POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2005, Vol. 14/55, No 2, pp. 107–116

ACYLATED ANTHOCYANINS AS STABLE, NATURAL FOOD COLORANTS – A REVIEW

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Key words: acylated anthocyanins, intramolecular copigmentation, natural food colorants, red cabbage, black carrot

There is a demand for food colorants from natural sources that can serve as alternatives to the use of synthetic dyes due to the consumer concerns over the use of synthetic additives. Documented health benefits of anthocyanin extracts intensified the interest in anthocyanin-rich food. Anthocyanins, between other benefits, are potent antioxidants and may be useful to cure noninsulin-dependent diabetes.

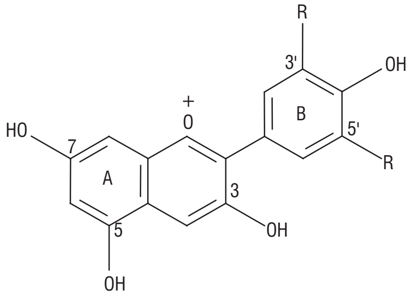
The finding that anthocyanin pigments containing acyl substituent are incredibly stable opens new opportunities for food producers. The stable acylated anthocyanins are present in large amounts in vegetables such as: red cabbage, black carrot, red radish, red potatoes or red corn. These pigments indicate a low sensibility to pH changes and an increased heat and light stability. For this reason, acylated anthocyanins are suitable to be applied not only for food with low pH but also for neutral and slightly alkaline products (dairy products, powdered and ready-to-eat desserts).

INTRODUCTION

An attractive and stable colour is important in the marketability of foods and beverages. Synthetic coloring agents have commonly been used in the food industry. The safety of synthetic dyes, however, has been questioned, leading to a reduction in the number of permitted colorants. Due to this limitation and the worldwide tendency towards the consumption of natural products, the interest in natural colorants has increased significantly. However, replacing synthetic dyes with natural colorants poses a challenge due to the higher stability of synthetic colorants with respect to light, oxygen, temperature and pH, among other factors.

ANTHOCYANIN COMPOUNDS

Anthocyanins demonstrate a high potential to be used as natural colorants due to their attractive orange, red and purple colours and water solubility that allows their incorporation into aqueous food systems [Brouillard, 1982; Mazza & Miniati, 1993; Shahidi & Naczk, 1995]. Anthocyanins belong to the widespread class of phenolic compounds collectively named flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salts (Figure 1) [Brouillard, 1982]. Anthocyanins occurring in nature contain several anthocyanidins or aglycones, but only six are common in foods – cyanidin, peonidin, pelargonidin, malvidin, delphinidin, and petunidin [Cooper-Driver, 2001; Horubała, 1996; Kong *et al.*, 2003]. In general, anthocyanidins are less soluble than their corresponding glycosides (anthocyanins) and they are not, therefore, found in nature [Brouillard, 1982; von Elbe & Schwartz, 1996]. With a few exceptions anthocyanins are always glycosylated at C-3 position [Takeoka & Dao, 2002].



Name	Substitution		Visible colour	Visible max (nm) in MeOH-HCL
	$R_{3'}$	$R_{5'}$		
Pelargonidin	H	H	red	520
Cyanidin	OH	H	magenda	535
Delphinidin	OH	OH	purple	546
Peonidin	OCH_3	H	magenda	532
Petunidin	OCH_3	OH	purple	543
Malvidin	OCH_3	OCH_3	purple	542

FIGURE 1. Basic structure of anthocyanins in flavylium cation (adopted from [Mazza & Miniati, 1993]).

Besides the C-3 position, other sugars can also be attached at any one of the hydroxyls at C-5, C-7, C-3', C-5', and even C-4' [Brouillard, 1982; Mazza & Miniati, 1993; Wilska-Jeszka, 1994; Zając & Wilska-Jeszka, 1991, 1994]. Usually anthocyanidin glycosides are 3-monosides and 3, 5-diglycosides. Sometimes 3, 7-diglycosides or 3-triglycosides are also known to occur [Clifford, 2000]. The most common sugar is glucose, but rhamnose, xylose, galactose, arabinose, and fructose as well as rutinose (6-O-α-L-rhamnosyl-D-glucose), sophorose (2-O-β-D-xylosyl-D-glucose), gentobiose (6-O-β-D-glucosyl-D-glucose), sambubiose (2-O-β-D-xylo-

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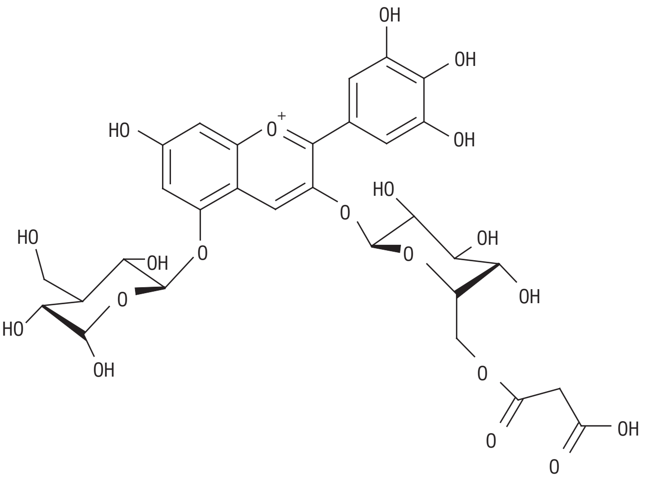


FIGURE 2. Chemical structure of delphinidin-3-malonylglucoside-5--glucoside isolated from flowers of *Scutellaria baicalensis* G [Oszmiański *et al*., 2004].

syl-D-glucose), xylosylrutinose and glycosylrutinose may also be present [Clifford, 2000; Shahidi & Naczk, 1995; Takeoka & Dao, 2002]. The sugar residues may be further acylated with organic acids [Giusti *et al.*, 1998b; Honda & Saito, 2002; Mahmoud *et al.*, 2001; Mazza & Miniatti, 1993; Stinzing *et al.*, 2002] (Figure 2). Common acylating agents include cinnamic acids such as caffeic, *p*-coumaric, ferulic and sinapic acid (Figure 3), as well as a range of aliphatic acids such as acetic, malic, malonic, oxalic, and succinic acid (Figure 4). Cinnamic acids may themselves bear glycosidic sugars. Aromatic and aliphatic acylation may occur in the same molecule, forming the polyacylated structure [Bloor, 1997, 2001; Bloor & Abrahams, 2002; Clifford, 2000; Giusti *et al.*, 1998a, b; Gonzales *et al.*, 2001; Harborne & Williams, 2000, 2001; Hosokawa *et al.*, 1995a,b; Nakatani *et al.*, 1995; Norbaek & Kondo, 1998, 1999, Norbaek *et al.*, 2002; Tatsuzawa *et al.*, 1997; Takeoka & Dao, 2002; Yoshida *et al.*, 1990]. Acyl substituents are usually bonded to the C-6 sugar [Giusti & Wrolstad, 2003; Honda & Saito, 2002; Otsuki *et al.*, 2002]. For a few pigments, they have been shown to be attached to the 2-hydroxy [Reiersen *et al.*, 2003; Strack *et al.*, 1992], 3-hydroxy [Andersen & Fossen, 1995] or 4-hydroxy [Fossen *et al.*, 2003].

