Anthocyanins: from plant to health

Sonia de Pascual-Teresa · Maria Teresa Sanchez-Ballesta

Abstract Anthocyanins are a group of natural occurring pigments responsible for the red-blue colour of many fruits and vegetables. Anthocyanins are of interest for two reasons because they cannot only be used in the technological field as natural colorants but also have important implications in the field of human health. Numerous studies indicate the potential effect that this family of flavonoids may have in reducing the incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases through the intake of anthocyanin-rich foods. This review examines existing literature in this area: from plant content and distribution to health implications, including the effect of agronomic and genetic modifications on the anthocyanin content of plants as well as other biotechnological factors and food processing. The bioavailability, metabolism, bioactivity, and epidemiology of anthocyanins will also be reviewed.

Keywords Bioavailability · Biological activity · Food processing and storage · Postharvest treatments

S. de Pascual-Teresa (✉) · M. T. Sanchez-Ballesta
Instituto del Frío (CSIC), Jose Antonio Novais, 10, 28040 Madrid, Spain
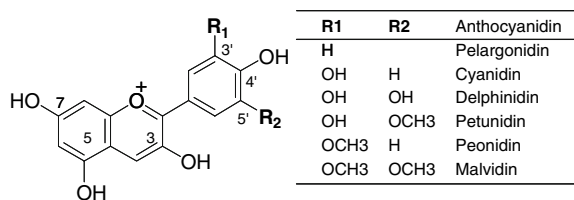e-mail: soniapt@if.csic.es

Introduction

The term anthocyanin is derived from the Greek words meaning flower and blue. Anthocyanins are natural pigments belonging to the flavonoid family. They are responsible for the blue, purple, red and orange colour of many fruits and vegetables. More than 500 different structures have been identified (Andersen 2001; Andersen and Markham 2006; Harborne 1993; Mazza and Miniati 1993). Anthocyanins are interesting for two reasons. The first being technological, namely, their impact on the organoleptic characteristics of food which may influence their technological behaviour during food processing and the second reason being anthocyanin implication in human health through different pathways, as it will be reviewed in the present article.

Anthocyanins are present in nature mainly in the form of heterosides. The aglycon form of anthocyanins, also called anthocyanidin, is structurally based on the flavilium ion or 2-phenylbenzopyrilium, and presents hydroxyl and methoxyl groups in different positions. Depending on the number and position of the hydroxyl and methoxyl substituents some dozen different anthocyanidins have been described in publications, of which six are commonly found in fruits and vegetables (Fig. 1).

Anthocyanidins are present in fruits and vegetables linked to one or more glycosidic units. Sugars may be linked as mono, di or triglycosides and may, in addition, be acylated with different organic acids.

Fig. 1 Structure of the anthocyanidins most commonly found in foods

R1	R2	Anthocyanidin
H		Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH3	Petunidin
OCH3	H	Peonidin
OCH3	OCH3	Malvidin

The glycosidic units may be linked to the anthocyanidin by α or β linkage, and always in position 3 of the aglycon. When additional sugars are present in the anthocyanin molecule, they are linked to positions 5 and 7, and less frequently to 3′ and 5′. Glucose and galactose, in hexoses, and rhamnose, arabinose and xylose, as pentoses, are the sugars most commonly encountered forming part of the anthocyanin. The most common acylating agents are cinnamic acids, frecuently *p*-cinnamic or caffeic acid, but also ferulic and sinapic acid. Acetic, malic, malonic, oxalic and succinic acid are found in aliphatic acids (Clifford 2000).

Anthocyanins are highly soluble in water and alcoholic solutions. When in water they are more stable at low pH. However, in aqueous solution they co-exist as four main equilibrium species: the flavylium cation, the quinonoidal base, the carbinol or pseudobase, and the chalcone C (Fig. 2). Depending on the pH of the solution and the structure of a particular anthocyanin the relative amounts of each equilibrium form vary. This characteristic is an important issue when analysing anthocyanins and should be considered when interpreting bibliographical data both, from the technological point of view and with respect to health related studies. At low pH the predominant form of the anthocyanin is the flavylium cation which is red in colour. As pH increases most of the flavylium ions change into other anthocyanin forms some of them blue in colour (quinonoidal forms) and others colourless (pseudobases and chalcones) (Brouillard and Dangles 1993). In general acylation of the anthocyanidin produces an increase in the relative proportion of the flavylium cation and thus protect the red colour at higher pH, increasing anthocyanin stability.

Anthocyanins are relatively unstable and easily oxidised. They are sensitive to many factors, apart from pH, that may affect their stability and colour
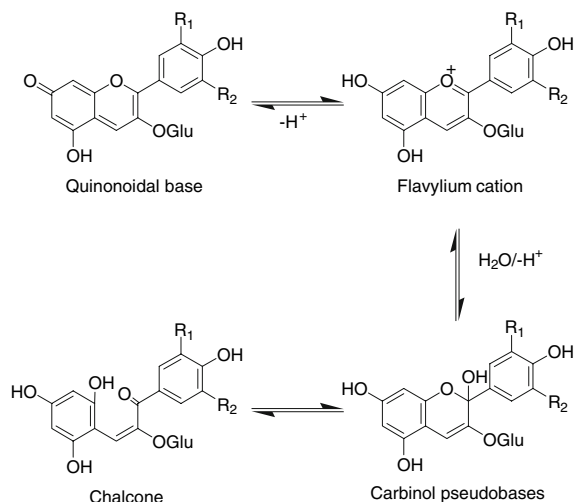
Fig. 2 Chemical transformations of anthocyanins

such as temperature and UV radiation. They are also affected by the presence in the medium of sulphur dioxide, ascorbic acid or methal ions among other food components and additives (Rivas-Gonzalo 2003).

Many studies in recent years have focused on the study of the health properties of anthocyanins from different perspectives. However it is important to note that anthocyanins levels in food products may be modified at different stages in the food production chain. These modifications could be introduced intentionally or not, by changes in agronomic and environmental conditions; hours of sun, kind of soil and humidity, among other factors, and would clearly affect the anthocyanin composition of fruits and vegetables. Biotechnology and postharvest treatments may increase the levels of this group of flavonoids in plant foods. Food processing and storage conditions may also have an effect on anthocyanins. In the present work we will analyse the effect of all these processes on anthocyanidin levels in the final product. In this way this review is aimed at helping us to understand what technology is available for designing functional foods enriched in this group of food components which in turn will have a beneficial effect on human health.

If anthocyanins are to be effective in preventing or treating a range of diseases, they must be bioavailable. In the present review we will analyse the factors which may alter anthocyanin bioavailability. Additionally,

we will briefly review the available bibliography on anthocyanins bioactivity, from antimutagenicity studies to anticancer and cardioprotective activities.

