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Original article

Betalains: properties, sources, applications, and stability – a review

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Summary Consumers are increasingly avoiding foods containing synthetic colourants, which lead food industries to replace them by natural pigments, such as carotenoids, betalains, anthocyanins and carminic acid. Betalains are water-soluble nitrogen-containing pigments, composed of two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. This review synthesises the published literature on basic chemistry of betalains, their sources and chemical stability. Moreover, several works are mentioned which have demonstrated the potent antioxidant activity of betalains, which has been associated with protection against degenerative diseases.

Keywords Antioxidants, beetroots, betalains, natural colourants, pigments.

Introduction

Colour is one of the most important attributes of foods, being considered as a quality indicator and determining frequently their acceptance. Many naturally coloured foods, such as fruit products, are submitted to colour losses during processing, requiring the use of colourants to restore their colour. Natural colourants have many disadvantages when compared to synthetic ones, including higher cost in-use and lower stability. However, people have increasingly avoided synthetic colourants, preferring natural pigments, which are considered to be harmless or even healthy. These requirements compelled numerous regulation changes worldwide. For instance, the USA permitted list of synthetic colourants was reduced from 700 to only seven until the beginning of the XXI Century (Downham & Collins, 2000). The current market for all food colourants is estimated at US$ 1 billion, with natural pigments responding for only one fourth of the total. However, the market for synthetic colourants has tended to decline in favour of natural ones (Fletcher, 2006).

Nature produces a variety of compounds adequate for food colouring, such as the water-soluble anthocyanins, betalains, and carminic acid, as well as the oil soluble carotenoids and chlorophylls. The health-benefit properties of natural pigments have been focussed by many works, especially those of carotenoids and anthocyanins, whose antioxidant properties have been extensively studied. Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments. These findings have helped to motivate utilisation of betalains as food colourants.

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Betalains as colourants

Betalains are water-soluble nitrogen-containing pigments, which are synthesised from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. Betalamic acid, whose structure is presented in Fig. 1a, is the chromophore common to all betalain pigments (Strack *et al*., 2003). The nature of the betalamic acid addition residue determines the pigment classification as betacyanin or betaxanthin (Fig. 1b and c, respectively).

The structural differences reflect in varying appearance of the betalain subgroups. Betacyanins contain a cyclo-3,4-dihydroxyphenylalanine (cyclo-Dopa) residue. The condensation with the closed structure of cyclo-Dopa extends the electronic resonance to the diphenolic aromatic ring. This extra conjugation shifts the absorption maximum from 480 nm (yellow, betaxanthins) to about 540 nm (violet, betacyanins) (Jackman & Smith, 1996; Strack *et al*., 2003).

Betanidin is the aglycone of most betacyanins; different substitution (glycosylation and acylation) patterns of one or both hydroxyl groups located at position 5 or 6 of betanidin result in the formation of the various betacyanins. Most of these are 5-*O*-glucosides, but 6-*O*-glucosides have also been detected. No betacyanin is

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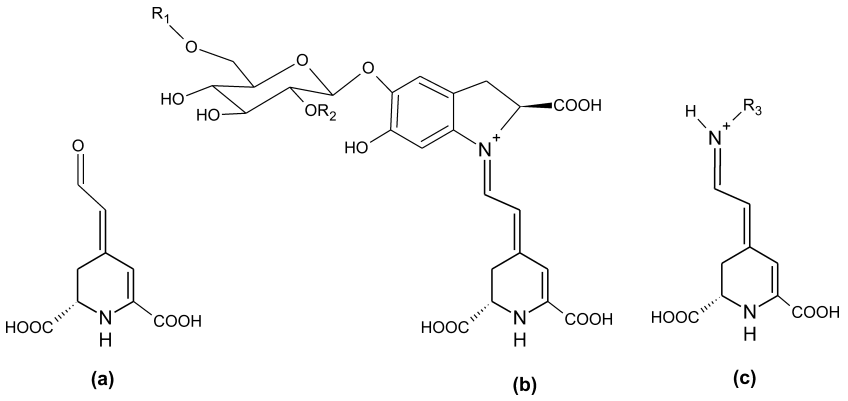

Figure 1 General structures of betalamic acid (a), betacyanins (b) and betaxanthins (c). Betanin: $R_1 = R_2 = H$. R_3 = amine or amino acid group (Strack *et al*., 2003).

known to have both positions substituted with sugar residues (Delgado-Vargas *et al*., 2000). Further glycosylation of the 5-*O*-glucoside is very common and so is esterification with hydroxycinnamic acids (Strack *et al*., 2003). The most common betacyanin is betanidin-5-*O*-β-glycoside (betanin), the major pigment in red beets (Stintzing & Carle, 2004). Betacyanins display two absorption maxima – one in the UV-range (270–280 nm) because of cyclo-Dopa and a second one in the visible range (535–540 nm, depending on the solvent). The red and violet colours result from different substitution patterns of betacyanins. Glycosylation of betanidin generally comes along with a hypsochromic shift of about 6 nm, while a second sugar moiety attached to the first one apparently did not greatly affect the colour (Cai *et al*., 1998; Stintzing & Carle, 2004). Acylation with hydroxycinnamic acids results in a third maximum (300–330 nm), whereas aliphatic acyl moieties do not alter the spectrum (Stintzing & Carle, 2004).

