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Anthocyanins: Antioxidant and/or anti-inflammatory activities

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ABSTRACT

Anthocyanins are polyphenols with known antioxidant activity which may be responsible for some biological activities including the prevention or lowering the risk of cardiovascular disease, diabetes, arthritis and cancer. Nevertheless such properties, their stability and bioavailability depend on their chemical structure. In the present work a brief review is made on chemical structures, bioavailability and antioxidant/anti-inflammatory of anthocyanins.

Key words: Anthocyanins, chemistry, stability, bioavailability, free radical scavenging.

INTRODUCTION

Anthocyanins are generally accepted as the largest and most important group of water-soluble pigments in nature (Harborne, 1998). They are responsible for the blue, purple, red and orange colors of many fruits and vegetables. The word anthocyanin derived from two Greek words: *anthos*, which means flowers, and *kyanos*, which means dark blue (Horbowicz et al., 2008). Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine (Mazza, 2007). They belong to the family of compounds known as flavonoids, but they are distinguished from other flavonoids due to their capacity to form flavylium cations (Fig. 1) (Mazza, 2007).
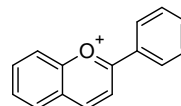
Fig 1. Flavylium cation.

They occur principally as glycosides of their respective aglycone anthocyanidin-chromophores with the sugar moiety generally attached at the 3-position on the C-ring or the 5-position on the A-ring (Prior and Wu, 2006). There are about 17 anthocyanidins found in nature, but only six (cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin, with cyanidin being the most common) (Fig. 2) are ubiquitously spread and of great importance in human diet (Harborne, 1998; Jaganath and Crozier, 2010). According to a recent review on the role of anthocyanins in cancer prevention, the daily intake of anthocyanins in the U.S. diet is estimated to be between 180 and 215 mg, amount significantly higher when compared to the intake of other

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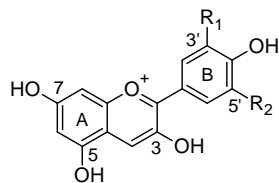
Anthocyanidin	R_1	R_2	Colour
Pelargonidin	H	H	Orange
Cyanidin	OH	H	Orange-red
Delphinidin	OH	OH	Bluish-red
Peonodin	OCH_3	H	Orange-red
Petunidin	OCH_3	OH	Bluish-red
Malvidin	OCH_3	OCH_3	Bluish-red

Fig 2. Structures of major anthocyanidins (adapted from Jing, 2006).

dietary flavonoids such as genistein, quercetin and apigenin, which is only 20–25 mg/day (Wang and Stoner, 2008).

The diversity of anthocyanins are due to the number and position of hydroxyl and methoxy groups on the basic anthocyanidin skeleton; the identity, number, and positions at which sugars are attached; and the extent of sugar acylation and the identity of the acylating agent (Prior and Wu, 2006; Jaganath and Crozier, 2010). Intensity and type of the colour of anthocyanins is affected by the number of hydroxyl and methoxyl groups: if hydroxyl groups predominate, then the colour goes toward a more bluish shade; if more methoxyl groups prevail, then redness is increased (Heredia et al., 1998; Delgado-Vargas and Paredes-López, 2003; Horbowicz et al., 2008).

