 2002; Cai et al., 2003; Escribano et al., 1998; Kanner et al., 2001; Pedreno and Escribano, 2000). Some parameters measured in chemical environments are summarised in Table 1. The activity in biological models is reported in detail following Table 1.

Table 1. Trolox Equivalents Antioxidant Capacity (TEAC) and calculated inhibition constants of betanin and indicaxanthin towards oxidant species

	Betanin	**Indicaxanthin**
TEAC[a]	20.0±0.5[b]	1.7±0.1[b]
$K_{LOO^{\bullet}}$[c] ($M^{-1}s^{-1}$)	n.d.	$3.6x10^5$
K_{HOCl}[d] ($M^{-1}s^{-1}$)	$1.8x10^4$	$7.7x10^4$
$K_{MPO[Fe^{IV}=O]}$[e] ($M^{-1}s^{-1}$)	$1.5x10^6$	$1.1x10^6$
$K_{MPO[Fe^{IV}=O]}$[f] ($M^{-1}s^{-1}$)	$1.1x10^5$	$2.9x10^5$

[a] from Butera et al. (2002)
[b] Each value is the mean ± SD of four determinations performed in duplicate.
[c] lipoperoxyl radical, from Tesoriere et al. (2006b)
[d] from Allegra et al. (2005)
[e] Myeloperoxidase-compound I, from Allegra et al. (2005)
[f] Myeloperoxidase-compound II, from Allegra et al. (2005)
n.d. not determined

5.2. *Bioactive components and fruit processing*

Freshly harvested cactus-pear fruits are usually consumed. The short shelf life of the fruits, due to the very low acidity (pH varies between 5.3 and 7.1, Saenz 1996; Sepulveda and Saenz, 1990), prevents long-term storage. The juice might be a valuable alternative for fresh fruits; however, it is not very much considered worldwide, with the exception of Mexico and the southwestern United States of America.

Highly reactive molecules such as free radical-scavengers and antioxidants may be damaged during processing of food such as juice preparation. Recently, cactus-pear fruit juice has been prepared in Sicily from local fruits, and analysed for the changes in bioactive components (Figure 2). The industrially processed juice from whole fruit exhibited concentrations of cysteine and vitamin C five- and ten-fold lower, respectively, than in the edible fruit pulp, whereas beta-carotene and GSH were totally lost, which mainly appeared to be the result of thermal degradation. On the contrary, vitamin E which is less susceptible to heat (Machlin, 1991), and taurine appeared to be preserved. Interestingly, betalains were not noticeably lost during processing, in spite of their alleged thermal lability (Cai et al., 2001; Castellar et al., 2003; Reynoso et al., 1997). Other authors (Mosshammer et al., 2005) who addressed nutritional changes of cactus pear upon processing reported very similar findings. In addition, polyphenolics decreased after 6 days, while the content of vitamin C did not change, during storage of minimally processed cactus fruits at 4°C (Piga et al., 2003).

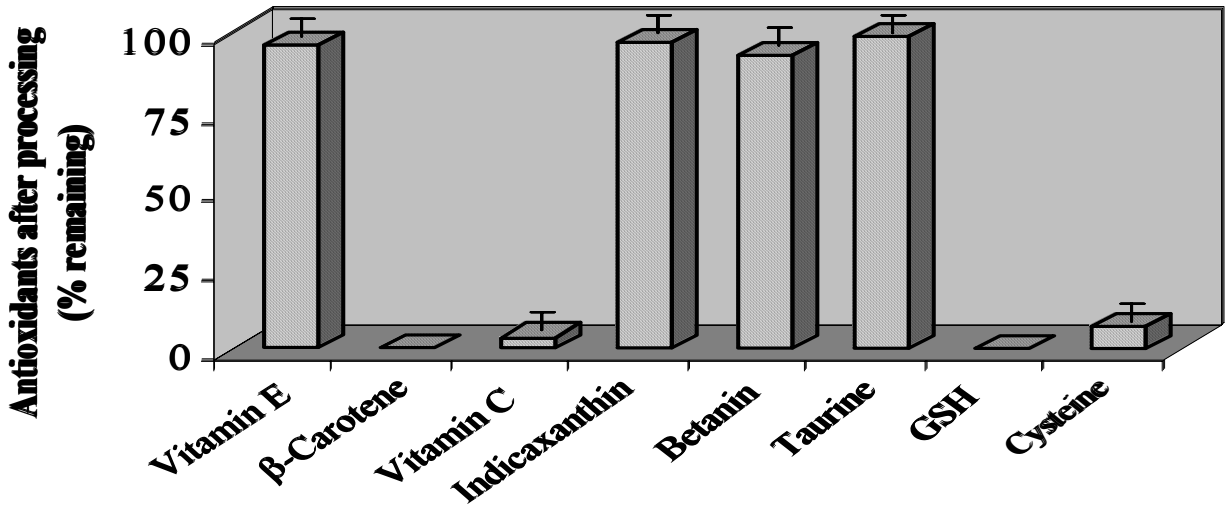

Figure 2. Effect of pasteurization on the antioxidant content of cactus fruit juice. Adapted from Tesoriere et al., 2005b.