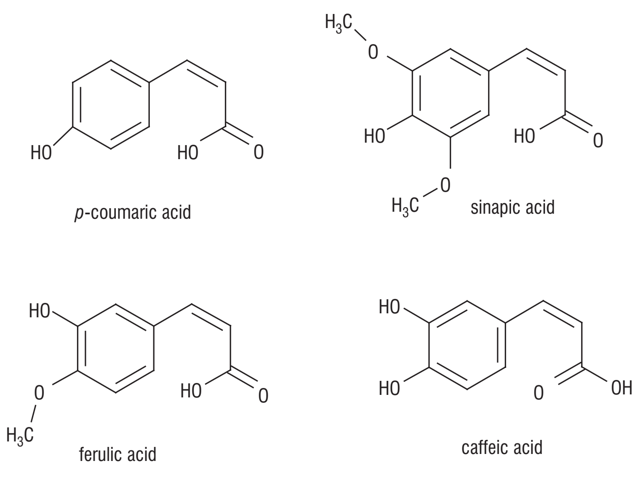


FIGURE 3. Common cinnamic acid acylated with sugar moieties of anthocyanins.

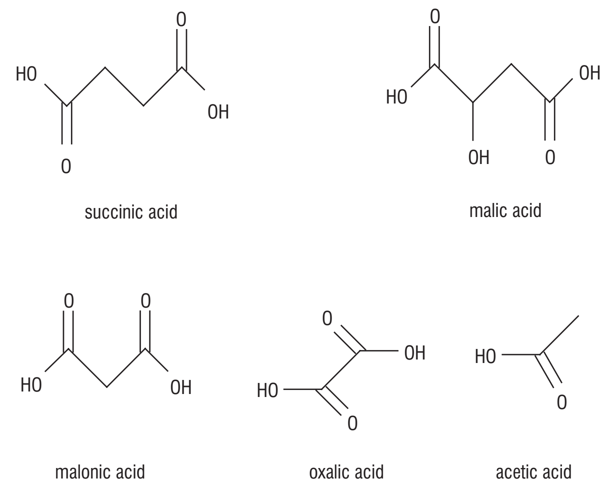


FIGURE 4. Common aliphatic acid acylated with sugar moieties of anthocyanins.

BIOLOGICAL ACTIVITY

Beside the colour attributes, interest in anthocyanins has intensified because of their possible health benefits. Health benefits associated with anthocyanins extracts include the enhancement of sight acuteness [Lamer--Zarawska & Oszmiański, 1991, 1994], anticancerogenic activity [Katsube *et al.*, 2003], antioxidant capacity [Gabrielska *et al.*, 1999; Kähkönen & Hainonen, 2003; Kähkönen *et al.*, 2003; Kong *et al.*, 2003; Lamer-Zarawska & Oszmiański, 1994; Wang *et al.*, 1997], antiulcer activity [Cristoni & Magistretti, 1987], the maintenance of normal vascular permeability (vitamin P) [Lamer-Zarawska & Oszmiański, 1991, 1994]. The results presented by Jayaprakasam *et al.* [2005] showed that isolated and purified anthocyanins from fruit and vegetable may be useful in the treatment of type 2-diebetes (noninsulin-dependent diabetes). The most significant function of anthocyanin extracts is their antioxidant activity. Ghiselli *et al.* [1998] investigated an antioxidant activity of anthocyanin fractions from Italian red wine. The results showed that the anthocyanins were the most effective both in scavenging reactive oxygen species and in inhibiting lipoprotein oxidation and platelet aggregation. Those authors suggested that anthocyanins could be the key component in red wine that protects against the cardiovascular disease. Another report on the antioxidative activity was published by Tamura and Yamagami [1994]. They found that anthocyanins acylated by *p*-coumaric acid are much better antioxidants than α-tocopherol or (+)-catechin. Besides the antioxidant activity, anthocyanins possess also free radical scavenging activity. Anthocyanin radical is more stable than other radicals generated in human body, hence duration of this radical is longer [Wolniak, 2002]. Wang and Mazza [2002] were first to report that anthocyanins had strong inhibitory effects on NO production. Anthocyanins with their 3', 4'-dihydroxy groups can rapidly chelate metal ions to form stable anthocyanin-metal complexes [Sarma *et al.*, 1997]. As a result, anthocyanins with the ortho-dihydroxyl groups have the potential to scavenge hydroxyl radical through the inhibition of ·OH generation by chelating iron [Noda *et al.*, 1998; 2000], and to prevent iron-induced lipid peroxidation [Wang *et al.*, 1999]. The ortho-dihydroxyl group also helps to form anthocyanin-metal-copigment complexes at physiological pH ranges with various organic compounds such as ascorbic acid [Sarma *et al.*, 1997] and partially through this mechanism to spare vitamin C. The results of a research described by Sarma and Sharma [1999] indicate that cyanidin-DNA copigmentation may be a potential defense mechanism against the oxidative damage of DNA and may have *in vivo* physiological functions attributable to the antioxidant activity of anthocyanins. It has been suggested that anthocyanins have the ability to stabilize DNA triple-helical complexes [Mas *et al.*, 2000].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that anthocyanin-containing extracts are of a very low order of toxicity, based on limited toxicological studies including mutagenicity, reproductive toxicity and teratogenicity. In a two-generation reproduction study, the no-observed-effect-level (NOEL) for young rats was determined to be 225 mg/kg body weight (diet containing 7.5% grape skin extract, or 3% anthocyanin pigments, equivalent to 7500 mg diet per kg body weight). Based on the above result, in 1982 the estimated acceptable daily intake (ADI) for man, calculated using the equation of ADI=NOEL/100 [Joint FAO/WHO Food Additives Series 17; Clifford, 2000] accounted for 2.5 mg/kg body weight. The presented health benefits make anthocyanins an attractive alternative to synthetic dyes.

