JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Anthocyanins from Bay (*Laurus nobilis* L.) Berries

LUIGIA LONGO AND GIUSEPPE VASAPOLLO*

Dipartimento di Ingegneria dell'Innovazione, Università di Lecce, via per Arnesano km 1, 73100 Lecce, Italy

Anthocyanin composition in the berries of *Laurus nobilis* L., a perennial tree or shrub typical of the Mediterranean region, was determined for the first time. The pigments were extracted from the berries with 0.1% HCl in methanol, purified on a C-18 solid-phase cartridge, and characterized by means of high-performance liquid chromatography (HPLC)-diode array detection (DAD)-mass spectrometry (MS) analysis. The major anthocyanins were characterized as cyanidin 3-*O*-glucoside (41%) and cyanidin 3-*O*-rutinoside (53%). Furthermore, two minor anthocyanins were detected and identified as 3-*O*-glucoside and 3-*O*-rutinoside derivatives of peonidin (5%). The two major pigments were also isolated by preparative HPLC and characterized by H^1 nuclear magnetic resonance (NMR) spectroscopy. The attractive color and the great abundance of the plant in the south of Italy make *Laurus nobilis* berries a new and very good source of natural pigments.

KEYWORDS: Anthocyanins; *Laurus nobilis*; HPLC-DAD-MS; berries; natural colorants; antioxidant agents

INTRODUCTION

Nowadays, there is an increasing interest in the development of colorants from natural sources, especially in food industries, favored essentially by the social trend toward the consumption of natural products instead of synthetic ones. Among the natural pigments, anthocyanins are the target of numerous studies, due to their characteristic colorant and antioxidant properties. Anthocyanins are phenolic plant metabolites belonging to the flavonoid family responsible for the red, blue, and purple colors of many fruits, berries, and flowers (*1*). They are nontoxic, water-soluble compounds and can find application in food industries as natural colorants (*2*). These pigments also have many human health beneficial effects, mainly due to their antioxidant properties (*3*−*5*). For this reason, new sources of anthocyanins with high colorant power, stability, and low cost are nowadays desired.

Laurus nobilis L., commonly known as bay, bay laurel, or sweet bay, is a perennial pyramid-shaped tree or a large shrub with aromatic, evergreen leaves and shiny gray bark of the family Lauraceae (*6*). It is native of the Mediterranean region and Asia Minor; currently, it is both cultivated and collected from the wild in many Mediterranean countries. The leaves are alternate, lanceolate, coriaceous, and shining with wavy margins. Clusters of small yellow flowers are produced in spring, followed, on the female plants, by shiny purplish black berries (about 1.3 cm long) in autumn. Bay leaves (fresh or dried), fruits, and essential oils are used extensively in the food industry for seasoning of soups, meat, fish, beverages, etc. (*7*) and as a food preservative, due to their antimicrobial and insecticide activities (*8*, *9*). The essential oil is used as a folk medicine, especially for rheumatism and dermatitis. It is also used by the cosmetic industry in creams, perfumes, and soaps; it was reported to be used in the preparation of hair lotions for its antidandruff activity and for the external treatment of psoriasis (*10*, *11*).

The chemical composition of bay leaves has been extensively studied. Flavones (apigenin and luteolin) and flavonols (kaempferol, myricetin, and quercitin) have been studied in bay leaves (*12*, *13*). Volatile constituents of leaves, buds, flowers, and fruits from bay have also been determined (*14*). In addition, the leaves contain tocopherols (*15*), tannins (*16*), sesquiterpenoid lactones, and isoquinoline alkaloids (*17*).

Anthocyanins have been studied in bay shoots and leaves with cyanidin being the main pigment (*18*, *19*). To our knowledge, the anthocyanin composition of bay berries has never been described. Continuing our work in this field (*20*, *21*), we report in this paper the profile of anthocyanins in bay berries for their potential application as natural colorants and antioxidant agents by food, pharmaceutical, and cosmetic industries.