Plant content and distribution

Anthocyanins are present in different plant organs, such as fruits, flowers, stems, leaves and roots (Brouillard 1982). These pigments are normally found dissolved uniformly in the vacuolar solution of epidermal cells. However, in certain species, the anthocyanins are localized in discrete regions of the cell vacuole, called anthocyanoplasts (Pecket and Small 1980). The main sources of anthocyanin (Table 1) are red fruits, mainly berries and red grapes, cereals, principally purple maize, vegetables and red wine among drinks (Harbone 1993; Escribano-Bailón et al. 2004).

Anthocyanin content may vary from fruit to fruit of the same type due to different external and internal factors, such as genetic and agronomic factors, intensity and type of light, temperature, processing and storage. For example, anthocyanin concentrations in red grapes are specially variable being able to reach values of up to 250 mg/100 g, while in red wines the concentrations vary in accordance with the varieties of grapes employed as well as with the type of vinification and, specially, with aging. However, a medium value could be established of around 500 mg/l anthocyanins in young wines. Moreover, it is important to note that anthocyanins are used as food additives in beverages, fruit fillings, snacks and dairy products.

The most common anthocyanidins in higher plants are: delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin. The glycosides of the three non-metylated anthocyanidins (delphinidin, cyanidin and pelargonidin) are the most abundant in nature, which represent 80% of leaf pigments, 69% in fruits and 50% in flowers. The distribution of the six most common anthocyanidins in the edible parts of plants is cyanidin (50%), pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%) and malvidin (7%). The most widespread anthocyanin in most fruit is cyanidin-3-glucoside (Kong et al. 2003). However, malvidin glycosides are the most characteristic anthocyanins in red grape and derived products (wine, juice, etc.) (Mazza and Miniati 1993).

Agronomic modifications and biotechnology

The development of *agronomic techniques* to enhance production of useful secondary metabolites provides an opportunity to optimize currently available cultivars. Environmental conditions are known to induce the accumulation of anthocyanin pigments in the major groups of higher plants, light and temperature factors being the most studied. Therefore, agronomic manipulation by the application of mild stress at defined points during the growing season may have generic effects on anthocyanin accumulation. Work with apples has shown the effectiveness of covering the orchard floor with foil and metalized film thus increasing light penetration into the canopy, which in turn correlates with an increase in anthocyanin concentration in fruit peel (Ju et al. 1999). Moreover, in the fruit of many crops such as grape, peach, strawberry, eggplants and lychee, anthocyanin synthesis is enhanced by sunlight (Dokoozlian and Kliewer 1996; Tyas et al. 1998; Matsuzoe et al. 1999; Bergqvist et al. 2001; Spayd et al. 2002; Jia et al. 2005). Likewise, the position of the fruit on the tree affects anthocyanin accumulation due to sunlight intensity. The skin of the apples from the top of the canopy contained the highest levels of cyanidin 3-galactoside, followed by fruit from the outside of the canopy, and then those from the canopy interior (Awad et al. 2001). Shading bunches of Cabernet Sauvignon, at veraison, reduced light intensity during the daytime and the resulting anthocyanin content (Jeong et al. 2004). Opaque boxes applied to bunches of Shiraz grapes prior to flowering had little effect on berry development, ripening and the accumulation of anthocyanin (Downey et al. 2004) and insignificant differences in anthocyanin content were also observed in Pinot clusters (Cortell and Kennedy 2006). However, this treatment reduced the levels of delphinidin, cyanidin, petunidin, and malvidin and increased peonidin glucosides (Cortell and Kennedy 2006). Likewise, in Reliance, a seedless *Vitis* hybrid, 95% shading decreased the percentage of delphinidin and cyanidin and increased the percentage of peonidin, malvidin, and acylated cyanidin derivatives compared to 55% shading or unshaded controls (Gao and Cahoon 1994).

Low temperatures have long been considered to promote, and high temperatures to reduce, anthocyanin synthesis (Saure 1990; Leng et al. 2000). Long-term

Table 1 Concentration of anthocyanins in foods

Food	Anthocyanin (mg/100g)	References
Apple, Red Delicious	1.7	Koponen et al. 2007
Bilberry	300–698	Mazza and Miniati 1993
Black Bean	24.1–44.5	Macz-Pop et al. 2006
Black Currant	130–476	Timberlake 1988; Wu et al. 2006
Black Olives	42–228	Mazza and Miniati 1993
Black Rice	10–493	Ryu et al. 1998
Blackberry	82.5–325.9	Torre and Barrit 1977; Wang and Lin 2000
Blueberry	25–495	Ballinge et al. 1972; Makus and Ballinger 1973
Bog whortleberry	154	Koponen et al. 2007
Cherry	2–450	Harbone and Hall 1964; Mazza and Miniati 1993; Gao and Mazza 1995
Chokeberry	410–1480	Wu et al. 2006; Koponen et al. 2007
Cranberry	67–140	Wu et al. 2006; Koponen et al. 2007
Crowberry	360	Koponen et al. 2007
Eggplant	8–85	Wu et al. 2006; Koponen et al. 2007
Elderberry	200–1816	Kaack and Austed 1998; Wu et al. 2006
Gooseberry	2.0–43.3	Wu et al. 2006; Pantelidis et al. 2007
Grapefruit	5.9	Koponen et al. 2007
Lettuce	2.2–5.2	Wu et al. 2006; Koponen et al. 2007
Nectarine	2.4	Koponen et al. 2007
Peach	4.2	Koponen et al. 2007
Pear	5–10	Mazza and Miniati 1993
Plum	2–25	Wu et al. 2006
Pomegranate (juice)	600–765	Kriventsov and Arendt 1981
Port Wine	14–110	Timberlake 1988
Purple Corn	1642	Cevallos-Casals and Cisneros-Zevallos 2003
Raspberry	20–687	Wang and Lin 2000; Wu et al. 2006
Red apple	1.3–12	Mazza and Velioglu 1992; Wu et al. 2006
Red Cabbage	322	Wu et al. 2006
Red currant	22	Koponen et al. 2007
Red Grape	30–750	Lamikanra 1989
Red Onion	23.3–48.5	Ferreres et al. 1996; Wu et al. 2006
Red Radish	100–154	Giusti and Wrolstad 1996; Wu et al. 2006
Red Wine	16.4–35	Bakker and Timberlake 1985; Teissedre and Landrault 2000; Frankel et al. 1995
Rhubarb	4–200	Timberlake 1988; Koponen et al. 2007
Rowanberry	14	Koponen et al. 2007
Saskatoon berry	234	Koponen et al. 2007
Strawberry	19–55	Lopez-da-Silva et al. 2007

seasonal changes in temperature affect anthocyanin levels. Thus, habitats with cool weather were associated with a rapid anthocyanin accumulation in the skin of 'Starkrimson' and 'Golden Delicious' apple, whereas the warm weather was associated with slower anthocyanin accumulation (Li et al. 2004). Cooling by

microsprinkler irrigation in apples increased their red colour and anthocyanin content especially when applied at sunset and at sunrise (Iglesias et al. 2002). In grapes, Mori et al. (2005) showed that anthocyanin synthesis, L-phenylalanine ammonia-lyase (PAL) activity and chalcone synthase 3 (*CHS3*) transcript

levels were markedly higher in berries grown under low night temperatures.

