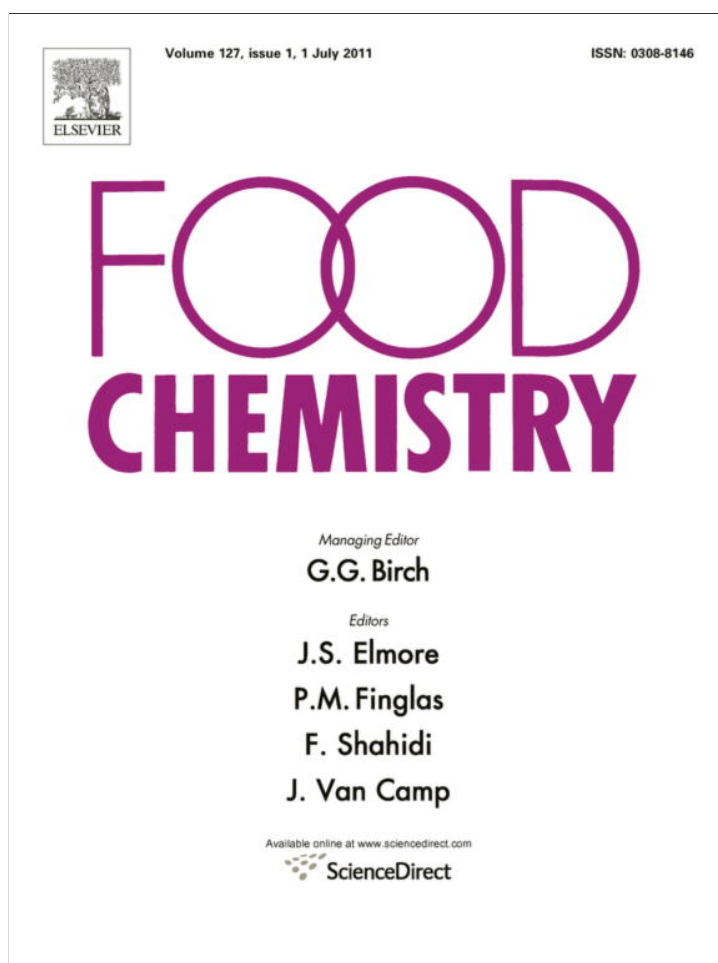


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Betalainic and nutritional profiles of pigment-enriched red beet root (*Beta vulgaris* L.) dried extracts

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ABSTRACT

Red beet root (*Beta vulgaris* L.) colourants (betalains) are available either as concentrates produced by evaporating beet juice under vacuum, or as powders made by spray-drying a concentrate. The degradation of the pigments is dependent upon temperature, duration of heat treatment, pH and water activity of a product. Recently, a new proprietary method of large-scale chromatographic purification of red beet root extract has been discovered and developed that allows the production of more concentrated betalain formulations. In order to trace the betalainic compositions of the new products in comparison to the currently in-use spray-, air- and freeze-dried concentrates, a chromatographic study on betalains and their degradation derivatives analysed by LC-DAD-ESI-MS/MS was performed. Additionally, nutritional characterisation of the extracts was accomplished.

Besides the most prominent betanin/isobetanin, elevated levels of neobetanin, which previously had been frequently detected in red beet roots, were also observed in the product prepared by the new method. The principal betanin/isobetanin pigments measured spectrophotometrically, comprised a major portion of the total betalain content (41%), the highest concentration of betalains reported in industrial products to date. The presence of other decarboxylated and dehydrogenated betanin derivatives in the new products is also discussed.

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1. Introduction

During the past decades, there has been significant growing consumer interest in natural foods without stabilisers or artificial additives. As a result, natural pigments of plant or animal origin are being used to replace artificial colourants (Henry, 1996). Therefore, new natural colourants are being sought which could be healthier, or at least not harmful, alternatives.

Of the numerous natural sources of betalains, red beet, amaranthus, *Hylocereus polyrhizus* (Weber) Britton and Rose, and prickly pear are food products that are richest in these pigments. Red beet (*Beta vulgaris* L.) roots have generally been the most popular source of betalain-based food colourants.

Red beet roots contain two groups of betalain pigments: red-violet betacyanins and yellow betaxanthins. The main betacyanin present in the roots is betanin (Fig. 1). The betacyanin and betaxanthin concentration ratio usually ranges from 1 to 3 and depends mainly on beet varieties, as well as on the respective technology

of juice or extract production. Mixing the yellow and violet pigments results in a range of red colour varieties. The average amount of betalains in red beet has been estimated as 1000 mg/100 g of total solids, or 120 mg/100 g of fresh weight (Marmion, 1991).

Red beet colourant is available as concentrates produced by evaporating beet juice under vacuum to a total solids content of 40–60%, or as powders made by spray-drying the concentrate (Marmion, 1991). The powdered products are more stable due to lower degradation rate of the pigments.

The thermal stability of betalains depends on numerous physicochemical conditions of which heating conditions are the most important factors. Recent comprehensive studies established the directions of thermal degradation of betalains in their aqueous preparations (Herbach, Stintzing, & Carle, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005) and the preliminary qualitative results on degradation of betacyanins in alcoholic solutions have been published (Wybraniec, 2005). Structural studies on thermal decarboxylation of betacyanins from red beet (*B. vulgaris* L.) (Herbach, Stintzing, & Carle, 2004; Wybraniec, 2005) and purple pitaya (*H. polyrhizus* (Weber) Britton and Rose) (Wybraniec & Mizrahi, 2005) preparations in aqueous solutions revealed several

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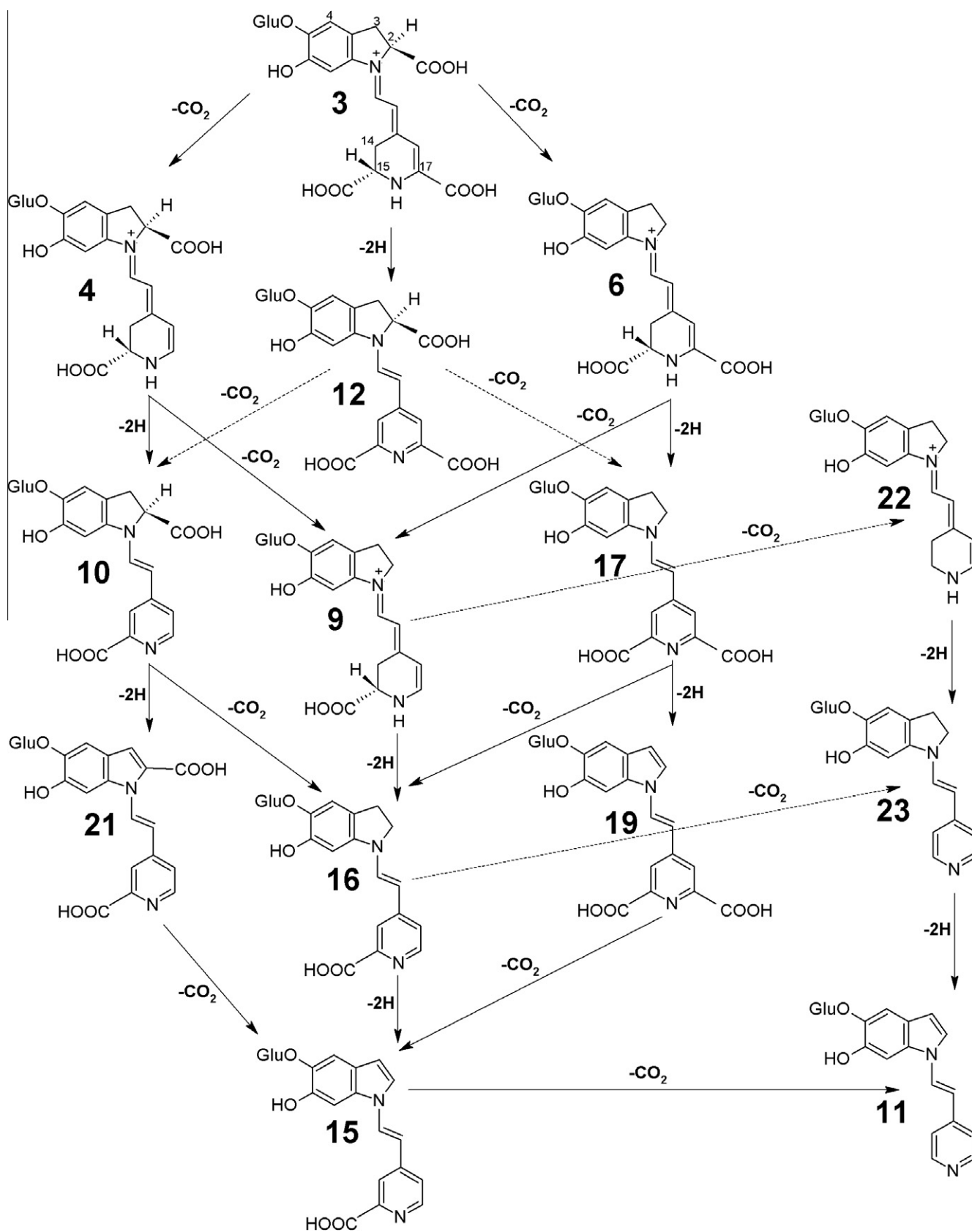


Fig. 1. Frequent routes of betanin 3 decarboxylation (starting at C-2 or C-17) and dehydrogenation (starting at C-14,15) possible to occur during the process of pigment enrichment in the red beet root extracts (compound designation in Table 1). Pigments 21, 22 and 23 were not detected in the analysed samples.

mono- and bidecarboxylated as well as 14,15-dehydrogenated betacyanins. Some of these pigments were analysed by LC-MS

for the first time in thermally obtained complex mixtures resulting in frequently overlapped chromatographic peaks of not yet identi-

fied compounds. Additional conditions influencing the stability of the pigments are the presence of light, oxygen, and heavy metal cations as well as other factors including water activity (Pasch & von Elbe, 1975) and the presence/absence of chelating agents or antioxidants (e.g. ascorbic acid).