STABILITY OF ANTHOCYANIN COMPOUNDS

The stability of anthocyanin pigments is determined by several factors, including structure and concentration of the pigment, pH, temperature, light intensity and quality, the presence of copigments, metal ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products and sulfur dioxide, among others [Brouillard, 1982; Mazza & Brouillard, 1990].

With respect to molecular structure, some anthocyanins are more stable than the others. Generally, increased hydroxylation decreases stability, whereas increased methylation increases it [Brouillard, 1982]. The colour of foods containing anthocyanins that are rich in pelargonidin, cyanidin, or delphinidin aglycones is less stable than that of food containing petunidin or malvidin aglycones. Moreover, anthocyanins containing galactose are more stable than those containing arabinose [von Elbe & Schwartz, 1996]. Recent researches have shown that anthocyanins with acylating substituents are more stable during processing and storage than other natural pigments [Cevallos-Casals & Cisneros-Zevallos, 2004; Fossen *et al.*, 1998; Giusti & Wrolstad, 2003; Honda & Saito, 2002; Inami *et al.*, 1996; Malien-Aubert *et al.*, 2001; Rodriguez-Saona *et al.*, 1998, 1999; Sapers *et al.*, 1981]. The improved stabilization has been attributed to the stacking of the acyl groups with the pyrylium ring of the flavylium cation, thereby reducing the susceptibility of nucleophile attack of water and subsequent formation of a pseudobase or a chalcone (intramolecular copigmentation) (Figure 5) [Brouillard *et al.*, 1982, 2003; Dangles *et al.*, 1992, 1993; Davies & Mazza, 1993; Figueiredo *et al.*, 1996, 1999; Mazza & Miniati, 1993; Osawa, 1982]. Full colour stabilization is best achieved when the anthocyanins bear aromatic than aliphatic ones [Giusti & Wrolstad, 2003]. In addition, more stable complexes are formed when the aromatic acids are substituted in ring B of flavylium cation than in ring A [Yoshida *et al.*, 2002]. Acylating substituents at C-3' and C-7 which are specific to the pigments of *Orchidaceae* and *Senecio* completely protect these anthocyanins against hydrolysis throughout the acidic to neutral pH range [Harborne & Williams, 2001]. Another important factor for colour stabilization is the free malonyl group attached to the glucose at the C-3 position. This malonic acid group preserves colour by increasing medium acidity in the cell vacuole. This function of the malonyl residue applies more generally to any anthocyanin pigments with 3-(6-malonylglucoside) substitution [Figueiredo *et al.*, 1999].

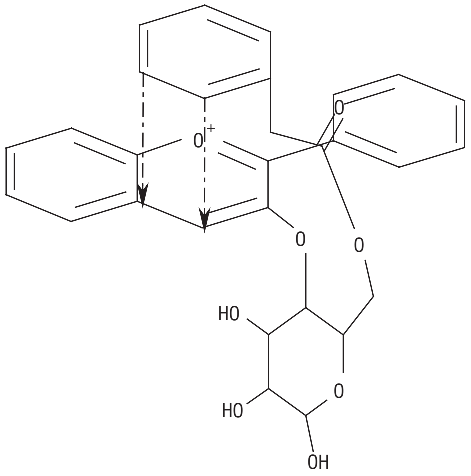


FIGURE 5. Intramolecular copigmentation of acylated anthocyanins [http://www.arches.uga.edu].

Acylated anthocyanins are also more resistant to colour fading with increased pH than their unacylated analogs. In aqueous media, four anthocyanin structures exist in equilibrium: flavylium cation, carbinol pseudobase, quinonoidal base and chalcone [Amić *et al.*, 1990; Baranac & Amić, 1990; Carbita *et al.*, 2000; Dao *et al.*, 1998; Mazza & Brouillard, 1987; Melo *et al.*, 2000; Mirabel *et al.*, 1998; Musoke, 2002]. In strongly acidic aqueous media (pH 1), the red-colored flavylium cation is the predominant species. Unacylated anthocyanins are only stable at pH values where the flavylium cation dominates [Heredia *et al.*, 1998; Zając & Wilska-Jeszka, 1994]. Between pH values of 2 and 4, the uncharged blue quinonoidal unstable species prevails, and if the pH is increased, the ionization of the hydroxyl groups forms the anionic blue quinonoidal unstable species. At pH 5 and 6, unacylated anthocyanins are unstable and decolorize quickly by hydration at the 2-position of the anthocyanidin skeleton (carbinol pseudobase and chalcone structures are formed) [Brouillard, 1982; Dao *et al.*, 1998; Mazza & Miniati, 1993]. Evidence has been provided that the chroma of some pelarginidin derivatives increased when the pH was further increased to neutral conditions [Giusti & Wrolstad, 2003]. Cevallos-Casals & Cisneros-Zevallos [2004] showed that colorants rich in acylated anthocyanins, such as sweet potato and purple carrot, were more resistant to the solution pH than colorants rich in unacylated anthocyanins such as red grape. The other researchers confirmed the unusual stability of acylated anthocyanins at pH over 5.0 [Idaka, 1991; Fossen *et al.*, 1998].

It is generally recognized that light accelerates degradation of anthocyanins. This adverse effect has been demonstrated in several fruit juices and red wines. In fruit juices it has been established that acylated anthocyanins are more stable than unacylated derivatives [Inami *et al.*, 1996]. In addition, the absorbance of juices tested was observed to increase after exposure to light. Further investigations demonstrated that anthocyanins containing cinnamoyl derivatives are able to isomerize from *trans* to *cis* form [George *et al.*, 2001; Yoshida *et al.*, 1990, 2000, 2002, 2003 a, b]. The reaction of isomerization evokes colour intensification and resistance to pyrilium ring hydration.

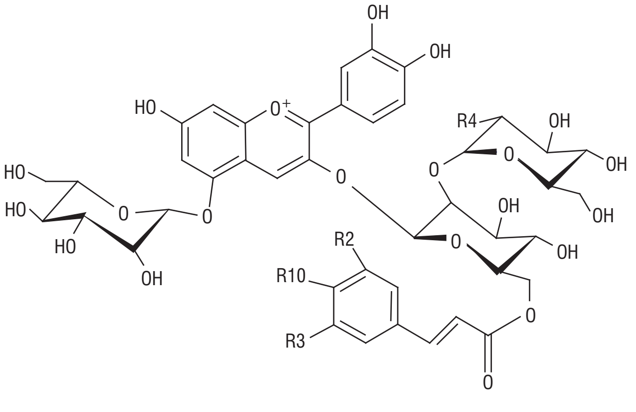
High temperature is another factor inducing degradation of anthocyanin colorants [Bąkowska *et al.*, 2003; Furtado *et al.*, 1993; Garcia-Viguera *et al.*, 1999; Horubała, 1996]. The results presented by Dyrby *et al.* [2001] showed that acylated anthocyanins from red cabbage were more stable than the unacylated anthocyanins obtained from red grape, black currant and elderberry. The high stability of red cabbage anthocyanins during heating at 80°C was confirmed by our unpublished data.

