Antioxidant, antimicrobial and antiproliferative activity of *Brassica oleracea* varieties broccoli, cauliflower and kohlrabi under organic and conventional cropping

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Summary

Although crops produced in organic cultivation are considered to be better for health and often have better sensory characteristic than conventionally produced foodstuffs, the influence of cropping practice on phytochemicals content and healing properties of plant-based food still needs clarification. This study compared nutrient quality and biological activity of *Brassica oleracea* vegetables grown under organic and conventional production systems. Total glucosinolate, phenolics and flavonoids concentrations, together with antioxidant capacity of vegetable juices against 1,1-diphenyl-2-picryl-hydrazyl radicals (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and cupric ion reagent were estimated. Antimicrobial activity against bacteria and fungi, together with antiproliferative activity in tumour cell lines were investigated. Organic cropping increased total glucosinolates concentration in broccoli (*B. oleracea* L., var. *italica* Plenck) and cauliflower (*B. oleracea* L. var. *botrytis* l.c.). Higher total flavonoids concentration was found in all conventionally grown vegetables. Juices of conventionally grown broccoli and cauliflower were more effective antioxidants, antimicrobials and cancer chemopreventive agents than their organically grown counterparts and those from kohlrabi (*B. oleracea* L. var. *gongylodes* l.c). Cropping system and vegetable variety together affected chemical composition and biological activity of *B. oleracea* vegetables. Thus, the combination of both factors must be considered when producing vegetables with health-promoting properties.

Keywords

Brassica oleracea; organic; antioxidant; antibacterial; antifungal; antiproliferative

The Brassicaceae (Cruciferae) vegetables are inexpensive, nutritious and rich in health-promoting compounds [1]. The antioxidant, antimutagenic, cytotoxic, antifungal and antiviral potential of *Brassica* spp. plants have been demonstrated [2]. Dietary fibre, flavonoids, sterols, phenolic acids and glucosinolates commonly found in these plants are considered to have a chemopreventive role and to be effective against free-radical damage to human body, including low-density lipoprotein (LDL) oxidation involved in pathogenesis of cardiovascular diseases, DNA damage and cancer [3].

In general, plants produced by organic cultivation are considered to be better for health, as they are free from pesticides and growth hormones, not genetically modified, have a higher content of beneficial and health-promoting compounds and often have a better flavour [4]. Due to the absence of synthetic pesticides and fertilizers, organic agri-

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culture could hypothetically result in higher exposure of the plant to stressful situations leading to enhancement of natural defence substances such as phenolic compounds [5]. In recent study of CRUZ-CARRIÓN et al. [6] on thirteen plant-based foods, organically cultivated vegetables displayed higher phenolics and anthocyanins content, which was reflected by higher antioxidant capacity than the non-organic counterparts. On the other hand, FALLER and FIALHO [7] found only few statistically significant differences in content of soluble and hydrolysable polyphenols between vegetables from organic and non-organic agriculture, with small variation among the analysed samples. Those findings suggested that farming systems may differentially modulate phenolic compounds composition and antioxidant capacity in different plant species.

Concerning the *Brassica* vagetables, it was found that tronchuda cabbages from organic culture had a higher content of phenolics than those from conventional systems, and that the organic fertilizers enhanced the yield of phenolic compounds, flavonoids and glucosinolates in broccoli [8, 9]. In the study of VALVERDE et al. [10], the contents of phenolic compounds were not significantly different between organic and conventional production of broccoli, while content of indolyl glucosinolates glucobrassicin and neoglucobrassicin were significantly higher in vegetables grown under organic management.

Those data implicated no clear evidence of differences in nutrient quality between organically and conventionally produced *Brassica* vegetables. Thus, the aim of this study was to further examine the influence of different cropping systems on phytochemicals content and biological activity of very closely related vegetables, broccoli (*B. oleracea* L., var. *italica* Plenck) and cauliflower (*B. oleracea* L. var. *botrytis* l.c.), whose edible portions are immature inflorescences, and kohlrabi (*B. oleracea* L. var. *gongylodes* l.c.), whose enlarged basal stems are usually eaten.

MATERIALS AND METHODS

Chemicals

Aluminium chloride, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), sodium carbonate, sodium acetate, potassium persulfate, ethylenediamine tetraacetic acid (EDTA), gallic acid, methyl-β-cyclodextrin, cupric chloride, Folin-Ciocalteu reagent, 2,9-dimethyl-1,10-phenathroline (neocuproine), ammonium acetate, sodium tetrachloropalladate (II), sodium chloride, potassium chloride, disodium phosphate, monopotassium phosphate, hydrochloric acid, quercetin, (–)-sinigrin hydrate, (±)-6-hydroxi-2,5,7,8-tetramethylchromane-2-carboxilic acid (Trolox) and sulforhodamine B were from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol and ethanol were from J. T. Baker (Phillipsburg, New Jersey, USA). Standards of compounds for liquid chromatography with tandem mass spectrometry (LC-MS/MS) were from Sigma-Aldrich or from ChromaDex (Los Angeles, California, USA). Nutrient culture media (Mueller-Hinton broth and Malt agar and broth) were obtained from Torlak Institute (Belgrade, Serbia). The Dulbecco's Modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from PAA Laboratories (Cölbe, Germany). Penicillin and streptomycin were obtained from Galenika (Belgrade, Serbia). Trypsin was obtained from Serva (Heidelberg, Germany.