Recent studies described preparation of spray-dried formulations containing betalains in extracts of red beet (Azeredo, Santos, Souza, Mendes, & Andrade, 2007), amaranthus (Cai & Corke, 2000) and cactus pear (Díaz, López, Kerstupp, Ibarra, & Scheinvar, 2009; Moßhammer, Stintzing, & Carle, 2006; Obón, Castellar, Alacid, & Fernández-López, 2009; Rodríguez-Hernández, González-García, Grajales-Lagunes, Ruiz-Cabrera, & Abud-Archilla, 2005; Saenz, Tapia, Chávez & Paz, 2009). The effects of various carrier agents on the resulting products, and the properties of the different spray-dried amaranthus pigments and their stabilities were investigated (Cai & Corke, 2000). The betacyanin degradation rates during the storage and the process of spray-drying were also studied for red beet extracts (Azeredo et al., 2007). Relatively more research has been recently published on spray-dried cactus pear, an important emerging source of betalains. Since cactus pear juice is rich in sugars, the addition of a carrier (maltodextrin) is necessary to avoid clogging (Moßhammer et al., 2006).

Extensive studies on cactus pear juice concentrates and powders produced by rotary evaporation and freeze drying at laboratory scale in comparison to column evaporation and spray-drying at pilot plant-scale were performed by Moßhammer et al. (2006). The research indicated comparable and acceptable overall pigment retentions of 71–83% in all preparations. Moreover, the initial colour characteristics were retained for juice concentrates and fruit powders. However, taking other quality parameters into account, non-enzymatic browning and 5-hydroxymethylfurfural formation were observed after concentration at pilot-plant scale and freeze drying in comparison to juice concentrates (Moßhammer et al., 2006).

Despite accelerating research on spray-drying of betalain preparations, and serious efforts to optimise the associated process, there are persistent issues which limit the concentration of betalains in the resulting products. The most important drawback of the existing methods is the presence of substantial quantities of sugars in the starting material as well as the presence of maltodextrins in the resultant spray-dried products. Consequently, there is still a need for large-scale purification of the pigments that can eliminate the bulk of unwanted balast of impurities. Such methods would make possible the production of high-pigmented formulations ready for food or medicinal use and would open further fields of applications.

Recently, a new method of a large-scale chromatographic method of purification of red beet root extract has been discovered and patented (Pietrzkowski & Thresher, 2008) which allows for production of more concentrated betalain formulations. In order to compare the betalainic compositions of new products produced by the new method with existing enriched extracts, a chromatographic study on betalains and their degradation derivatives analysed by LC-DAD-ESI-MS/MS was performed. For a better perspective, nutritional analyses were also conducted.

2. Materials and methods

2.1. Sample material

Spray- (SD-1, SD-2, SD-3 and SD-4), air- (AD-1, AD-2, AD-3 and AD-4) and freeze- (FD-1 and FD-2) dried samples prepared from red beet roots were obtained from companies in Europe, Asia and North America. Sample SD-1 was obtained from Ven Katesh Food Industires (India); sample SD-2 was from Jaskulski Aromaty (Poland); sample SD-3 was from Diana Naturals (France); sample

SD-4 was from Celiko S.A. (Poland); sample AD-1 was from Howenia Enterprise CO., LTD (Taiwan); sample AD-2 was from FutureCeuticals, Inc. (Illinois, USA); sample AD-3 was from Hungarian Food Ingredients (Hungary); sample AD-4 was from Bioritm, Inc. (Russia); sample FD-1 was from Alberts Organics, Inc.(USA) and made from Wisconsin fresh red beets; sample FD-2 was from FutureCeuticals, Inc. (USA) and made from Illinois fresh red beets. A betalain-rich red beet extract (FC-1), a novel and proprietary food-based extract prepared from red beet roots, was obtained from FutureCeuticals, Inc. USA, where it was produced using a patent-pending technology (Pietrzkowski & Thresher, 2008), that does not require use of organic solvents, and that significantly reduces amounts of sugars in the final material and consequently enriches total betalains up to 41%. All samples were coded for betalain and nutritional analyses.

2.1.1. Sample material preparation procedures

2.1.1.1. Spray-drying process. In general, the spray-drying was performed according to the following procedure. Raw material beet root liquid concentrate was mixed with 50% maltodextrin (10 DE) as a carrier agent. The mixture was sprayed into the drying air and entered the chamber with an input temperature ca. 150 °C and output temperature ca. 68 °C. Final product moisture was within a range of 3–5% on all tested samples. Material was dried and ground to –40 USS mesh powder using a hammer mill.

2.1.1.2. Freeze-drying process. In general, for the freeze-drying, red beet roots were washed with water, dried and then sliced to 1/8" thickness. Sliced product was subsequently placed into freeze-drying trays at a depth of 0.5 inches and frozen to ca. –23 °C. Frozen drying trays were loaded into a commercial freeze-dryer and dried at a shelf temperature of ca. 60 °C for 24 h. Vacuum pressure during the drying cycle was within a range of 0.5–1.0 mm Hg. Final product moisture was 1.9% - 2.3% on all tested products. Dried product was then ground to a –40 USS mesh powder using a hammer mill.

2.1.1.3. Air-drying process. Air-drying process was performed generally by the following procedure. Beet root was washed with water, dried and then sliced to 1/8" thickness. Sliced product was subsequently placed on a stainless steel mesh belt and dried on a commercial forced-air dryer at ca. 60 °C and 40% relative humidity. Drying time was 6 h and maximum product temperature was ca. 60 °C. Final product moisture was within a range of 3–5.5% on all tested samples. Dried product was then ground to a –40 USS mesh powder using a hammer mill.

2.1.1.4. Betalain-rich red beet extraction process. Extraction started with commercially available beet juice (about 65 brix; obtained from SVZ International BV, Etten Leur, The Netherlands) having a total betalain content of about 0.6 wt.% on dry basis. The juice was filtered to remove particulates and the filtrate was used without further modification for chromatography. A column was packed with a hydrophobically modified silica resin (commercially available as Resin HSI-564 from VDF Futureceuticals; Momence, IL USA), and the filtered juice was passed through the column at between 1 and 50 bed volumes per hour at a loading of between 1 and 20 bed volumes. The pass fractions were discarded, and the betalains were eluted from the resin using a mild buffer at slightly basic pH (e.g., 0.1–0.2 M ammonium acetate in water, pH 8.2–8.4, at a temperature of 43 °C). The so-obtained eluate was freeze-dried without further modification to a dry product. Quantitative analysis of the product revealed a total betalain content of about 41 wt.%. The dry product was then ground using a rotating blade grinder to form a powder product that was passed through a 60 mesh sieve. The product was stable over at least 2 weeks storage

at 24 °C and 50% relative humidity without any observable changes in composition, free-flowability, or other parameters, and did not aggregate to larger particles or clumps.