6. BIOAVAILABILITY AND DISTRIBUTION OF BETALAINS FROM CACTUS PEAR IN HUMANS

Exact data on oral bioavailability of phytochemicals are difficult to obtain. Indeed, the extent of absorption varies greatly because of many factors, including the dietary source, instability of the molecules in the digestive environment, bacterial degradation in the gut, and mechanisms of absorption. Plasma kinetics and urinary excretion of betalains from *Opuntia ficus-indica* have recently been studied in healthy volunteers that ingested a single portion of 500 g of fruit pulp containing 28 mg and 16 mg of indicaxanthin and betanin, respectively (Tesoriere et al., 2004a). Plasma peak concentrations of both phytochemicals were reached 3 h after the ingestion, whereas both compounds disappeared from plasma within 12 h from the fruit meal (Figure 3 A, B). The plasma decline of both molecules appeared to be mono-exponential, suggesting there was not a selective tissue storage. The urinary recovery of indicaxanthin and betanin at 12 h represented about 75% and 3.5%, respectively, of the ingested compound. Information about the bioavailability of betalains from other sources have been obtained by measurement of urinary excretion of betanin after ingestion of beetroot juice (Frank et al., 2005; Kanner et al., 2001), or beetroot extracts (Watts et al., 1993). These papers show that the bioavailability of betanin was no more than 1% of the administered compound. Though cactus-pear fruits seem to be a better source of bioavailable betanin, it is not possible at this stage to definitely assess if interindividual differences between test persons and/or food matrix may play a major role.

The post-absorption distribution of betalains in humans has been assessed in red blood cells and low-density lipoproteins (LDL), after an intake of 500 g fruit pulp (Tesoriere et al., 2004b; 2005a). Both compounds were recovered in the cells and LDL to an extent that appeared to be a reflection of their plasma concentration (Figure 3, A, B). The amphiphilic character of betalains is to be considered an important factor to allow binding to LDL particles (Tesoriere et al., 2003; 2004b), as well as partition between plasma and RBCs (Tesoriere et al., 2005a; 2006a).

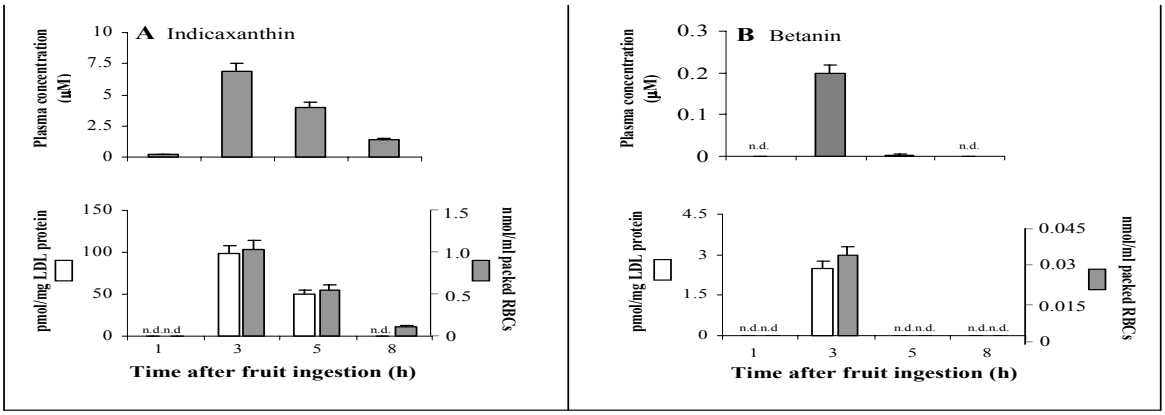

Figure 3. Plasma kinetics and distribution in LDL and RBCs of indicaxanthin (A), and betanin (B), in healthy individuals after a cactus-pear fruit meal containing 28 mg and 16 mg of indicaxanthin and betanin, respectively. n.d., not detectable. Adapted from Tesoriere et al., 2004b; 2005a.

7. ACTIVITY OF BETALAINS IN BIOLOGICAL MODELS

The radical-scavenging properties of phytochemicals are less important, unless these compounds are bioavailable and reach body compartments to a suitable concentration. In this context, the findings from studies on bioavailability of betanin and indicaxanthin, stimulated various researches on the potential antioxidant activity in biological environments.

7.1. *Microsomal membranes*

The affinity of betanin/betanidin for microsomal membranes has been shown by evaluating the rate of migration of the molecule(s) through a dialysis tube, either in the absence or in the presence of microsomes (Kanner et al., 2001). The antioxidant activity of various concentrations of betanin in this environment was assessed by submitting the membranes to either $FeCl_2$/ascorbate or H_2O_2-activated myoglobin. However, due to its electron-donating activity, low concentrations of betanin (<12.5 µM) were pro-oxidant in the system catalyzed by iron/ascorbate because of the reduction of ferric to the pro-oxidant ferrous iron (Halliwell, 1999). An effective inhibition of lipid peroxidation required higher concentrations (25 µM).