Samples

Five randomly selected plants of broccoli, cauliflower and kohlrabi were collected from a certified organic and a conventional producer in Serbia (Tab. 1) in the fall of 2013. The voucher specimens were prepared, identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium) at University of Novi Sad (Novi Sad, Serbia). The plants were stored in plastic bags at 4 °C and processed

Tab. 1. *Brassica oleracea* vegetables studied.

Trivial name	Botanical name	Cultivar	Origin	Growing conditions	Location	Voucher No.
Broccoli	*B. oleracea* L. var. *italica* Plenck	Corvet	Commercially cultivated	Organic	Kisač	2-1518
		Unknown*	Commercially cultivated	Conventional	Ruma	2-1519
Cauliflower	*B. oleracea* L. var. *botrytis* l.c.	Snowball	Commercially cultivated	Organic	Kisač	2-1521
		Santa Maria	Traditionally grown	Conventional	Ruma	2-1520
Kohlrabi	*B. oleracea* L. var. *gongylodes* l.c.	Unknown*	Traditionally grown	Organic	Kisač	2-1517
		Unknown*	Traditionally grown	Conventional	Ruma	2-1522

* – producer did not provide information.

within 7 days. Fresh plant material (400 g) was passed through a domestic centrifugal juice extractor and then through 4 gauze layers under gravity. The remainder was manually squeezed to obtain extra liquid and the solid residue was discarded. The juice was centrifuged at 13 700 ×*g* at 4 °C for 15 min, the supernatant was filtered through a membrane filter (Captiva Econo Syringe Filter, regenerated cellulose, 0.2 μm pore size; Agilent Technologies, Santa Clara, California, USA) to remove all possible particles including microorganisms, divided into small aliquots, frozen at –20 °C and kept until use for a maximum of 45 days. The obtained vegetable juices were used in all analyses except for glucosinolate determination.

LC-DAD-MS/MS analysis

Juices were analysed by high-performance liquid chromatography (HPLC) with photodiode-array detection and tandem mass spectrometry (LC-DAD-MS/MS) using 1 μl of sample, filtered through regenerated cellulose membrane syringe filter (0.45 μm pore size, Agilent Technologies) to remove possible precipitates after thawing, and analysed by 1200 series chromatograph coupled to 6410A series triple quadrupole mass spectrometer with an electrospray ion source (ESI-QqQ-MS/MS; Agilent Technologies). The system was controlled by MassHunter Workstation software, version B.03.01 (Agilent Technologies). Components were separated on Zorbax XDB-C18 column (50 mm × 4.6 mm, 1.8 μm particle size; Agilent Technologies) at 50 °C. The flow rate of the mobile phase was 1.0 ml·min^{-1}. The mobile phase consisted of 0.1% (v/v) aqueous formic acid and methanol. The gradient elution started with 30% (v/v) methanol, ramping to 100% (v/v) methanol during 10 min, held at 100% (v/v) methanol for additional 2 min, and the column was re-equilibrated with 30% (v/v) methanol for 3 min. The photodiode-array detector (DAD) recorded full spectra in 190–700 nm range. In mass spectrometry (MS), analytes were ionized using the electrospray ion source (ESI) with N_2 as drying gas (350 °C, 9.5 l·min^{-1}) and nebulizer gas (340 kPa), with capillary voltage of 4.0 kV. The MS2Scan mode, with m/z range of 120–1 000, scan time of 200 m and fragmentor voltage of 135 V, was used for preliminary screening and selection of precursor ions for tandem mass spectrometry (MS^2). Data were acquired in positive and negative modes. Product Ion Scan mode was used to obtain MS^2 spectra for components identification. The $[M+H]^+$ and $[M–H]^–$ ions of selected abundant compounds were used as precursors for collision-induced dissociation (CID), using high-purity N_2 as the collision gas and collision energies of 5–35 V (in 10 V increments). The product ions were scanned in *m/z* range from 30 units to 2 units above precursor *m/z*. The instrument was controlled by MassHunter B.03.03 software (Agilent Technologies). Data were analysed using MassHunter Workstation Qualitative Analysis B.06.00 software (Agilent Technologies), in conjunction to NIST MS Search 2.0d software (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) equipped with locally created MS libraries of Laboratory for Investigation of Natural Resources of Pharmacologically and Biologically Active Compounds (LAFIB) at University of Novi Sad.