2.2. Chromatographic betalain analysis

Concentrated red beet root (3–20 mg) preparations were shaken with 1 ml of water for 10 min under argon in 2 ml glass vials. The samples were centrifuged and the supernatant was analysed directly by HPLC without any purification. A Gynkotek HPLC system with UVD340U, Gynkotek HPLC pump Series P580 and thermostat (Gynkotek Separations, H.I. Ambacht, Netherlands) was used for chromatographic analysis. A Luna C-18(2) 250 × 3 mm I.D., 5 µm (Phenomenex, Torrance, CA, USA) analytical column was used. The following gradient system was used for separation of analytes: 3% (v/v) A in B at 0 min; gradient to 16% A in B at 17 min; gradient to 50% A in B at 30 min (A, acetonitrile; B, 2% formic acid in water). The injection volume was 10 µl and a flow rate of 0.5 ml/min was applied. Detection was generally performed at $\lambda = 538$ nm with a UV-Vis detector or a DAD (diode array detection) system at 538, 505, 480, and 310 nm, respectively. The column was thermostated at 35 °C. Positive ion electrospray mass spectra were recorded on a ThermoFinnigan LCQ Advantage mass spectrometer (Thermo Finnigan LLC, San Jose, CA, USA) at electrospray voltage 4.5 kV, capillary 250 °C and sheath gas: N₂ coupled to a ThermoFinnigan LC Surveyor pump applying in the same HPLC gradient system as LC-DAD. The MS was controlled and total ion chromatograms and mass spectra were recorded using ThermoFinnigan Xcalibur software (Thermo Finnigan LLC, San Jose, CA, USA). The relative collision energies for the CID experiments (MS/MS) were set at 30% (according to a relative energy scale). Helium was used to improve trapping efficiency and as the collision gas for the CID experiments.

2.2.1. Identification of betalains

For the pigment identification, authentic standards from previous betalain studies were used (betanin and its derivatives (Wybraniec, 2005; Wybraniec & Mizrahi, 2005; Wybraniec, Nowak-Wydra, & Mizrahi, 2006)) or were semi-synthesised (betaxanthins, according to Stintzing, Schrieber, and Carle (2002)); otherwise, the status of a pigment was assigned as “tentatively identified” (Table 1) supported by the spectrophotometric and mass spectrometric data. In addition, betanidin and its decarboxylated derivatives were semi-synthesised by enzymatic hydrolysis of betanin and decarboxy-betanins catalysed by β -glucosidase (Sigma, Chemical Co., St. Louis, MO).

2.3. Total betalain quantification

Quantification of betalains was performed by a spectrophotometric multiple-component method of Nilsson (1970) using a Shimadzu 1650 PC UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The determination of betalain concentration, i.e., violet and yellow pigments, was calculated in terms of betanin and vulgaxanthin-I, respectively. Total pigment content was expressed as the sum of violet and yellow components. Pigment content calculations were based upon the absorptivity values $A^{1\%}_{1\text{cm}}$ 1120 for betanin (at 538 nm) and 750 for vulgaxanthin-I (at 470 nm) (Piattelli & Minale, 1964).

2.4. Nutritional composition of dried extracts

2.4.1. Proximate analysis

Moisture content of RBE was determined according to USP loss-on-drying (LOD) method (Díaz et al., 2009). Sample was heated in a

vacuum oven at 70 °C for 7 h. Total protein content was determined by the Kjeldahl method (Bradstreet, 1965; Official AOAC Method 979.09, 2005) based on nitrogen content ($N \times 6.25$). Nitrogen was determined as a difference of total and non-protein nitrogen (NPN). The principle of non-protein-nitrogen assay was based on determination of both inorganic and organic nitrogen-containing compounds from sources other than high molecular weight proteins. Samples were homogenised, extracted in water, treated with trichloroacetic acid (TCA) to precipitate the protein and filtered. An aliquot from the soluble nitrogen portion was then analysed for non-protein-nitrogen via Kjeldahl methodology. Nitrogen adjusted for NPN has been used for protein calculation. The RSD values for total nitrogen, non-protein nitrogen, and adjusted nitrogen were 0.9%, 1.3%, and 1.2%, respectively. Available carbohydrates were calculated by deducting the sum of crude protein, crude fat, ash and moisture from 100% of the DM. Ash content was determined by igniting the sample at 550 °C in electric furnace, AOAC 923.03 (Official Method 923.03, 2005). The RSD values for moisture, ash, and protein by Kjeldahl were 1.0%, 5.0%, and 1.3%, respectively.

2.4.2. Mineral analysis

The 1.2 g sample test portion was dry ashed at 500 ± 50 °C for 8 h and treated with HNO₃. The resultant ash was treated with concentrated hydrochloric acid (5%, v/v), dried, and redissolved in hydrochloric acid solution (Official Method 923.03, 2005). The amount of each element (Al, Ba, B, Ca, Cu, Fe, K, Mg, Mn, Na, Zn) was determined by comparing the emission of the unknown sample against the emission of each element from standard solutions using Inductively Coupled Plasma Atomic Emission Spectroscopy ICAP-61E-Trace (Thermo Jarrell Ash Corp., Franklin, MA) according to Official Method 985.01 (2005). All standard solutions used were obtained from Inorganic Ventures (Christiansburg, VA, USA) and were of analytical-reagent grade. The RSD for analysis of each element was 5.0%.

2.4.3. Sugar analysis

The sugars (sucrose, glucose, fructose, maltose, lactose, and galactose) were extracted from an accurately weighed sample with 80% ethanol by allowing it to stand for 24 h with occasional swirling. Aliquots were dried under inert gas and reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl- β -D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoroacetic acid (TFA) treatment and subsequently analysed by gas chromatography Agilent 6890 with a flame ionisation detector FID (Agilent Technology Inc., Palo Alto, CA USA) (Brosbt, 1972; Mason & Slover, 1971). The RSD values for fructose, glucose, maltose and sucrose were 3.2%, 5.8%, 5.8%, and 2.6%, respectively.

2.4.4. Total dietary fibre analysis

Determination of total dietary fibre (TDF) was based on the methods of Lee, Prosky, and De Vries (1992) and Prosky et al. (1988). Duplicate samples were cooked at ~ 100 °C with 50 µl of heat stable α -amylase (Megazyme International Ireland Limited, Ireland) to give gelatinisation, and then digested with 100 µl of protease (Megazyme International Ireland Limited, Ireland) and 200 µl of amyloglucosidase (Megazyme International Ireland Limited, Ireland) in a phosphate buffer to break down starch and some proteins. Ethanol was added to each sample to precipitate any soluble fibre. The samples were filtered, and residues were rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fibre in the sample was calculated after

Table 1
Chromatographic, spectrophotometric and mass spectrometric data of the analysed pigments found in the red beet dried samples.

Peak no.	Compound	Retention time (min)	λ_{\max}	m/z [M+H] ⁺	m/z from MS/MS of [M+H] ⁺
<i>Betacyanins and their derivatives</i>					
1	Prebetanin	14.6	536	631	551, 389
2	2'-O-glucosyl-betanin	14.7	535	713	551, 389
3	Betanin	14.9	536	551	389
4	17-decarboxy-betanin	15.8	505	507	345
1'	Isoprebetanin	15.8	536	631	551, 389
2'	2'-O-glucosyl-isobetanin	15.9	535	713	551, 389
3'	Isobetanin	16.1	536	551	389
4'	17-Decarboxy-isobetanin	17.2	506	507	345
5	15-Decarboxy-betanin ^a	17.7	528	507	345
6	2-Decarboxy-isobetanin	19.3	– ^b	507	345
6'	2-Decarboxy-betanin	19.4	– ^b	507	345
7	Betanidin	19.5	540	389	345
8	17-Decarboxy-betanidin	19.8	– ^b	345	–
9/9'	2,17-Bidecarboxy-betanidin/isobetanidin	20.1	507	463	301
10	17-Decarboxy-neobetanin ^a	20.6	444	505	343, 297
7'	Isobetanidin	20.7	540	389	345
8'	17-Decarboxy-isobetanidin	21.4	– ^b	345	–
11	2,15,17-Tridecarboxy-2,3-dehydro-neobetanin ^a	21.5	– ^b	415	253
12	Neobetanin	21.8	464	549	387
13	15-Decarboxy-betanidin ^a	23.0	– ^b	345	–
14/14'	2-Decarboxy-betanidin/isobetanidin	23.9	– ^b	345	–
15	2,17-Bidecarboxy-2,3-dehydro-neobetanin ^a	24.0	– ^b	459	297
16	2,17-Bidecarboxy-neobetanin ^a	25.2	462	461	299
17	2-Decarboxy-neobetanin ^a	26.0	– ^b	505	343, 297
18	6'-O-feruloyl-2'-O-glucosyl-betanin ^a	27.0	– ^b	889	713, 551
18'	6'-O-feruloyl-2'-O-glucosyl-isobetanin ^a	27.2	– ^b	889	713, 551
19	2-Decarboxy-2,3-dehydro-neobetanin ^a	28.2	420	503	341
20/20'	6'-O-feruloyl-betanin/isobetanin	28.3	– ^b	727	551
<i>Betaxanthins</i>					
B1	Glutamine-bx (Vulgaxanthin I)	8.1	471	340	323, 277
B2	Glutamic acid-bx (Vulgaxanthin II)	10.3	470	341	297
B3	γ -Aminobutyric acid-bx	13.3	462	297	–
B4'	Proline-isobx (Isoindicaxanthin)	14.8	– ^b	309	291
B4	Proline-bx (Indicaxanthin)	15.1	– ^b	309	291
B5	Dopamine-bx (Miraxanthin V)	18.6	457	347	303, 211
B6'	Tyrosine-isobx (Isoportulacaxanthin II)	19.1	– ^b	375	–
B6	Tyrosine-bx (Portulacaxanthin II)	19.4	– ^b	375	–
B7'	Valine-isobx	21.6	466	311	267
B7	Valine-bx	22.4	466	311	267
B8'	Isoleucine-isobx	27.2	470	325	281
B8	Isoleucine-bx	27.6	470	325	281
B9'	Leucine-isobx (Isovulgaxanthin IV)	27.6	470	325	281
B10'	Phenylalanine-isobx	27.6	– ^b	359	315
B9	Leucine-bx (Vulgaxanthin IV)	27.9	470	325	281
B10	Phenylalanine-bx	27.9	– ^b	359	315
B11	Tryptophan-bx	28.0	472	398	354, 269

^a Tentatively identified.