According to the pH of the medium, anthocyanins may change from intensely red or orange under acidic conditions (pH<2) due to the presence of eight conjugated double bonds carrying a positive charge (Horbowicz et al., 2008). At pH values between 2 and 4, the quinoidal blue species prevailed.
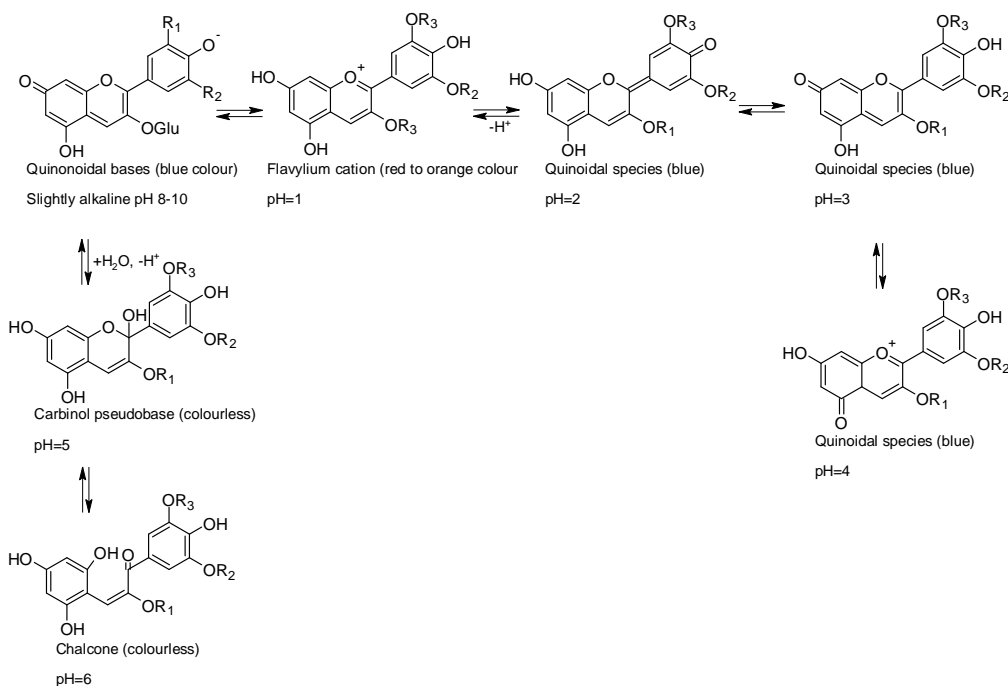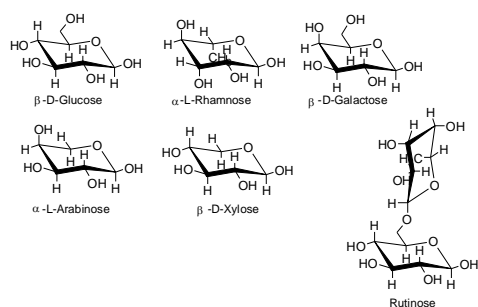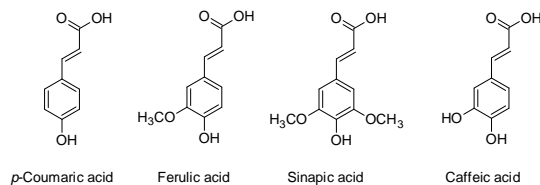
At pH values between 5 and 6 only two colourless species can be found (carbinol pseudobase and chalcone, respectively) (Fig 3). At pH values higher than 7, the anthocyanins are degraded depending on their substituent groups (Castañeda-Ovando et al., 2009). Colour stability then decreases towards neutrality but some anthocyanins showed a stability increase culminating at local maxima around 8-9. Examples include the 3-glucosides of malvidin, peonidin and pelargonidin which showed their most bluish colours at this interval of pH values. The presence of methoxyl groups and the absence of ortho-dihydroxylation on the B-ring seems to favour this blue colour in the alkaline region, according to the results of some authors (Cabrita et al., 1999). The anthocyanidins stability is influenced by the ring B substituents and the presence of additional hydroxyl or methoxyl groups decreases the aglycon stability in neutral media; therefore, pelargonidin is the most stable anthocyanidin. In contrast with aglycons, monoglycosides, and mostly, diglycosides derivatives are more stable in neutral pH conditions. Such may be explained by the sugar moieties which avoid the degradation of instable intermediates into phenolic acid and aldehyde compounds (Castañeda-Ovando et al., 2009).

Generally anthocyanidin glycosides are 3-monoglycosides and 3,5-diglycosides. The most common sugar of anthocyanidin glycosides is glucose, nevertheless rhamnose, xylose, galactose, arabinose and rutinose (6-O-L-rhamnosyl-D-glucose) (Fig. 4) can also occur (Horbowicz et al., 2008). Although very rare, glycosylation at the 3', 4', or 5' positions of the B ring is also possible (Wu and Prior, 2005). The sugar moiety may be acylated by aromatic acids, generally hydroxycinnamic acids (caffeic, ferulic, p-coumaric or sinapic acids) (Fig. 5) and sometimes by aliphatic acids, namely succinic, malic, malonic, oxalic and acetic acids (Fig. 6). The acyl moieties are normally linked to the sugar at C-3 (Jing, 2006; Pereira et al., 2009).

Fig 3. Predominant structural forms of anthocyanins present at different pH levels (R_1=H or glycoside; R_2 and R_3=H or methyl group) (adapted from Castañeda-Ovando et al., 2009).

Fig 4. The most common glycosyl units of anthocyanins.