MATERIALS AND METHODS

Reagents and Standards. Trifluoroacetic acid (TFA) (99.9%) was purchased from Romil Ltd. (Cambridge, United Kingdom), and formic acid (98−100%) and sodium tetraborate (99%) were from Aldrich (Steinheim, Germany). Hydrochloric acid (36−38%), acetonitrile, methanol, and water of high-performance liquid chromatography (HPLC) grade and methanol of analytical grade were provided by J. T. Baker (Deventer, Holland). Methanol-d_4 (99.8 Atom%D) was purchased from Armar Chemicals (Döttingen, Germany), and cyanidin 3-*O*-glucoside was from Extrasynthese (Genay, France). Deionized water was used to prepare all solutions.

Samples. Wild grown *Laurus nobilis* berries were hand harvested in Lecce, Italy, during November 2004, placed in polyethylene bags, and stored at −20 °C until use. The plant was classified at the

* Author to whom correspondence should be addressed [telephone +39-(0)832-297252; fax +39-(0)832-297252; e-mail giuseppe.vasapollo@unile.it].

10.1021/jf051400e CCC: $30.25
Published on Web 09/07/2005

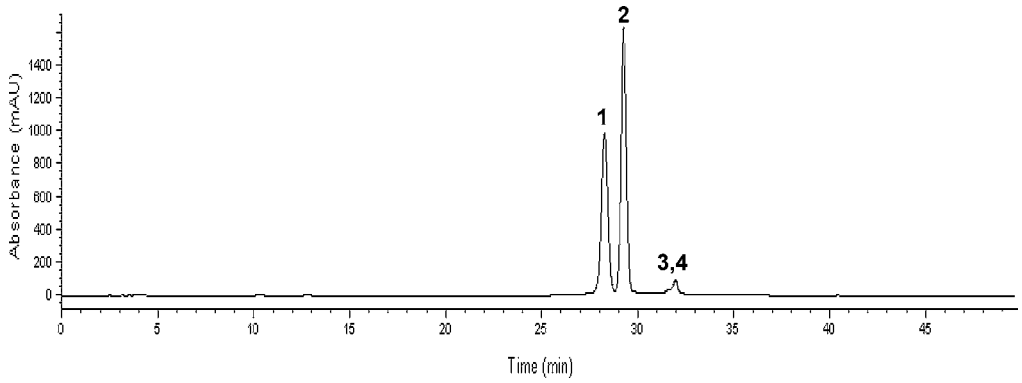

Figure 1. HPLC-DAD chromatogram recorded at 520 nm corresponding to the purified extract of *Laurus nobilis* berries.

Systematic Botanic and Vegetable Ecology Laboratory of the Department of Biological and Environmental Science and Technology, University of Lecce (Italy), as *Laurus nobilis* L.

Extraction of Anthocyanins. The stoned berries (16 g) were extracted in the dark by stirring with 100 mL of 0.1% HCl (v/v) in methanol for 20 h at room temperature. The samples were filtered on a Buchner funnel, and the solid residue was washed with an additional 50 mL of 0.1% HCl (v/v) in methanol. Filtrates were combined and dried using a rotary evaporator at 30 °C. The remaining solid was dissolved in 0.01% HCl (v/v) in deionized water and successively purified.

Purification of Anthocyanins. The anthocyanin aqueous solution obtained from the extraction procedure described before was passed through a 1 g sorbent weight C-18 Sep-Pak cartridge (Waters Corp., Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (v/v). Anthocyanins and other polyphenolics were adsorbed onto the Sep-Pak column, while sugars, acids, and other water-soluble compounds were removed by washing the cartridge with 2 volumes of 0.01% aqueous HCl (v/v). Less polar polyphenolics were subsequently eluted with 2 volumes of ethyl acetate. Anthocyanins were then eluted with methanol containing 0.01% HCl (v/v). The acidified methanol solution was evaporated using a rotary evaporator at 30 °C. The remaining solid was dissolved in 0.01% HCl (v/v) aqueous solution to have a known concentration solution (3 mg/mL) and immediately analyzed. This solution was stored at −20 °C until used for successive acid and alkaline hydrolyses.