Betaxanthins, on the other hand, contain different amino acid or amine side chains (Strack *et al*., 2003). Structural modifications in betaxanthins produce hypso- or bathochromic shifts. Amine conjugates display a lower absorption maximum than their respective amino acid counterparts (Stintzing *et al*., 2002b).

Betalains have several applications in foods, such as desserts, confectioneries, dry mixes, dairy and meat products. The concentration of pure pigment required to obtain the desired hue is relatively small, rarely exceeding 50 $mg^{-1}kg$, calculated as betanin (Delgado-Vargas *et al*., 2000). According to the Codex Alimentarius Commission (2004), betalain use is limited only by Good Manufacturing Practice. The food colourant known as 'beetroot red', extracted from beetroots, is commercialised in European Union and USA as food colourant (Castellar *et al*., 2006). Commercial beet colourants are available as either juice concentrates (produced by vacuum-concentration of beet juice to 60–65% total solids) or powders (produced by freeze- or spray-drying), containing from 0.3% to 1% of pigment (Cerezal *et al*., 1994; Cerezal & Núñez, 1996).

Cai & Corke (1999) compared *Amaranthus* betacyanins and commercial colorants in terms of their colour characteristics and stability at different temperatures in model food systems. Betacyanins exhibited brighter red colour than the red radish anthocyanin. Both pigments showed similar colour stability at 14 °C and 25 °C, but betacyanin colour was less stable than red radish anthocyanin at 37 °C. A synthetic colourant was also tested, and was more stable than betacyanins under most storage conditions.

Plant sources of betalains

Anthocyanins and betalains have never been reported in the same plant, seeming to be mutually exclusive in the plant kingdom (Stafford, 1994). Betalains can be found in roots, fruits and flowers (Strack *et al*., 2003). They absorb visible radiation over the range of 476–600 nm with a maximum at 537 nm at pH 5.0. The few edible known sources of betalains are red and yellow beetroot (*Beta vulgaris* L. ssp. *vulgaris*), coloured Swiss chard (*Beta vulgaris* L. ssp. *cicla*), grain or leafy amaranth (*Amaranthus* sp.) and cactus fruits, such as those of *Opuntia* and *Hylocereus* genera (Cai *et al*., 1998; Stintzing *et al*., 2002b; Kugler *et al*., 2004; Vaillant *et al*., 2005).

The major commercially exploited betalain crop is red beetroot (*Beta vulgaris*), which contains two major soluble pigments, betanin (red) and vulgaxanthine I (yellow). According to Nilsson (1970), the betacyanin and betaxanthin contents of red beetroots vary within the ranges 0.04–0.21% and 0.02–0.14%, respectively, depending on the cultivar (Von Elbe, 1975), although some new varieties produce higher betalain contents (Pszczola, 1998; Gaertner & Goldman, 2005).

Gasztonyi *et al*. (2001) analysed five red beet varieties ('Bonel', 'Nero', 'Favorit', 'Rubin' and 'Detroit') in terms of their pigment composition. In all cases, the major red-violet pigments were betanin, isobetanin, betanidin and isobetanidin, and the major yellow components were vulgaxanthin I and vulgaxanthin II.

'Bonel', 'Favorit' and 'Rubin' exhibited the highest betacyanin contents (around 0.08%), while the 'Nero' variety showed the poorer betacyanin content (near 0.06%). The variety 'Rubin' showed the highest betacyanin/betaxanthin ratio (2.08), so was considered as the most suitable for food colourant production.