Nutrient deficiencies, especially of phosphorus (P) and nitrogen (N), commonly induce the accumulation of anthocyanin in many plant species (Cobbina and Miller 1987; Hodges and Nozzolillo 1996; Close et al. 2000). The anthocyanin formation in *Spirodela polyrhiza* is mainly a response to the perturbed C:N ratio in plants, an apparent consequence of N depletion in the nutrient medium (Kumar and Sharma 1999). In bean plants cultured on P-deficient media, the higher concentrations of anthocyanins found in leaves could protect the plant from oxidative stress (Juszczuk et al. 2004). In tomato, in addition to increasing flavonoid content, N stress also produces differential effects on expression of genes encoding anthocyanin biosynthetic enzymes (Bongue-Bartelsman and Phillips 1995). By contrast, an increased application of nitrogen in cranberry resulted in a decreased content of red pigments (Eaton 1971), and Eck (1976) detected a significant decrease in anthocyanin content as a response to increased N application every third year. Similar results were observed in black chokeberry when a combined nitrogen-phosphorus-potassium fertilizer was used (Jeppsson 2000).

Preharvest benzothiadiazole (BTH) treatment of grapevine not only increased the content of *trans*-resveratol and anthocyanins significantly, but also induced systemic acquired resistance to *Botrytis cinerea* infection (Iriti et al. 2004). BTH triggers a significant increase in anthocyanin content, including five monoglucosides of the anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin) as well as the corresponding acetylated and *p*-coumaroyl derivatives (Fumagalli et al. 2006). These changes in BTH-treated berry skins are closely accompanied by a significant improvement in the NO-releasing capacity of berry skin extracts.

Plant hormones also affect anthocyanin biosynthesis. McGlasson et al. (1978) suggested that abscisic acid (ABA) promotes biosynthesis of anthocyanin. Poor coloration of fruit is improved by the application of ABA, which increases the anthocyanin content in grape skin (Jeong et al. 2004; Peppi et al. 2007) and cherry skin (Kondo and Inoue 1997). Ethylene has been shown to influence the biosynthesis of light-induced anthocyanin formation (Craker and Wetherbe 1973; Faragher and Brohier 1984).

Application of ethephon, an ethylene-generating compound, improves colour development in apples and increases anthocyanin content (Larrigaudiere et al. 1996; Awad and de Jager 2002; Li et al. 2002). Preharvest foliar application of AVG, an inhibitor of ethylene synthesis, in apple (*Malus domestica* Borkh.) fruit retards several key maturation events but does not affect the levels of anthocyanin (Silverman et al. 2004).

Genetic engineering techniques, by introduction of genes encoding novel anthocyanin enzyme activities and transcription factors or inactivation of endogenous genes, have been used to modify flower colour and plant coloration as well as to analyze the potential of the anthocyanin pathway in plant defence response (Fig. 3). Several important structural genes and regulatory elements required for anthocyanin biosynthesis have been cloned and characterized in a variety of plant species over the past few years, including some with direct practical applications. With the generation of transgenic petunia plants using a dihydroflavonol-4-reductase (*DFR*) gene from maize a novel flower colour was created by genetic engineering for the first time (Meyer et al. 1987). DFR which converts dihydrokaempferol (DHK) to leucopelargonidin enables the production of pelargonidin in petunia and leads to a change in flower colour from pale pink to brick red. Similar results have been observed in petunia by overexpressing heterologous *DFR* genes from gerbera (Helariutta et al. 1993) and rose (Tanaka et al. 1995). An approach involving the co-suppresion of the *CHS* gene has been used to prevent the formation of flower pigments in petunia and tobacco (van der Krol et al. 1988), gerbera (Elomaa et al. 1993) and lisianthus (Deroles et al. 1998). However, by contrast, overexpression of the *CHS* gene has been used to reduce flower pigments in petunia (Napoli et al. 1990) and chrysanthemum (Couryney-Gutterson et al. 1994). White and blue-white transgenic varieties of *Torenia hybrida* taken from the blue variety cv. Summerwave were obtained by cosuppressing the expression of both the *CHS* and *DFR* genes (Suzuki et al. 2000).

Flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′,5′-hydroxylase (F3′5′H), members of the cytochrome P450 family, play a key role in determining the pattern of anthocyanin. Whereas F3′H is necessary for the synthesis of 3′-hydroxylated anthocyanins (e.g., cyanidin), F3′5′H participates in the synthesis of
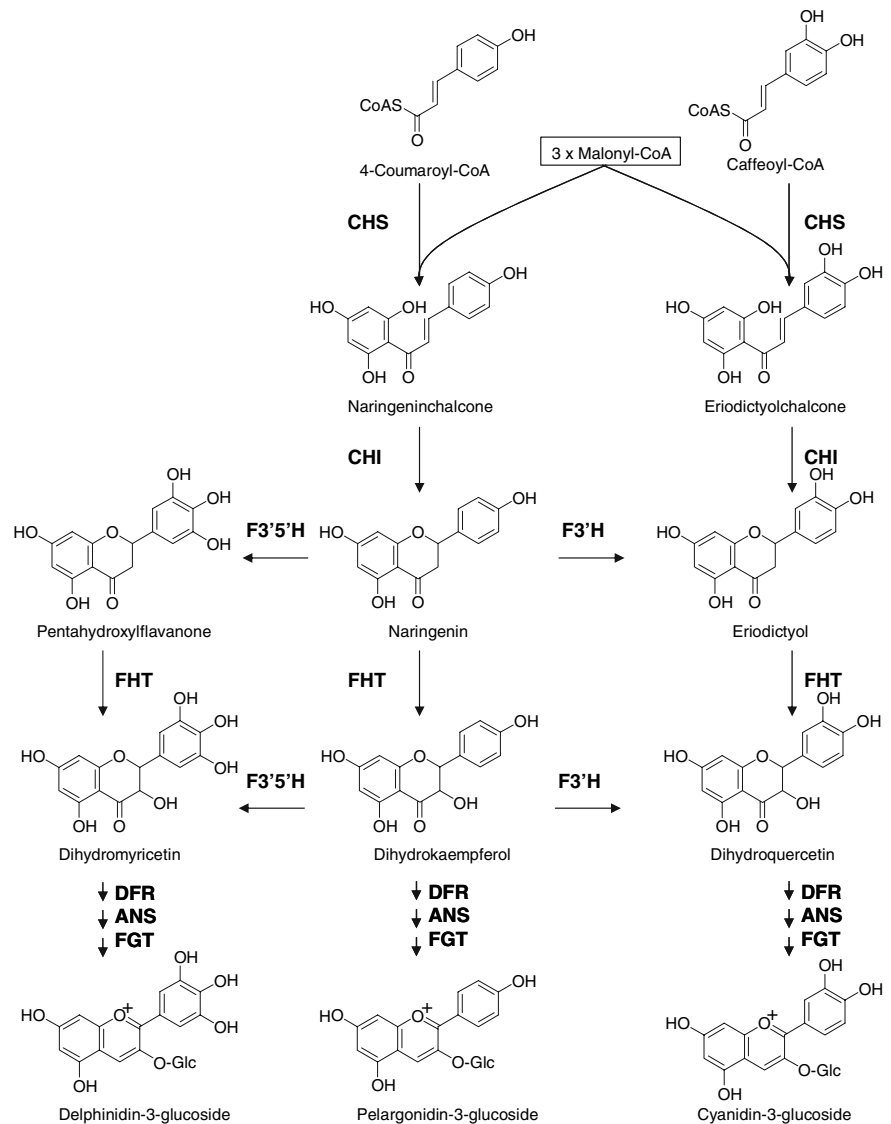
3′5′-hydroxylated anthocyanins (e.g., delphinidin). Overexpression of *F3′5′H* produced purple to violet transgenic flower colours due to the induction of the synthesis of delphinidin-derivatives (Fukui et al. 2003; reviewed in Tanaka et al. 2005) and a dramatic change of flower colour from pink to magenta with a high content of 3,5′-hydroxylated anthocyanins was observed in petunia (Shimada et al. 1999). However, transgenic torenia expressing *F3′H* cDNA exhibited redder flower colour due to the increase in 3′-hydroxylated anthocyanins (Ueyama et al. 2002).

Another useful strategy for introducing or increasing anthocyanin production in target ornamental species is the alteration of the balance between flavonoid enzyme activities by genetic modification. The dihydroflavonols are the direct substrates for colourless flavonol and coloured anthocyanin biosynthesis. Thus, the introduction of a *DFR* sense transgene in white-flowered lines of petunia, which accumulates flavonol, resulted in a pink-flowered phenotype. Furthermore, inhibition of flavonol biosynthesis by cosuppression of the flavonol synthase (*FLS*) also led to anthocyanin production and a pink-flowered

phenotype (Davies et al. 2003). In torenia the inactivation of the *DFR* gene by genetic transformation caused the accumulation of flavones, possible copigments, resulting in a copigmentation likely to make the torenia flowers bluer (Aida et al. 2000).

Advances are also being made in understanding the action mechanisms of regulators in anthocyanin biosynthesis. A general role for the basic-helix-loop-helix (bHLH) MYB, and WD40 repeat proteins as regulators of the anthocyanin pathway has been conserved in a wide variety of species (reviewed in Winkel-Shirley 2001). The overexpression of the *R* gene, encoding a bHLH, from maize enhanced the expression of anthocyanin in Arabidopsis, petunia, tobacco and *Caladium bicolor* (Lloyd et al. 1992; Quattrocchio et al. 1993; Li et al. 2005). Overexpression of *bHLH* from *Antirrhinum* and *Perilla frutescens* also increased pigmentation in the whole tomato plant and in the tobacco flower (Mooney et al. 1995; Gong et al. 1999). A recent study shows that mutation of *PH4*, which encodes a MYB domain protein, results in a bluer petunia flower colour, increased pH in petal extracts, and, in certain genetic backgrounds, the disappearance of anthocyanins and fading of the flower colour (Quattrocchio et al. 2006).

Transgenic approaches uncovered a strong relationship between structural and/or regulatory anthocyanin gene expression and plant defence response levels. It has been suggested that anthocyanin functions as photoprotective pigments (Li et al. 1993) and that its accumulation can reportedly be induced in many plants by biotic and abiotic stresses, such as UV-B radiation, pathogen infection or low temperature (Mancinelli 1983; Winkel-Shirley 2001; Treutter 2006). Anthocyanin accumulation appears to be a general response to cold stress (Christie et al. 1994). However, evidence suggests that anthocyanin itself is not essential for freezing tolerance (Leyva et al. 1995), although some responses essential to freezing tolerance may be governed by a regulatory pathway or biochemical level that is also involved in the anthocyanin response (McKown et al. 1996).

Different studies using transgenic plants showed the implication of anthocyanin pathway in plant response to pathogen attack. A transgenic line of *Arabidosis thaliana* constitutively expressing a conserved MYB transcription factor resulted in purple leaves showing increased anthocyanin accumulation and resistance to leaf feeding by insects (*Spodptera*

frugiperda) (Johnson and Dowd 2004). Transgenic maize plants carrying the *C2* (*coloured-2*, encoding chalcone synthase) gene under the control of the maize *Ubiquitin* promoter were generated and preliminary studies revealed an increase in resistance to the rice blast fungus *Magnaporthe grisea* (Gandikota et al. 2001).

The ectopic expression of anthocyanin 5-O-glucosyltransferase (*5-UGT*) cDNA in potato increased anthocyanin diglucoside levels and improved the resistance of transgenic lines to *Erwinia carotovora* subsp. Carotovora. An increase in starch as well as a decrease in sucrose levels were also detected in transgenic tubers (Lorenc-Kukuła et al. 2005). Likewise, simultaneous expression of genes encoding CHS, chalcone isomerase (CHI), and DFR in potato resulted in a significant increase in trisaccharide derivatives of pelargonidin and petunidin. However, transgenic lines showed a decrease in starch levels, accompanied by an increase in sucrose levels (Lukaszewicz et al. 2004), suggesting that the limitation of flavonoid synthesis might depend on the availability of carbohydrates for phenolic compounds synthesis.