Determination of glucosinolate concentration

Total glucosinolate concentration (GLS) was determined in vegetable aqueous extracts by the method of Kestwal et al. [11] with small modifications. Vegetables were freeze-dried over 72–96 h using freeze dryer Alpha 2-LDplus (Martin Christ, Osterode am Harz, Germany). Then they were ground with mortar and pestle into a fine powder. A sample of this (0.5 g) was mixed with 15 ml of phosphate-buffered saline solution (PBS, pH 7) and placed in a boiling water bath for 10 min to inactivate the enzyme myrosinase. After cooling to room temperature, extraction was done in an ice bath with constant shaking for 1 h. The obtained homogenate was centrifuged at 13 700 ×*g* for 10 min at 4 °C and the supernatant preserved at –20 °C for a maximum of 1 week. An aliquot (20 μl) of the vegetable extract was spiked with different volumes of 2.5 mmol·l^{-1} sinigrin (0, 5, 10, 15 and 20 μl) and the volume adjusted to 150 μl. To the obtained mixture, 150 μl of 4 mmol·l^{-1} sodium tetrachloropalladate (II) was added and the reaction mixture was left to stand for 30 min at 25 °C. Then, absorbance was measured at 450 nm. GLS was calculated by standard addition method and expressed in millimoles of sinigrin equivalents (SE) per litre of the extract.

Determination of antioxidants concentration

Total phenolics concentration (*TPC*) was evaluated in vegetable juices by the Folin-Ciocalteu colorimetric method adapted for microplates [12]. A standard calibration curve was constructed using gallic acid and the results were expressed in milligrams of gallic acid equivalents (GAE) per litre of vegetable juice.

Total flavonoids concentration (*TFC*) was determined by the colorimetric method with aluminium chloride adapted for microplates [13,

14]. A standard calibration curve was constructed using quercetin as a standard and the results were expressed in milligrams of quercetin equivalents (QE) per litre of vegetable juice.

Antioxidant activity determination

The antioxidant activity of vegetable juices was determined by three different assays, namely, scavenging capacity towards 2,2-diphenyl-1-picrylhydrazyl radical (DPPH assay), scavenging capacity towards 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS assay) and cupric ion reducing antioxidant capacity (CUPRAC assay).

The capability of juices to neutralize DPPH radicals was determined according to SILVA et al. [15]. The reaction mixture contained 25 μl of appropriately diluted fresh vegetable juice and 200 μl of 120 mmol·l^{-1} DPPH solution in methanol. After 30 min at 25 °C in the dark, absorbance was read at 515 nm against a reagent blank.

The ABTS assay was done according to ARNAO et al. [16]. To generate ABTS$^{\bullet+}$ radicals, a stock solution was prepared by reaction of 7 mmol·l^{-1} ABTS solution in water and 2.45 mmol·l^{-1} potassium persulfate solution. The mixture was left at 25 °C in the dark for 16 h and then diluted with ethanol. An aliquot of 10 μl of fresh plant juice was added to 290 μl of diluted ABTS solution. The reaction mixture was left to stand at 25 °C in the dark and after 6 min, absorbance was read at 734 nm against a reagent blank.

The CUPRAC assay was done according to APAK et al. [17] adapted for microplates [18]. Briefly, 27.5 μl of vegetable juice, dissolved in 70 g·l^{-1} macrocyclic oligosaccharide, methyl β-cyclodextrin (M-β-CD), and 27.5 μl of distilled water, were added to wells containing 50 μl of 20 mmol·l^{-1} cupric chloride solution, 50 μl of 7.5 mmol·l^{-1} neocuproine solution in 96% (v/v) ethanol and 50 μl of 1 mol·l^{-1} ammonium acetate buffer solution, pH 7 (19.27 g of ammonium acetate dissolved in 250 ml of distilled water). The reaction solution was thoroughly mixed and left at 25 °C in the dark for 30 min, after which absorbance was read at 450 nm against a reagent blank.

Trolox was used as a standard compound in all three antioxidant activity assays and the results were expressed as Trolox equivalents antioxidant capacity (*TEAC*) for each assay ($TEAC_{DPPH}$ for DPPH assay, $TEAC_{ABTS}$ for ABTS assay and $TEAC_{CUPRAC}$ for CUPRAC assay) in micromoles per litre of vegetable juice.

Antimicrobial activity determination

Antimicrobial activity of vegetable juices was screened against reference bacterial strains (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Bacillus subtilis* ATCC 6633; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella eneritidis* ATCC 13076), as well as against fungi (*Candida albicans* – isolate from oral cavity (strain I), *Aspergillus* sp. and *Penicillium* sp. from the Department of Biology and Ecology, University of Novi Sad; *Candida albicans* – human clinical vaginal specimens (strains II and III) from the Department of Obstetrics and Gynecology, University of Novi Sad). Bacterial and fungal suspensions were prepared according to the Kirby-Bauer procedure with a turbidity of 0.5 McFarland using a photoelectric photometer (MA Colorimeter 9504; Metrix, Paris, France). The level of antimicrobial effect was assessed using a microdilution broth assay in 96-well microplates according to established protocols [19–21]. In brief, 100 μl of diluted vegetable juice and 10 μl of bacterial or *Candida* suspension or 2 μl of filamentous fungi suspension were added to wells with 100 μl of Mueller-Hinton broth for bacteria or Malt broth for fungi. Plates with bacteria were incubated at 37 °C for 24 h, while plates with fungi at 26 °C for 48 h. Microbial growth was determined by measuring of turbidity. The minimum inhibitory concentration (*MIC*) representing the lowest concentration of the juice that inhibits the visible growth of tested microorganism (exhibit no turbidity) was determined. In order to determine minimum bactericidal concentration (*MBC*) and minimal fungicidal concentration (*MFC*), 100 μl of the broths used for *MIC* determination, were subcultured on fresh agar plates and incubated at 37 °C for 24 h for bacteria or at 26 °C for 48 h for fungi. *MBC* and *MFC* were determined as the lowest concentrations of the juice that reduce the viability of the initial inocula by ≥ 99.9 %.