7.2. *Human LDL*

Spiking of human plasma with either purified betanin or indicaxanthin, followed by isolation of LDL, was the strategy used to obtain a betalain-enriched LDL fraction (Tesoriere et al., 2003). Both compounds showed a maximum binding of about 0.50 nmoles per mg LDL protein. The enriched LDL were more resistant to a copper-induced oxidation, with indicaxanthin twice as effective as betanin, a finding possibly explained by synergistic interactions of indicaxanthin with the LDL-vitamin E. Importantly, the kinetic studies did not provide evidence of pro-oxidant effects over a large concentration range of either betanin or indicaxanthin in this system.

One pathway for LDL oxidation may involve myeloperoxidase (MPO), a heme protein secreted by activated phagocytes as a part of the defense process against invading pathogens (Hazell and Stocker, 1993; Leeuwenburgh et al., 1997), and nitrite, the final oxidation product of nitric oxide metabolism, acting as a substrate for the enzyme (Burner et al., 2000; Van der Vliet et al., 1997). Nitrogen dioxide radical ($NO_2^{\cdot}$), the one-electron oxidation product of nitrite by peroxide-activated MPO, has been

proposed as the reactive species to start massive oxidation of the LDL lipids (Byun et al., 1999; Kostyuk et al., 2003). Recent studies (Allegra et al., 2006) showed that betanin, at micromolar concentrations as low as those attained in human plasma after ingestion of cactus-pear fruits, inhibits the production of lipid hydroperoxides in human LDL submitted to a MPO/nitrite-induced oxidation. Kinetic measurements suggest that the antioxidant effect is possibly the result of various actions, including scavenging of the initiator radical nitrogen dioxide, and of lipoperoxyl radicals. Interestingly, it was noted that yet unidentified oxidation product(s) of betanin by MPO/nitrite can also inhibit the MPO/nitrite-induced LDL oxidation as effectively as the parent compound.

7.3. *Human endothelial cells*

Vascular endothelial cells are a direct target of pro-inflammatory stimuli that remarkably affect a number of redox-mediated signalling pathways, leading to production of chemotactic factors, lipid mediators and cytokines (D'Alessio, 2002). Adhesion molecules such as the adhesion molecule-1 (ICAM-1) are characteristically expressed by vascular endothelial cells under inflammatory conditions (Carman et al., 2003). An *in vitro* model of inflammation consisting of umbilical-vein endothelial cells (HUVEC) stimulated with the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α), has been used to test the activity of betanin and indicaxanthin in modulating the expression of ICAM-1 (Gentile et al., 2004). Both pigments were able to slightly inhibit the ICAM-1 expression at micromolar concentrations. This activity, in connection with the antioxidant properties of the molecules, may be of potential pharmacological interest in pathologies such as atherosclerosis, a complex process to a great extent mediated by cytokines, growth factors, adhesion molecules, and compounds involved in redox-sensitive regulatory mechanisms, as well as in other inflammatory diseases characterized by tissue degeneration due to endothelial dysfunction such as atherothrombosis, lower-limb ischemia, and stroke (Badimon et al., 2006; D'Alessio, 2004).

7.4. *Human red blood cells*

As a consequence of high oxygen tension and large amounts of iron, a transition metal promoting the formation of oxygen free radicals (Halliwell, 1999), erythrocytes are highly susceptible to oxidation, leading to impairment of the cell function. In addition, the oxidative alterations of the RBC membrane can even ensue in injury to the endothelium cells. Bioavailable phytochemicals that might prevent and/or mitigate the effects of the oxidative stress in red blood cells are actively researched. It has recently been reported that, *ex vivo* spiking of blood from healthy humans with purified either betanin or indicaxanthin, resulted in a saturable incorporation of both betalains in the RBCs to a comparable extent (approximately 1 nmole/mL packed cell). The betalain-enriched erythrocytes turned out to be more resistant to the cumene hydroperoxide-induced oxidative haemolysis than the homologous not-enriched erythrocytes, with a significant correlation between the increase of resistance and the amount of the incorporated either betanin or indicaxanthin (Tesoriere et al., 2005a).

7.5. *Thalassemia red blood cells*

Beta-thalassemia is a genetic haemolytic disorder characterised by an increased generation of reactive oxygen species, first caused by haemoglobin auto-oxidation and precipitation. This is associated to depletion of the RBC antioxidant defence, which results in damage to cell components, impairment of morphology and function of cell membrane and accelerated RBC destruction (Chiu et al., 1996; Grinberg et al., 1995; Rund and Rachmilewitz, 2005; Scott et al., 1993;Van Dyke and Saltman, 1996). Antioxidant vitamins and phytochemicals may be helpful to treat β-thalassemia (Das et al., 2004; Grinberg and Rachmilewitz, 1994; Tesoriere et al., 2001). Protective effects of indicaxanthin on both membrane and soluble compartments of β-thalassemic RBCs submitted to an *in vitro* oxidation by cumene hydroperoxide have been demonstrated (Tesoriere et al., 2006a). The betalain enhanced the resistance to haemolysis, prevented lipid and haemoglobin oxidation, and retarded vitamin E and GSH depletion, dose-dependently. The same work it has been reported that spiking of blood from thalassemia patients with indicaxanthin resulted in its incorporation in the RBCs, indicating that the pathological alterations to the

membrane do not affect a trans-bilayer movement of this phytochemical. The finding that indicaxanthin can be incorporated in the redox machinery of β-thalassemia RBCs, suggests opportunities of therapeutic interest for this phytochemical.