Acid Hydrolysis of Anthocyanins. First, 5 mL of 2 N HCl was added to 1 mL of the purified anthocyanin solution (3 mg/mL) in a screw-cap test tube, flushed with nitrogen, and capped. The pigments were hydrolyzed for 1.5 h at 100 °C; then, the solution was immediately cooled in an ice bath (*22*). The hydrolysate was purified by using a 500 mg sorbent weight C-18 Sep-Pak cartridge (Waters) as previously described.

Alkaline Hydrolysis of Anthocyanins. First, 1 mL of the purified anthocyanin solution (3 mg/mL) was saponified in a screw-cap test tube with 5 mL of 10% KOH for 8 min in the dark at room temperature (*22*). The solution after neutralization with 2 N HCl was purified by using a 500 mg sorbent weight C-18 Sep-Pak cartridge (Waters) as previously described.

Selective Elution of Anthocyanins with Alkaline Borate Buffer. The purified anthocyanins were adsorbed onto a 500 mg sorbent weight C-18 Sep-Pak cartridge (Waters), previously activated with methanol followed by 0.01% aqueous HCl. Alkaline borate buffer (0.1 N sodium tetraborate, ca. pH 9) was passed through the column until the eluant was colorless. The anthocyanins were reconverted into their red oxonium salt form by passing 2 N HCl through the column (*23*). Washing with borate buffer and HCl was repeated one additional time. The anthocyanins remaining on the cartridge were subsequently eluted with 0.01% HCl (v/v) in methanol. The acidified methanol solution was evaporated using a rotary evaporator at 30 °C. The anthocyanins were then dissolved in 0.01% HCl (v/v) aqueous solution and immediately analyzed.

Analytical HPLC-DAD-MS System. The high-performance liquid chromatography (HPLC)-diode array detection (DAD)-mass spectrometry (MS) analytical analyses were performed using an Agilent 1100 Series LC/MSD system with a diode array detector (DAD) coupled to a mass spectrometer (quadrupole analyzer) equipped with an electrospray ionization interface (ESI, Agilent). Chromatographic separation was carried out using a 150 × 4.6 mm i.d., 5 μm SS Wakosil C18 with a 4 × 3 mm i.d. Phenomenex C18 guard cartridge both thermostated at 32 °C. The mobile phase was composed of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) at a flow rate of 1 mL/min. The following gradient was utilized: 0 min, 10% B; 0−20 min, 10% B; 30 min, 20% B; 50 min, 30% B. Absorbance spectra were recorded every 2 s, between 250 and 600 nm, with a bandwidth of 4 nm, and chromatograms were acquired at 520, 440, 310, and 280 nm. MS parameters were as follows: capillary voltage, 4000 V; fragmentor, 160 V; drying gas temperature, 350 °C; gas flow (N_2), 10 L/min; nebulizer pressure, 50 psig. The instrument was operated in positive ion mode scanning from *m*/*z* 100 to 800 at a scan rate of 1.43 s/cycle. The wavelength used for quantification was 520 nm. The calibration curve was produced by the integration of absorption peaks generated from the analysis of dilution series of cyanidin 3-glucoside.

Preparative HPLC System. The major anthocyanins were isolated by a preparative HPLC system consisting of a Shimadzu model LC-8A pump, a Shimadzu SCL-10A VP system controller, a manual injector fitted with a 1 mL sample loop, and a Shimadzu model SPD-10A UV/vis detector equipped with a preparative flow cell. A 250 × 21.2 mm i.d., 7 μm Zorbax Stable Bond-C18 preparative column, coupled to a 50 × 21.2 mm i.d., 5 μm Zorbax Stable Bond-C18 guard column, was used for the separation and isolation of anthocyanins. The mobile phase was formic acid/water/methanol (10:75:15 v/v). The isocratic flow rate was 20 mL/min, and the detector was set at 520 nm. The anthocyanin fractions of interest were isolated, and their purity was checked by analytical HPLC-DAD-MS analysis as previously described. Each fraction was evaporated using a rotary evaporator at 30 °C to remove completely the methanol and then loaded on a 5 g sorbent weight C-18 Sep-Pak cartridge (Waters) previously activated with methanol followed by 0.01% aqueous HCl (v/v). The cartridge was rinsed with 5 volumes of 0.01% aqueous HCl (v/v), and the adsorbed anthocyanin was eluted with 0.1% HCl (v/v) in methanol. The eluent solution was evaporated to dryness using a rotary evaporator at 30 °C, and the resulting solid was resolubilized with the suitable solvent for NMR analysis.