ACYLATED ANTHOCYANINS AS COLORANTS FOR THE FOOD INDUSTRY

The confirmed large stability of anthocyanins, including acidic substituent, and their wide colour range caused that searches for acylated anthocyanin-rich material have begun.

Taking into consideration low prices, big surface of tillage and high amounts of anthocyanins, red cabbage (*Brassica oleracea* L) is the most valuable source of the stable form of anthocyanins for the Polish food industry. Red cabbage contains about 15 anthocyanins (mostly diacylated) being derivatives of cyanidin-3-diglucoside-5-glucoside acylated with ferulic, sinapic and/or *p*-coumaric acids (Figure 6) [Baublis *et al.*, 1994; Giusti *et al.*, 1999; Mazza & Miniati, 1993; Murai & Wilkins, 1990].

The extract of red cabbage anthocyanins is one of the most expensive anthocyanin concentrates, but its high cost is offset by a high tinctorial strength. Available in a liquid or sprayed-dried form, the extracts are water-soluble and, because of the presence of acylated anthocyanins, demonstrate improved stability to heat and light [Giusti, 2002]. Slight changes in pH have a large effect on the resulting hue, and therefore, pH control of the final product becomes crucial.



1. R1 = R2 = R3 = R4 = H
2. R1 = R3 = R4 = H R2 = OCH_3
3. R1= R4 = H R2 = R3 = OCH_3
4. R1 = R2 = R3 = H R4 = sinapic acid
5. R1 = R3 = H R2 = OCH_3 R4 = sinapic acid
6. R1 = H R2 = R3 = OCH_3 R4 = sinapic acid

FIGURE 6. Chemical structure of main acylated anthocyanins from red cabbage (*Brassica oleracea*) [Mazza & Miniati, 1993].

The commercialization of red cabbage anthocyanins was pioneered by the Overseal Natural Ingredients Ltd under their brand name Magento™ [http://www.overseal.co.uk]. The interest in and success of red cabbage–derived anthocyanins lie in the fact that they: produce a bright pink shade at low pH on clear bases and pink/mauve shades in white bases; provide mauve/blue shades at more neutral pH values; have no unpleasant taste due to minimized odour notes; demonstrate superior stability to heat and light over anthocyanins from more traditional sources such as grapeskin and elderberry; are naturally low in polyphenols reducing the risk of hazing with proteins; are available all year round; and demonstrate potential health benefits when included into the diet [http://www.overseal.co.uk].

Giusti and Wrolstad [2003] tested the viability of using acylated anthocyanins from red cabbage to colour of dairy products such as yogurt or sour cream, with pH levels of about 4.2–4.5. These anthocyanins gave an attractive purple colour, resembling the colour of blueberries.

Other excellent source of acylated anthocyanins is black carrot (*Daucus carota* L). It has been grown and consumed in the East for at least 3,000 years. Historical records indicate that it was cultivated in parts of Europe in the 12th century and in Holland by the 14th century. It was commonly consumed until about 1750 when Dutch breeders developed the orange variety which we are all familiar with [Pszczola, 1999]. The black carrot is still consumed in some parts of the world, such as Spain, Pakistan, Afghanistan, Turkey, and Egypt. Nowadays black carrots are also cultivated in Europe (including Poland) on a large scale.

The colour of this vegetable is determined by 5 main anthocyanins, being derivatives of cyanidin-3-rutynoside--glucoside-galactoside acylated with one cinnamic acid (*p*--coumaric, ferulic or sinapic) (Figure 7) [Dougall *et al.*, 1997, 1998; Gakh *et al.*, 1998; Giusti, 2002; Giusti & Wrolstad, 2003; Glässgen *et al.*, 1992; Kammerer *et al.*, 2004; Lazcano *et al.*, 2001; Narayan & Venkatarman, 2000]. It was the GNT group that pioneered the development of food colorants from black carrot [Pszczola, 1999]. The coloring extract sold under EXBERRY® brand name is pleasantly fruity in taste and free of off flavours. The black carrot extract is also produced by the Overseal Foods Ltd under its brand name Carantho® [Downham & Collins, 2000]. EXBERRY® and Carantho® extracts offer the following features and benefits: they provide an excellent bright strawberry red shade in acidic products, up to pH 4.5; they exhibit mauve to blue tones under neutral pH conditions; they are an excellent vegetarian and kosher alternative to carmine; they contain low levels of polyphenols, which are naturally present in grapeskin anthocyanins and which can cause hazing and precipitation problems; and finally they offer improved stability to heat, light and SO_2 [http://www.overseal.co.uk].

Other permitted sources of stable acylated anthocyanins are also under evaluation. One of them is red radish (*Raphanus sativus* L). This vegetable contains 12 acylated

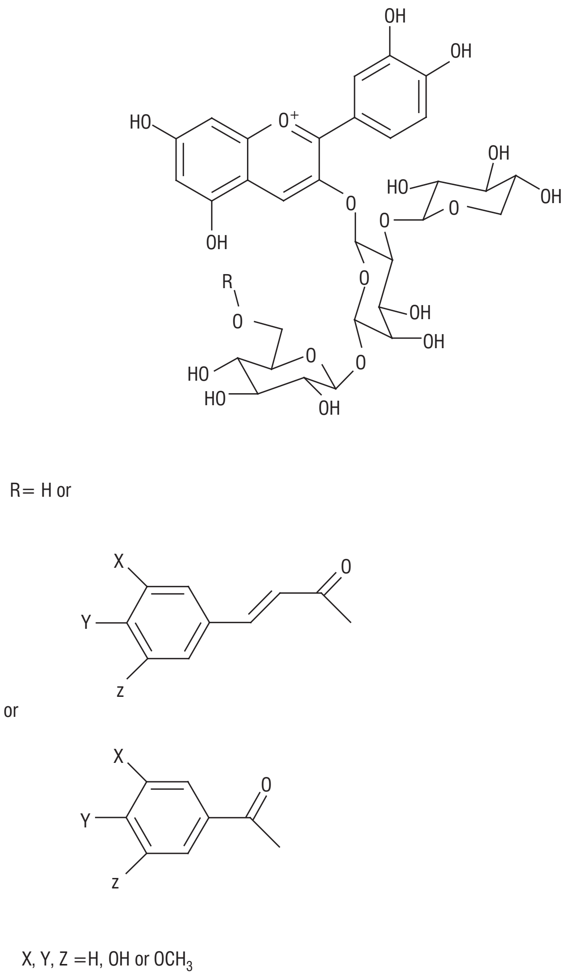


FIGURE 7. Chemical structure of main acylated anthocyanins from black carrot (*Daucus carota*) [Glässgen *et al*., 1992].