Since the betalain spectrum of red beets is restricted mainly to betanin, colour variability is poor. Moreover, an adverse earthy-like flavour because of geosmin and some pyrazines is undesirable when applying beet extracts to, for instance, dairy products (Lu *et al*., 2003; Stintzing & Carle, 2004). Therefore, attempts have been made to explore alternative sources of betalains. The most promising family among betalain-bearing plants is the Cactaceae. Among those, cactus pears (genus *Opuntia*) and pitayas (genera *Cereus*, *Hylocereus* and *Selenicereus*) are most commonly cultivated as fruit crops and best suited to be studied as betalain sources for colouring food (Mizrahi *et al*., 1997; Stintzing *et al*., 2003; Stintzing & Carle, 2006). Cactus fruits, in contrast to red beetroot, may be used in food without negative flavour impacts as those derived from beetroot extracts. On the contrary, the faint flavour tends to impair the market potential for plain cactus fruits, making their utilisation for colouring applications more promising. The betalains in cactus fruits also cover a broader colour spectrum from yellow–orange (*Opuntia* sp.) to red–violet (*Hylocereus* sp.) compared to red beet and thus may open new windows of colour diversification. The yellow-orange cactus fruits are of particular interest, because of the scarceness of yellow water-soluble pigments (Moβhammer *et al*., 2005). Moreover, the low levels of colourless phenolic compounds in cactus fruits make them very promising, since potential interactions of betalains with these phenolics are avoided (Stintzing *et al*., 2001). An additional advantage of cactus fruits are their minimal soil and water requirements, being regarded as alternative cultures for the agricultural economy of arid and semi-arid regions (Castellar *et al*., 2006). Fruit pulps of *Hylocereus* cacti contain high concentrations of betacyanins (0.23–0.39%), both non-acylated and acylated, and (in contrast with beetroots) contain no detectable betaxanthins (Wybraniec *et al*., 2001; Stintzing *et al*., 2002a; Vaillant *et al*., 2005). On the other hand, *Opuntia* fruits cover a broad colouring range, from bright yellow to red-violet, depending both on the betacyanin/betaxathin ratio and their absolute concentrations (Moβhammer *et al*., 2005; Stintzing *et al*., 2005). Stintzing *et al*. (2005) reported broad ranges of betacyanin (0.001–0.059%) and betaxanthin (0.003–0.055%) contents in different *Opuntia* clones. The betaxanthin/betacyanin ratios vary widely in cactus pears (Butera *et al*., 2002; Stintzing *et al*., 2003), but fruits containing exclusively betaxanthins are not known (Stintzing & Carle, 2006).

Castellar *et al*. (2003) analysed the betalains of three *Opuntia* species. Both betacyanins and betaxanthins were identified in *Opuntia undulata* and *O. ficus-indica*, while in *O. stricta* only betacyanins were detected. Among the three species, *O. stricta* seemed to be the most promising one, with the highest betacyanin content (0.08%). Moreover, *O. stricta* has thinner peel and less seeds than the other species, making pigment extraction easier.

Biotechnological production of betalains

Some authors have investigated beet cell cultures for producing betalains (Leathers *et al*., 1992; Akita *et al*., 2000). With this technology, it would be easier to control quality and availability of pigments independently of environmental changes (Dörnenburg & Knorr, 1997). However, these cultures are unable to compete with the beetroot, which is an abundant and inexpensive crop which may produce up to 0.5 g of betanin per kg of roots (Gasztonyi *et al*., 2001). The low productivity of the current bioreactor systems available and the high cost of the process impair its economic feasibility. Then, the selection of a bioreactor and cultivation techniques for optimal culture growth and betalain production is one of the most important issues to be solved (Jimenez-Aparicio and Gutierrez-Lopez, 1999).

Betalain extraction

Betalain-containing materials are generally macerated or ground. Pigments can be water-extracted, although, in most cases, the use of methanol or ethanol solutions (20–50%) is required to complete extraction (Delgado-Vargas *et al*., 2000). Nevertheless, Castellar *et al*. (2006) reported that water extracted higher levels of pigments from *Opuntia* fruits than ethanol:water. García Barrera *et al*. (1998) reported higher betalain extraction with ethanol–HCl (v/v ratio, 99:1) than that with water. However, the aqueous extraction promoted better stability of the pigments. Slight acidification of the extraction medium enhances betacyanin stability and avoids oxidation by polyphenoloxidases (Schliemann *et al*., 1999; Strack *et al*., 2003).

A previous enzyme inactivation by a short heat treatment of the extract is desirable, to avoid betalain enzymatic degradation (Delgado-Vargas *et al*., 2000). Enzymatic treatments for degradation of hydrocolloids may also favour the pigment extraction (Moβhammer *et al*., 2005). Fermentation of extracts may reduce free sugars, increasing the betacyanin content (Pourrat *et al*., 1988).

The degree of cell membrane permeabilisation is a major factor to determine the extraction efficiency. Pulsed electric field treatments increase cell permeability (Rastogi *et al*., 1999; Ade-Omowaye *et al*., 2001),

enhancing betalain extraction efficiency (Chalermchat *et al*., 2004; Fincan *et al*., 2004), with relatively low levels of tissue damage and low energy consumption (Fincan *et al*., 2004). Nayak *et al*. (2006), using gamma-irradiation as a pre-treatment to a solid–liquid extraction of betanin from red beets, observed that the extraction efficiency increased with the irradiation doses (0–10 kGy); this effect was attributed to the cell permeabilisation. On the other hand, irradiation also increased betanin degradation rates.