^b λ_{\max} was not determined due to co-elution with another compound or low concentration.

adjustment for the protein and ash values. All of these analyses were performed twice with RSD 6.8%.

2.4.5. Amino acid analysis

A sample of RBE was hydrolysed in hydrochloric acid (HCl) and adjusted to pH 2.2 for all amino acids except tryptophan. Tryptophan samples were hydrolysed in sodium hydroxide and adjusted to pH 5.2. Individual amino acids were determined by comparison using an automated amino acid analyzer Hitachi L-8900 (Hitachi Ltd., Japan) (Official Method 982.30, 2005). The RSD value for amino acid analysis was 1.9%.

2.4.6. Fatty acid profile analysis

Fat and fatty acids were extracted from RBE samples by a hydrolytic method based on (Official Method 996.06, 2005). Pyrogalllic acid was added to minimise oxidative degradation of fatty acids during analysis. A triglyceride, triundecanoin (C11:0), was added as internal standard. Fat was extracted into diethyl ether–petroleum ether mixture, then methylated to fatty acid

methyl esters (FAMES) using BF₃ in methanol. FAMES were quantitatively measured by capillary gas chromatography (GC) in Agilent 6890A GC (Agilent Technology, Inc., Palo Alto, CA USA) against a C11:0 internal standard. Total fat was calculated as the sum of individual fatty acids and expressed as triglyceride equivalents. Saturated and monounsaturated fats were calculated as the sum of respective fatty acids. The RSD value for analysis of total fatty acids was 3.6% and the RSD values for saturated, monounsaturated, polyunsaturated and trans fatty acids were 1.2%, 1.6%, 1.4% and 1.8%, respectively.

2.4.7. Vitamin analysis

Vitamin C in the RBE sample was extracted, oxidised, and reacted with *o*-phenylenediamine to produce a fluorophor (Official Method 967.22, 2005). Sample was weighed and extracted by homogenising sample in metaphosphoric acid–acetic acid solution (15 g HPO₃ and 40 ml HOAc in 500 ml H₂O). The filtered or centrifuged sample extract was diluted appropriately to a final concentration of 10–100 mg of ascorbic acid/100 ml. The vitamin C

content was determined by comparison of the sample extract fluorescence to the fluorescence of a known standard (Sigma, Chemical Co., St. Louis, MO) with an RSD value 6.3%. Beta carotene analysis was performed by reverse-phase HPLC Agilent 1100 system equipped with UV detection (Agilent Technology Ins., Palo Alto, CA) and compared to a standard curve (Quackenbush, 1987). The RSD value for beta carotene analysis was 7.4%.

2.4.8. Energy value calculation

The energy value was calculated from the concentration of proteins, fat and carbohydrates using the energy conversion factors reported in Southgate and Durnin (1970). The protein concentration expressed as the total amino acids was taken for the calculations.

3. Results and discussion

3.1. Betalainic composition of dried concentrates

In order to compare betalainic compositions of different formulations produced from red beet root concentrates, and extracts produced by air-drying, spray-drying, freeze-drying and also by the above-described proprietary method, total betacyanin and betaxanthin concentrations were evaluated by the spectrophotometric method (Nilsson, 1970). Additionally, the analysis of the particular betalains as well as their derivatives was performed by LC-DAD-ESI-MS/MS. The basic chromatographic, spectrophotometric and mass spectrometric qualitative results for all analysed pigments are included in Table 1. The degradation routes of the principal pigment (betanin) are shown in Fig. 1. The relative HPLC peak area profiles of betacyanins with their derivatives and betaxanthins are presented in Tables 2 and 3, respectively. The results of the total quantification of the violet (mostly betacyanins) and yellow

(mostly betaxanthins and betacyanin derivatives) pigments are also presented in Table 3.

Because the Nilsson method of pigment total concentration calculation was previously devoted to formulations containing only betacyanins and betaxanthins having known extinction coefficients (Piattelli & Minale, 1964), the results obtained in this study, especially for the yellow components, should be regarded as a rough estimation due to the presence of various betacyanin derivatives of unknown extinction coefficients. These derivatives were recently tentatively identified in heated red beet root formulations (Herbach et al., 2004, 2006; Pasch & von Elbe, 1975; Wybraniec, 2005; Wybraniec & Mizrahi, 2005) and their presence in the analysed samples confirms the partial degradation of betacyanins.

Because the profiles of the compounds could change during a purification process and some further pigment degradation was possible, it was decided to analyse the samples directly after extraction with water. As a result of low concentration of some betaxanthins and betanin derivatives, their UV-Vis spectra were not clear, however, obtained mass spectra and chromatographic data made their identification feasible (Table 1). The resulting chromatograms of the FC-1 sample containing the highest amount of betalains, and SD-1, monitored at 538 and 480 nm, are comparatively presented in Fig. 2.

The comparison of the results of the total betalain concentration measurements in SD, AD and FD samples confirmed the declared values by the producers (0.46–1.26%). The high concentration of betalains in FC-1 (41%) (Table 3) results from a novel method of cleanup of red beet root extract eliminating the bulk of sugars (Pietrzkowski & Thresher, 2008). This methodology creates a new quality product ready for various applications in food technology, nutritional supplements and pharmaceuticals. The lack of sugar in the material opens the

Table 2

The relative HPLC peak areas of betacyanins and their derivatives (numbered according to Table 1) obtained for analysed enriched red beet extracts.