Fig 5. Common hydroxycinnamic acids acylated with sugar moieties in anthocyanins.
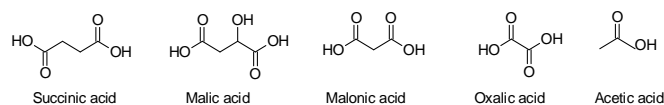
Fig 6. Common aliphatic acids acylated with sugar moieties in anthocyanins.

Anthocyanin-rich foods are becoming more popular. Epidemiologic studies suggest that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis and cancer, due to their antioxidant anti-inflammatory properties (Rechner and Kroner, 2005; Wang and Stoner, 2008). Visual acuity can also be markedly improved with the administration of anthocyanins because these pigments enhance night vision or overall vision (Lila, 2004).

However to achieve any effect in a specific tissue, these bioactive compounds must be bioavailable, that is, effectively absorbed from the intestine into the circulation and delivered to the appropriate local for reaching the target (McDougall et al., 2005). Oral administration of anthocyanin-rich fruits, extracts or pure compounds has proved to be effective in preventing or suppressing diseased states (Ramirez-Tortosa, 2001; Tsuda et al., 2003; McDougall et al., 2005). Many of the activities reported for anthocyanins are attributed to their antioxidant activity and/or to their metabolites. Nevertheless, some studies revealed that the anthocyanin concentrations were too low which poorly could contribute to *in vivo* quenching of reactive oxygen species but could be adequate to influence signal transduction and gene signal transduction and gene expression pathways (Milbury et al., 2010). Some topics on biodisponibility and antioxidant activity as well as signal transduction of anthocyanins are reported in the present work.

BIOAVAILABILITY OF ANTHOCYANINS

In vitro studies on the antioxidant activity of anthocyanins are abundant; nevertheless after ingestion of foods in which these pigments are present they are very probably metabolized in the

organism with the possible change of activity. Information on the absorption, metabolism, tissue and organ distribution and excretion of anthocyanins in human subjects is scarce because they are complex, expensive and lengthy, and sometimes with contradictory results (Kay et al., 2004; McDougall et al., 2005).

In vivo experiment using rats showed that malvidin-3-glucoside appeared in both portal and systemic plasma after only 6 min and by this time an apparent steady state was reached. This finding suggested to the authors that this anthocyanin may permeate the gastric mucosa. (Passamonti et al., 2003). Other study revealed that anthocyanin glycosides are also rapidly and efficiently absorbed from the small intestine of rats being furthermore quickly metabolized and excreted into bile and urine as intact glycosides as well as methylated forms and glucuronidated derivatives (Talavéra et al., 2004). Other studies also referred that human subjects were the capacity to metabolize cyanidin-3-glycosides in the respective glucuronide conjugates, as well as methylated and oxidised derivatives of cyanidin 3-galactoside and cyanidin glucuronide (Kay et al., 2004) and strawberry anthocyanins were glucuro- and sulfo-conjugated in humans and that the main metabolite of strawberry anthocyanins in human urine was a monoglucuronide of pelargonidin (Felgines et al., 2003). In another study, these authors concluded the importance of the aglycone structure on anthocyanin metabolism, because in an experiment using rats, the authors found that pelargonidin-3-glucoside was rapidly absorbed from both stomach and small intestine, similarly to cyanidin-3-glucoside. Nevertheless, pelargonidin-3-glucoside was glucuronidated to a larger extent than cyanidin-3-glucoside (Felgines et al., 2007).

In contrast there are other authors, which found in their experiments that raspberry anthocyanins in rats were poorly absorbed, and that substantial amounts pass from the small to the large intestine where they were degraded by colonic bacteria (Borges et al., 2007). These authors also stress the absence of knowledge about either the metabolism or absorption of the pseudobases or the quinoidal base of anthocyanins in the gastrointestinal tract due to the absence of adequate analytical procedures. According to the same authors, it could be that after anthocyanins leave the stomach, the colourless carbinol pseudobase becomes the main form, in the small intestine where it undergoes very limited absorption. Consequently, significant amounts pass into the large intestine where degradation, to as yet undetermined products, occurs due to the action of colonic bacteria. Later on, the same authors also studying the bioavailability of raspberry anthocyanins in human subjects found the presence of anthocyanins in ileal fluid indicating that despite the impact of pH on the stability of anthocyanins in the small intestine, in the subjects with an intact colon, large amounts have passed from the small intestine to the large intestine without biotransformation (González-Barrio et al., 2010). Microbiota found in this part of the gastro-intestinal tract could metabolize anthocyanins into phenolic compounds. In fact, Aura (2005) has already detected the formation of protocatechuic acid (3,4-dihydroxybenzoic acid) as the major metabolite after