On a laboratory scale, betalains may be extracted by various methods, such as diffusion-extraction (Wiley & Lee, 1978), solid–liquid extraction (Lee & Wiley, 1981), reverse osmosis (Lee *et al*., 1982) and ultrafiltration (Bayindirli *et al*., 1988; Real & Cerezal, 1995). These processes are more efficient on recovering betalains from beet tissue when compared to conventional hydraulic techniques (Real & Cerezal, 1995). Since approximately 80% of beet juice solids consist of fermentable carbohydrates and nitrogenous compounds, fermentation processes have been applied to remove these materials (Drdák *et al*., 1992), thus increasing betalain concentrations.

Betalain analysis

Betalain analysis has been carried out basically on UV-visible spectroscopy. Betacyanins absorb around $\lambda_{máx}$ = 540 nm, and betaxanthins, at $\lambda_{máx}$ = 480 nm. The first studies of betalain identification were based on this methodology. Structural modifications of betalains have also been followed by UV-visible spectroscopy (Piattelli, 1981). Nowadays, chemical characterisation must be carried out considering at least HPLC separation and UV-visible, mass spectrometry, and NMR spectroscopy, for identification of individual compounds (Strack *et al*., 1993; Stintzing *et al*., 2004).

Stintzing *et al*. (2004) developed a solvent system which improved data acquisition at almost neutral pH. The authors were successful in submitting four non-carboxylated betacyanins (betanin, isobetanin, phyllocactin and hylocerenin) to ^{1}H and ^{13}C NMR characterisation. Later, Wybraniec *et al*. (2006) elucidated structures of mono- and di-decarboxylated betacyanins by using both techniques. Until then, a highly acidic media was considered as necessary for an adequate data acquisition during NMR measuring. Since betalains are unstable under such conditions, betalain structure elucidation by NMR spectroscopy was scarce and limited to ^{1}H NMR (Wybraniec *et al*., 2001; Stintzing *et al*., 2002a). The only ^{13}C NMR betacyanin spectrum then available (Alard *et al*., 1985) was that of neobetanin (14,15-dehydrobetanin), thanks to its higher stability to acidic conditions when compared to other betacyanins. Betaxanthin structure elucidation was also exclusively based on ^{1}H NMR data (Piattelli *et al*., 1964, 1965; Wyler & Dreiding, 1984; Hilpert *et al*., 1985; Strack *et al*., 1987; Trezzini & Zr, 1991), until Stintzing *et al*. (2006), applying only slightly acidic conditions, were successful in reporting the first ^{13}C NMR data of two betaxanthins (indicaxanthin and miraxanthin).

Factors affecting chemical stability of betalains

Betalain degradation may occur by different mechanisms, which were detailed by Herbach *et al*. (2006b). Several factors, both intrinsic and extrinsic, affect betalain stability, and need to be considered to ensure optimum pigment and colour retention in foods containing betalains.

Structure and composition

Concerning structural aspects, betacyanins have been reported to be more stable than betaxanthins, both at room temperature (Sapers & Hornstein, 1979) and upon heating (Singer & von Elbe, 1980; Herbach *et al*., 2004a). Comparing stability of different betacyanins, glycosylated structures are more stable than aglycons, probably because of the higher oxidation–reduction potentials of the former (von Elbe & Attoe, 1985). However, stability does not seem to be enhanced by further glycosylation (Huang & von Elbe, 1986). Some studies have indicated increasing betacyanin stability resulting from esterification with aliphatic acids (Reynoso *et al*., 1997; García Barrera *et al*., 1998), as well with aromatic acids, especially at the 6-O position (Heuer *et al*., 1994; Schliemann & Strack, 1998). However, Herbach *et al*. (2006c) found interesting results when monitoring thermal degradation of betanin and acylated betacyanins (phyllocactin and hylocerenin) by spectrophotometric and high-performance liquid chromatography-diode array detection (HPLC-DAD) analyses. They observed that betanin were more stable than the acylated structures, but the tinctorial stability of phyllocactin and especially hylocerenin solutions was favoured by the formation of red degradation products with high colour retention. Hence, spectrophotometric analyses were reported to be insufficient to assess structure-related stability characteristics of betacyanins.

Red beets have several endogenous enzymes such as β-glucosidases, polyphenoloxidases and peroxidases, which if not properly inactivated by blanching may account for betalain degradation and colour losses (Lee & Smith, 1979; Martínez-Parra & Muñoz, 2001; Escribano *et al*., 2002). The optimum pH for enzymatic degradation of both betacyanins and betaxanthins was reported to be around 3.4 (Shih & Wiley, 1981). The degradation products are similar to those of thermal, acid or alkaline degradation (Martínez-Parra & Muñoz, 2001; Escribano *et al*., 2002; Stintzing & Carle, 2004). Betacyanins are more susceptible than betaxanthins to

degradation by peroxidases, while the latter are more oxidised by hydrogen peroxide, since the presence of catalase almost thoroughly suppressed betaxanthin oxidation (Wasserman *et al*., 1984). Some attempts have been made to take advantage of endogenous β-glucosidase activity to extend available shades offered by red beets. The transformation of betanin glycosides into their respective aglycones produces a bathochromic shift of 4–6 nm. However, these aglycones are more labile and prone to further oxidation which results in red colour losses and subsequent browning (Stintzing & Carle, 2004).