No.	SD-1 – India ^a	SD-2 – Poland ^a	SD-3 – France ^a	FD-1 – USA ^a	FD-2 – USA ^a	AD-1 – Taiwan ^a	AD-2 – USA ^a	AD-3 – Poland ^a	AD-4 – Russia ^a	FC-1 – USA ^a
1	0.16 ± 0.022	0.94 ± 0.18	0.17 ± 0.021	0.68 ± 0.079	1.22 ± 0.23	0.59 ± 0.11	0.62 ± 0.091	0.22 ± 0.031	0.38 ± 0.042	0.26 ± 0.041
2	0.31 ± 0.038	0.61 ± 0.083	0.5 ± 0.060	0.63 ± 0.089	0.37 ± 0.070	0.34 ± 0.059	0.3 ± 0.039	0.23 ± 0.039	0.11 ± 0.016	0.25 ± 0.031
3	26.1 ± 2.1	28.2 ± 2.9	38.3 ± 3.3	74.5 ± 5.6	68.0 ± 6.8	44.9 ± 4.3	49.5 ± 4.4	32.4 ± 2.3	53.3 ± 5.4	23.8 ± 1.4
4	19.7 ± 1.9	4.9 ± 0.33	5.7 ± 0.44	0.92 ± 0.096	0.36 ± 0.024	5.32 ± 0.34	1.4 ± 0.17	12.4 ± 0.75	5.2 ± 0.31	3.6 ± 0.37
1'	0.15 ± 0.026	0.71 ± 0.13	0.11 ± 0.019	0.13 ± 0.020	0.17 ± 0.025	0.34 ± 0.064	0.49 ± 0.062	0.18 ± 0.034	–	0.38 ± 0.047
2'	0.11 ± 0.017	0.91 ± 0.18	0.35 ± 0.061	0.3 ± 0.058	0.06 ± 0.007	0.19 ± 0.030	0.44 ± 0.087	0.17 ± 0.037	–	0.51 ± 0.086
3'	15.7 ± 1.1	32.5 ± 3.5	42.0 ± 4.6	15.6 ± 0.95	19.5 ± 1.4	31.0 ± 2.2	34.3 ± 3.2	26.8 ± 2.4	17.3 ± 1.9	30.3 ± 2.8
4'	15.5 ± 1.3	4.6 ± 0.31	5.5 ± 0.50	0.62 ± 0.083	0.34 ± 0.068	4.4 ± 0.38	1.4 ± 0.24	11.4 ± 0.95	4.6 ± 0.32	4.7 ± 0.42
5	10.9 ± 1.0	3.7 ± 0.52	2.5 ± 0.37	1.6 ± 0.31	1.5 ± 0.18	7.8 ± 0.75	1.5 ± 0.21	10.9 ± 1.5	7.4 ± 0.67	5.1 ± 0.62
6	–	0.46 ± 0.092	0.26 ± 0.040	–	–	–	0.54 ± 0.063	–	0.29 ± 0.041	0.09 ± 0.012
6'	–	0.51 ± 0.075	0.33 ± 0.062	–	–	–	0.17 ± 0.018	–	0.20 ± 0.040	0.15 ± 0.024
7	0.9 ± 0.18	7.6 ± 1.5	–	2.0 ± 0.26	1.2 ± 0.22	0.58 ± 0.074	0.68 ± 0.14	–	–	2.4 ± 0.41
8	0.23 ± 0.041	–	–	–	–	–	–	–	–	0.15 ± 0.022
9/9'	0.4 ± 0.080	0.21 ± 0.027	–	–	–	–	–	0.18 ± 0.021	0.07 ± 0.013	0.03 ± 0.003
10	4.6 ± 0.72	2.2 ± 0.20	1.2 ± 0.19	0.31 ± 0.041	0.41 ± 0.046	0.76 ± 0.13	0.9 ± 0.18	1.4 ± 0.23	4.2 ± 0.45	2.6 ± 0.35
7'	0.51 ± 0.063	6.5 ± 0.97	–	0.09 ± 0.017	–	0.17 ± 0.023	–	–	–	2.3 ± 0.32
8'	0.24 ± 0.032	–	–	–	–	–	–	–	–	0.03 ± 0.006
11	0.22 ± 0.039	–	–	–	–	–	–	0.11 ± 0.015	0.18 ± 0.025	0.07 ± 0.012
12	1.2 ± 0.15	1.8 ± 0.38	1.2 ± 0.13	0.07 ± 0.009	0.46 ± 0.056	0.85 ± 0.15	1.2 ± 0.19	2.2 ± 0.34	3.8 ± 0.58	16.8 ± 1.17
13	0.27 ± 0.029	–	–	–	–	–	–	–	–	0.13 ± 0.019
14/14'	0.75 ± 0.092	1.3 ± 0.227	–	–	–	–	–	–	–	0.59 ± 0.072
15	0.8 ± 0.16	0.34 ± 0.065	0.18 ± 0.026	0.21 ± 0.031	0.26 ± 0.045	0.56 ± 0.069	0.77 ± 0.14	0.37 ± 0.060	1.3 ± 0.25	0.89 ± 0.18
16	0.41 ± 0.069	0.26 ± 0.029	–	–	–	–	–	0.17 ± 0.033	0.95 ± 0.19	0.13 ± 0.015
17	–	–	–	–	–	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	0.05 ± 0.008
18'	–	–	–	–	–	–	–	–	–	0.09 ± 0.011
19	0.84 ± 0.16	1.4 ± 0.20	1.7 ± 0.27	1.9 ± 0.23	5.3 ± 0.92	2.2 ± 0.32	5.0 ± 0.95	0.87 ± 0.13	0.58 ± 0.11	3.4 ± 0.65
20	0.35 ± 0.053	–	–	0.44 ± 0.063	0.85 ± 0.092	–	0.79 ± 0.13	–	0.14 ± 0.016	1.2 ± 0.22
Ratio	1:0.60	1:1.15	1:1.10	1:0.21	1:0.29	1:0.69	1:0.69	1:0.83	1:0.32	1:1.27
Bt:IBt										

^a Relative HPLC peak areas (for extracted ions analysed in LC-MS (Table 1)) expressed as percentage of the total peak area. Averages of three replicates ± SD.

Table 3
The relative HPLC peak areas of betaxanthins (numbered according to Table 1) obtained for analysed enriched red beet extracts.

No.	SD-1 – India ^a	SD-2 – Poland ^a	SD-3 – France ^a	FD-1 – USA ^a	FD-2 – USA ^a	AD-1 – Taiwan ^a	AD-2 – USA ^a	AD-3 – Poland ^a	AD-4 – Russia ^a	FC-1 – USA ^a
B1/B1'	29.8 ± 4.1	26.2 ± 2.4	27.8 ± 2.6	66.1 ± 9.5	49.0 ± 4.5	46.0 ± 5.8	44.6 ± 6.3	31.9 ± 4.7	37.5 ± 4.9	–
B2/B2'	3.7 ± 0.71	8.6 ± 1.9	4.9 ± 0.69	5.8 ± 0.81	10.8 ± 2.7	5.2 ± 1.1	7.6 ± 1.7	5.6 ± 1.4	4.7 ± 0.82	–
B3/B3'	6.0 ± 1.1	14.1 ± 3.1	22.0 ± 2.1	6.2 ± 0.86	7.9 ± 1.8	19.0 ± 3.3	10.9 ± 2.3	13.1 ± 2.1	4.5 ± 0.58	–
B4'	8.8 ± 2.1	6.1 ± 0.88	8.9 ± 1.2	0.78 ± 0.19	0.39 ± 0.083	1.3 ± 0.22	0.18 ± 0.026	3.0 ± 0.53	0.64 ± 0.11	6.6 ± 1.2
B4	29.1 ± 4.3	23.8 ± 3.5	13.9 ± 1.5	3.6 ± 0.69	9.6 ± 1.9	4.9 ± 0.65	9.8 ± 1.3	8.2 ± 1.3	7.6 ± 1.8	18.0 ± 3.3
B5/B5'	14.3 ± 3.4	–	1.0 ± 0.22	–	–	2.1 ± 0.41	0.83 ± 0.13	9.9 ± 1.7	22.6 ± 2.5	50.5 ± 7.3
B6'	–	–	–	–	0.40 ± 0.092	–	0.35 ± 0.055	0.46 ± 0.076	0.09 ± 0.022	–
B6	–	–	–	1.0 ± 0.14	1.3 ± 0.23	–	1.4 ± 0.31	1.2 ± 0.27	1.5 ± 0.27	–
B7'	–	2.9 ± 0.44	1.6 ± 0.32	–	–	1.5 ± 0.32	1.4 ± 0.27	3.6 ± 0.66	0.81 ± 0.11	6.0 ± 1.1
B7	–	3.7 ± 0.72	3.2 ± 0.55	4.1 ± 1.2	4.7 ± 0.95	3.6 ± 0.49	6.2 ± 0.93	4.7 ± 0.91	4.6 ± 0.86	4.1 ± 0.65
B8'	–	3.6 ± 0.82	4.4 ± 0.85	–	–	0.41 ± 0.077	2.1 ± 0.42	2.9 ± 0.72	0.56 ± 0.13	2.4 ± 0.46
B8/B9'	–	–	7.6 ± 1.8	5.4 ± 0.84	7.5 ± 1.5	7.7 ± 1.7	8.2 ± 1.3	7.7 ± 1.2	6.9 ± 1.6	1.8 ± 0.41
B9	–	3.4 ± 0.73	3.6 ± 0.52	4.2 ± 0.73	4.6 ± 0.79	4.2 ± 1.1	5.0 ± 1.16	4.0 ± 0.64	5.4 ± 0.71	2.6 ± 0.35
B10'	–	–	–	–	–	0.14 ± 0.019	–	0.34 ± 0.081	0.17 ± 0.031	–
B10	8.3 ± 1.2	–	–	0.82 ± 0.12	1.3 ± 0.19	0.76 ± 0.15	0.74 ± 0.16	2.1 ± 0.32	1.6 ± 0.21	–
B11/B11'	–	–	–	2.0 ± 0.32	2.1 ± 0.44	1.5 ± 0.37	1.6 ± 0.36	1.3 ± 0.21	0.83 ± 0.12	8.0 ± 1.2
Total concentration of betalains, violet pigments and yellow pigments [%]										
Betalains ^b	0.46 ± 0.06	0.24 ± 0.05	0.43 ± 0.07	1.26 ± 0.06	0.89 ± 0.06	0.61 ± 0.04	0.57 ± 0.08	0.59 ± 0.06	0.56 ± 0.07	41.0 ± 0.08
Violet p. ^c	0.28 ± 0.06	0.18 ± 0.04	0.32 ± 0.08	0.89 ± 0.05	0.57 ± 0.06	0.33 ± 0.03	0.35 ± 0.09	0.32 ± 0.05	0.34 ± 0.06	23.0 ± 0.09
Yellow p. ^d	0.18 ± 0.07	0.06 ± 0.06	0.12 ± 0.06	0.37 ± 0.06	0.32 ± 0.07	0.28 ± 0.05	0.23 ± 0.08	0.28 ± 0.06	0.23 ± 0.07	18.1 ± 0.07
Ratio V:Y ^e	1:0.64	1:0.33	1:0.38	1:0.42	1:0.56	1:0.85	1:0.66	1:0.88	1:0.68	1:0.79

^a Relative HPLC peak areas (for extracted ions analysed in LC-MS (Table 1)) expressed as percentage of the total peak area.