7.6. *Tumor cell lines*

Betanin, at concentrations ranging 12.5 μg/mL (25 μM) to 200 μg/mL (340 μM) showed a dose-dependent growth inhibition against breast, colon, stomach, central nervous system, and lung tumor cells, with IC_{50} of 162, 142, 158, 164, and 147 μg/mL, respectively (Reddy et al., 2005). Though betanin showed potent inhibition of cell growth, its combination with anthocyanin caused a remarkable reduction of efficacy, a finding to be considered in evaluating eventual biological activity deriving *in vivo* from dietary consumption of both these substances.

The so-called phase-II enzymes, such as quinone reductase, are essential to detoxify electrophilic carcinogens in cells, during the initial stages of a cancerous process (Talalay, 1989). Studies with murine hepatoma cells showed that betanin may act as a quinone reductase inducer (Lee et al., 2005)

7.7. *Biomimetic membrane models*

Liposomes are considered suitable models for studying reactions relevant to biological membranes under controlled conditions in order to achieve chemical and/or physical parameters of reactivity and interactions of various compounds with lipid bilayers.

Indicaxanthin prevented lipid oxidation when incorporated in liposomal bilayers of phosphatidylcholine that underwent oxidation by the water-soluble azo-compound AAPH (Tesoriere et al., 2006b). The kinetic study suggested that location of indicaxanthin in the bilayer may allow scavenging of radicals from the aqueous phase, as well as lipoperoxyl radicals formed in the lipid. In addition, as a peculiar feature of the antioxidant mechanism, regeneration of indicaxanthin from its radical, involving reaction with unsaturated lipids, may strongly enhance the antioxidant potential of the phytochemical in this system.

A liposomal oxidation model of 1-stearoyl-2-linoleoyl-sn-glycerol-3-phosphocholine (Wang et al., 1999), using fluorescence spectroscopy, was used to test the antioxidant activity of betanin. The oxidation was initiated by $FeCl_2$. With respect to known commercial antioxidants (BHA, BHT, TBHQ, all at a 10 μM level), used as positive controls, betanin, at a 180 μM level, showed 71% inhibition.

8. INTERACTIONS OF BETALAINS WITH HEME-PROTEINS

Functional heme-proteins in the body are of pivotal importance, among others, in oxygen transportation, as well as in biochemical pathways involved in inflammation. The appropriate redox status of the heme-iron allows these molecules to accomplish their function. Due to their peroxidase or peroxidase-like activity in the presence of hydrogen peroxide or alkyl hydroperoxides (Everse, 1998; Furtmuller et al., 2000), heme-proteins have been suggested as candidates for inducing oxidative stress. In addition, under extreme conditions, these proteins damage themselves thus impairing their function. Reaction of heme-proteins with oxygen donors gives rise to highly reactive intermediates with hypervalent iron, that can subsequently be repaired by two sequential electron transfer reactions to the heme edge from suitable electron-rich substrates. A number of redox molecules, either endogenous or exogenous may interfere with the activity of heme-proteins by affecting the redox status of the heme iron. The interaction of betalains with heme proteins was first reported in studies that characterized oxidation products from the reaction of betanin and betanidin with purified horseradish peroxidase (Martinez-Parra and Munoz, 1997; 2001). In the light of the bioavailability of betalains, studying their interaction with highly oxidised forms

of human heme-proteins can provide interesting approaches to the health-promoting potential of these molecules.

Though protected by the protein architecture, the haemoglobin (Hb) heme-iron can be oxidised under strongly oxidant pathological conditions, and it was recently reported to be oxidatively modified even in blood from healthy individuals (Vollaard et al., 2005). The so-called perferryl-Hb is an intermediate in the oxidative degradation of haemoglobin (Everse, 1998). A spectrophotometric study showed that on a molar basis the reducing activity of indicaxanthin towards the hypervalent heme iron of perferryl-Hb is one order of magnitude higher than that exhibited by well-known reductants such as ascorbate and Trolox, the latter being a water-soluble analog of vitamin E (Tesoriere et al., 2006b). Dietary phytochemicals may also act in the gastro-intestinal tract (Halliwell et al., 2001; Kanner and Lapidot, 2001; Scalbert et al., 2002; Ursini and Sevanian, 2002). With regard to heme-proteins, scavenging of highly oxidising hypervalent-iron myoglobin (perferryl-Mb) formed during meat digestion (Halliwell et al., 2001) may preserve oxidable lipids and avoid formation of potentially toxic lipid hydroperoxides.

MPO is a human heme-enzyme that plays key roles in the defense against invading pathogens by oxygen-dependent antimicrobial action (Klebanoff, 1975). By converse, MPO has an enormous potential to inflict damage to host tissues through its ability to catalyse the production of a complex array of reactive oxidants, including hypochlorous acid, nitrogen dioxide, organic free radicals and drug metabolites (Hazell et al., 1996; Heinecke, 1998; Klebanoff, 2005; Leeuwenburgh et al., 1997). Betanin and indicaxanthin, at very low micromolar concentrations, were shown to interfere with the catalytic cycle of MPO by reducing the hypervalent-heme iron formed upon oxidation of ferric MPO by hydrogen peroxide. In addition, both betalains were able to scavenge HOCl (Allegra et al., 2005). The constants for the reaction with these highly oxidising species are reported in Table 1. From these data, the impact of betalains on inflammatory events may be supposed.