pH

Although altering their charge upon pH changes, betalains are not as susceptible to hydrolytic cleavage as the anthocyanins. Betalains is relatively stable over the broad pH range from 3 to 7 (Jackman & Smith, 1996), which allows their application to low acidity foods. Below pH 3.5, the absorption maximum shifts toward lower wavelengths, and above pH 7 the change is toward upper ones; out of the pH range 3.5–7.0 the intensity of the visible spectra decreases. Optimal pH range for maximum betanin stability is 5–6 (Huang & von Elbe, 1985, 1987; Castellar *et al*., 2003; Vaillant *et al*., 2005). Alkaline conditions cause aldimine bond hydrolysis, while acidification induces recondensation of betalamic acid with the amine group of the addition residue (Schwartz & von Elbe, 1983). At low pH values, C_{15} isomerisation (Wyler & Dreiding, 1984) and dehydrogenation (Mabry *et al*., 1967) were observed. Von Elbe *et al*. (1974) observed that, under fluorescent light, betanin degradation rate was three-fold higher at pH 3 than at pH 5. However, according to Herbach *et al*. (2006b), any of the degradation mechanisms elucidated so far are able to explain the shift in betanin maximum absorbance at pH values below 3.0 and the slight increase in absorbance at 570–640 nm (Jackman & Smith, 1996).

Although activation energy for betacyanin degradation decreases with pH, this does not impairs pigment application to most foods undergoing ordinary thermal treatments. As an example, betacyanin losses in pitaya juice acidified to pH 4 is less than 10% during pasteurisation at 80 °C for 5 min (Vaillant *et al*., 2005).

Some factors affect betalain stability upon pH. Havlíková *et al*. (1983) reported that high temperatures shifted the optimum pH for betacyanin stability toward 6. According to Huang & von Elbe (1987), anaerobic conditions favour betanin stability at lower pH (4.0–5.0).

Water activity (a_w)

Betalain stability is exponentially affected by a_w, which is a key factor determining the pigment susceptibility to aldimine bond cleavage (Saguy *et al*., 1984; Herbach *et al*., 2006b). The a_w effect on betalain stability may be attributed to a reduced mobility of reactants or limited oxygen solubility (Delgado-Vargas *et al*., 2000).

Kearsley & Katsaboxakis (1980) reported that a_w reduction improved betanin stability, especially below 0.63. Cohen & Saguy (1983) observed an increase of about one order of magnitude in betalain degradation rates when a_w increased from 0.32 to 0.75. Stability of betacyanins was reported to increase after being submitted to methods to reduce water activity, such as concentration (Castellar *et al*., 2006) and spray-drying (Cai & Corke, 2000). In a stability study of encapsulated beetroot pigments, greatest betanin degradation occurred at $a_w = 0.64$ (Serris & Biliaderis, 2001); this value was attributed by the authors to the decreasing mobility of reactants at lower and the dilution effects at higher a_w values.

Oxygen

Betalains react with molecular oxygen (Attoe & von Elbe, 1985). The storage of betanin solutions under low oxygen levels results in decreased pigment degradation than under air atmosphere, since low oxygen levels favour the pigment to be partially recovered after degradation (Von Elbe *et al*., 1974; Huang & von Elbe, 1987). A deviation from the first-order degradation kinetics of betanin in absence of oxygen was attributed to reaction reversibility. Betalain stability has been reported to be improved by antioxidants (Attoe & von Elbe, 1985; Altamirano *et al*., 1992; Han *et al*., 1998) or by a nitrogen atmosphere (Attoe & von Elbe, 1982, 1985; Drunkler *et al*., 2006).

Light

Betalain stability was reported to be impaired by light exposure (Von Elbe *et al*., 1974; Attoe & von Elbe, 1981; Cai *et al*., 1998). Attoe & von Elbe (1981) showed an inverse relationship between betalain stability and light intensity (in the range 2200–4400 lux). UV or visible light absorption excites π electrons of the pigment chromophore to a more energetic state (π^*), increasing reactivity or lowering activation energy for the molecule (Jackman & Smith, 1996). Betalain light-induced degradation is oxygen dependent, because the effects of light exposure are negligible under anaerobic conditions (Attoe & von Elbe, 1981; Huang & von Elbe, 1986).

Metals

Some metal cations, such as iron, copper, tin and aluminium were reported to accelerate betanin degradation (Pasch & von Elbe, 1979; Attoe & von Elbe, 1984; Czapski, 1990; Sobkowska *et al*., 1991). Metal-pigment

complexation can occur, followed by bathochromic and hypochromic shifts. Results by Czapski (1990) indicate that beet juice is less susceptible to the negative impact of metal ions when compared to solutions of pure betanin, probably because of the presence of metal-complexing agents in the juice. Chelating agents, such as citric acid and EDTA were reported to stabilize betanin against metal-catalysed degradation (Savolainen & Kuusi, 1978; Pasch & von Elbe, 1979; Attoe & von Elbe, 1984; Han *et al*., 1998; Herbach *et al*., 2006a).