bioconversion of cyanidin-3-rutinoside by human faecal microbiota *in vitro*. This metabolite was also reported by Vitaglione et al. (2007) in humans after ingestion of cyanidin-3-glucoside, accounting for about 40% of the ingested cyanidin-3-glucoside in 6-h post-consumption bloodstream. It was also found in faecal samples. Protocatechuic acid was considered by these authors as the major human metabolite of cyanidin-3-glucoside. That phenolic compound could even explain the several biological properties given to anthocyanins, including antioxidant, antiobesity, cardiovascular-protective, and anti-inflammatory activities.
After cleavage of the 3-glycosidic moiety, the released cyanidin is very unstable under physiological conditions and can be metabolized by the bacteria or degraded by a chemical reaction without the action of bacteria to phenolic acids and aldehydes (Galvano et al., 2007).

Other phenolic compounds can arise from anthocyanin biotransformation by microflora present in large intestine. Examples include 3-*O*-methylgallic acid, syringic acid, and 2,4,6-trihydroxy-benzaldehyde after the incubation in the large intestinal contents of three freshly slaughtered pigs of an extract from Cabernet Sauvignon grape anthocyanins, which contained delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside (Forester and Waterhouse, 2008). Some authors consider that the numerous health benefits attributed to dietary anthocyanin consumption may be explained by the presence of the phenolic metabolites (Vitaglione et al., 2007; Forester and Waterhouse, 2008).

Only incipient information can be found on the presence and distribution of anthocyanins and/or their metabolites in internal tissues, which is a key for understanding the mechanisms of their effects. Espín et al. (2007) in a review article referred some works approaching this issue, referring that anthocyanins, their aglycones and both methylated and glucuronide derivatives of anthocyanins could be detected in tissues such as stomach, small intestine, liver, bile, kidney, lung and eye, although in some organs (eye and brain) detection of anthocyanins was very fast and, in some cases, in very small amounts.

McGhie and Wlaton (2007) have compiled, in a review article, the results obtained by various authors on the processes of absorption and metabolism of anthocyanins. From this work they could report that anthocyanin glycosides can be rapidly absorbed from the stomach after ingestion, and they enter the systemic circulation after passing through the liver. Methylation and glucuronidation reactions can occur in this organ and some of the metabolites are transported to the intestine as bile. Anthocyanin glycosides which were not absorbed from the stomach move into the small intestine, converting to a combination of chalcone and quinonoidal forms due to the pH of this organ. Further absorption seems to take place in the jejunum, being the transport mechanism unknown. Absorbed anthocyanins enter the systemic circulation after passage though the liver and may be metabolized. Anthocyanins that reach the colon are exposed to a microbial population able to transform them to sugar and phenoilic compounds, with further degradation by disruption of the C-ring to yield phenolic acids and aldehydes. The same authors present in their review article a scheme which depicts very well the possible processes of absorption and metabolism of anthocyanins according to the scientific information found by them.

BIOLOGICAL PROPERTIES OF ANTHOCYANINS

Food may have beneficial effects on human health in addition to its nutritional value. Anthocyanins present in some food and beverages has shown to play an important role in the prevention of diverse diseases such as cancer; cardiovascular diseases involving mechanisms of antioxidant activity, detoxification activity, anti-proliferation, induction of apoptosis, and anti-angiogenic activity; anti-inflammatory activity; inhibition of digestive enzymes (α-glucosidase, α-amylase, protease, and lipase), which is a clinical therapic target for controlling type II diabetes and obesity; improvement of the immune system; improvement of night vision as well as in the retardation of the aging process, reducing, for example, the risk of degenerative disorders, such as Alzheimer's disease (Ames et al., 1993; Jing, 2006; Nikkhah et al., 2008). Antioxidant activities which can explain some of the beneficial effects of anthocyanins are approached in the present work.