Cyclooxygenase enzymes, COX-1 and COX-2, catalyze the conversion of arachidonic acid to generate chemical mediators of inflammation (Simmons et al., 2004). Micromolar concentrations of betanin (170 μM) were found to inhibit the COX-1 and COX-2 activities of 33% and 97%, respectively (Reddy et al., 2005). Reported data do not allow drawing conclusions about the molecular interaction between betanin and the enzyme protein. It may be interesting to mention that, when assayed in combination with anthocyanin, the inhibitory effect and COX selectivity decreased.

9. CONCLUSIONS

“Let food be your medicine” Hippocrates recommended as far as 2,500 years ago. The so-called “nutraceuticals” and the “functional food” make this old tenet a new reality. Coined in Japan in the eighties, these terms refer to natural mostly vegetal products, some components of which (phytochemicals) can support the genuine antioxidant machinery of the human body (Kalra, 2003). Because of political, industrial, and economical interests, and of an increased information of consumers on a health-promoting nutritional behaviour, functional foods and their ingredients are now strongly claimed and promoted. However, sound scientific research is needed to confirm the benefits of any particular food or component, to document health effects and claims.

Interdisciplinary efforts on a molecular basis are required to integrate nutrition-related health and disease research. In this context, interest and studies on phytochemicals and their biological effects have increased immensely during the past decade. The present review focused on the documented beneficial effects of the fruits from *Opuntia ficus-indica* and of its peculiar betalain pigments. The data reported so far in biological models *in vitro* and *in vivo*, suggest that these phytochemicals may be considered as potentially effective bio-molecules for improving human health and preventing diseased states. Because

of nutrients, vitamins, and mineral composition, cactus-pear fruits have a nutritive value. In addition, because of its particular bioactive components the fruit has a remarkable added value. In the light that investigation in humans showed that consumption of fresh fruits from *Opuntia ficus-indica* can decrease the body oxidative stress in healthy individuals (Tesoriere et al., 2004a), and that betanin and indicaxanthin are highly bioavailable (Tesoriere et al., 2004b), the cactus-pear fruit may be well considered a functional food.

10. LITERATURE

Allegra, M., Furtmuller, P.G., Jantschko, W., Zederbauer, M., Tesoriere, L., Livrea, M.A., Obinger, C. (2005) Mechanism of interaction of betanin and indicaxanthin with human myeloperoxidase and hypochlorous acid. Biochem. Biophys. Res. Commun. 332:837-844.

Allegra, M., Tesoriere, L., Livrea, M.A. (2006) Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins. Free Radic. Res. in press.

Ames, B.; Shigenaga, M.K.; Hagen, T.M. (1993) Oxidants, antioxidants and the degenerative disease of aging. Proc. Nat. Acad. Sci., 90:7915-7922.

Badimon, L., Martinez-Gonzalez, J., Llorente-Cortes, V., Rodriguez, C., Padro, T. (2006) Cell biology and lipoproteins in atherosclerosis. Curr. Mol. Med. 5:439-456.

Burner, U., Furtmuller, P.G., Kettle, A.J., Koppenol, W.H., Obinger, C. (2000) Mechanism of reaction of myeloperoxidase with nitrite. J. Biol. Chem. 275:20597-20601.

Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A.M.; Kohen, R.; Livrea, M.A. (2002) Antioxidant activities of Sicilian prickly pear (*Opuntia ficus-indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agric. Food Chem. 50:6895-6901.

Byun, J., Mueller, D.M., Fabjan, J.S., Heinecke, J.W. (1999) Nitrogen dioxide radical generated by the myeloperoxidase-hydrogen peroxide-nitrite system promotes lipid peroxidation of low density lipoprotein FEBS Lett. 455:243-246.

Cai, Y., Sun, M., Corke, H. (2003) Antioxidant activity of Betalains from plants of the Amaranthaceae. J. Agric. Food Chem. 51:2288-2294.

Cai, Y., Sun, M., Schliemann W., Corke, H. (2001) Chemical stability and colorant properties of betaxanthin pigments from *Celosia argentea*. J. Agric. Food Chem. 49:4429-4435.

Carman, C.V., Jun, C.D., Salas, A., Springer, T.A. (2003) Endothelial cells proactively form microvilli-like membrane projections upon intracellular adhesion molecule-1 engagement of leukocyte LFA-1. J. Immunol. 171:6135-6144.

Castellar, R., Obon, J.M., Alacid, M., Fernandez-Lopez, J.A. (2003) Color properties and stability of betacyanins from Opuntia fruits. J. Agric. Food Chem. 51:2772-2776.

Chiu, D.T., van den Berg, J., Kuypers, F.A., Hung, I.J., Wie, J.S., Liu, T.Z. (1996) Correlation of membrane lipid peroxidation with oxidation of hemoglobin variants: possibly related to the rates of hemin release. Free Radic. Biol. Med. 21:89-95.

Cornett, J. (2000) How Indians used desert plants. Nature Trails Press.

D'Alessio, P. (2002) Endothelium as a pharmacological target. Curr. Op. Invest. Drugs 2:1720-1724.