Antioxidants

Some food antioxidants, especially ascorbic and isoascorbic acids, have been described to enhance betalain stability (Attoe & von Elbe, 1982; Cai & Corke, 1999; Máriássyová & Šilhár, 2000; Herbach *et al*., 2006a). However, Pasch & von Elbe (1979) observed a pro-oxidant effect of ascorbic acid a concentration of 1000 mg/kg. A possible explanation, given by Savolainen & Kuusi (1978), refers to bleaching effects by hydrogen peroxide during ascorbic acid degradation. There are also discrepancies regarding the replacement of ascorbic with isoascorbic acid. Some studies reported that isoascorbic acid had a better effect on betanin stability than ascorbic acid (Bilyk & Howard, 1982; Attoe & von Elbe, 1985; García Barrera *et al*., 1998), while results by Herbach *et al*. (2006a) indicated a higher pigment retention with ascorbic than with isoascorbic acid (Herbach *et al*., 2006a). The ineffectiveness of phenolic antioxidants, which act by terminate free radicals, indicates that betanin oxidation does not involve a free radical chain mechanism (Attoe & von Elbe, 1985).

Temperature

Temperature is the most important factor on betalain stability during food processing and storage. Some studies reported increasing betalain degradation rates resulting from increasing temperatures (Saguy *et al*., 1978; Havlíková *et al*., 1983; García Barrera *et al*., 1998). Thermal betacyanin degradation in betanin solutions as well as in red beet and purple pitaya juices was reported to follow first-order reaction kinetics (Von Elbe *et al*., 1974; Saguy *et al*., 1978; Saguy, 1979; Herbach *et al*., 2004b).

During heat processing, betanin may be degraded by isomerisation, decarboxylation or cleavage (by heats or acids, as indicated in Fig. 2), resulting in a gradual reduction of red colour, and eventually the appearance of a light brown colour (Huang & von Elbe, 1985; Drdák & Vallová, 1990). Dehydrogenation of betanin leads to neobetanin formation, bringing about a yellow shift. Cleavage of betanin and isobetanin, which can also be induced by bases (Schwartz & von Elbe, 1983; Schliemann *et al*., 1999), generates the bright yellow betalamic acid and the colourless cyclo-Dopa-5-*O*-glycoside. Herbach *et al*. (2004a) proposed that while betanin colour is maintained upon C_{15}-isomerisation or decarboxylation, C_{17}-decarboxylation causes a hypsochromic shift of the absorption peak from 538 to 505 nm, resulting in an orange-red colour.

Altamirano *et al*. (1993) evaluated thermal degradation of betanin in three model systems (water/glycerol, water/ethylene glycol and water/ethanol), at temperatures ranging from 60 °C to 86 °C. They reported that the lowest stability of betanin was in the water/ethanol system, supporting the hypothesis that the first step of the thermal betanin degradation is the nucleophilic attack on the aldimine bond, since ethanol has a high electron density on the oxygen atom. More recent studies (Wybraniec, 2005; Wybraniec & Mizrahi, 2005) reported a fast degradation of betacyanins in ethanolic solutions, leading to single and double decarboxylation. Moreover, the identification of different monodecarboxylation products in ethanolic and aqueous betacyanin solutions suggests different decarboxylation mechanisms depending on the solvent.

Betacyanins with different decarboxylation levels were identified together with their corresponding neo-derivatives as heating degradation products of betacyanins from red beetroot juice (Wybraniec, 2005) and purple pitaya extract (Wybraniec & Mizrahi, 2005). Later, Wybraniec *et al*. (2006) elucidated the structure of all mono- and bidecarboxylated betacyanins generated from heating red beet and purple pitaya preparations. Herbach *et al*. (2005) monitored the heat-degradation products from betanin, phyllocactin (malonylbetanin) and hylocerenin (3-hydroxy-3-methylglutarylbetanin) isolated from purple pitaya juice. Hydrolytic cleavage was the predominant degradation mechanism in betanin, while decarboxylation and dehydrogenation predominated in hylocerenin. Phyllocactin degradation was most complex, involving decarboxylation of the malonic acid moiety, betanin generation (by demalonylation) and subsequent degradation of betanin. Upon prolonged heating, the degradation products presented an additional double bond at C_2–C_3.

Betanin may be regenerated from their primary degradation products, because the extracts are kept for some time under temperature below 10 °C and pH around 5.0 (Huang & von Elbe, 1985, 1987). Betanin regeneration, which consists in a partial resynthesis of betanin from its hydrolysis products, involves a condensation of the amine group of cyclo-Dopa-5-*O*-glycoside with the aldehyde group of betalamic acid; betanin is rapidly formed when both compounds are mixed in solution (Huang & von Elbe, 1985).