Antioxidant activity

Living organisms have a redox system trying to keep human life to be at a healthy balance. Free radicals are necessary for the living state of the cells and organisms (Nagy, 2001; Droge, 2002). Some free radicals such as nitric oxide, superoxide radical anion, and related reactive oxygen species (ROS) and or reactive nitrogen species (RNS) mediate cells in signalling processes (Droge, 2002; Jing, 2006). However the redox homeostasis could be off balance. And an oxidative stress occurs. Oxidative stress is an imbalanced state where excessive quantities of ROS/RNS overcome endogenous antioxidant capacity, leading to oxidation of enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Dai and Mumper, 2010). Antioxidants may be defined as being compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. In an oxidative sequence, antioxidants can act at different levels by: (a) decreasing localized oxygen concentrations; (b) preventing chain initiation by scavenging reactive oxygen and/or nitrogen species (ROS/RNS), e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite; (c) binding metal ions in such a manner that they will not generate species such as $HO^{\bullet}$, ferryl or $Fe^{2+}/Fe^{3+}/O_2$, and/or decompose lipid peroxides to peroxyl and alkoxyl radicals; (d) decomposing peroxides by converting them to non-radical products, such as alcohols; (e) chain-breaking, i.e. scavenging intermediate radicals, such as peroxyl and alkoxyl radicals, to prevent continued hydrogen abstraction (Dai and Mumper, 2010; Miguel, 2010).

The antioxidant properties of different plant extracts, food and beverages can be evaluated using various *in vitro* assays.

Antioxidant assays in foods and biological systems can be divided in two groups: those that evaluate lipid peroxidation, and those that measure free radical scavenging ability (Miguel, 2010). In assessing lipid peroxidation, several lipid substrates can be used and the antioxidant activity in these systems can be detected by measuring the substrate and the oxidant consumption, and the intermediates or the final products formation. For measuring free radical scavenging ability, there are methods which can be grouped in two sets, according to the chemical reactions involved: electron transfer- and hydrogen transfer-based assays. In addition, tests evaluating effectiveness against several reactive oxygen species and nitrogen reactive species ($O_2^{\bullet-}$, $HO^{\bullet}$, $ONOO^-$, $NO^{\bullet}$, H_2O_2) are also needed and generally performed. (Apac et al., 2007; Miguel, 2010).

The antioxidant capacity of anthocyanins present in diverse fruits has been demonstrated with a wide variety of assay methods: oxygen radical absorbance capacity (ORAC), a hydrogen transfer-based assay (Wang et al., 1997; Prior et al., 1998; Wang and Lin, 2000; Zheng and Wang, 2003; Steed and Truong, 2008); ferric reducing antioxidant potential (FRAP), trolox equivalent antioxidant capacity (TEAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, all of them electron transfer-based assays (Moyer et al., 2002; Lee et al., 2004; Einbond et al., 2004; Nakajima et al., 2004; Solomon et al., 2006; Koca and Karadeniz, 2009); scavenging activity towards superoxide (Costantino et al., 1992); peroxynitrite ($ONOO^-$) scavenging activity (Muselík et al., 2007); inhibition of human low-density lipoprotein (LDL and liposome oxidation) (Heinonen et al., 1998); inhibition of lipid peroxidation (Muselík et al., 2007); ability to bind heavy metals such as iron, zinc and copper (Havsteen, 1983); and induction of antioxidant enzymes such as gluthatione-*S*-transferase (GST), gluthatione reductase (GR), gluthatione peroxidise (GPx) and superoxide dismutase (Fiander and Schneider, 2000; Turner, 2009).

However, this activity is greatly dependent on the chemical structure of anthocyanins and not all of them possess similar activities for scavenging diverse ROS and RNS. The antioxidant ability of anthocyanins depends on the basic structural orientation of the compound because the ring orientation will determine the ease by which a hydrogen atom from a hydroxyl group can be donated to a free radical as well as the capacity of the anthocyanin to support an unpaired electron (Kay, 2004).

In addition, the efficacy to scavenge diverse ROS differs from one anthocyanin to another, for instance, delphinidine is the most active against the superoxide anion (being followed by cyanidin and pelargonidin) and pelargonidin is the most efficient against the hydroxyl radical (Tsuda et al., 1996; Antal et al., 2003). Generally the antioxidant activity of anthocyanins is associated with the number of free hydroxyls around the pyrone ring. Greater number of hydroxyls greater antioxidant activity. Anthocyanins with their 3',4'-dihydroxy groups can quickly chelate metal ions to form stable anthocyanin-metal complexes (Sarma et al., 1997). Anthocyanins at pH 2-4 mostly exist in the form of flavylium cations and because of the charge distribution they are susceptible

to nucleophilic attack on positions 2 and 4. According to those authors, it can be postulated that the hydroxylation of an anthocyanin at these positions enhances its chelating capacity, protecting, for example, ascorbic acid from metal-induced oxidation (Fig. 7).
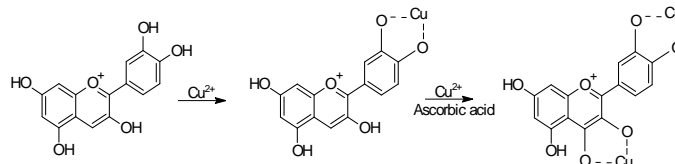

Fig. 7. A possible mechanism of increased metal chelaring by cyanidin in presence of copper and ascorbic acid.