D'Alessio, P. (2004) Aging and the endothelium. Exp. Gerontol. 36:165-171.

Das, N., Chowdhury, T.D., Chattopadhyay A., Datta, A.G. (2004) Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. Polish J. Pharmacol. 56:85-96.

Devamanoharan, P.S.; Ali, A.H.; Varma, S.D. (1998) Oxidative stress to rat lens in vitro: protection by taurine. Free Radic. Res. 29:189-195.

Escribano, J., Pedreno, M.A., Garcia-Carmona, F., Munoz, R. (1998) Characterization of the antiradical activity of betalains from Beta vulgaris L. roots. Phytochem. Anal. 9:124-127.

Everse J. (1998) The structure of heme proteins Compounds I and II: some misconceptions. Free Radic. Biol. Med. 24:1338-1346.

Fernandez-Lopez, J.A., Almela, L. (2001) Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. J. Chromatogr. A 913:415-420.

Forni, E., Polesello, A., Montefiori, D., Maestrelli, A. (1992) High-performance liquid chromatographic analysis of the pigments of blood-red prickly pear (*Opuntia ficus-indica*). J. Chromatogr. 593:177-183.

Frank, T., Stinzing, F.C., Carle, R., Bitsch, I., Quaas, D., Strass, G., Bitsch, R., Netzel, M. (2005) Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. Pharmacol. Res., 52:290-297.

Furtmuller, P.G., Obinger, C., Hsuanyu, Y., Dunford, H.B. (2000) Mechanism of reaction of myeloperoxidase with hydrogen peroxide and chloride ion. Eur. J. Biochem. 267:5858-5864.

Galati, E.M., Mondello, M.R., Giuffrida, D., Dugo, G., Miceli, N., Pergolizzi, S., Taviano, M.F. (2003) Chemical characterization and biological effects of sicilian *Opuntia ficus-indica* [L.] Mill. Fruit juice: antioxidant and antiulcerogenic activity. J. Agric. Food Chem. 51:4903-4908.

Galati, E.M., Mondello, M.R., Lauriano, E.R., Taviano, M.F., Galluzzo, M., Miceli, N. (2005) *Opuntia ficus-indica* [L] Mill. fruit juice protects liver from carbon tetrachloride induced injury. Phytother. Res. 19:796-800.

Gentile, C., Tesoriere, L., Allegra, M., Livrea, M.A., D'Alessio, P. (2004) Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. Ann. N.Y. Acad. Sci. 1028:481-486.

Grinberg, L.N., Rachmilewitz, E.A., Newmark, H. (1994) Protective effects of rutine against hemoglobin oxidation. Biochem. Pharmacol. 48:643-649.

Grinberg, L.N., Rachmilewitz, E.A., Kitrossky, N., Chevion, M. (1995) Hydroxyl radical generation in beta-thalassemic red blood cells. Free Radic. Biol. Med. 18:611-615.

Ha, H.J., Kwon, Y.S., Park, S.M., Shin, T.K., Park J.H., Kim, H.C., Kwon, M.S., Wie, M.B. (2003) Quercetin attenuates oxygen-glucose deprivation-and excitotoxin-induced neurotoxicity in primary cortical cell cultures. Biological and Pharmaceutical Bull. 26:544-549.

Halliwell, B., Gutteridge, J.M.C. (1999) Free Radical in Biology and Medicine. 3rd edn. Oxford University Press.

Halliwell, B., Zhao, K., Whiteman, M. (2001) The gastrointestinal tract: a major site of antioxidant action? Free Radic Res. 33:819-80.

Hazell, L.J., Arnold, L., Flowers, D., Waeg, G., Malle, E., Stocker, R. (1996) Presence of hypochlorite-modified proteins in human atherosclerotic lesions. J. Clin. Invest. 97:1535-1544.

Hazell, L.J., Stocker, R. (1993) Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. Biochem. J. 290:165-172.

Heinecke, J.W. (1998) Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis 141:1-15.

Janero, D.R. (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9:515-540.

Kalra, E.K. (2003) Nutraceutical - Definition and Introduction. *AAPS Pharm. Sci.* 5:article 25. DOI: 10.1208/ps050225.

Kanner, J., Harel, S., Granit, R. Betalains-A new class of dietary cationized antioxidants. (2001) J. Agric. Food Chem. 49:5178-5185.

Kanner, J., Lapidot, T. (2001) The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radic. Biol. Med. 31:1388-1395.

Kim, J.H., Park, S.M., Ha, H.J., Moon, C.J., Shin, T.K., Kim, J.M., Lee, N.H., Kim, H.C., Jang K.J., Wie, M.B. (2006) *Opuntia ficus-indica* attenuates neuronal injury in in vitro and in vivo models of cerebral ischemia. J. Ethnopharmacol. 104:257-262.

Klebanoff, S.J. (1975) Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. Seminar Haematol. 12:117-142.

Klebanoff, S.J. (2005) Myeloperoxidase: friend and foe. J. Leukoc Biol. 77:598-625.

Knishinsky, R. (1971) Prickly pear cactus medicine. Healing Art Press, Rochester, Vermont.