Antiproliferative activity determination

Antiproliferative activity of vegetable juices was determined in cell lines of cervical cancer (HeLa, ECACC93021013), breast adenocarcinoma (MCF 7, ECACC 86012803), colon adenocarcinoma (HT-29, ECACC 91072201), Burkitt's lymphoma (Raji, ECACC 85011429) and fetal human fibroblasts derived from healthy tissue (MCR-5, ECACC84101801). Cells were grown in DMEM medium with 45 g·l^{-1} glucose, supplemented with 100 ml·l^{-1} heat-inactivated FBS, 100 IU·ml^{-1} of penicillin and 100 mg·ml^{-1} of streptomycin at 37 °C in a humidified atmosphere with 5% (v/v) CO_2 and sub-cultured twice a week. Single cell suspension was obtained using 1 g·l^{-1} trypsin with 0.4 g·l^{-1} ethylenediamine tetraacetic acid (EDTA). Vege-

table juices were diluted in 9 g·l^{-1} sodium chloride and sterilized by filtration through syringe membrane microfilters, pore size 0.22 μm. Volumes of 20 μl of diluted juices were added in 180 μl of medium. Antiproliferative activity was evaluated by colorimetric sulforhodamine B (SRB) assay [22]. Levels of cytotoxicity towards individual cells was expressed as concentration that caused 50 % of growth inhibition (IC_{50}). The non-tumour/tumour IC_{50} ratio (NT/T) was calculated according to equation (1) for each juice. High NT/T values indicate higher efficacy of the vegetable juice towards cancer cells compared to healthy tissue cells.

$$\frac{NT}{T} = \frac{IC_{50(NT)}}{IC_{50(T)}} \quad (1)$$

where $IC_{50(NT)}$ is IC_{50} for non-tumour cells and $IC_{50(T)}$ is IC_{50} for tumour cells.

Statistical methods

LC-DAD-MS/MS determinations were done without replication. Antimicrobial and antiproliferative activity assays were done in quadruplicate and other experiments in triplicate. Data were presented by mean values and analysed using two-way ANOVA (main effect or factorial), followed by Tukey's honestly significant difference (HSD) test. Differences were considered significant if the p-value was less than 0.05. Statistical analysis was done using statistical software Statistica (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Qualitative LC-DAD-MS/MS analysis of vegetable juices resulted in total or tentative identification of nine compounds in all analysed samples (Tab. 2). Citric acid, phenylalanine and tryptophan with retention times (Rt) of 0.560 min, 0.700 min and 0.760 min, respectively, were identified by comparison of MS^2 spectra with reference standards and Human Metabolome Database [23]. Ascorbigen (Asc) and 4-methoxyascorbigen (MeAsc) with Rt of 1.200 min and 2.730 min, respectively were identified according to their UV spectra (Fig. 1A, Fig. 1B), molecular weight difference, fragmentation and literature data [24, 25]. These compounds were already found in *Brassica* species [26]. Their relative abundance in the juices according to the peak areas in extracted ion chromatograms in the positive ionization mode is presented on Fig. 1C. Asc was more readily formed in organic samples of investigated plants whereas MeAsc was more abundant in conventional samples of broccoli and cauliflower but also in an organic sample of kohlrabi. Based on the losses in precursor ion MS^2 spectra, UV-spectrum and aglycone molecular weight, the compound with Rt of 1.160 min possibly represented rutinosyl indole carboxylate, although further confirmation is needed.

3-O-*p*-Coumaroylquinic acid and 4-O-*p*-coumaroylquinic acid (peaks at 0.751 min and 1.022 min, respectively, with identical molecular weights) were identified by comparison of their spectral and literature data [27]. Only one flavonoid was detected in MS^1 chromatograms (Rt of 1.590 min, Tab. 2) and ions observed in MS^2 spectra indicated kaempferol or luteolin as the likely aglycone moiety. Based on UV-spectrum and fragmentation, the compound was thus either kaempferol 3-O-hexosylhexoside or kaempferol 3,x-di-O-hexoside. Kaempferol diglucoside was previously identified as minor glucoside of broccoli florets [28].