Anthocyanins with the *ortho*-dihydroxyl groups have the potential to scavenge hydroxyl radicals through the inhibition of $HO^{\bullet}$ generation by chelating iron (Noda et al., 1998; 2000; Bąkowska-Barczak, 2005).

In addition to the degree and position of hydroxyl groups in the B ring on the antioxidant activity of anthocyanins, the degree and position of methoxyl groups also influenced the stability and reactivity of these pigments, consequently their antioxidant activities (Muselík et al., 2007). For example and according to the results of these authors, also corroborated by Kähkönen and Heinonen (2003), the anthocyanins malvidin-3-glucoside and petunidin-3-glucoside showed lower efficiency compared to cyanidin-3-rutinoside and delphinidin-3-glucoside. Figure 8 depicts a free radical mechanism for the semiquinone stabilization formed from the cyanidin oxidation proposed by Castañeda-Ovando et al. (2009).
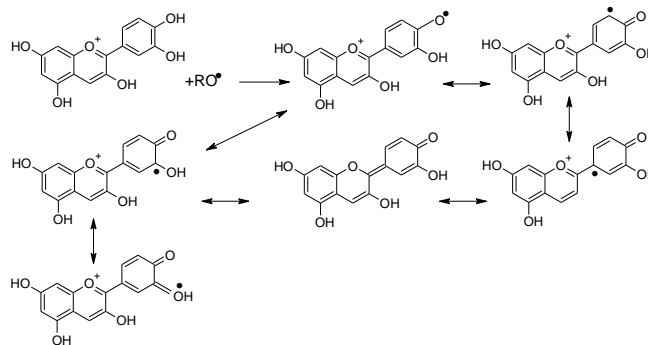

Fig. 8. Proposed mechanism for the stabilization of the cyanidin semiquinone radical (resonance) (adapted from Castañeda-Ovando et al., 2009).

Nevertheless those activities are dependent on type of reactive species. For example, Muselík et al. (2007) reported that for the FRAP and TEAC assays the methoxylation of hydroxyl groups in 5´ (petunidin-3-monoglucoside) or 3´ and 5´ positions (malvidin-3-monoglucoside) significantly reduced the antioxidant activity. However, the antioxidant activity showed by malvidin-3-monoglucoside in peroxynitrite mediated tyrosine nitration was the same as that of delphinidin-3-monoglucoside and cyanidin-3-rutinoside activities. The activity of anthocyanins in preventing tyrosine nitration decreased in the following order: cyanidin-3-rutinoside > malvidin-3-monoglucoside ≈ delphinidin-3-

monoglucoside > petunidin-3-monoglucoside (Muselík et al., 2007).

One mechanism proposed by Tsuda et al. (2000) for ONOO⁻ scavenging activity of pelargonidin (anthocyanidin) consists firstly in the break of this pigment by the radical with the formation of *p*-hydroxybenzoic and secondly the reaction of this acid with ONOO⁻ resulting in the formation of 4-hydroxy-3-nitrobenzoic acid (Fig. 9).
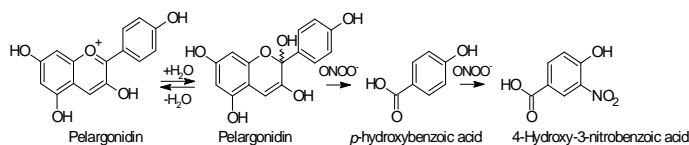
Fig 9. Proposed mechanism for the ONOO⁻ scavenging activity of pelargonidin (adapted from Tsuda et al., 2000).

Depending on the pH, the relative proportions of protonated, deprotonated, hydrated, and isomeric forms of anthocyanins exist. These forms may play an important role in the antioxidant activity. In addition, the relative proportions of peroxynitrite anion (ONOO⁻) and its conjugate acid (HOONO), with different reactivities, are too strongly dependent on pH. The peroxynitrite scavenging activity of anthocyanins at pH 7.4 ($\approx 80\%$ of peroxynitrite was in the anionic form) decreased in the following order: cyanidin-3-rutinoside > malvidin-3-monoglucoside $\approx$ delphinidin-3-mono-glucoside > petunidin-3-monoglucoside (Muselík et al., 2007).