NMR Analysis. ^{1}H NMR spectral data were recorded on a Bruker Advance 400 instrument operating at 400 MHz in CD_3OD with TMS as internal standard. Sample temperature was stabilized at 25 °C.

RESULTS AND DISCUSSION

The anthocyanin composition of bay berries was determined by means of HPLC-DAD-MS analysis. The chromatogram of the purified anthocyanin extract from bay berries, recorded at 520 nm, is shown in **Figure 1**. As can be seen, there are three peaks in the chromatogram, but the MS analysis revealed that the last peak corresponded to two different pigments (compounds **3** and **4**) that coeluted under the conditions employed

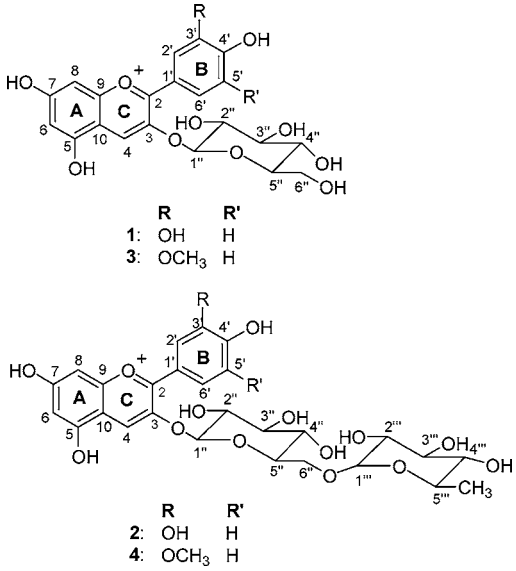

Figure 2. Chemical structures of the anthocyanins identified in *Laurus nobilis* berries: **1**, cyanidin 3-*O*-glucoside; **2**, cyanidin 3-*O*-rutinoside; **3**, peonidin 3-*O*-glucoside; **4**, peonidin 3-*O*-rutinoside.

Table 1. Chromatographic, Spectroscopic, and Spectrometric Characteristics of the anthocyanins found in *Laurus nobilis* Berries

peak no. (**Figure 1**)	t_R (min)	λ_{max} (nm)	M^+ (*m*/*z*)	$M^+ - X$ (*m*/*z*)	peak assignment
1	28.2	280, 516	449	287 ($M^+ -$ glu)	cyanidin 3-*O*-glucoside
2	29.2	280, 520	595	287 ($M^+ -$ rut)	cyanidin 3-*O*-rutinoside
3	31.6	278, 520	463	301 ($M^+ -$ glu)	peonidin 3-*O*-glucoside
4	31.9	280, 520	609	301 ($M^+ -$ rut)	peonidin 3-*O*-rutinoside

in this work, indicating the presence of four different anthocyanins in bay berries. These four anthocyanins, the structures of which are shown in **Figure 2**, were identified by comparison of HPLC retention times, elution order, photodiode array UV/vis spectroscopic, and ESI-MS spectrometric data (**Table 1**) with our anthocyanin library and published data (*2*, *22*, *24*, *25*). Chromatographic and spectroscopic characteristics of the co-eluted anthocyanins **3** and **4** were determined by analyzing the initial and the end part of the corresponding peak, respectively.