GLS in aqueous extracts was within a range of 1.93-4.93 mmol·l^{-1} (expressed as SE). Two-way ANOVA analysis (Tab. 3) showed that GLS was significantly affected by vegetable variety ($F(1,7) = 11.5559$, $p = 0.0114$) and cropping system ($F(1,7) = 16.2134$, $p = 0.0050$) but there was no significant interaction effect of vegetable variety and cropping system ($F(1,7) = 1.0$, $p > 0.05$). Tukey's HSD showed that significantly higher GLS could be found in extracts of broccoli than in extracts of cauliflower ($p < 0.01$; Tab. 4). Significantly higher GLS could be found in organically grown vegetables than in conventionally grown ($p < 0.01$; Tab. 5). These results were consistent with previously published results [29], but not with other studies showing variable influence of cropping practice on GLS [10, 30].

TPC (expressed as GAE) in the juices ranged from 338 mg·l^{-1} to 744 mg·l^{-1}. Two-way ANOVA (Tab. 3) showed that vegetable variety was the major factor influencing phenolic compounds in the investigated plants ($F(2,9) = 864.5386$, $p = 0.0000$). The increased *TPC* in samples from conventional cropping compared to organic cropping (overall averages of 600 mg·l^{-1} and 582 mg·l^{-1}, respectively) was not significant ($F(1,9) = 0.20$, $p > 0.05$) and there was no significant interaction effect of vegetable variety and cropping system ($F(2,9) = 2.93$, $p > 0.05$). Furthermore, kohlrabi showed significantly lower *TPC* (398 mg·l^{-1}) than broccoli and cauliflower (696 mg·l^{-1} and 686 mg·l^{-1}, respectively; $p < 0.001$, Tab. 4).

On the other hand, significant influence of vegetable variety ($F(2,10) = 363.098$, $p = 0.0000$) and cropping system ($F(1,10) = 281.179$, $p = 0.0000$) on

Tab. 2. Phytochemical profile of vegetable juices acquired by high performance liquid chromatography with UV-Vis and tandem mass spectrometric detection.

Rt [min]	*Mw* [g·mol^{-1}]	UV-Vis absorbance peak position [nm]	MS^2 data			Identity
			I	*CE* [V]	*m/z* (in brackets percentage of base peak)	
0.560	192	none	NI	5	191 (6), 173 (5), 129 (6), 111 (100), 87 (18), 85 (15)	Citric acid
			NI	15	191 (5), 111 (100), 87 (39), 85 (26), 67 (5)	
			NI	25	111 (48), 111 (50), 87 (100), 85 (54), 67 (45), 57 (24)	
			NI	35	87 (63), 57 (100)	
0.700	165	320–355 (broad), 256, 206, 198	PI	5	120 (100)	Phenylalanine
			PI	15	120 (100), 103 (16)	
			PI	25	120 (85), 103 (100), 93 (15), 91 (12), 79 (10), 77 (25)	
			PI	35	120 (16), 103 (100), 93 (8), 91 (27), 79 (12), 77 (96), 51 (8)	
			NI	5	164 (18), 147 (100), 103 (32), 72 (10)	
			NI	15	147 (52), 103 (100), 91 (7), 72 (17)	
			NI	25	103 (92), 72 (100)	
0.751	338	310, shoulder 290	NI	5	191 (6), 163 (100)	3-O-*p*-coumaroylquinic acid
			NI	15	191 (14), 163 (100), 119 (36), 111 (5)	
			NI	25	191 (19), 163 (64), 119 (100)	
			NI	35	163 (11), 119 (100)	
0.760	204	278, 272, 217, 198	PI	5	120 (100)	Tryptophan
			PI	15	120 (100), 103 (16)	
			PI	25	120 (85), 103 (100), 93 (15), 91 (12), 79 (10), 77 (25)	
			PI	35	120 (16), 103 (100), 93 (8), 91 (27), 79 (12), 77 (96), 51 (8)	
			NI	5	164 (18), 147 (100), 103 (32), 72 (10)	
			NI	15	147 (52), 103 (100), 91 (7), 72 (17)	
			NI	25	103 (92), 72 (100)	
1.022	338	310, shoulder 290	NI	5	173 (100), 163 (20)	4-O-*p*-coumaroylquinic acid
			NI	15	173 (100), 163 (14), 93 (8)	
			NI	25	173 (100), 163 (11), 119 (49), 93 (66)	
			NI	35	119 (58), 93 (100)	
1.160	469	286, 220 (impure)	PI	5	324 (100), 162 (29)	Rutinose indole-3-carboxylate
			PI	15	324 (16), 162 (100)	
			PI	25–35	162 (100)	
			NI	5–35	145 (100)	
1.200	305	280, approx. 272, 218, 196	PI	5–35	130 (100)	Ascorbigen
			NI	5–35	116 (100)	
1.590	610	348, 264	PI	5	448 (11), 287 (100)	Kaempferol-3,*x*-di-O-hexoside or kaempferol-3-O-hexosylhexoside
			PI	15–35	287 (100)	
			NI	5–15	609 (100)	
			NI	25	285 (33), 284 (100)	
			NI	35	285 (42), 284 (100)	
2.730	335	289, 276, 220, 197	PI	5	305 (12), 304 (32), 160 (100)	Methoxyascorbigen
			PI	15	160 (100), 145 (7), 130 (8)	
			PI	25	160 (100), 145 (48), 130 (12), 128 (16), 117 (18)	
			PI	35	160 (56), 145 (100), 130 (25), 129 (21), 128 (53), 117 (83)	

Rt – retention time, *Mw* – molecular weight, MS^2 – second-order mass spectral data, given as ion polarity, *I* – ionization (PI – positive ionization, NI – negative ionization), *CE* – collision energy.