Apart from heat treatment, lactic fermentation was also reported to promote betanin isomerisation and dehydrogenation. Still, aglycons can be formed from

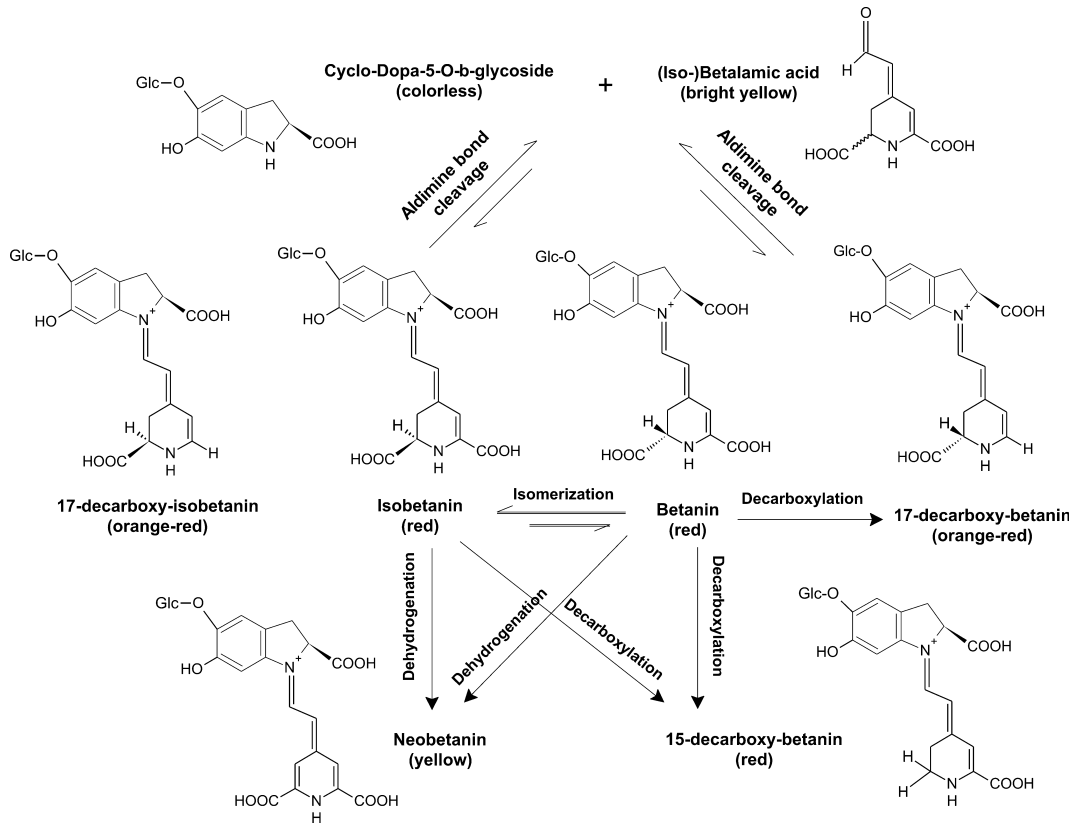

Figure 2 Some degradation pathways of betanin. (Herbach *et al*., 2004a).

betanin by fermentation, betanidin/betanin ratios depending on the endogenous β-glucosidase activity of the cultivar (Czyżowska *et al*., 2006).

Antioxidant properties of betalains

There is increasing evidence that oxidative stress leads to biochemical changes, which contribute to the development of several degenerative diseases, such as cancer and cardiopathies. Antioxidant molecules, some of which are derived from the diet, protect against the potentially injurious effects of oxidative stress (Kanner *et al*., 2001).

Several works have demonstrated the potent antiradical scavenging activity of betalains *in vitro* (Escribano *et al*., 1998; Kanner *et al*., 2001; Kujala *et al*., 2001; Butera *et al*., 2002; Pavlov *et al*., 2002; Wettasinghe *et al*., 2002; Cai *et al*., 2003). Some reports based on *in vitro* tests rank beet among the ten most potent vegetables with respect to their antioxidant activity (Vinson *et al*., 1998; Halvorsen *et al*., 2002; Ou *et al*., 2002), betalains responding at least in part for these beneficial properties (Pedreño & Escribano, 2001; Wettasinghe *et al*., 2002). Vaillant *et al*. (2005) observed that the *in vitro* antioxidant capacity of red pitaya pulp is as high as that of beetroot. Wu *et al*. (2006) reported that the peel of red pitaya, with higher betacyanin levels when compared to the flesh, presented higher antioxidant activity and was a stronger *in vitro* inhibitor of proliferation of melanoma cells, suggesting the contribution of betacyanins. Data on antioxidant activity of cactus pear was reviewed by Feugang *et al*. (2006) and Stintzing & Carle (2006).