^b The sum of the total concentrations of violet^c and yellow^d pigments.

^c In betanin equivalents.

^d In vulgaxanthin I equivalents.

^e The ratio of the total concentrations of violet^c and yellow^d pigments.

way for further efficient cleanup for large scale isolation of betanin or other minor betalainic constituents.

It was also interesting to compare the total composition of the violet (mainly betanin/isobetanin **3/3'** but also the less prominent betanidin/isobetanidin **7/7'** and other betacyanins) and yellow (betaxanthins, neobetainin **12** and other betanin decarboxylated and/or dehydrogenated derivatives) pigments in the samples. Except for FC-1, the highest violet pigment concentrations (up to 0.89%) were found for the freeze-dried products (FD-1 and FD-2). The lowest concentrations were observed in the spray-dried materials. This is likely a result of the addition of maltodextrins to the sprayed red beet juice, as well as being due to the influence of temperature (Herbach et al., 2004, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005). For estimation of betaxanthin concentrations from spectrophotometric results it is necessary to take into account the presence of yellow betanin derivatives. Most of the formulations were characterised by a ratio of violet to yellow pigments close to 1:0.5; however, a higher fraction of yellow constituents was found in AD-1 (1:0.85) because of the impact of betaxanthins, as well as for FC-1 (1:0.79) due to the overwhelming expression of betanin derivatives. This would be a good indicator of some drastic conditions used for a specific method of FC-1 preparation; however, taking into account high betanin concentration in comparison to its decarboxylated derivatives (Table 2), it can be deduced that the conditions of the enrichment didn't result in excessive betanin degradation. Interestingly, the spray-dried SD-2 sample contained low quantities of yellow betanin derivatives in contrast to another spray-dried product (SD-1) in spite of the degradative temperature influence on betanin during the pre-concentration process. Some meaningful differences were also found in the group of air-dried samples where AD-3 was enriched with more betanin degradation derivatives presumably due to the action of higher temperature or longer exposure to oxygen during the drying.

Besides the most prominent peaks of betanin/isobetanin **3/3'**, other betacyanins, usually present in *B. vulgaris* L. roots, were

detected: betanin/-isobetanin 6'-O-sulphate **1/1'**, 2'-O-glucosyl-betanin/-isobetanin **2/2'**, 6'-O-feruloyl-2'-O-glucosyl-betanin/-isobetanin **18/18'** and 6'-O-feruloyl-betanin/-isobetanin **20/20'** (Kujala, Loponen, Klika, & Pihlaja, 2000). A prominent peak of neobetainin **12** which had been frequently detected in *B. vulgaris* L. roots (Alard, Wray, Grotjahn, Reznik, & Strack, 1985; Strack, Engel, & Wray, 1987) was also observed in the chromatograms. The presence of excessive amounts of **12** in betacyanin-bearing samples had often been attributed to the degradation of betanin during processing of the samples (Herbach et al., 2004, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005). The presence of **12** in FC-1 at high concentration, should be a result of betanin dehydrogenation favoured by the basic conditions (pH 8.2–8.4) which can proceed in betalain analogs (Hilpert & Dreiding, 1984).

The high concentration ratio of betanin **3** to isobetanin **3'** (1:0.20–1:0.29) (Table 2) must be a consequence of the gentle conditions of the freeze-drying process (FD-1 and FD-2) in contrast to the conditions of the other methods except of AD-4. The betanin isomerisation takes place usually during the long-term betalainic material elaboration and can be accelerated by temperature increase or an action of some introduced compounds (Strack, Vogt, & Schliemann, 2003). This can be applicable also to the FC-1 production (long chromatographic process and elution with slightly basic eluent) because of the lowest Bt:IBt ratio (1:1.3).

Further inspection of the chromatograms and mass ion traces revealed already known decarboxylated betacyanins **4/4'** and **6/6'** (Herbach et al., 2004, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005; Wybraniec et al., 2006) present at low levels in the concentrated extract (Table 2). This is obviously a result of the betanin degradation process (Herbach et al., 2004, 2006; Wybraniec, 2005; Wybraniec & Mizrahi, 2005). It should be mentioned that different derivatives of betanin are formed at different rates; additionally, they degrade during the process and consequently these potentially indicative pigments may have quite different enrichment factors. The high proportion of **4** and **4'** in SD-1 confirms a big influence of heating on betanin decarboxylation, whereas a

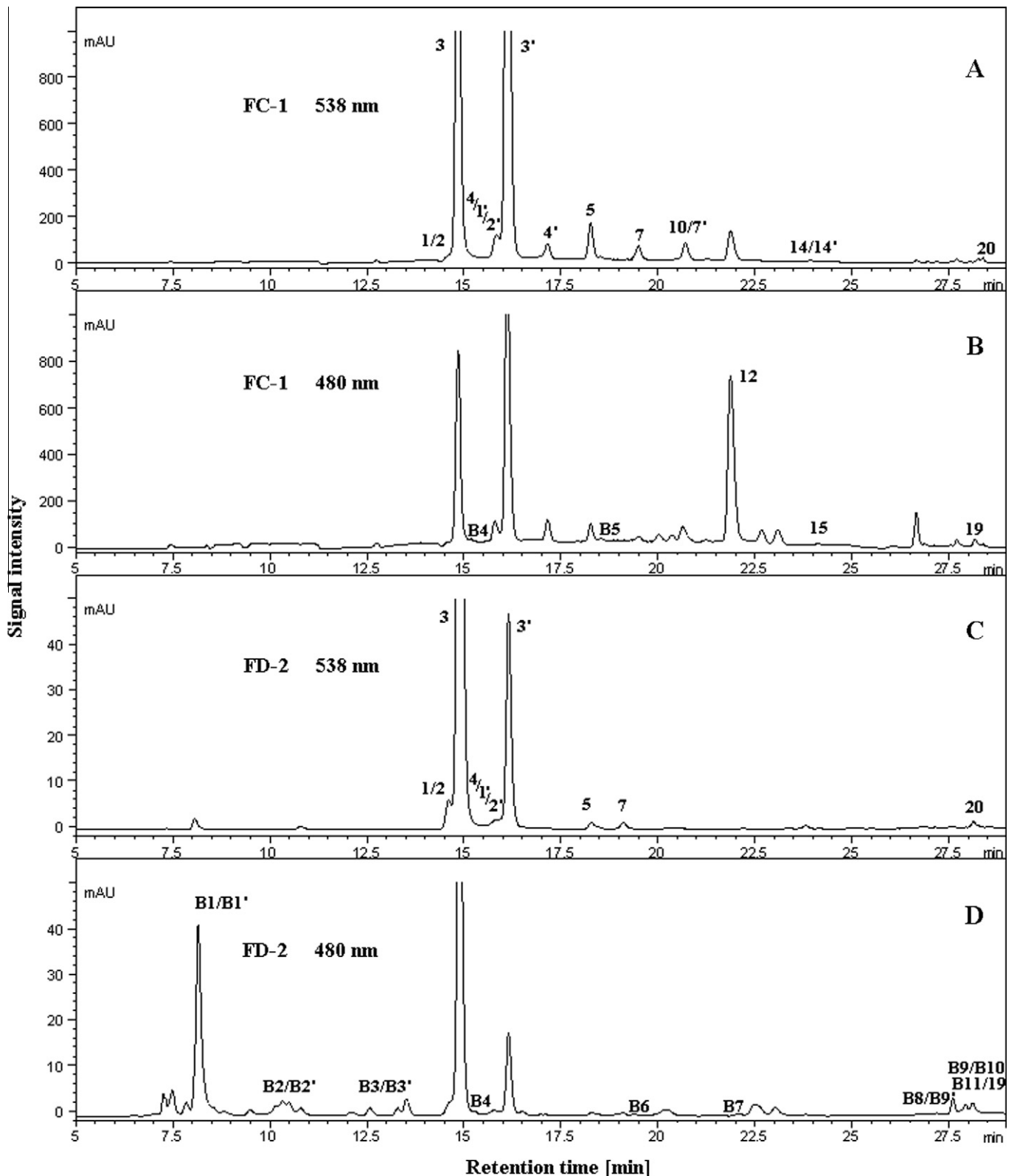


Fig. 2. HPLC chromatograms of the FC-1 and FD-2 samples monitored at 480 and 538 nm.

low concentration of neobetanin can be explained by its parallel decomposition. In contrast, lower decarboxylation of betanin as well as the highest proportion of betanidin 7/7' in SD-2 indicates a very interesting gentle method of spray-drying. Betanidin is significantly labile at any physicochemical conditions because of the lack of the protecting glucosyl moiety in the structure (Herbach

et al., 2006), therefore, it is a very good indicator of hard and aggressive environment.