Generally anthocyanins prevail in fruits and vegetables and glycosylation seems decreasing the antioxidant capacity of anthocyanins by reducing free hydroxyls and metal chelation sites. However, some authors (Kähkönen and Heinonen, 2003) considered that depending on the anthocyanidin and lipid oxidation models used for antioxidant analysis, different glycosylation patterns either enhanced or diminished the antioxidant power. In their experiment, for the most part, the activities of the glycosides and the aglycons did not differ remarkably in emulsion, whereas in LDL the aglycons showed in general higher activities than the glycosides. In bulk oil, to the contrary, the glycosides were more effective than the aglycons assayed by them. Therefore, the *in vitro* effect of glycosylation on antioxidant activity also depends on the environment in which oxidations is occurring (Kay, 2004).

Different number of sugar residues and their position in the anthocyanidin may also have different effects on the antioxidant activity of an anthocyanin (Wang et al., 1997; Antal et al., 2003; Kähkönen and Heinonen, 2003). The number of sugar residues at the C_3 position seems to be very important for antioxidant activity. The smaller the number of sugar units at C_3, the higher the antioxidant activity. For example, those authors reported that delphinidin and cyanidin-3-rutinoside are less active in the DPPH scavenging activity than the corresponding monoglucosides. Nevertheless some authors consider that such effect is very much dependent on the method used (Kähkönen and Heinonen, 2003; Muselík et al., 2007). An example is that of the glycosylation of malvidin-3-monoglucoside to malvidin-3,5-diglucoside which significantly reduces the antioxidant ability when measured through the TEAC method, but without significant effect on the inhibition of tyrosine nitration (ONOO⁻ scavenging), or being better as scavenging free radicals when measured through the FRAP assay when compared to malvidin-3-monoglucoside (Muselík et al., 2007).

The presence of acyl groups also shows having influence on the antioxidant activity, nevertheless and as reported for sugar moieties, the scavenging activity is highly dependent on the free radical type. Some authors reported that the pyranoanthocyanins of cyanidin, petunidin, malvidin and pelargonidin showed a high capacity to scavenge superoxide anion radicals but did not scavenge hydroxyl radicals (García-Alonso et al., 2005), although some other authors have found that the incorporation of pyruvic acid into delphinidin-3-monoglucoside and malvidin-3-monoglucoside caused a significant decrease in antioxidant activity in aqueous phase assays (Muselík et al., 2007).

Anti-inflammatory activity

The marketing around the health-promoting effects of anthocyanins and berries is increasing being one of the main claims of their properties the high level of antioxidant capacity. However, this activity identified *in vitro* for anthocyanins (Wang et al., 1999; Zheng and Wang, 2003; Galvano et al., 2004; Miguel et al., 2007), conflicts sometimes with data obtained *in vivo* on the antioxidant capacity of plasma after the consumption of anthocyanin-rich foods by human subjects (Pedersen et al., 2000). This does not exclude the possibility that very low concentrations of anthocyanins may modulate cell signalling, gene regulation and other biological processes by non-antioxidant mechanisms, which may explain the health benefits of anthocyanins. In fact recently, some studies have demonstrated that anthocyanidins possess anti-inflammatory activities by inhibition of cyclooxygenase-2 (COX-2) expression in lipopolysaccharide (LPS)-activated RAW 264 cells or inhibiting inducible nitric oxide (iNOS) protein and mRNA expression in LPS-activated murine J774 macrophages (Hou et al., 2005; Hämäläinen et al., 2007) and such activities appear to be structure-dependent. COX-2 seems to be involved in many inflammatory processes. Some antioxidants inhibit the expression of COX-2 by interfering with the signalling mechanisms that regulate the COX-2 gene (Hou et al., 2005). In this gene, four transcription factors including nuclear factor-κB (NF-κB), CCAAT/enhancer-binding protein (C/EBP), activator protein 1 (AP-1) and CRE-binding protein (CREB) have been identified as regulators of COX-2 transcription. C/EBP is considered to regulate COX-2-production, whereas AP-1 and CREB are essential for both basal and induced COX-2 transcription (Hou et al., 2005).