Tomato fruits (*Lycopersicum esculentum*) are not usually reported to contain anthocyanin. However, tomato accession LA1996 with the *Anthocyanin fruit* (*Aft*) gene has elevated anthocyanin levels in the skin and outer pericarp tissues of the fruit produced by the glycosylated anthocyanidins of petunidin, malvidin, and delphinidin (Jones et al. 2003). These authors proposed that the introduction of the *Anthocyanin fruit* characteristic into carotenoid-rich tomatoes would provide the opportunity for developing new cultivars rich in water- and lipid-soluble antioxidants.

Effect of postharvest treatments, food processing and storage

Anthocyanins are unstable and especially sensitive to changes in pH, temperature or atmospheric composition. Another characteristic anthocyanin reaction is the ability to chelate metals such as Fe, Cu, Al, and Sn, present in the media or packaging, this is generally undesirable since it result in a change of pigment colour. The rate of anthocyanin destruction is pH dependent, being greater at higher pH values. This chemical instability characteristic of anthocyanins has

to be kept in mind when analysing the effect of postharvest and processing treatments in anthocyanin-containing foods.

The goal of postharvest storage technology is to manipulate fruit and vegetable metabolism during storage so as to extend product shelf life. Postharvest treatments such as low temperatures, high CO_2 concentrations, controlled atmosphere (CA) and modified atmosphere packaging (MAP) involve slowing down the velocity of many metabolic processes which leads to a natural deterioration and loss of quality. However, some of these treatments affect the anthocyanin content adversely, with negative consequences in fruit colour and nutritional value. It is already known that anthocyanin synthesis continues after harvest and also during long-term cold storage, but it is inhibited in fruits stored with high CO_2 concentrations. An increase in anthocyanin content by low temperature postharvest storage has been reported for different fruit, such as strawberries (Gil et al. 1997; Kalt et al. 1999), cranberries (Kalt et al. 1999), blueberries (Connor et al. 2002), cherries (Gonçalves et al. 2005), oranges (Lo Piero et al. 2005) and grapes (Sanchez-Ballesta et al. 2007). However, in the case of apple fruit the levels of anthocyanins do not appear to be stable throughout low temperature storage (Ju et al. 1996). In pomegranates, the increase in anthocyanin content was correlated with the activity of anthocyanin biosynthetic pathway enzymes: PAL and UDPglucose:flavonoid-3-O-glucosyltransferase (UFGT) (Holcroft et al. 1998). In red oranges stored at 4°C for up to 75 days, the expression of anthocyanin biosynthetic genes, including PAL, CHS, DFR, and UFGT was strongly induced, parallel to the accumulation of anthocyanin (Lo Piero et al. 2005).

Different studies showed that treatment of harvested fruit with CO_2 applied as CA or MAP inhibits the increase of anthocyanin by affecting its biosynthesis, degradation or both (Holcroft et al. 1998; Holcroft and Kader 1999; Artés-Hernández et al. 2003; Remon et al. 2004). In strawberry, a remarkable decrease in internal anthocyanin content was observed, particularly at 20 and 40% CO_2 but not in external tissues suggesting that most of the anthocyanin degradation takes place in the internal tissues (Gil et al. 1997). The colour loss of strawberry fruit has been related to the degradation of anthocyanin pigments by condensation with quinones, formed from endogenous phenolics due to the action of

polyphenol oxidase (PPO) (Wesche-Ebeling and Montgomery 1990). In red raspberry cultivars CA storage did not affected anthocyanin content (Haffner et al. 2002). Nevertheless, Veazie and Collins (2002) reported that total monomeric anthocyanin in Navaho blackberries held in CA storage at 2°C increased during the first 3 days, but decreased thereafter. Application of a 3 days high CO_2 pre-treatment at 0°C in table grapes increased the anthocyanin content when fruits were transferred to air at a lower temperature (Romero et al. 2007).

Heat treatments, such as air oven, vapour, water immersion, and hot water rinsing and bruising, can control insect pests, prevent pathogen infection, induce resistance to chilling injury, slow fruit ripening, extend postharvest shelf life in different fruit (reviewed in Lurie 1998) and also affect the anthocyanin content. Heat treatments at 42 and 48°C for 3 h prevented fungal development and reduced anthocyanin content and PAL activity in strawberries (Civello et al. 1997) and a residual effect of heat treatment on anthocyanin accumulation was still present after 7–14 days of refrigerated storage (Vicente et al. 2002). In contrast, prestorage heat treatments (hot water bath immersion) inhibited postharvest anthocyanin synthesis and retained the overall quality of white asparagus spears (Siomos et al. 2005). Some increases in total anthocyanin content were detected in cycles of intermittent warming applied to reduce chilling symptoms in pomegranate, due mainly to cyanidin and pelargonidin 3-glucoside (Artés et al. 2000).

Chitosan, a high molecular weight polymer coating had the potential to prolong storage life and control decay of many fruits (El Ghaouth et al. 1991; Romanazzi et al. 2003). Although anthocyanin levels decreased in chitosan treated and non-treated litchis during storage at low temperature, the decrease was lower in chitosan treated fruit (Zhang and Quantick 1997). Maintaining the skin colour of the litchi fruit by chitosan coating may account for the higher levels of anthocyanin content in the skin resulting from the inhibition of PPO activity (Jiang et al. 2005). El Ghaouth et al. (1991) have observed retention of firmness, higher titratable acidity and a reduced rate of anthocyanin production in chitosan coated strawberries.

Minimal processing of fruits and vegetables may affect anthocyanidins by affecting their chemical stability or by inducing or inhibiting their

biosynthesis. It has been shown that refrigerated storage of shredded red onion for up to 7 days produces a decrease in anthocyanin levels. This loss of pigments depends on the anthocyanin structure, acylated derivatives being more stable than the non-acylated glycosides (Ferreres et al. 1996).

Ngo et al. (2007) have studied the effect of processing and storage on strawberry anthocyanins. Freezing strawberries (−37°C followed by 1 month storage at −23°C) produced a slight increase in the anthocyanin content. This is in agreement with the results obtained by de Ancos et al. (2000) who also found an increase of total anthocyanins in raspberries after frozen storage at −24°C for one year. Canning of strawberries with sucrose syrup at 95°C and subsequent immersion of the cans in boiling water for 15 min. resulted in losses of around 70% of antho-cyanin content after 60 days storage at room temperature (Ngo et al. 2007). Processing strawberry jams under different conditions produced losses of 40–70% of the initial anthocyanin content (Garcia-Viguera et al. 1999; Ngo et al. 2007). In jams, storage temperature has proved to be crucial for anthocyanins stability. Storage of strawberry jams for up to 9 weeks at 38°C doubled anthocyanin losses when compared with those at 21°C for the same length of time.