The two major anthocyanins corresponding to peaks **1** and **2** (**Figure 1**) represented about 41% and 53%, respectively, of the total peak area revealed at 520 nm. Peak **1** was identified as cyanidin 3-*O*-glucoside on the basis of its λ_{max} of 516 nm and a mass spectrum comprising a M^+ at *m*/*z* 449 and a fragment ion at *m*/*z* 287 resulting from the loss of the glucose molecule ($M^+ - 162$). Peak **2** had a λ_{max} of 520 nm and a mass spectrum consisting of a M^+ at *m*/*z* 595 and a fragment ion at *m*/ *z* 287 resulting from the loss of the rutinose molecule ($M^+ - 308$). It corresponded therefore to cyanidin 3-*O*-rutinoside. The last peak (**Figure 1**), as previously mentioned, contained two different anthocyanins (compounds **3** and **4**). The ESI-MS profiles of these compounds presented the molecular ions M^+ at *m*/*z* 463 (**3**) and 609 (**4**) and the fragment ions at *m*/*z* 301 resulting from the loss of the glucose and rutinose molecules, respectively, and corresponding to the molecular ion of the aglycone peonidin. Compounds **3** and **4** were therefore identified as peonidin 3-*O*-glucoside and peonidin 3-*O*-rutinoside, respectively. The absorbance spectra of these compounds confirmed their identity.

The $Abs_{440}/Abs_{\lambda max}$ ratio values calculated for each anthocyanin, ranging from 31% to 32%, indicated a substitution in the C-3 position of the flavylium ring (*26*). It is well known that anthocyanins with glycosidic substitutions at position 3 exhibit a ratio of the absorbance at 400−440 nm to the absorbance at the visible maximum wavelength (520 nm) that is almost twice as large as for anthocyanins with glycosidic substitution at position 5 or both 3 and 5 (*27*). In addition, the obtained $Abs_{280}/Abs_{\lambda max}$ (67−100%) and $Abs_{310}/Abs_{\lambda max}$ (13−22%) ratios confirmed that bay berry anthocyanins were simple anthocyanin molecules without acylation of glycoside with aromatic acids (*28*, *29*). Alkaline hydrolysis of the purified anthocyanins produced a chromatographic profile similar to that reported in **Figure 1** confirming that the anthocyanins of bay berries were not acylated (*23*).

Acid hydrolysis of the purified anthocyanins produced two peaks (**5** and **6**), as shown in the chromatogram of **Figure 3**. The ESI-MS profiles of these compounds presented the molecular ions M^+ at *m*/*z* 287 (**5**) and 301 (**6**) corresponding to the molecular ions of cyanidin and peonidin, respectively. The absorbance spectra of these compounds confirmed their identity.

Figure 4 shows the chromatogram of the fraction of bay berry anthocyanins that remained on the C-18 Sep-Pak cartridge after elution with alkaline borate solution. It is known that borate ions react with phenols possessing *o*-dihydroxy groups, forming negatively charged complexes (*30*). In the treatment of nonacylated anthocyanins with borate, the compounds possessing *o*-dihydroxy groups (cyanidin derivatives) formed a charged borate complex, resulting in more hydrophilic species that were preferentially eluted from the C-18 cartridge, while anthocyanins not containing *o*-dihydroxy groups (peonidin derivatives) did not complex with borate ions and were concentrated on the cartridge (*23*). This technique provided therefore an additional method for the characterization of bay berry anthocyanins.

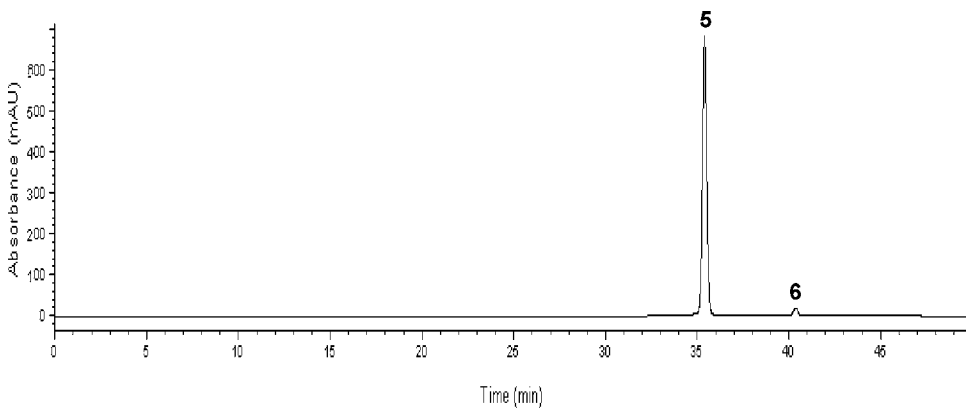

Figure 3. HPLC-DAD chromatogram recorded at 520 nm corresponding to the purified extract of *Laurus nobilis* berries after acid hydrolysis.