Kostyuk, V.A., Kraemer, T., Sies, H., Schewe, T. (2003) Myeloperoxidase/nitrite-mediated lipid peroxidation of low-density lipoprotein as modulated by flavonoids. FEBS Lett. 537:146-150.

Kuti, J.O. (2004) Antioxidant compounds from four Opuntia cactus pear fruit varieties. Food Chem. 85:527-533.

Lampe, J.W. (1999) Health effects of vegetables and fruits: assessing mechanism of action in human experimental studies. Am. J. Clin. Nutr. 70:475-490.

Lee, J., Koo, N., Min, D.B. (2004) Reactive oxygen species, aging, and antioxidant nutraceuticals. Comprehens. Rev. Food Sci. Food Safety 3:21-33.

Lee, C.H., Wettasinghe, M., Bolling, B.W., Ji, L.L., Parkin, K.L. (2005) Betalains, phase II enzyme-inducing components from red beetroot (Beta vulgaris L.) extracts. Nutr. Cancer 53:91-103.

Leeuwenburgh, C., Hardy, M.M., Hazen, S.L., Wagner, P., Oh-ishi, S., Steinbrecher, U.P., Heinecke, J.W. (1997) Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. J. Biol. Chem. 272:1433-1436.

Livrea, M.A., Tesoriere, L. (2004) Antioxidant Activities of Prickly Pear (*Opuntia ficus-indica*) Fruit and Its Betalains: Betanin and Indicaxanthin. in: Packer, L., Ong, C.N., Halliwell, B. (Eds.) Herbal and Traditional Medicine: Molecular Aspects of Health. Marcel Dekker, Inc., pp. 537-556.

Machlin, L.J. (1991) Vitamin E. in: Handbook of Vitamins. Machlin, L.J. (Ed.) Marcel Dekker, Inc. New York, pp. 99-144.

Martínez Parra, J., Munoz, R. (1997) An approach to the characterization of betanine oxidation catalyzed by horseradish peroxidase. J. Agric. Food Chem. 45:2984-2988.

Martinez-Parra, J., Munoz R. (2001) Characterization of betacyanin oxidation catalyzed by a peroxidase from Beta vulgaris L. roots. J. Agric. Food Chem. 49:4064-4068.

Miller, N.J., Rice-Evans, C.A. (1996) Spectrophotometric determination of antioxidant activity. Redox Report 2:161-171.

Mosshammer, M.R., Stinzing, F.C., Carle, R. (2005) Development of a process for the production of a betalain-based colouring foodstuff from cactus pear. Innovative Food Science & Emerging Technologies 6:221-231.

Navab, M., Ananthramaiah, G.M., Reddy, S.T., Van Lenten, B.J., Ansell, B.J., Fonarow, G.C., Vahabzadeh, K., Hama, S., Hough, G., Kamranpour, N., Berliner, J.A., Lusis, A.J., Fogelman, A.M. (2004) The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J. Lipid Res. 45:993-1007.

Pedreno, M.A., Escribano, J. (2000) Studying the oxidation and the antiradical activity of betalain from beetroot. J. Biol. Educ. 35:49-51.

Peterson, J., Dwyer, J. (1998) Flavonoids: dietary occurrence and biochemical activity. Nutr. Res. 12:1995-2018.

Piattelli, M. (1981) The betalains: structure, biosynthesis and chemical taxonomy. In The Biochemistry of Plants: A Comprehensive Treatise. Conn EE (Ed) Academic Press, New York, vol.7, pp. 557-575.

Piga, A., Del Caro, A., Pinna, I., Agabbio, M. (2003) Changes in ascorbic acid, polyphenol content and antioxidant activity in minimally processed cactus pear fruits. Lebensm-Wiss Technol 36:257-262.

Reddy, M.K., Alexander-Lindo R.L., Nair, M.G. (2005) Relative inhibition of lipid peroxidation, cycloxygenase enzymes, and human tumor cell proliferation by natural food colors. J. Agric. Food Chem. 53:9268-9273.

Reynoso, R., Garcia, F.A., Morales, D., Gonzalez de Mejia E. (1997) Stability of betalain pigments from a cactacea fruit. J. Agric. Food Chem. 45:2884-2889.

Rice-Evans, C.A., Miller, N.J. (1985) Antioxidants: the case of fruit and vegetables in the diet. Brit Food J. 97:35-40.

Roberts, L.J., Morrow, J.D. (2000) Measurement of F_2-isoprostanes as an index of oxidative stress in vivo. Free Radic. Biol. Med. 28:505-513.

Ross, R. (1999) Atherosclerosis-an inflammatory disease. New Engl. J. Med. 340:115-126.

Ross, J.A., Kasum C.M. (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22:19-34.

Rund, D., Rachmilewitz, E. (2005) Beta-thalassemia. New Engl. J. Med. 353:1135-46.

Saenz, C. (1996) Food products from cactus pear (*Opuntia ficus-indica*). Food Chain 18:10-11.

Scalbert, A., Morand, C., Manach, C., Remesy, C. (2002) Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother. 56:276-282.

Scott, M.D., van den Berg, J.J., Repka, T., Rouyer-Fessard, P., Hebbel, R.P., Beuzard, Y., Lubin, B.H. (1993) Effect of excess alpha-hemoglobin chains on cellular and membrane oxidation in model beta-thalassemic erythrocytes. J. Clin. Invest. 91:1706-1712.