In general, high hydrostatic pressure has a negative effect on anthocyanins, when compared with unpro-cessed fruit. However, it has a protective effect on anthocyanins during storage under refrigeration when fruit is processed at 400 MPa in the case of black-urrant and at 200 or 800 MPa for raspberries (Kouniaki et al. 2004; Suthanthangjai et al. 2005).

The effect of gamma irradiation on anthocyanin content in packed grape pomace has been studied by Ayed et al. (1999). Irradiation in the presence of SO_2 gave, in every case, better results when compared with air packaging. The highest anthocyanin content was obtained by irradiation at a dose of 6 kGy.

It is generally accepted that ascorbic acid has a negative effect on anthocyanin stability, and the same happens with Maillard reaction products and sugars (Wrolstad et al. 2005). Kaack and Austed (1998) have studied the effect of vitamin C on flavonoid degradation under conditions similar to those applied for Sambucus nigra juice processing. Under conditions where high concentrations of oxygen were present anthocyanin content decreased dramatically during juice processing and storage. However, when oxygen was eliminated from the atmosphere, by nitrogen purging, anthocyanins were protected against degradation by the presence of ascorbic acid in the media.

If there is a product in which anthocyanin modifi-cations have been studied in deep, this is wine. Wine making has a crucial effect on the final anthocyanin composition of red wine. The initial content on anthocyanins of the grape is also a pivotal aspect and may be modified by agricultural, environmental and genetic modifications. The use of different varieties of grapes is already being used in wine industry in order to enhance the quality of the wine by modifying the starting anthocyanin composition of the must (Mona-gas et al. 2006). However, during red wine aging, there is a loss of anthocyanins and other pigments are formed through various mechanisms (Mateus et al. 2002). This change in anthocyanin composition may cause quality changes. In many cases these new formed molecules have the property of stabilising the red wine colour. In others, the new molecules are pigments them-selves that will contribute to the changes in colour characteristics of red wine aging.

The social trend toward the consumption of natural products instead of synthetic ones has increased the interest in the development of colorants from natural sources in the food industry. Anthocyanins are among this group of natural colorants one of the groups with more applicability. The production of anthocyanin extracts may, however, modify the anthocyanin composition of the original anthocyanin-rich source. By instance, it as been shown that alcoholic extrac-tion at medium to high temperatures may accelerate the formation of new pigments by acylation of the original anthocyanin (de Pascual-Teresa et al. 2002). This modification in the structure of anthocyanins will have an influence in the stability of the natural colorant through out the food processing process and later during the storage time. Moreover structure modifications will also influence the bioavailability, metabolism and biological properties of the pigments.

Intake, bioavailability and metabolism

Until very recently the only available data on anthocyanin intake by humans was that of Kuhnau (1976). In the aforementioned study the intake of

anthocyanins in humans was estimated to be 180–215 mg/day in the United States. More recently Chun et al. (2007) have calculated the mean daily anthocyanidin intake based on 24-h dietary recalls and the USDA database for the "Flavonoid content of Selected Foods" (Agricultural Research Service 2003), and have arrived at a much lower figure of 3 mg/day. This is in accordance with the value obtained by Wu et al. (2006) also for the US population of 12 mg/day. However, since anthocyanins are restricted to berries, red fruits and wine it should be assumed that there are big variations between individuals due to differences in the intake of these anthocyanin-rich products. In Finland, where the consumption of berries is widely extended, the average intake of anthocyanins has been estimated to be 82 mg/day (Heinonen 2001). In any case, the intake of anthocyanins can be considered to be between a few milligrams and 100 mg.

As recently reviewed by Manach et al. (2005), existing literature in the field of anthocyanin bioavailability shows that this group of pigments is less well absorbed than other flavonoids. However it should be noted that until now most studies have focused on the analysis in plasma and urine of anthocyanin metabolites derived from glucuronidation and sulphation metabolism. It has been suggested that anthocyanins may be metabolised by intestinal microflora producing a set of new products that have not yet been totally identified, not to mention quantified. This fact, together with the difficulties inherent in anthocyanin analysis may explain the low bioavailability encountered in the different studies performed until now.

Anthocyanins are rapidly absorbed, with maximum plasma concentration (Cmax) of between 1.4 and 200 nM for doses of 10–720 mg of anthocyanins. The Cmax is reached from between 45 min to 4 h after ingestion of an anthocyanin containing meal, depending on the conditions of the trial. When the anthocyanins are ingested alone and after an overnight fast the Cmax is reached after only 1 h (Matsumoto et al. 2001; Cao et al. 2001; Nielsen et al. 2003). However, when anthocyanins are ingested together with other food items absorption is retarded by up to 1.5 h (Nielsen et al. 2003), and even 4 h in the case of meals with a very high fat content (Mazza et al. 2002). Plasma half-life for anthocyanins is from between 2 h, for cyanidin glucoside and sambubioside, to around 3.3 h for the rutinosides of cyanidin and delphinidin.

In urine the maximum concentration of anthocyanins is reached after 1.5–6 h of ingestion (Felgines et al. 2003, 2005; Frank et al. 2005). The percentage of anthocyanins encountered in urine represents between 0.004 and 0.2% of the quantity ingested. Only in the case of two published studies is this percentage of anthocyanins in urine substantially higher, reaching 5% in the study by Lapidot et al. (1998) and around 2% in that of Felgines et al. (2003).

The nature and position of the glycosidic groups in the molecule of anthocyanins may influence their absorption. It has been shown that the 3-monoglucosides of anthocyanidins are less bioavailable than their corresponding rutinosides (McGhie et al. 2003; Nielsen et al. 2003; Matsumoto et al. 2001).

The structure of the anthocyanidin may also have an influence on anthocyanin absorption. Nielsen et al. (2003) did not find any difference in the bioavailability of delphinidin and cyanidin. However, McGhie et al. (2003) did find differences between the galactosides of malvidin and petunidin and that of delphinidin in terms of absorption, suggesting that an increase in the number of hydroxyl groups in the molecule decreases its bioavailability. Felgines et al. (2003, 2005) have shown that the relative quantity of anthocyanins recuperated in urine after 24 h is 10 times higher for glycosides of pelargonidin than for those of cyanidin. However it should be noted that there were substantial differences in the quantity of anthocyanidins ingested in both studies, cyanidin ingestion being almost six times that of pelargonidin. In line with this, Kurilich et al. (2005) have postulated the existence of a saturation effect in the absorption mechanisms of anthocyanins.

Acylation of the anthocyanin molecule produces a decrease in its absorption (Kurilich et al. 2005; Mazza et al. 2002). The ingestion of anthocyanins with alcohol accelerates their absorption, while sugar has the opposite effect (Bub et al. 2001; Bitsch et al. 2004; Mülleder et al. 2002).