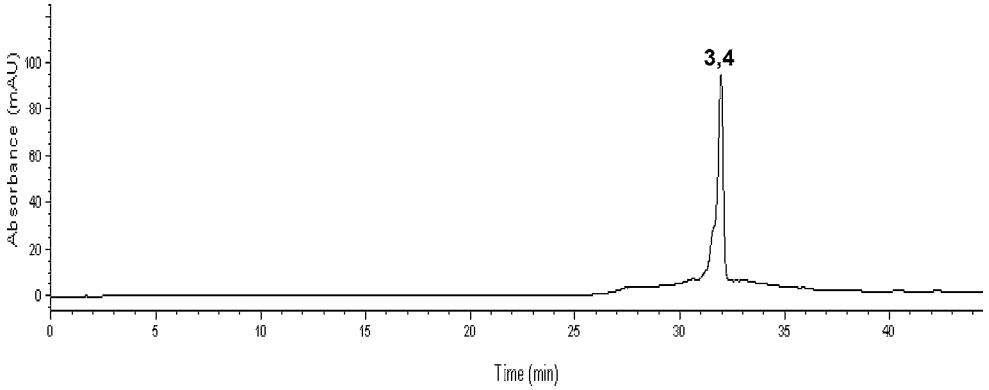

Figure 4. HPLC-DAD chromatogram recorded at 520 nm corresponding to the fraction of the anthocyanins of *Laurus nobilis* berries remaining on a C-18 Sep-Pack cartridge after elution with alkaline borate buffer.

Table 2. H^1 NMR Spectroscopic Data of Two Anthocyanins (**1**, **2**) Isolated from the Extract of *Laurus nobilis* Berries (δ in CD_3OD at 25 °C)

anthocyanin	**1** δ (*J* in Hz)	**2** δ (*J* in Hz)
aglycone		
H-4	9.03 s	8.96 s
H-6	6.66 d (2.0)	6.68 d (1.9)
H-8	6.91 brs	6.91 brs
H-2′	8.05 d (2.4)	8.04 d (2.4)
H-5′	7.03 d (8.7)	7.03 d (8.7)
H-6′	8.28 dd (2.4, 8.7)	8.29 dd (2.4, 8.7)
3-glucosyl		
H-1″	5.30 d (7.8)	5.29 d (7.6)
6″-rhamosyl		
H-1‴		4.65 brs

Among the four pigments detected in bay berries, the two major anthocyanins (compounds **1** and **2**, **Figure 1**) were isolated by preparative HPLC and their identity and structure were confirmed on the basis of H^1 NMR spectroscopic data. The chemical shifts (δ) obtained from the H^1 NMR analysis of anthocyanins **1** and **2** were reported in **Table 2**. Signals in the downfield of the spectra between δ 6.7 and 9.0 ppm were clearly attributable to the aromatic protons (A and B rings) of the aglycone molecule as previously reported (*31*, *32*) for these compounds. The signal doublets at δ 5.3 corresponded to the protons on the anomeric carbon from the glucose residues, confirming that they were in position C-3 as also indicated by the $Abs_{440}/Abs_{\lambda max}$ ratio values. The β-configuration of this moiety was confirmed from the magnitude ($J = 7.6$ Hz) of the $J_{1''2''}$ coupling constant in the H^1 NMR spectra (*33*, *34*). The spectrum of the compound **2** presented a doublet at δ 5.29 (d, $J = 7.6$ Hz, H-1 glucose) and a broad singlet at δ 4.65 (brs, H-1 rhamnose) confirming the presence of rutinose as sugar moiety (*32*, *35*). It was not possible to confirm by means of NMR analysis the identity of the anthocyanins **3** and **4** (**Figure 1**), which were characterized as glucoside and rutinoside derivatives of peonidin, respectively, by means of HPLC-DAD-MS analysis; their low concentration in the bay berry extract did not enable us to preparatively isolate these minor pigments for their NMR characterization.