Some authors have reported inhibition of cyclooxygenase by cyanidin glycosides present in cherries and berries (Seeram et al., 2001). However, other authors reported that the anthocyanidin delphinidin was the most potent inhibitor of COX-2 expression at both mRNA and protein levels, suppressing LPS-stimulated activation of transcription factors including C/EBP , AP-1 and NF-κB, but not CREB (Hou et al., 2005).

NF-κB is a transcriptional regulator that consists of homo- and heterodimers of proteins (p65 or RelA, p50/p105, c-Rel, p52/p100 and RelB). NF-κB is maintained as a latent form in the cytoplasm of cells where it is complexed to IκB inhibitor protein. Seven members of the IκB family of proteins have been identified and includes IκB-α. Upon activation of NF-κ, IκB-α is phosphorylated by IκB kinases (IKK) leading to proteasome-dependent degradation of IκB, which allows a rapid translocation of NF-κB into the nucleus where it binds to DNA. The most predominant NF-κB dimmer activated is p65:p50. Translocation of p65:p50 to the nucleus results in the transcription of several proinflammatory genes, such as cytokines (TNF-α, IL-1β, IL-6) and inducible enzymes (iNOS and COX-2) (Heininger et al., 2000; Yoshimura, 2006). Some studies have revealed that the anthocyanidin delphinidin had an inhibitory effect on degradation of IκB-α and nuclear translocation of p65 (Hou et al., 2005).

In macrophage and other types of cells, LPS activates three subclasses of mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinase (ERK), c-Jun-terminal kinase (JNK) and p38. Studies have revealed that some compounds are able to suppress pro-inflammatory cytokines and NF-κB, to inhibit the COX-2 and iNOS expression inhibiting some of these MAPKS (Weinstein et al., 1992; Wadsworth and Koop, 2001; Hou et al., 2005). For example, some authors considered delphinidin a potent inhibitor of COX-2 due to the blockage of activation of all of three kinases (Hou et al., 2005), in a structure-dependent manner.

Signal transducer and activator of transcription (STAT-1) is another transcriptional factor for iNOS and some authors (Hämäläinen et al., 2007) reported the capacity of some flavonoids to inhibit STAT-1 activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. The sole anthocyanidin assayed (pelargonidin) by the same authors, such ability was not observed.

Therefore, anthocyanin, or anthocyanin-extract, inhibits the expression and biological activity of some pro-inflammatory cytokines *in vitro* by suppressing NF-κB through down-regulation of MAPK pathways (Wang et al., 1999; Pergola et al., 2006). These actions are likely to be attributed to the antioxidant property of anthocyanins. However, some authors have reported that anthocyanins can exert a significant anti-inflammatory activity with almost no change to the antioxidative *status in vivo* (Xia et al., 2006; Wang et al., 2007). Thus, Wang et al. (2008) suggested that there were other signalling pathways involved in the anti-inflammatory action induced by anthocyanins aside from that involved in scavenging of ROS. They demonstrated such in their work for cyanidin-3-glucoside which was able to inhibit iNOS and COX-2 expression by inducing liver X receptor alpha activation in THP-1 macrophages. Some studies have reported that anthocyanins effectively up-regulates the signalling pathway of the nuclear receptors, such as liver X receptor α (LXRα) and peroxisome proliferator-activated receptor γ (PPARγ) (Xia et al., 2005). In addition, it seems that activation of these nuclear receptors significantly antagonizes inflammatory gene expression *in vitro* (Chawla et al., 2001; Walcher et al., 2006).

CONCLUSIONS

High level of antioxidant ability is reported for anthocyanins. Nevertheless, food and beverages in which phenolic compounds can be found may greatly change depending of several factor such as: 1. related to plant, agronomic and technological conditions (plant species, varieties, climatic and agronomical conditions in which plants grow, post-harvesting conditions of vegetable and fruits, technological treatment of rich-anthocyanin foods); 2. related to human beings (age, gender, health status, genetics and the presence of some pathologies which is determinant in the biotransformation, interaction between anthocyanins and other foods ingested at the same time or within a short interval of time) are only some examples which hampered studies concerning the effect of anthocyanins in human health. Nevertheless and according to some authors (Espín et al., 2007), the absence of evaluations of biological activities attributed to anthocyanins by FDA, does not permit to consider these products as good for curing, mitigating, treating or preventing any disease. Therefore a more suitable and accurate survey on the type, levels, doses and real benefits of such products urges to be performed. Only after this effort, health care professionals can advice with certitude consumers which are looking for information about such products for taking a decision about its inclusion in their dietary as supplement, nutraceutical, or other form to improve health, prevent or retard some diseases.

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