Anthocyanins have been described in human plasma not only as the original glycosides, but also as glucuronidated and methylated metabolites. And only in one study has the presence of the aglycon cyanidin been reported in plasma (Cao et al. 2001).

The presence of the parent compounds, the glycosides, together with methylated and glucuronidated metabolites have been reported in human urine

(Wu et al. 2002). Kay et al. (2004) showed that after ingestion of a high dose of cyanidin glucosides, the original anthocyanins represented 75% of the total anthocyanins excreted in the urine, methylated metabolites 10% and the remaining 15% was eliminated as glucuronidates. However, Felgines et al. (2005) obtained totally different results after ingestion of cyanidin glycosides. In this study monoglucuronides represented 64% of the total anthocyanins excreted in urine, 19% as glycosides, 10% as aglycons and 1.2% as diglucuronides.

As has been mentioned before, there is a second via of metabolism for anthocyanins involving the action of intestinal microflora. In vitro studies have shown that anthocyanin monoglucosides (3 glucosides of cyanidin, malvidin and peonidin) and diglucosides (3,5-diglucosides of cyanidin and malvidin, and cyanidin rutinoside) are de-glycosilated by the action of colonic microflora in a 20 min to 2 h period, depending on their structure (Aura et al. 2005; Keppler and Humpf 2001). The aglycons are further degradated and their degradation products, mainly phenolic acids, appear after 20 min of incubation with intestinal microflora. Due to the high structural similarity of the anthocyanidin ring-A between the different anthocyanidins, their degradation produces the phenolic acid corresponding to the anthocyanidin ring-B together with the aldehyde phloroglucinol (Fleschhut et al. 2006).

Anthocyanin distribution in animal tissues has also been described. The presence of pelargonidin and some of its metabolites have been described in stomach, kidney, liver, brain and lung of rats after 2 h of ingestion (El Mohsen et al. 2006). Furthermore, Matsumoto et al. (2006) have reported the presence of intact anthocyanins in several ocular tissues after administration of a blackcurrant extract to rats or rabits.

Biological activity

Many beneficial activities have been attributed to anthocyanins and the majority of work investigating these activities focuses on the antioxidant characteristics of anthocyanin rich diets and the enhancement of cardiovascular health. Most of them are in vitro studies and a few of them were carried out in vivo, in general by measuring total antioxidant capacity in plasma after consumption of a source rich in anthocyanins.

Anthocyanins are able to prevent oxidative damage to DNA, proteins, lipids and other macromolecules caused by reactive oxygen species (ROS). It has been postulated that anthocyanins act on two levels. They have a systemic action, since they are absorbed and circulate in the blood, and it is in this circulating form that they act upon different target tissues in the human body. Anthocyanins may also act locally, in the gut, if they are not absorbed by intestinal mucosa. Finally, they may also act as topical agents, for example by protecting the skin from UV radiation.

In the case of the gut, it is not yet clear if these local effects are caused by the original anthocyanin or by the metabolites formed by the colonic microflora. The formation of these degradation products may be involved in the protective action of anthocyanins against colon and rectum cancer.

Even if anthocyanins are excreted mainly in their intact form, i.e., as glycosides; it has been shown that once absorbed, anthocyanins are metabolised generating sulphates, glucuronides and methylated derivatives. Up to now, the biological effects of these anthocyanin metabolites in humans are largely unknown. Moreover, it has been reported that the gut microflora plays an important role in the biotransformation of anthocyanins.

Free radical damage contributes to the aetiology of many chronic diseases and thus antioxidants may have beneficial effects on human health at different levels. Anthocyanins have shown to be potent antioxidant and free radical scavengers in different in vitro models. Within the assayed anthocyanins the 3 glucoside of delphinidin, which is the only anthocyanin containing the 3′, 4′ and 5′-hydroxy group (gallocatechol structure) in the B-ring, has shown the highest antioxidant capacity (Garcia-Alonso et al. 2005).

The biological activity of isolated anthocyanins and anthocyanidins, or of anthocyanin rich foods can be manifested at different levels:

Prevention of cardiovascular disease: This is possibly the most studied effect and the one for which a great quantity of epidemiological evidence exists. Anthocyanins are capable of acting on different cells involved in the development of atherosclerosis, one of the leading causes to cardiovascular dysfunction. The chemokine monocyte chemotactic protein 1 (MCP-1) is known to mediate in the recruitment of macrophages to sites of infection or inflammation, and direct involvement of MCP-1 on atherogenesis has been established. Anthocyanins have been shown to have a

protective effect against TNF-α induced MCP-1 secretion in primary human endothelial cells (Garcia-Alonso et al. 2004). Vascular endothelial growth factor (VEGF) is a major pro-angiogenic and pro-atherosclerotic factor but anthocyanins have been shown to prevent the expression of VEGF in vascular smooth muscle cells (Oak et al. 2006). In macrophages, blackberry anthocyanins inhibit LPS induced nitric oxide biosynthesis (Pergola et al. 2006). Anthocyanins also inhibit TRAP-induced platelet aggregation but do not influence platelet reactivity when faced with strong agonists such as collagen and ADP (Rechner and Kroner 2005). Anthocyanin extracts from chokeberry, bilberry, and elderberry have shown endothelium-dependent relaxation capacity in porcine coronary arteries (Bell and Gochenaur 2006). Moreover, anthocyanins have an effect on cholesterol distribution, protecting endothelial cells from CD40-induced proinflammatory signalling (Xia et al. 2007).

Anticancer, antitumor and antimutagenic activity: It has been suggested that anthocyanins possess antiinflammatory as well as chemopreventive properties. Distinct cell growth and cell cycle related pathways have already been identified as targets for anthocyanins in in vitro and laboratory animal studies. It has been shown that the anthocyanin-containing fraction of red wines is capable of suppressing proliferation of HCT-15 human colon cancer cells and gastric adenocarcinoma (AGS) cells (Kamei et al. 1998; Shih et al. 2005). Administering purple corn, with a high anthocyanin content, to rats decreases the incidence and multiplicity of colorectal adenomas and carcinomas induced by 1,2-dimethylhydrazine (DMH) and 2-amino-1-methyl-6-phenylimidazo(4,5-β)pyridine (PhIP) (Hagiwara et al. 2001). Liu et al. (2002) have shown that the proliferation of human hepatocellular liver carcinoma cells (HepG2) is inhibited by raspberry extracts, containing among other flavonoids anthocyanins. Marko et al. (2004) have reported an inhibitory effect of the anthocyanidins, delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin on cell proliferation in human vulva carcinoma cells (A431) and human colon carcinoma cells (HT29). Moreover the authors showed that depending on the substitution pattern of their B-ring, anthocyanidins may interfere with different signalling cascades involved in the regulation of cell growth. It has been shown that cyanidin-3-glucoside, as well

as different anthocyanin-rich extracts induce apoptosis in different human leukaemia cell lines (Fimognari et al. 2004). This same effect has been reported in liver hepatoma cells treated with (Yeh and Yen 2005).