anthocyanins (8 diacylated) [Otsuki *et al.*, 2002]. A natural colour based on red radish has been recently launched by the RFI Ingredients as the newest addition to the company's ColorPure™ line of natural colorants. Giusti and Wrolstad [1996] reported that acylated pelargonidin derivatives from red radish could impart red colour to maraschino cherries extremely close to that of synthetic colorant FD&C Red # 40 at pH 3.5. The recent research has shown that this synthetic colorant can be replaced also by a natural one obtained from red potatoes (*Solanum tuberosum*) [Cevallos-Casals & Cisneros-Zevallos, 2004; Rodriguez-Saona *et al.*, 1998]. Depending on a variety, red potatoes contain from 4 to 7 monoacylated anthocyanins [Fossen *et al.*, 2003; Rodriguez-Saona *et al.*, 1998; Terahara *et al.*, 2004]. The coloring extract of monoacylated red potato anthocyanins is less stable than that obtained from red radish containing diacylated anthocyanins [Rodriguez-Saona *et al.*, 1998, 1999]. Purple corn (Zea Mays) [Aoki *et al.*, www.ffcr.or.jp] and oxalis (*Oxalis triangularis*) [Pazmino-Duran *et al.*, 2001] are also under consideration as a potential food colour sources.

Extracts of acylated anthocyanins, like other anthocyanin colorants, are suitable to be applied for foods with a low pH level, including soft drinks, confectionery, table jellies, conserves, sauces, water ice cream [http://www.overseal.co.uk]. Results of many researches indicate that other potential applications for certain acylated anthocyanins may be in neutral or slightly alkaline products, such as ice cream, powdered and ready-to-eat desserts, panned products and milk drinks [Giusti, 2002; Giusti & Wrolstad, 2003; http://www.overseal.co.uk].

CONCLUSIONS

Acylated anthocyanins have been shown to be a promising alternative to synthetic colorants in food systems. Depending on anthocyanin structure and pH of food matrix, a wide variety of hues can be achieved. Acylation of anthocyanins improves colour and pigment stability. There is an increased market for nutraceutical or medicinal food and consumers are interested in foods that may help to prevent diseases. Identification of health promoting properties in anthocyanin extracts would increase the demand for these natural pigments, and would open a new window of opportunities for their use in a variety of food applications.

REFERENCES

1. Amić D., Baranac J., Vukadinović V., Reactivity of some flavylium cations and corresponding anhydrobases. J. Agric. Food Chem., 1990, 38, 936–940.
2. Andersen O.M., Fossen T., Anthocyanins with an unusual acylation pattern from stem of *Allium victorialis*. Phytochem., 1995, 40, 1809–1812.
3. Anthocyanins: alternatives to synthetic food dyes [http://www.arches.uga.edu].
4. Aoki H., Noriko K., Yoshiaki K., Anthocyanins isolated from purple corn (*Zea mays* L.) [http://www.ffcr.or.jp].
5. Baranac J., Amić D., Structural transformations of apigenidin-type flavylium salts. J. Agric. Food Chem., 1990, 38, 2111–2115.
6. Baublis A., Spomer A., Berber-Jimenez M.D., Anthocyanin pigments: comparison of extract stability. J. Food Sci., 1994, 59, 1219–1221.
7. Bąkowska A., Kucharska A.Z., Oszmiański J., The effect of heating, UV irradiation, and storage stability of anthocyanin-polyphenol copigment complex. Food Chem., 2003, 81, 349–355.
8. Bloor S.J., Blue flower colour derived from flavonol-anthocyanin co-pigmentation in *Ceanothus papillosus*. Phytochem., 1997, 45, 1399–1405.
9. Bloor S.J., Deep blue anthocyanins from blue *Dianella* berries. Phytochem., 2001, 58, 923–927.
10. Bloor S.J., Abrahams S., The structure of the major anthocyanins in *Arabidopsis thaliana*. Phytochem., 2002, 59, 343–346.
11. Brouillard R., Chemical structure of anthocyanins. 1982, *in*: Anthocyanins as Food Color (ed. P. Markakis.). Academic Press, New York, pp. 1–40.
12. Brouillard R., Chassaing S., Fougerousse A., Why are grape/fresh wine anthocyanins so simple and why is it that red wine color lasts so long? Phytochem., 2003, 64, 1179–1186.
13. Carbita L., Fossen T., Andersen O.M., Colour and stability of six common anthocyanin 3-glucosides in aqueous solutions. Food Chem., 2000, 68, 101–107.
14. Cevallos-Casals B.A., Cisneros-Zevallos L., Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chem., 2004, 86, 69–77.