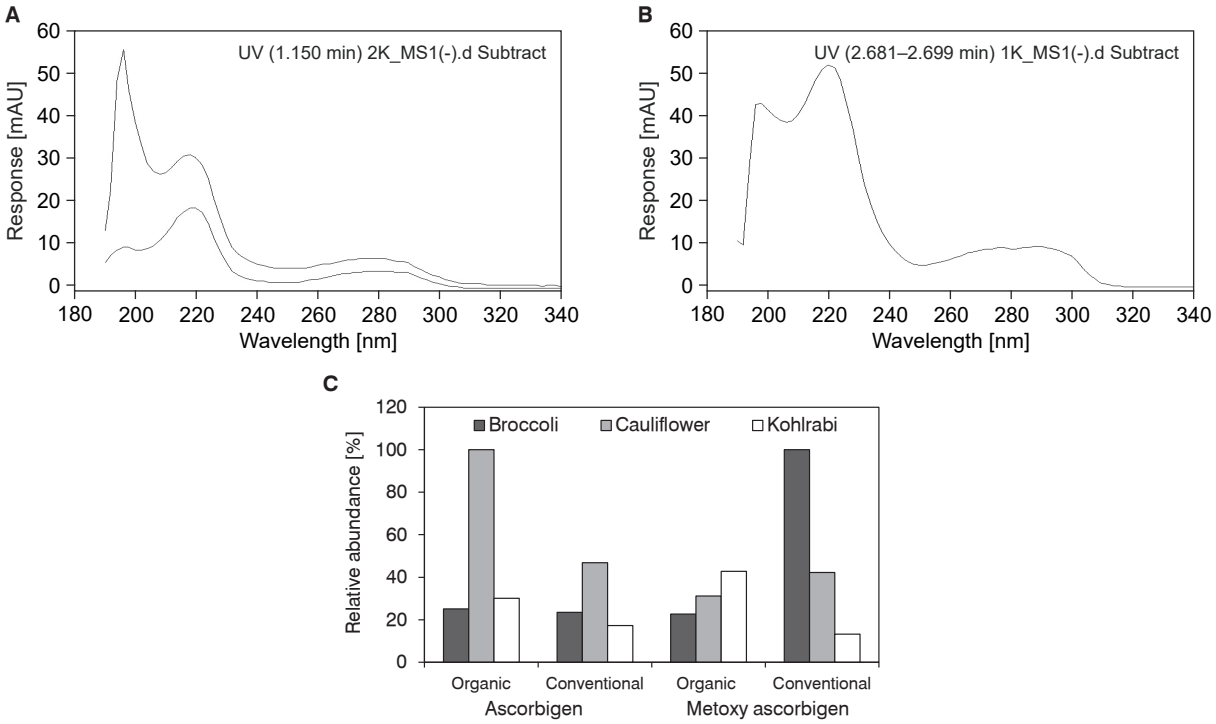

Fig. 1. UV-Vis spectral data and abundance of ascorbigen and 4-methoxyascorbigen in vegetable juices.

A – UV-Vis spectrum of ascorbigen, B – UV-Vis spectrum of methoxyascorbigen, C – relative abundance of ascorbigen and methoxyascorbigen in vegetable juices.
Relative abundance data based on peak areas in extracted ion chromatograms in the positive ionization mode are presented.

the production of flavonoid compounds affected all plants under the study. No significant interaction effect of vegetable variety and cropping system was observed ($F(2,10) = 2.95$, $p > 0.05$; Tab. 3). *TFC* (expressed as QE) ranged from 17.59 mg·l^{-1} to 71.69 mg·l^{-1}. It was significantly different in individual vegetables ($p < 0.001$, Tab. 4) and particularly high in broccoli (63.84 mg·l^{-1}, Tab. 4). *TFC* was significantly higher in juices of conventionally grown vegetables (50.52 mg·l^{-1}) than in juices of their organically grown counterparts (30.15 mg·l^{-1}, $p < 0.001$, Tab. 5). These are results opposite to those obtained by Naguib et al [9]. Our results showed that vegetable variety was the major factor that influenced the content of both *TPC* and *TFC*, while cropping practice predisposed only *TFC*. As suggested previously, environmental factors (geographic region, climate, soil, etc.) have potentially greater influence on the nutritive value of plants than cropping practice [31].