Thanks to the increasing interest in the antioxidant properties of betalains, some researches have focused on their health benefits. Betaxanthins have been used as a food supplement in order to fortify processed food products with essential amino acids, giving rise to an 'essential dietary colourant' (Leathers *et al*., 1992). Kapadia *et al*. (1996) showed a significant inhibitory effect of beetroot towards skin and lung cancer in mice. Years latter, Kapadia *et al*. (2003) demonstrated the efficacy of betanin for long-term local suppression of skin and liver tumours induced by different chemical carcinogens in mice. Kanner *et al*. (2001) reported the ability of both betanin and betanidin at very small concentrations to inhibit lipid peroxidation and heme decomposition *in vitro*. Findings by Tesoriere *et al*. (2005) provide evidence that human red blood cells incorporate dietary betalains, which may protect the

cells and avoid oxidative hemolysis. Despite being hydrophilic, indicaxanthin and betanin have been shown also to bind to human low-density lipoproteins *in vitro* (Tesoriere *et al*., 2003) and *in vivo* (Tesoriere *et al*., 2004a), increasing their resistance to oxidation. Gentile *et al*. (2004) observed the ability of betalains to protect an *in vitro* model of endothelial cells from oxidation, related to inflammatory response. Allegra *et al*. (2005) reported the effectiveness of betanin and indicaxanthin to scavenge hypoclorous acid (HClO), the most powerful antioxidant produced by human neutrophils. In addition, both betalains were able to act as reductants of the redox intermediates of myeloperoxidase, which catalyzes the production of HClO. Lee *et al*. (2005) reported the ability of betalains to induce quinone reductase, a potent detoxification enzyme associated with cancer chemoprevention. Zou *et al*. (2005) reported growth inhibition of cervical, ovarian and bladder cancer cells *in vitro* and mice ovarian cancer model *in vivo* by cactus pear extract, whose antioxidant activity was suggested to be partially due to betalains. *In vivo* tests carried out by Tesoriere *et al*. (2004b) suggested that cactus pear fruit decreases oxidative damage to lipids, and improves antioxidant status in healthy humans. Comparative tests with vitamin C supplementation suggested that some component other than ascorbic acid has helped to decrease lipid oxidation. Findings by Stintzing *et al*. (2005) indicated that the contribution of betalains on *in vitro* antioxidant activity of cactus pear is even much greater than that provided by ascorbic acid. Netzel *et al*. (2005) reported that the ingestion of a single oral dose of red beet juice resulted in a significantly increased urinary excretion of antioxidant compounds, both phenolics and 'other' antioxidants, which probably include betalains.

According to results reported by Escribano *et al*. (1998) and Pedreño & Escribano (2001), the *in vitro* antiradical activity of betacyanins was greater than that of the betaxanthins. The structure–activity relationships of betalains with respect to free radical scavenging capacities were studied by Cai *et al*. (2003, 2005). In betaxanthins, they found that the free radical scavenging activity usually increases with the numbers of hydroxyl and imino residues, depending also on the position of hydroxyl groups. In betacyanins, glycosylation reduced activity while acylation generally improved the antioxidant activity. Furthermore, 6-*O*-glycosylated betacyanins presented higher antioxidant values than 5-*O*-glycosylated ones; more glycosylation reduces activity. The C-5 position of the hydroxyl group on aglycones improves antioxidant activity.

Final considerations and future prospects

Anthocyanins are the most widespread and most used natural pigments covering the red-purple colour range. However, the instability of anthocyanins at pH values above 3 (Stintzing & Carle, 2004) makes betacyanins the natural pigments of choice to provide red-purple colour shades to low acid foods. Moreover, betalains can effectively be stabilised by ascorbic acid, which on the other hand, impairs anthocyanin stability (Shenoy, 1993). Hence, application of betalains instead of anthocyanins for colouring foods with high ascorbic acid contents may be interesting (Herbach *et al*., 2006b).

Regarding the yellow-orange colour range, carotenoids are the natural pigments of choice in foods. Nevertheless, they have an important technological limitation, namely their poor solubility in water. Although betaxanthin sources are much scarcer worldwide, their water solubility could propel their application as yellow-orange food colourants in situations when the water solubility is crucial.

The commercial exploitation of cactus fruits as alternative sources of food colourants may not only provide a wider colour spectrum than the red beet without its negative sensory impacts, but also contribute to the sustainable development of the usually underdeveloped semi-arid regions which could supply markets with cactus fruits.

Some aspects related to betalain research are important to be further investigated, named: generation of crops with improved properties, such as beets with increased betalain levels and less or no earth-like flavour; further studies on betalain sources alternative to red beets, especially cactus fruits, as potential food colourants; improvements in productivity by bioreactors used for betalain cell cultures, as well as reducing costs of the process; betalain production under controlled conditions at industrial level; further investigation of the health benefits promoted by betalain consumption.

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