15. Clifford M.N., Anthocyanins-nature, occurrence and dietary burden. J. Sci. Food Agric., 2000, 80, 1063–1072.
16. Cooper-Driver G.A., Contributions of Jeffrey Harborne and co-workers to the study of anthocyanins. Phytochem., 2001, 56, 229–236.
17. Cristoni A., Magistretti M.J., Antiulcer and healing activity of *Vaccinum padifolium* anthocyanosides. Farmaco. Ed. Prat., 1987, 42, 29–43.
18. Dangles O., Wigand M.C., Brouillard R., Polyphenols in plant pigmentation: the copigmentation case. J. Agric. Food Chem., 1992, 41, 209–216.
19. Dangles O., Saito N., Brouillard R., Kinetic and thermodynamic control of flavylium hydration in the pelargonidin-cinnamic acid complexation. Origin of the extraordinary flower color diversity of *Pharbitis nil*. J. Am. Chem. Soc., 1993, 115, 3125–3132.
20. Dao L.T., Takeoka G.R., Edwards R.H., Berrios J., De J., Improved method for stabilization of anthocyanidins. J. Agric. Food Chem., 1998, 46, 3564–3569.
21. Davies A.J., Mazza G., Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. J. Agric. Food Chem., 1993, 41, 716–720.
22. Dougall D.K., Baker D.C., Gakh E.G., Redus M.A., Biosynthesis and stability of monoacylated anthocyanins. Food Technol., 1997, 51, 69–71.
23. Dougall D.K., Baker D.C., Gakh E.G., Redus M.A., Whittemore N.A., Anthocyanins from wild carrot suspension cultures acylated with supplied carboxylic acids. Carbohydrate Res., 1998, 310, 177–189.
24. Downham A., Collins P., Colouring our foods in the last and next millenium. Inter. J. Food Sci. Tech., 2000, 35, 5–22.
25. Dyrby M., Westergaard N., Stapelfeldt H., Light and heat sensitivity of red cabbage extract in soft drink model systems. Food Chem., 2001, 72, 431–437.
26. von Elbe M., Shwartz R., Colorants. 1996, *in*: Food Chemistry (ed. O.R. Fennema). Marcel Dekker, Inc., New York, pp. 681–694.
27. Figueiredo P., Elhabiri M., Toki K., Saito N., Dangles O., Brouillard R., New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for colour loss? Phytochem., 1996, 41, 301–308.
28. Figueiredo P., George F., Tatsuzawa F., Toki K., Saito N., Brouillard R., New features of intramolecular copigmentation by acylated anthocyanins. Phytochem., 1999, 51, 125–132.
29. Fossen T., Carbrita L., Andersen Ø.M., Colour and stability of pure anthocyanins influenced by pH including the alkaline region. Food Chem., 1998, 63, 435–440.
30. Fossen T., Øvstedal D.O., Slimestad R., Andersen Ø.M., Anthocyanins from a Norwegian potato cultivar. Food Chem., 2003, 81, 433–437.
31. Furtado P., Figueiredo P., Neves H.C., Pina F., Photochemical and thermal degradation of anthocyanins. J. Photochem. Photobiol. A, 1993, 75, 113–118.
32. Gabrielska J., Oszmiański J., Komorowska M., Langner M., Anthocyanin extracts with antioxidant and radical scavenging effect. Z. Naturforsch., 1999, 54, 319–324.
33. Gakh E.G., Dougall D.K., Baker D.C., Proton nuclear magnetic resonance studies of monoacylated anthocyanins from the wild carrot: Part 1. Inter- and intra-molecular interaction in solution. Phytochem. Anal., 1998, 9, 28–34.
34. Garcia-Viguera C., Zafrilla P., Romero F., Abellan P., Artes F., Tomas-Barberan F.A., Color stability of strawberry jam as affected by cultivar and storage temperature. J. Food Sci., 1999, 64, 243–247.
35. George F., Figueiredo P., Toki K., Tatsuzawa F., Saito N., Brouillard R., Influence of *trans-cis* isomerisation of coumaric acid substituents on colour variance and stabilisation in anthocyanins. Phytochem., 2001, 57, 791–795.
36. Ghiselli A., Nardini M., Baldi A., Scaccini C., Antioxidant activity of different phenolic fractions separated from an Italian red wine. J. Agric. Food Chem., 1998, 46, 361–367.
37. Giusti M.M., Wrolstad R.E., Radish anthocyanins extract as a natural red colorant for marachino cherries. J. Food Sci., 1996, 61, 688–694.
38. Giusti M.M., Ghanadan H., Wrolstad R.E., Elucidation of the structure and confirmation of red radish (*Raphanus sativus*) anthocyanins using one- and two-dimensional Nuclear Magnetic Resonance techniques. J. Agric. Food Chem., 1998a, 46, 4858–4863.
39. Giusti M.M., Rodriguez-Saona L.E., Baggett J.R., Reed G.L., Durst R.W., Wrolstad R.E., Anthocyanin pigment composition of red radish cultivars as potential food colorants. J. Food Sci., 1998b, 2, 219–224.
40. Giusti M.M., Rodriguez-Saona L.E., Wrolstad R.E., Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J. Agric. Food Chem., 1999, 47, 4631–4637.
41. Giusti M.M., Applications of acylated anthocyanins as natural food colourants. Business Briefing: Innovative food ingredience, 2002, 45–48.
42. Giusti M.M., Wrolstad R.E., Acylated anthocyanins from edible sources and their applications in food systems. Biochem. Eng. J., 2003, 14, 217–225.
43. Glässgen W.E., Wrag V., Strack D., Metzeger J.W., Anthocyanins from cell suspension cultures of *Daucus carota*. Phytochem., 1992, 31, 1593–1597.
44. Gonzales E., Fougerousse A., Brouillard R., Two diacetylated malvidin glycosides from *Petunia hybrida* flowers. Phytochem., 2001, 58, 1257–12612.
45. Harborne J.B., Williams C.A., Advances in flavonoid research since 1992. Phytochem., 2000, 55, 481–504.
46. Harborne J.B., Williams C.A., Anthocyanins and other flavonoids. Nat. Prod. Rep., 2001, 18, 310–333.
47. Heredia F.J., Francia-Aricha E.M., Rivas-Gonzalo J.C., Vicario I.M., Santos-Buelga C., Chromatic characterization of anthocyanins from red grapes – I. pH effect. Food Chem., 1998, 63, 491–498.
48. Honda T., Saito N., Recent progress in the chemistry of polyacylated anthocyanins as flower color pigments. Heterocycles, 2002, 56, 633–692.
49. Horubała A., The changes of colour of fruit juices in technological processes, their receipt and storage. Przem. Ferm. Owoc. Warzyw., 1996, 8, 31–34 (in Polish).
50. Hosokawa K., Fukunaga Y., Fukushi E., Kawabata J., Seven acylated anthocyanins in the blue flowers of *Hyacinthus orientalis*. Phytochem., 1995a, 38, 1293–1298.
51. Hosokawa K., Fukushi E., Kawabata J., Fuji C., Ito T., Yamamura S., Three acylated cyanidin glucosides in pink flowers of Gentiana. Phytochem., 1995b, 40, 941–944.
52. http://http://www.overseal.co.uk/magneto3.htm Preparat Magento^{TM}.