The presence of 4/4' and 6/6' has been frequently attributed to decarboxylation during heating of the betanin solution; however, the degradation at room temperature cannot be excluded. Interestingly, another decarboxylated pigment 5, was tentatively identified

as 15-decarboxy-betanin, with λ_{\max} 528 nm. The chromatographic peak of **5** was very well separated from 17-decarboxy-betanin/isobetanin **4/4'** and 2-decarboxy-betanin/isobetanin **6/6'**. The presence of a single peak of **5** at the applied chromatographic conditions suggested the lack of the configurational isomer at C-15 carbon, supporting the conclusion of 15-decarboxylation.

The presence and the level of concentration of **5** and **4/4'** were very similar, presumably resulting from the degradation of betanin at the same rate. The presence of **5** supported by its m/z and λ_{\max} values was not reported in the recent degradation studies; however, it was postulated by Schwartz and von Elbe (1983) after their pioneering chromatographic experiments on betanin decarboxylation in aqueous solutions. In light of recent publications (Wybraniec, 2005; Wybraniec & Mizrahi, 2005), the conclusions of Schwartz and von Elbe (1983) resulted rather from the lack of separation between **6** and **6'** peaks, therefore, based on λ_{\max} (535 nm) measured in experiments of Schwartz and von Elbe (1983) for the peak, it is possible that the peak should have been assigned as 2-decarboxy-betanin/isobetanin **6/6'**. The enrichment of **5** in the concentrated extract was at similar level as for **4/4'**, suggesting the same formation rate.

Low levels of **4/4'**, **5** and **6/6'** in the FC-1 sample in comparison to betanin as well as a very low level of 2,17-bidecarboxy-betanin (the more stable product of recent thermodegradation experiments (Wybraniec, 2005; Wybraniec & Mizrahi, 2005), in the material (Table 2) suggest that the enrichment procedure is gentle enough to prevent the decomposition of betanin at any meaningful rate. A low enrichment of betanidin/isobetanidin **7/7'** in FC-1 in light of its known instability is unsurprising (Herbach et al., 2006), especially taking into account the basic conditions (pH 8.2–8.4).

Dehydrogenated betanin derivatives were tentatively identified in the samples which were presumably formed as a result of betanin degradation (Tables 1 and 2). The most prominent was usually

2-decarboxy-2,3-dehydro-neobetanin **19**, a recently-detected and mostly hydrophobic degradation product of betanin (Herbach et al., 2004; Wybraniec & Mizrahi, 2005). Another frequently reported derivative was 17-decarboxy-neobetanin **10**, which in many cases was eluted before neobetanin **12** (Herbach et al., 2004; Wybraniec, 2005) even if the overall hydrophobicity of **10** should be somewhat increased as a result of decarboxylation of **12**. Other tentatively identified pigments present at low levels were 2,17-bidecarboxy-2,3-dehydro-neobetanin **15** and 2,17-bidecarboxy-neobetanin **16**. In addition, 2-decarboxy-neobetanin **17** (Fig. 1) was not detected in any sample. The lack of direct correlation between the retention times and the structures of these dehydrogenated derivatives is still hindering the proper designation of the pigments. These structures are currently under investigation.

In general, the betaxanthins based on the following amino acids were present in the tested samples: glutamine (**B1**), glutamic acid (**B2**), γ -aminobutyric acid (**B3**), proline (**B4**), dopamine (**B5**), tyrosine (**B6**), valine (**B7**), isoleucine (**B8**), leucine (**B9**), phenylalanine (**B10**), and tryptophan (**B11**) (Tables 1 and 2). Depending on the HPLC conditions, some of the diastereomers of betaxanthins are not well separated (Schliemann, Kobayashi, & Strack, 1999); therefore, for the unresolved chromatographic peaks, the sum of their relative peak areas was calculated (Table 3).

Vulgaxanthin I **B1**, the principal betaxanthin present in red beet, was the most prominent pigment in this group in all analysed samples (with the exception of FC-1) and it reached the highest relative peak area of 66.1% for FD-1. Other betaxanthins found at increased relative levels in most of the samples included vulgaxanthin II **B2** (3.7–10.8%), γ -aminobutyric acid-bx (**B3**) (4.5–19.0%), proline-bx (**B4**) (3.6–29.1%), and leucine-bx (**B9**) (2.6–5.4%).

Interestingly, **B1**, **B2** and **B3** were not at all present in the betanin-rich FC-1 sample. Furthermore, it was noticed that also other

Table 4
Comparative nutritional characterisation of air-dried (AD), freeze-dried (FD), spray-dried (SD) concentrates and enriched by a special method (FC-1) red beet root extract.

Nutrient (RSD) ^a	Units	Sample								
		AD-3	AD-1	AD-2	FD-2	FD-1	SD-3	SD-4	FC-1	
Energy value	cal/100 g	348	333	339	354	334	366	364	102	
Energy value from fat	cal/100 g	14.3	2.34	51.5	3.42	4.93	<1.0	<1.0	<1.0	
Total carbohydrates	g/100 g	76.6	75.7	76.1	81.7	75.4	89	88.6	23.6	
Total dietary fibre (RSD 6.8%)	g/100 g	19.5	18	20.8	17.2	21.8	<1.0	<1.0	4.55	
<i>Sugar profile</i>										
Fructose (RSD 3.8%)	g/100 g	1.3	0.9	1	0.8	1.2	1.9	1.7	<0.1	
Glucose (RSD 5.8%)	g/100 g	1.3	1.1	1.2	1.2	1.6	1.9	2.0	<0.1	
Sucrose (RSD 2.6%)	g/100 g	53	51.5	55.9	60.5	50.7	26.2	33.2	0.3	
Lactose	g/100 g	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
Maltose (RSD 5.8%)	g/100 g	<0.1	<0.1	<0.1	<0.1	<0.1	0.8	0.7	<0.1	
Galactose	g/100 g	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
Total Sugars	g/100 g	55.6	53.5	58.1	62.5	53.5	30.8	37.6	0.3	
Protein as total nitrogen ($N \times 6.25$) by Kjeldahl method (RSD 1.3%)	g/100 g	10.3	10.3	8.13	8.63	12.4	3.81	4.13	64.7	
Protein as nitrogen adjusted for NPN ($N^* \times 6.25$)	g/100 g	2.70	2.80	1.85	2.34	4.28	0.09	0.38	1.58	
Protein as amino acids (RSD 1.9%)	g/100 g	6.77	6.88	5.33	6.05	7.03	2.41	2.44	1.99	
Total amino acids (RSD 1.9%)	g/100 g	6.77	6.88	5.33	6.05	7.03	2.41	2.44	1.99	
Total Nitrogen (N) (RSD 0.9%)	%	1.64	1.65	1.30	1.38	1.99	0.61	0.66	10.4	
Non-protein nitrogen (NPN) (RSD 1.3%)	%	1.21	1.20	1.00	1.00	1.31	0.60	0.60	10.1	
Nitrogen adjusted for NPN ($N^* = N - \text{NPN}$) (RSD 1.2%)	%	0.43	0.45	0.30	0.37	0.68	0.01	0.06	0.25	
<i>Fatty acid profile</i>										
Saturated fatty acids (RSD 1.2%)	g/100 g	1.26	0.073	0.754	0.090	0.129	0.019	<0.007	<0.007	
Monounsaturated fatty acids (RSD 1.6%)	g/100 g	0.048	0.047	0.290	0.063	0.061	0.022	<0.007	<0.007	
Polyunsaturated fatty acids (RSD 1.4%)	g/100 g	0.072	0.067	0.401	0.115	0.333	0.021	<0.007	<0.007	
Trans fatty acids (RSD 1.8%)	g/100 g	0.139	0.061	0.02	0.097	<0.007	<0.007	<0.007	<0.007	
Total fatty acids (RSD 3.6%)	g/100 g	1.52	0.248	1.46	0.365	0.523	0.065	<0.007	<0.007	
Total vitamin A	IU/100 g	<100	<100	<100	<100	<100	<100	<100	<100	
Vitamin A from carotenes	IU/100 g	<35.0	<35.0	<35.0	<35.0	<35.0	<35.0	<35.0	<35	
Beta carotene	mg/100 g	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	
Vitamin C (RSD 6.3%)	mg/100 g	<1.0	1.9	7.7	21.7	14.7	10.7	55.7	615	
Ash (RSD 5.0%)	g/100 g	5.62	6.08	5.32	5.82	6.14	2.17	2.79	1.18	
Moisture (RSD 1.0%)	g/100 g	5.3	6.2	8.4	2.3	4.5	3.84	4.12	5.84	

^a The relative standard deviation (RSD) for the applied method of measurement.

betaxanthins that can usually be found in red beet (the more hydrophobic **B6** and **B10**) were not present in FC-1. The lack of the polar betaxanthins **B1–B3** as well as the hydrophobic **B10** in FC-1 is a direct result of their chromatographic separation from other betalains (data not shown). In contrast, the betaxanthins left in the concentrated extract FC-1 were very highly enriched. As a consequence, the dopamine-based compound **B5** was the prevailing betaxanthin in this material.