The results on antioxidant activity (*TEAC*) of vegetable juices were in the following ranges: 0.36–0.76 mmol·l^{-1} in DPPH assay, 1.31–3.96 mmol·l^{-1} in ABTS assay and 2.51–7.03 mmol·l^{-1} in CUPRAC assay. Using two-way analysis of variance, significant interaction effect of vegetable variety and cropping system to $TEAC_{DPPH}$, $TEAC_{ABTS}$ and $TEAC_{CUPRAC}$ was found ($F(2,8) = 14.1714$, $p = 0.002348$; $F(2,11) = 13.376$, $p = 0.0011$ and $F(2,11) = 72.071$, $p = 0.0000$, respectively; Tab. 6). When the means were subjected to Tukey's multiple comparison, conventionally grown cauliflower showed significantly lower $TEAC_{DPPH}$ (0.36 mmol·l^{-1}) than conventionally grown broccoli (0.76 mmol·l^{-1}; $p < 0.01$) and organically grown cauliflower and kohlrabi (0.65 mmol·l^{-1} and 0.70 mmol·l^{-1}, respectively; $p < 0.050$; Tab. 6). The highest $TEAC_{DPPH}$ shown by conventionally grown broccoli was not significantly different from the mean values estimated for the rest of samples excluding conventionally grown cauliflower (Tab. 6).

According to p values obtained by Tukey's HSD test, cauliflower showed $TEAC_{ABTS}$ superior to other vegetables (Tab. 6). It was 7.03 mmol·l^{-1} and 6.08 mmol·l^{-1} in conventionally and organically grown vegetables, respectively ($p > 0.05$). Also, no significant differences in antioxidant capacity of broccoli produced in both cropping system and organically grown kohlrabi were observed ($p > 0.05$). At the same time, conventionally grown kohlrabi showed significantly lower $TEAC_{ABTS}$ than all

other samples (2.51 mmol·l^{-1}, Tab. 6). Therefore, influence of cropping system on $TEAC_{ABTS}$ can be neglected for broccoli and cauliflower, but not for kohlrabi, whose $TEAC_{ABTS}$ was significantly decreased due to conventional cropping.

After analysis of interaction effect by Tukey's HSD test, reducing power of juices of conventionally grown broccoli and cauliflower was found to be significantly higher than reducing power of other samples ($p < 0.001$, Tab. 6). All conventionally grown vegetables showed significantly different $TEAC_{CUPRAC}$ ($p < 0.001$) but very similar reducing power was shown by all organically grown vegetables ($p > 0.05$, Tab. 6). Conventionally grown broccoli showed the highest reducing power (3.96 mmol·l^{-1}) whereas conventionally grown kohlrabi showed the lowest one (1.31 mmol·l^{-1}). Hence, both vegetable variety and cropping system have to be taken into consideration when attempting to produce food rich in antioxidants capable to react with cupric reagent.

By summarizing the results obtained from the three assays, the effect of the cropping system on antioxidant activity varied among the *Brassica* vegetables studied. Specifically, it had an effect on broccoli, but not consistently on kohlrabi and cauliflower. Contrary to the results obtained by Naguib et al. [9], results of our study revealed that conventional cropping enchanced antioxidant activity of broccoli.

All juices affected the growth of tested microbial strains (Tab. 8). Juices of conventionally grown vegetables showed the highest inhibitory activity against fungal pathogens. It was particularly high against clinical isolate II of a human vaginal specimen of *C. albicans* (with both *MIC* and *MFC*

Tab. 3. Statistical significance of main effects on concentrations of antioxidants.

	GLS	*TPC*	*TFC*
Vegetable variety	*	***	***
Cropping system	**	ns	***

GLS – total glucosinolate concentration, *TPC* – total phenolics concentration, *TFC* – total flavonoids concentration.
ns – not significant; *, **, *** – significant at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively.

Tab. 4. Concentrations of antioxidants in vegetable juices or extracts.

	GLS [mmol·l^{-1}]	*TPC* [mg·l^{-1}]	*TFC* [mg·l^{-1}]
Broccoli	4.61 ^{a}	696 ^{a}	63.84 ^{a}
Cauliflower	3.63 ^{b}	686 ^{a}	27.90 ^{b}
Kohlrabi	ND	398 ^{b}	35.82 ^{c}

Means followed by different lowercase superscript letters in a column are significantly different (Tukey's HSD test).
GLS – total glucosinolate concentration (expressed as millimoles of sinigrin equivalents), *TPC* – total phenolics concentration (expressed as milligrams of gallic acid equivalents), *TFC* – total flavonoids concentration (expressed as milligrams of quercetin equivalents), ND – not determined.

Tab. 5. Total glucosinolate and total flavonoids in vegetable juices or extracts.

	GLS [mmol·l^{-1}]	*TFC* [mg·l^{-1}]
Organic	4.61 ^{a}	30.15 ^{a}
Conventional	3.43 ^{b}	50.52 ^{b}

Means followed by different lowercase superscript letters in a column are significantly different (Tukey's HSD test).
GLS – total glucosinolate concentration (expressed as millimoles of sinigrin equivalents), *TFC* – total flavonoids concentration (expressed as milligrams of quercetin equivalents).

Tab. 6. Statistical significance of main effects on antioxidant activity.