The total amount of anthocyanins in bay berries, determined on the cyanidin 3-glucoside basis, was 217 mg/g of stoned fresh berries. Cyanidin 3-*O*-rutinoside was the most predominant anthocyanin (116 mg/g) followed by cyanidin 3-*O*-glucoside (90 mg/g). The total amount of peonidin 3-*O*-glucoside and peonidin 3-*O*-rutinoside was 10.6 mg/g.

To our knowledge, this is the first time that the anthocyanin composition of *Laurus nobilis* berries has been described. Because these berries have traditionally been used in human diet and they are in great abundance in the Mediterranean region, they could be used for the extraction of anthocyanins to be employed as food colorants and antioxidant agents by food, pharmaceutical, and cosmetic industries.

ACKNOWLEDGMENT

We thank PIERRE CHIMICA S.r.l (Galatina, Lecce, Italy) and Consorzio INCA (Venice, Italy).

LITERATURE CITED

(1) Cooper-Driver, G. A. Contributions of Jeffrey Harborne and co-workers to the study of anthocyanins. *Phytochemistry* **2001**, *56*, 229−236.

(2) Pazmiño-Duràn, E. A.; Giusti, M. M.; Wrolstad, R. E.; Glòria, M. B. A. Anthocyanins from banana bracts (*Musa* X paradisiaca) as potential food colorants. *Food Chem.* **2001**, *73*, 327−332.

(3) Wang, H.; Nair, M.; Strasburg, G.; Chang, Y.; Booren, A.; Gray, J.; DeWitt, D. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *J. Nat. Prod.* **1999**, *62*, 294−296.

(4) Wang, S.; Jiao, H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J. Agric. Food Chem.* **2000**, *75*, 5677−5684.

(5) Tamura, H.; Yamaganci, A. Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. *J. Agric. Food Chem.* **1994**, *42*, 1612−1615.

(6) Leung, A. Y.; Foster, S. Alloro. *Enciclopedia delle Piante Medicinali*; Aporie: Roma, Italy, 1999; pp 30−31.

(7) Bauer, K.; Garbe, D. *Common Fragrance and Flavor Materials, Preparation, Properties and Uses*; VCH: Weinheim, Germany, 1985.

(8) Hammer, K. A.; Carson, C. F.; Rley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985−990.

(9) Saim, N.; Meloan, C. E. Compounds from leaves of bay (*Laurus nobilis* L.) as repellents for *Tribolium* castaneum wheat added to white flour. *J. Stored Prod. Res.* **1986**, *22*, 141−144.

(10) Hafizoglu, H.; Reunanen, M. Studies on the components of *Laurus nobilis* from Turkey with special references to laurel berry fat. *Fat Sci. Technol.* **1993**, *95*, 304−308.

(11) Furia, T. E.; Bellance, N. *Feranoli's Handbook of Flavor Ingredients*, 2nd ed.; CRC Press: Cleveland, OH, 1975; Vol. I.

(12) Škerget, M.; Kotnik, P.; Hadolin, M.; Hraš, A. R.; Simonič, M.; Knez, Ž. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* **2005**, *89*, 191−198.

(13) Knackstedt, J.; Herrmann, K. Flavonol glycosides of bay leaves (*Laurus nobilis* L.) and star anise fruits (*Illicium verum Hook. fil.*). 7. Phenolics of spices. *Z. Lebensm.-Unters.-Forsch.* **1981**, *173*, 288−290.