53. Idaka E., Acylated anthocyanin and process for producing the same as well as pigment composition containing the same. Suntory Limited, Patent USA No 4,999,423, 1991.
54. Inami O., Tamura I., Kikuzaki H., Nakatani N., Stability of anthocyanins of *Sambuskus canadensis* and *Sambuskus nigra*. J. Agric. Food Chem., 1996, 44, 3090–3096.
55. Jayaprakasam B., Vareed S.K., Olson K.L., Nair M.G., Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. J. Agric. Food Chem., 2005, 53, 28–31.
56. Joint FAO/WHO Expert Committee on Food Additives. Anthocyanins (WHO Food Additives Series 17). [http://www.inchem.org].
57. Kammerer D., Carle R., Schieber A., Quantification of anthocyanins in black carrot extracts (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) and evaluation of their color properties. Eur. Food Res. Technol., 2004, 219, 479–486.
58. Katsube N., Iwashita K., Tsushuda T., Yamaki K., Kobori M., Induction of cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. J. Agric. Food Chem., 2003, 51, 68–75.
59. Kähkönen M.P., Heinämäki J., Ollilainen V., Heinonen M., Berry anthocyanins: isolation, identification and antioxidant activities. J. Sci. Food Agric., 2003, 83, 1403–1411.
60. Kähkönen M.P., Heinonen M., Antioxidant activity of anthocyanins and their aglycons. J. Agric. Food Chem., 2003, 51, 628–633.
61. Kong J.M., Chia L.S., Goh N.K., Chia T.F., Brouillard R., Analysis and biological activities of anthocyanins. Phytochem., 2003, 64, 923–933.
62. Lamer-Zarawska E., Oszmiański J., Meaning of vegetable dyes as food components. Wiad. Ziel., 1991, 10, 10–11 (in Polish).
63. Lamer-Zarawska E., Oszmiański J., New investigations of flavonoids bioactivity. Wiad. Ziel., 1994, 3, 11–13 (in Polish).
64. Lazcano C.A., Yoo K.S., Pike L.M., A method for measuring anthocyanins after removing carotenes in purple colored carrots. Sci. Hort., 2001, 90, 321–324.
65. Mahmoud I., Marzouk M.S.A., Moharram F.A., El-Gindi M.R., Hassan A.M.K., Acylated flavonol glycosides from *Eugenia jambolana* leaves. Phytochem., 2001, 58, 1239–1244.
66. Malien-Aubert C., Dangles O., Amiot M.J., Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. J. Agric. Food Chem., 2001, 49, 170–176.
67. Mas T., Susperregui J., Berke B., Cheze C., Moreau S., Nuhrich A., Vercauteren J., DNA triplex stabilization property of natural anthocyanins. Phytochem., 2000, 53, 679–687.
68. Mazza G., Brouillard R., Recent developments in the stabilization of anthocyanins in food products. Food Chem., 1987, 25, 207–225.
69. Mazza G., Brouillard R., The mechanism of co-pigmentation of anthocyanins in aqueous solutions. Phytochem., 1990, 29, 1097–1102.
70. Mazza G., Miniati E., Introduction. 1993, *in*: Anthocyanins in fruits, vegetables and grain CRC Press, Inc. Boca Raton, Florida, pp. 1–28.
71. Melo M.J., Moncada M.C., Pina F., On the red colour of raspberry (*Rubus Idaeus*). Tetrahedron Lett., 2000, 41, 1987–1991.
72. Mirabel M., Kahn N., Lagunde-Ammirati L., Glories Y., The copigmentation case in model wine solution: application to red wines. 1998, *in*: Polyphenol Communications XIXth International Conference on Polyphenols, 1998, Lille, France, pp. 341–343.
73. Murai K., Wilkins D., Natural red color derived from red cabbage. Food Technol., 1990, 44, 131.
74. Musoke B.E., The uses of plant colours (anthocyanin pigments) and methods to isolate them from plants. 2002, *in*: Materials of The World's Women Congress, 2002, Makerere University, Uganda, pp. 212–214.
75. Nakatani N., Kikuzaki H., Hikida J., Ohba M., Inami O., Tamura I., Acylated anthocyanins from fruits of *Sambuscus canadensis*. Phytochem., 1995, 3, 755–757.
76. Narayan M.S., Venkataraman L.V., Characterization of anthocyanins derived from carrot (*Daucus carota*) cell culture. Food Chem., 2000, 70, 361–363.
77. Noda Y., Kaneyuki T., Igarashi K., Mori A., Packer L., Antioxidant activity of nasunin, an anthocyanin in eggplant. Mol. Pathol. Pharmacol., 1998, 102, 175–187.
78. Noda Y., Kneyuki T., Igarashi, K., Mori, A., Packer, L., Antioxidant activity of nasunin, an anthocyanin in eggplant peels. Toxicology, 2000, 148, 119–123.
79. Norbaek R., Kondo T., Anthocyanins from flowers of *Crocus* (Iridaceae). Phytochem., 1998, 47, 861–864.
80. Norbaek R., Kondo T., Further anthocyanins from flowers of *Crocus antalyensis* (Iridaceae). Phytochem., 1999, 50, 325–328.
81. Norbaek R., Nielsen K., Kondo T., Anthocyanins from flowers of *Cichorium intybus*. Phytochem., 2002, 60, 357–359.
82. Osawa Y., Copigmentation of anthocyanins. 1982, *in*: Anthocyanins as Food Color (ed. P. Markakis). Academic Press, New York, pp. 41–69.
83. Oszmiański J., Bąkowska A., Piacente S., Thermodynamic characteristics of copigmentation reaction of acylated anthocyanin isolated from blue flowers of *Scutellaria baicalensis* Georgi with copigments. J. Sci. Food Agric., 2004, 84, 1500–1506.
84. Otsuki T., Matsufuji H., Takeda M., Toyoda M., Goda Y., Acylated anthocyanins from red radish (*Raphanus sativus* L.). Phytochem., 2002, 60, 79–87.
85. Pazmino-Duran E.A., Giusti M.M., Wrolstad R.E., Gloria M.B.A., Anthocyanins from *Oxalis triangularis* as potential food colorants. Food Chem., 2001, 75, 211–216.
86. Reiersen B., Kiremine B.T., Byamukama R., Andersen Ø.M., Anthocyanins acylated with gallic acid from chenille plant, *Acalypha hipsida*. Phytochem., 2003, 64, 867–871.
87. Rodrigez-Saona L.E., Giusti M.M., Wrolstad R.E., Anthocyanin pigment composition of red-fleshed potatoes. J. Food Sci, 1998, 63, 458–465.
88. Rodrigez-Saona L.E., Giusti M.M., Wrolstad R.E., Color and pigment stability of red radish and red-fleshed potato anthocyanins in juice model systems. J. Food Sci., 1999, 3, 451–456.
89. Pszczola D., Emerging ingredients: believe it or not! Food Technol., 1999, 53, 98–104.