Sepulveda, E., Saenz, C. (1990) Chemical and physical characteristics of prickly pear (*Opuntia ficus-indica*) pulp. Revista de Agroquimica y tecnologia de alimentos 30:551-555.

Sies, H. (1991) Oxidative stress II. Oxidants and antioxidants. Academic Press, London.

Simmons, D.L., Botting, R.M., Hla, T. (2004) Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 56:387-437.

Steglich, W., Strack, D. (1990) Betalains. In: The Alkaloids, Chemistry and Pharmacology. Brossi A (Ed) Academic Press, London, pp. 1-62.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., Witztum, J.L. (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. New Engl. J. Med. 320:915-924.

Stintzing, F.C., Schieber, A., Carle, R. (1999) Amino acid composition and betaxanthin formation in fruits from *Opuntia ficus-indica*. Planta Med. 65:632-635.

Stintzing, F.C., Schieber, A., Carle, R. (2002) Identification of betalains from yellow beet (Beta vulgaris L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem. 50:2302-2307.

Talalay, P. (1989) Mechanisms of induction of enzymes that protect against chemical carcinogenesis. Adv. Enzyme Regul. 28:237-250.

Tesoriere, L., D'Arpa, D., Butera, D., Allegra, M., Renda, D., Maggio, A., Bongiorno, A., Livrea, M.A. (2001) Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Radic. Res. 34:529-540.

Tesoriere, L., Butera, D., D'Arpa, D., Di Gaudio, F., Allegra, M., Gentile, C., Livrea, M.A. (2003) Increased resistance to oxidation of betalain-enriched human low density lipoproteins. Free Radic. Res. 37:689-696.

Tesoriere, L.; Butera, D., Pintaudi, A.M., Allegra, M., Livrea, M.A. (2004a) Supplementation with cactus pear (*Opuntia ficus-indica*) fruits decreases oxidative stress in healthy humans. A comparative study with vitamin C. Am. J. Clin. Nutr. 80:391-395.

Tesoriere, L., Allegra, M., Butera, D., Livrea, M.A. (2004b) Absorption, excretion, and distribution in low density lipoproteins of dietary antioxidant betalains. Potential health effects of betalains in humans. Am. J. Clin. Nutr. 80:941-945.

Tesoriere, L., Butera, D., Allegra, M., Fazzari, M., Livrea, M.A. (2005a) Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo-induced oxidative hemolysis in humans. J. Agr. Food Chem. 53:1266-1270.

Tesoriere, L., Fazzari, M., Allegra, M., Livrea, M.A. (2005b) Biothiols, taurine, and lipid-soluble antioxidants in the edile pulp of Sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. J. Agric. Food Chem. 53:7851-7855.

Tesoriere, L., Allegra, M., Butera, D., Gentile, C., Livrea M.A. (2006a) Cytoprotective effects of the antioxidant phytochemical indicaxanthin in β-thalassemia red blood cells. Free Radic. Res. 40:753-761.

Tesoriere, L., Allegra, M., Butera, D., Gentile, C., Livrea, M. A. (2006b) Kinetics of the lipoperoxyl radical-scavenging activity of indicaxanthin in solution and unilamellar liposomes. Free Radic. Res., in press.

Trichopoulou, A., Vasilopoulou, E. (2000) Mediterranean diet and longevity. Br. J. Nutr. 84:S205-209.

Ursini, F., Sevanian, A. (2002) Wine polyphenols and optimal nutrition. Ann. N.Y. Acad. Sci. 957:200-209.

Van der Vliet, A., Eiserich, J.P., Halliwell, B., Cross, C.E. (1997) Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. J. Biol. Chem. 72:7617-7625.

Van Dyke, B., Saltman, P. (1996) Hemoglobin: a mechanism for the generation of hydroxyl radicals. Free Radic. Biol. Med. 20:985-989.

Vollaard, N.B., Reeder, B.J., Shearman, J.P., Menu, P., Wilson, M.T., Cooper, C.E. (2005) A new sensitive assay reveals that hemoglobin is oxidatively modified in vivo Free Radic. Biol. Med. 39:1216-1228.

Wang, H., Nair, M.G., Strasburg, G.M., Chang, Y.C., Booren, A.M., Gray, J.I., DeWitt, D.L. (1999) Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J. Nat. Prod. 62:802-807.

Watts, A.R., Lennard, M.L., Tucker, G.T., Woods, H.F. (1993) Beeturia and the biological fate of beetroot pigments. Pharmacogenetics 3:302-311.

Weiss, S.J., Klein, R., Slivka, A., Wei, M. (1982) Chlorination of taurine by human neutrophils. J. Clin. Invest. 70:598-607.

Wie, M.B. (2000) Protective effects of *Opuntia ficus-indica* and *Saururus chinensis* on free radical-induced neuronal injury in mouse cortical cell cultures. Kakhak Hoiji 44:613.

Wyler, H. (1969) Die Betalaine. Chiuz 3:146-151.

Zou, D.M., Brewer, M., Garcia, F., Feugang, J.M., Wang, J., Zang, R., Liu, H., Zou C. (2005) Cactus pear: a natural product in cancer chemoprevention. Nutr. J. 4:25-36.