(14) Kilic, A.; Hafizoglu, H.; Kollmannsberger, H.; Nitz, S. Volatile constituents and key odorants in leaves, buds, flowers, and fruits of *Laurus nobilis* L. *J. Agric. Food Chem*. **2004**, *52*, 1601−1606.
(15) Gòmez-Coronado, D. J. M.; Barbas, C. Optimized and validated HPLC method for α- and γ-tocopherol measurement in *Laurus nobilis* leaves. New data on tocopherol content. *J. Agric. Food Chem*. **2003**, *51*, 5196−5201.
(16) Sakar, M. K.; Rolf, E. Tannin producing monomeric and dimeric substances in bay leaves (*Laurus nobilis* L.). *Z. Lebensm.-Unters.-Forsch.* **1985**, *180*, 494−495.
(17) Bruneton, J. *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2nd ed.; Lavoisier Publishing: Paris, 1999.
(18) Grigoryants, L. G. Dynamics of anthocyanins in Grecian laurel leaves and shoots. *Tr. Sukhum. Bot. Sada* **1980**, *26*, 60−68.
(19) Grigoryants, L. G. Anthocyanins in Grecian laurel shoots. *Byull. Gl. Bot. Sada* **1981**, *119*, 51−54.
(20) Longo, L.; Vasapollo, G. Determination of anthocyanins in *Ruscus aculeatus* L. berries. *J. Agric. Food Chem.* **2005**, *53*, 475−479.
(21) Longo, L.; Vasapollo, G.; Rescio, L. Identification of anthocyanins in *Rhamnus alaternus* L. berries. *J. Agric. Food Chem.* **2005**, *53*, 1723−1727.
(22) Chaovanalikit, A.; Thompson, M. M.; Wrolstad, R. E. Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *J. Agric. Food Chem.* **2004**, *52*, 848−852.
(23) Hong, V.; Wrolstad, R. E. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *J. Agric. Food Chem*. **1990**, *38*, 708−715.
(24) Ordaz-Galindo, A.; Wesche-Ebeling, P.; Wrolstad, R. E.; Rodriguez-Saona, L.; Argaiz-Jamet, A. Purification and identification of Capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chem*. **1999**, *65*, 201−206.
(25) De Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. LC-MS analysis of anthocyanins from purple corn cob. *J. Sci. Food. Agric.* **2002**, *82*, 1003−1006.
(26) Giusti, M. M.; Rodrìguez-Saona, L. E.; Wrolstad, R. E. Molar absorptivity and color characteristics of acylated and nonacylated pelargonidin-based anthocyanins. *J. Agric. Food Chem.* **1999**, *47*, 4631−4637.
(27) Harborne, J. B. *Comparative biochemistry of the flavonoids*; Academic Press: New York, 1976.
(28) Woodall, G. S.; Stewart, G. R. Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? *J. Exp. Bot.* **1998**, *49*, 1447−1450.
(29) Mozetic, B.; Trebse, P.; Hribar, J. Determination and quantification of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Gorica Region (Slovenia). *Food Technol. Biotechnol.* **2002**, *40*, 207−212.
(30) Pridham, J. B. Paper Electrophoresis of Phenolic Compounds. *Methods of Polyphenol Chemistry*; Pergamon Press: New York, 1964.
(31) Kuskoski, E. M.; Vega, J. M.; Rios, J. J.; Fett, R.; Troncoso, A. M.; Asuero A. G. Characterization of anthocyanins from the fruits of baguaçu (*Eugenia umbelliflora* Berg). *J. Agric. Food Chem*. **2003**, *51*, 5450−5454.
(32) Frøytlog, C.; Slimestad, R.; Andersen, Ø. M. Combination of chromatographic techniques for the preparative isolation of anthocyanins − applied on blackcurrant (*Ribes nigrum*) fruits. *J. Chromatogr., A* **1998**, *825*, 89−95.
(33) Pale, E.; Nacro, M.; Vanhaelen, M.; Vanhaelen-Fastrè, R. Anthocyanins from bambara groundnut (*Vigna* subterranea). *J. Agric. Food Chem*. **1997**, *45*, 3359−3361.
(34) Gonnett, J. F.; Fenet, B. "Cyclamen red" colors based on a macrocyclic anthocyanin in Carnation flowers. *J. Agric. Food Chem.* **2000**, *48*, 22−26.
(35) Fossen, T.; Øvstedal, D. O.; Slimestad, R.; Andersen, Ø. M. Anthocyanins from a Norwegian potato cultivar. *Food Chem*. **2003**, *81*, 433−437.

Received for review June 13, 2005. Revised manuscript received July 22, 2005. Accepted July 22, 2005. We acknowledge MIUR (Rome, Italy) for financial support (progetto PON "Estrazione di antocianine e altri principi attivi mediante CO_2 supercritica da substrati vegetali").

JF051400E