Angiogenesis plays a critical role in the development of cancer. Liu et al. (2005) have found that anthocyanins from black raspberry can reduce tumour development by inhibiting angiogenesis.

Mutagenicity is closely related to carcinogenicity. Yoshimoto et al. (1999) have observed that mutagenicity of certain carcinogenic compounds, such as heterocyclic amines, in *Salmonella typhimurium* TA 98, is decreased by aqueous extracts of the roots of different varieties of coloured sweetpotatoes (*Ipomoea batatas*). Juices from anthocyanin rich fruits such as strawberry, blueberry, and raspberry significantly inhibited mutagenesis caused by the direct-acting mutagen methyl methanesulfonate and the metabolically activated carcinogen benzo(a)pyrene (Hope Smith et al. 2004).

Sarma and Sharma (1999) have observed that co-pigmentation between cyanidin derivatives and DNA had a protective effect on both molecules against the action of hydroxyl radicals generated by the Fenton reaction. Russo et al. (2005) have shown that the treatment of human fibroblasts with cyanidin-3-glucoside prevents ochratoxin A-induced DNA damage.

Beneficial effects in diabetes: Basically anthocyanins may have a beneficial effect on diabetes at two levels. On one hand they can interfere with glucose absorption and on the other hand they may have a protective effect on pancreatic cells. It has been shown that the simultaneous and daily administration to rats of anthocyanins from red wine together with streptozotocin, a diabetogenic compound with beta-cytotoxic effects, decreases glucose concentration in urine and serum. Additionally, anthocyanins from red wine prevent the generation of free oxygen radicals, and decreases lipid peroxidation (reviewed by Kong et al. 2003). Jayaprakasam et al. (2005) have shown that anthocyanins are also able to stimulate insulin secretion from rodent pancreatic beta-cells. Of all the anthocyanins the 3 monoglucosides of cyanidin and delphinidin are the most potent stimulators and in anthocyanidins, pelargonidin.

Protective effect against hepatic damage: Anthocyanins from *Hibiscus sabdariffa L.* have shown to

protect rat liver from hepatotoxicity induced by tert-butyl hydroperoxide (t-BHP) by lowering the serum levels of alanine and aspartate aminotransferase and reducing oxidative liver damage (Wang et al. 2000). In rats in which hepatic injury was induced by the administration of D-galactosamine red potato extracts had a protective effect (Han et al. 2006).

Gastric effects: The protective effect of anthocyanins on gastric damage is closely related with the capacity of this group of flavonoids to prevent or ameliorate the inflammatory process. Inflammation is implicated, at least initially, in processes of gastric injury and in this regard, it has been shown that cyanidin protects gastric mucosa from the damage caused by aspirin (Galvano et al. 2004).

Ocular effects: Consumption of black currant-based extracts or concentrates of anthocyanins has a positive effect on night vision, i.e., dark adaptation (Nakaishi et al. 2000), though the positive effect on human vision of *Vaccinium myrtillus* (bilberry) extracts is controversial and still needs to be clarified (reviewed by Canter and Ernst 2004). However, recent studies in animal models suggest that long-term supplementation with bilberry extracts is effective in the prevention of macular degeneration and cataract (Fursova et al. 2005).

In subjects with myopia, it has been shown that the administration of anthocyanins for 4 weeks improved subjective symptoms and objective contrast sensitivity (Lee et al. 2005).

Antimicrobial and antiviral activity: Even though many studies have shown that isolated anthocyanins or plant extracts rich in this group of flavonoids have an effect on microbial growth, results are not yet conclusive. Puupponen-Pimia et al. (2001) showed that extracts from different berries had an inhibitory effect on gram-negative bacterial growth, but had no effect on gram-negative bacteria. Moreover, they showed that blackcurrant extracts stimulated the growth of different strains of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* but inhibited those of *Escherichia coli*. Recent studies have demonstrated that blackcurrant concentrates inhibit the growth of different strains of *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecium* but stimulate the growth of *Saccharomyces cerevisiae* (Werlein et al. 2005).

The effect of anthocyanins on microbial pathogens has not been studied in depth up to now. Some few studies exist, however, on the effect of berry extracts on pathogens. Among the berries and bacteria assayed by Puupponen-Pimia et al. (2005), cloud-berry and raspberry were the best inhibitors, and *Staphylococcus* and *Salmonella* the most sensitive bacteria. In another study by Nohynek et al. (2006), they showed that of all the pathogens assayed the most sensitive bacteria to berry phenolics were *Helicobacter pylori* and *Bacillus cereus*.

Different cyanidin glycosides present in extracts of *Ribes nigrum L.* have shown antiviral properties towards influenza A and B viruses and herpes-1 virus (Knox et al. 2003; Suzutani et al. 2003).

Effects on neurodegenerative processes: Joseph et al. (1999) have shown that anthocyanin-rich fruits may be beneficial in reversing the course of neuronal and behavioural aging. Moreover, by using transgenic mice as a model for Alzheimer's disease Joseph et al. (2003) have reported on the beneficial effect of blueberry extracts on the outcome of this neurodegenerative illness.

Conclusions

As reviewed, a lot of work has been done in the field of anthocyanins up to now. Much of it has been on their analysis, which, as it has been briefly outlined presents many difficulties due to the lack of stability of anthocyanins. These difficulties in analysis may be the cause of many of the limitations in intake, bioavailabilty and metabolism analysis. Studies on absorption and distribution are still needed; especially those targeted to study the effect of intestinal microflora on anthocyanin structure and to identify gut flora metabolites. The effect of long term exposure to anthocyanins is still largely unknown. The existing literature on biological activities gives enough evidences to think that anthocyanin rich products, such as berries or red wine, may have a protective effect on human health, especially by preventing cardiovascular diseases and some types of cancer. However more studies are needed in order to establish the real implications of anthocyanins in these health promoting properties, since most studies have been done by using fruit extract or wine and thus other substances may be totally or partially responsible for the mentioned biological activities.

Biotechnology seems a promising tool in order to increase anthocyanin levels in starting products. A lot

has been done in flowers and other non-edible plant tissues, however very few studies have been aimed to study the effect of genetic engineering on anthocyanin composition in plant foods. The selective activation of those metabolic pathways conducting to the synthesis of more stable or bioactive final products will open a promising perspective for the design of functional foods or new foods with health related claims.

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