90. Sapers G.M., Taffer I., Ross L.R., Functional properties of food colorant prepared from red cabbage. J. Food Sci., 1981, 46, 105–109.
91. Sarma A.D., Sharma R., Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. Phytochem., 1999, 52, 1313–1318.
92. Sarma A.D., Sreelakshimi Y., Sharma R., Antioxidant ability of anthocyanins against ascorbic acid oxydation. Phytochem., 1997, 45, 671–674.
93. Shahidi F., Naczk M., Contribution of phenolic compounds to sensory characteristics of foods. 1995, *in*: Food Phenolics: Sources, Chemistry, Effects, Applications. A Technomic Publishing Comp. Inc., Lancaster, Pennsylvania, pp. 199–233.
94. Stintzing F.C., Stintzing A.S., Carle R., Wrolstad R.E., A novel zwitterionic anthocyanin from evergreen blackberry (*Rubus laciniatus* Willd.). J. Agric. Food Chem., 2002, 50, 396–399.
95. Strack D., Wray V., Metzger J.W., Grosse W., Two anthocyanins acylated with gallic acid from the leaves of *Victoria amazonica*. Phytochem., 1992, 3, 989–991.
96. Takeoka G.R., Dao L.T., Anthocyanins. 2002, *in*: Methods of Analysis for Functional Foods and Nutraceuticals (ed. W.J. Hurst). CRC Press, Inc. Boca Raton, Florida, pp. 219–241.
97. Tamura H., Yamagami A., Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. J. Agric. Food Chem., 1994, 42, 1612–1615.
98. Tatsuzawa F., Saito N., Seki H., Hara R., Yokoi M., Honda T., Acylated cyanidin glycosides in the red-purple flowers of *Phalaenopsis*. Phytochem., 1997, 45, 173–177.
99. Terahara N., Konczak I., Ono H., Yoshimoto M., Yamakawa O., Characterization of acylated anthocyanins in callus induced from storage root of purple-fleshed sweet potato, *Ipomea batatas* L. J. Biomed. Biotech., 2004, 5, 279–286.
100. Wang H., Cao G., Prior R., Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem., 1997, 45, 304–309.
101. Wang H., Nair M.G., Strasburg G.M., Chang Y., Booren A.M., Gray, J.I.. DeWitt D.L., Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J. Nat. Prod. 1999, 62, 294–296.
102. Wang J., Mazza G., Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-γ-activated RAW 2647 macrophages. J. Agric. Food Chem., 2002, 50, 850–857.
103. Wilska-Jeszka J., Different natural components of food. 1994, *in*: Food Chemistry – Composition, Transformation and Properties of Food (ed. E. Sikorski). WNT, Warszawa, pp. 491–515 (in Polish).
104. Wolniak M., Antioxidative mechanisms of antocyanidin and their meaning for alive organisms. Farmacja Polska, 2002, 58, 931–934 (in Polish).
105. Yoshida K., Kondo T., Kameda K., Goto T., Structure of anthocyanins isolated from purple leaves of *Perilla ocimoides* L. var. *crispa benth* and their isomerization by irradiation of light. Agric. Biol. Chem., 1990, 54, 1745–1751.
106. Yoshida K., Toyama Y., Kameda K., Kondo T., Contribution of each caffeoyl residue of the pigment molecule of gentiodelphin to blue color development. Phytochem., 2000, 54, 85–92.
107. Yoshida K., Okuno R., Kameda K., Kondo T., Prevention of UV-light induced E,Z-isomerization of caffeoyl residues in the diacylated anthocyanin, gentiodelphin, by intramolecular stacking. Tetrahedron Lett., 2002, 43, 6181–6184.
108. Yoshida K., Mori M., Kawachi M., Okuno R., Kameda K., Kondo T., A UV-B resistant polyacylated anthocyanin, HBA, from blue petals of morning glory. Tetrahedron Lett., 2003a, 44, 7875–7880.
109. Yoshida K., Okuno R., Kameda K., Mori M., Kondo T., Influence of E,Z-isomerization and stability of acylated anthocyanins under the UV irradiation. Biochem. Eng. J., 2003b, 14, 163–169.
110. Zając K.B., Wilska-Jeszka J., Anthocyanins – natural colorants for food industry. Part I. Structure, properties and methods of isolation – a review. Przem. Ferm. Owoc. Warz., 1991, 12, 21–24 (in Polish).
111. Zając K.B., Wilska-Jeszka J., Anthocyanins – the factors influence on colorants stability and colour. 1994, *in*: Materials of Conference on Natural Colorants and Aromatic Dyes, Łódź, Poland, pp. 13–17 (in Polish).

Received February 2005. Revision received and accepted March 2005.

ANTOCYJANY ACYLOWANE JAKO STABILNE, NATURALNE BARWNIKI ŻYWNOŚCI – ARTYKUŁ PRZEGLĄDOWY

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Wraz ze wzrostem świadomości konsumentów rośnie ich zainteresowanie produktami spożywczymi, które oprócz zaspokojenia głodu, spełniają dodatkowe funkcje fizjologiczno-żywieniowe, wpływając na poprawę zdrowia lub zapobiegając chorobom takim jak: nowotwory, cukrzyca, miażdżyca, nadciśnienie czy próchnica. Te szczególne potrzeby konsumentów zmuszają producentów żywności do rezygnacji ze stosowania barwników syntetycznych i zastosowania naturalnych, aktywnych biologicznie barwników, m.in. antocyjanowych, do barwienia żywności. Związane to jest jednak ze znacznymi ograniczeniami, gdyż naturalne barwniki antocyjanowe są związkami bardzo niestabilnymi. Odkrycie antocyjanów acylowanych o dużej stabilności budzi nadzieję, że barwniki te nadadzą pożądany, trwały kolor produktom żywnościowym. Wysoka stabilność barwników acylowanych związana jest z utworzeniem kompleksu w formie „kanapki", który osłania cząsteczkę przed hydratacją w pozycji C-2 i C-4. Kompleks ten powstaje w wyniku utworzenia wiązania pomiędzy resztą aromatyczną grupy acylowej a pierścieniem pyryliowym kationu flawyliowego (rys. 5).

Antocyjany acylowane występują w wielu kwiatach, owocach i warzywach, jednak zastosowanie w przemyśle może znaleźć jedynie kilka z nich. Są to: czarna kapusta zawierająca w większości poliacylowane antocyjany (rys. 6), czarna marchew (zawierająca pojedynczo acylowane antocyjany (rys. 7), czerwona rzodkiewka czy czerwona kukurydza. Preparaty acylowanych antocyjanów otrzymane z wymienionych surowców cechują się małą wrażliwością na zmiany pH, wysoką stabilnością podczas ogrzewania i naświetlania oraz małą zawartością polifenoli, dzięki czemu dodatek tych preparatów do produktów zawierających białka nie powoduje zmętnień.

Te wyjątkowe zalety preparatów antocyjanów acylowanych stwarzają realne nadzieje na wykorzystanie ich do barwienia produktów o obojętnym lub lekko zasadowym odczynie, jak produkty mleczne, desery w proszku czy polewy lukrowe.