The spray-dried products (SD-1 and SD-2) were almost deprived of less polar betaxanthins (**B6–B11**). This must be the effect of increased temperature which is set during the concentration process. The freeze- and air-dried products contained mostly a full range of typical red beet betaxanthins (Schliemann et al., 1999; Stintzing et al., 2002) at various proportions indicating their lower degradation at less rigid conditions.

3.2. Nutritional composition of red beet dried concentrates

For the comparison of basic nutritional compositions in the investigated red beet powders, the analyses of proximate, minerals, sugars, total dietary fibre, fatty acids, and vitamins profiles in most of the samples were performed (Table 4). Additional results for amino acids (Table 5) and mineral elements (Table 6) were obtained for all investigated samples.

The most interesting differences between the samples were observed for the sugar profiles (Table 4). Whereas the air- and freeze-

dried samples contained an overwhelming proportion of sucrose (>50%), the betalain-rich FC-1 sample was almost free from sugars due to a special chromatographic cleanup protocol (the sugars are not bound to the chromatographic bed during the solvent movement). Decreased concentration of sucrose in spray-dried extracts (by ca. 50%) is entirely a result of the dilution of the material with maltodextrin rather than a result of any purification. Interestingly, the levels of glucose and fructose were higher in the spray-dried material. In addition, the total carbohydrate analysis revealed a much higher concentration in comparison to FC-1.

The application of the special purification method used to produce FC-1 resulted in diminished ash content results (1.2% in comparison to 5–6% in air- and freeze-dried samples), a lack of fatty acids and, accordingly, a lack of measurable energy value from fat. Interestingly, fatty acids were also absent in spray-dried material (SD-2). Energy value analysis of all air-dried, freeze-dried and spray-dried samples gave similar results (330–370 cal/100 g) but was significantly lower for FC-1 (102 cal/100 g) due to low amount of carbohydrates. The production of FC-1 also resulted in lowering of the total dietary fibre (4.6%), whereas in the spray-dried material dietary fibre was completely removed.

In the group of amino acids (Table 5), the highest concentrations of the total amino acids were found for air- and freeze-dried samples (6000–7000 mg/100 g). For spray-dried and FC-1 samples a lower level of total amino acids was obtained (2000–2400 mg/100 g). For all samples the highest concentration was found for

Table 5
Amino acid profiles of AD, SD, FD red beet concentrates and extract FC-1.

Amino acid	Concentration (mg/100 g) ^a							
	AD-3	AD-1	AD-2	FD-2	FD-1	SD-3	SD-4	FC-1
Aspartic acid	0.643	0.579	0.502	0.603	0.557	0.248	0.282	0.132
Theonine	0.210	0.230	0.186	0.200	0.238	0.049	0.037	0.049
Serine	0.349	0.329	0.261	0.261	0.332	0.103	0.123	0.057
Glutamic acid	2.81	2.84	2.00	2.41	2.78	1.39	1.60	0.741
Proline	0.177	0.191	0.141	0.167	0.191	0.027	0.016	<0.01
Glycine	0.226	0.247	0.179	0.197	0.247	0.048	0.034	0.297
Alanine	0.519	0.458	0.452	0.432	0.407	0.128	0.119	0.059
Valine	0.290	0.288	0.237	0.267	0.317	0.071	0.051	0.057
Isoleucine	0.238	0.246	0.211	0.213	0.268	0.076	0.065	0.026
Leucine	0.269	0.287	0.228	0.260	0.360	0.072	0.046	0.040
Tyrosine	0.188	0.200	0.166	0.196	0.197	0.015	<0.01	<0.01
Phenylalanine	0.137	0.157	0.114	0.132	0.186	0.020	<0.01	0.045
Lysine	0.242	0.309	0.249	0.257	0.328	0.057	<0.01	0.062
Histidine	0.120	0.136	0.104	0.126	0.140	0.023	0.016	0.046
Arginine	0.192	0.193	0.152	0.172	0.260	0.038	0.032	0.386
Cystine	0.038	0.041	0.034	0.035	0.051	<0.01	<0.01	0.195
Methionine	0.058	0.084	0.063	0.052	0.099	0.019	0.017	0.068
Tryptophan	0.068	0.069	0.056	0.068	0.077	0.023	<0.01	0.644

^a The relative standard deviation (RSD) for the applied method of measurement was at the level of 1.9%.

Table 6
Mineral profiles of AD, SD, FD red beet concentrates and extract FC-1.

Mineral	Concentration (mg/100 g) ^a							
	AD-3	AD-1	AD-2	FD-2	FD-1	SD-3	SD-4	FC-1
Aluminium	0.47	3.88	0.84	1.37	5.47	2.07	0.59	3.71
Barium	1.27	2.72	1.64	3.09	1.72	<0.10	<0.10	0.72
Boron	0.97	0.91	0.74	0.69	0.31	<0.20	<0.20	0.21
Calcium	224	167	97.9	98.5	106	39.7	61.0	74.8
Copper	0.39	0.43	0.82	0.86	0.60	0.10	<0.05	6.11
Iron	3.66	6.95	2.47	3.43	9.20	2.05	1.30	28.9
Magnesium	135	195	191	204	130	54.9	75.4	152.6
Manganese	2.23	2.44	4.76	4.64	2.93	1.19	1.22	13.41
Potassium	1730	1030	2120	2090	1790	1010	1090	124
Sodium	192	1320	297	315	196	125	158	16.0
Zinc	1.70	1.58	2.17	2.18	2.89	0.28	0.35	11.8

^a The relative standard deviation (RSD) for the applied method of measurement was at the level of 5.0%.

glutamic acid; however, no glutamine was detected in spite of the highest glutamine-based betaxanthin (vulgaxanthin I) concentrations. It is interesting that FC-1 tryptophan, a precursor to the key neurotransmitter serotonin, (known to exert a calming effect), was found to be the second most-highly concentrated amino acid component (644 mg/100 g). The percentage of essential amino acids to the total amino acids for AD, FD and extract FC-1 was approximately the same (29–31%) and was significantly lower for spray dried samples (10–18%).

Eleven elements (Al, Ba, B, Ca, Cu, Fe, Mg, Mn, K, Na, Zn) were compared among the differently concentrated red beet samples. For all samples, potassium, calcium, and magnesium were the most concentrated. The air- and freeze-dried samples contained the highest amount of these elements. Some interesting differences were noticed, especially between FC-1, AD and FD samples. While iron was enriched in FC-1, the potassium, calcium, magnesium and sodium were significantly diminished. Similar to previous analytical results, the FD samples were characterised by medium concentrations of Ca and Na. Further mineral analysis for FC-1 revealed high magnesium (152 mg/100 g) and potassium (124 mg/100 g) concentrations (Table 6). Manganese and zinc were also found in higher amounts in extract sample FC-1.

A meaningful difference was observed for nitrogen concentration. The FC-1 sample was characterised by an elevated level of non-protein nitrogen (10.1%) and as a result the total nitrogen content (10.4%) was much higher than the content of the other dried concentrates (total nitrogen: 0.61–0.66% for SD, 1.00–1.21% for AD and 1.00–1.31% for FD). This level can be partly explained by the increased concentration of betalains and their derivatives (Table 1) as well as other undetected products of betalain decomposition. The difference of protein content when calculated as non-protein nitrogen content and when calculated as total amino acids value is explained by the differences in testing methodologies. Total protein as total amino acids was used to calculate energy value.

With the exception of vitamin C, other vitamins were found in all of the extracts. In general, vitamin C was detected at diminished concentrations in the air-dried samples (1–7 mg/100 g) but was enriched in FC-1 (615 mg/100 g).

4. Conclusion

This is the first report on the comparison of betalainic composition that includes the dehydrogenated and decarboxylated betalain derivatives in red beet root extracts concentrated by different methods that have been the most commonly-used sources of betalains. Most of these products are frequently used in the food industry and comprise a large percentage of the betalain consumption in tandem with the natural sources of these pigments. In addition, betalains, primarily used as food colourants, have strong antioxidant properties. The introduction of a more highly concentrated betalain material can provide new opportunities for the use of these healthy pigments in the food and pharmaceutical industries. Further studies on FC-1 highly concentrated betalainic extract shall also characterise its biological activity.

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