	Statistical significance		
	DPPH	ABTS	CUPRAC
Vegetable variety	ns	*	***
Cropping system	ns	ns	***
Interaction effect	***	***	***

DPPH – scavenging capacity towards 2,2-diphenyl-1-picrylhydrazyl radical, ABTS – scavenging capacity towards 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC – cupric ion reducing antioxidant capacity.
ns – not significant; *, **, *** – significant at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively (two-way ANOVA).

Tab. 7. Antioxidant capacity of vegetable juices.

	TEAC [mmol·l^{-1}]		
	DPPH	ABTS	CUPRAC
Organic cropping			
Broccoli	0.50 ^{ab}	3.94 ^{a}	1.85 ^{a}
Cauliflower	0.65 ^{a}	6.08 ^{b}	1.72 ^{ad}
Kohlrabi	0.70 ^{a}	4.24 ^{a}	1.57 ^{ad}
Conventional cropping			
Broccoli	0.76 ^{a}	4.55 ^{a}	3.96 ^{b}
Cauliflower	0.36 ^{b}	7.03 ^{b}	2.74 ^{c}
Kohlrabi	0.52 ^{ab}	2.51 ^{c}	1.31 ^{d}

Mean values of three replicates are presented. Means followed by different lowercase superscript letters in a column are significantly different (Tukey's HSD test).
TEAC – Trolox equivalent antioxidant capacity, DPPH – scavenging capacity towards 2,2-diphenyl-1-picrylhydrazyl radical, ABTS – scavenging capacity towards 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC – cupric ion reducing antioxidant capacity.

Tab. 8. Antimicrobial activity of vegetable juices.

	Broccoli				Cauliflower				Kohlrabi			
	O	C	O	C	O	C	O	C	O	C	O	C
Bacteria	*MBC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]		*MBC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]		*MBC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]	
Staphylococcus aureus	50	25	– [a]	25	50	–	–	–	–	–	–	–
Enterococcus faecalis	–	–	–	–	50	–	–	–	–	–	–	–
Bacillus subtilis	25	25	–	–	50	25	–	–	25	25	–	–
Escherichia coli	25	25	50	25	50	12.5	–	12.5	25	50	50	–
Pseudomonas aeruginosa	–	50	–	–	–	25	–	25	–	–	–	–
Fungi	*MFC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]		*MFC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]		*MFC* [ml·l^{-1}]		*MIC* [ml·l^{-1}]	
S. enteritidis	50	50	–	50	–	50	–	50	50	50	–	–
Candida albicans (I)	25	12.5	25	25	6.25	25	50	25	12.5	12.5	12.5	25
Candida albicans (II)	50	25	–	25	–	25	–	25	50	50	50	50
Candida albicans (III)	50	6.25	50	6.25	50	6.25	50	6.25	25	6.25	25	6.25
Penicillium sp.	49.5	24.7	49.5	24.7	–	12.4	–	12.4	–	49.5	–	49.5
Aspergillus sp.	–	49.5	–	49.5	–	12.4	–	24.7	49.5	49.5	49.5	–

O – organic cropping, C – conventional cropping, *MBC* – minimum bactericidal concentration; *MFC* – minimum fungicidal concentration; *MIC* – minimum inhibitory concentration.
I – isolate from oral cavity, II, III – human clinical vaginal specimens, a – > 50 ml·l^{-1}.

at 6.25 ml·l^{-1}) and required 8 folds (broccoli, cauliflower) or 4 folds dilution (kohlrabi) in comparison to samples of organically grown vegetables. The juice of conventionally grown cauliflower was the most successful in inhibiting the growth of *E. coli* and *P. aeruginosa* (with both *MIC* and *MBC* at 12.5 ml·l^{-1} and 25 ml·l^{-1}, respectively) and filamentous fungi (*Penicillium* sp., both *MIC* and *MFC* at 12.4 ml·l^{-1}; *Aspergillus* sp., *MIC* at 12.4 ml·l^{-1} and *MFC* at 24.7 ml·l^{-1}), while organic sample was the most effective against *C. albicans* isolated from the oral cavity (strain I) and was the only one active against *E. faecalis* (Tab. 8). Juice of conventionally grown broccoli exhibited the highest inhibitory effect on *Staph. aureus* (Tab. 8). The least active against the bacterial strains were the juice extracted from fresh stems of kohlrabi, probably due to a lower concentration of phenolics and flavonoid compounds which are well known antimicrobial agents [32].

All investigated juices showed considerable antiproliferative activity against the most of the studied cancer cell lines. Two-way ANOVA showed significant interaction effect of vegetable variety and cropping system on antiproliferative activity against HeLa, MCF 7 and HT-29 cells ($F(1,12) = 20.46$, $p = 0.0007$; $F(2,15) = 301.15$, $p = 0.0000$ and $F(1, 9) = 78.57$, $p = 0.0000$, respectively; Tab. 9). According to Tukey's HSD test, conventionally grown broccoli showed significantly higher antiproliferative activity against HeLa cells (IC_{50} of 1.04 ml·l^{-1}) than organically grown broccoli (IC_{50} of 1.98 ml·l^{-1}, $p < 0.01$; Tab. 10) and conventionally grown kohlrabi (IC_{50} of 1.90 ml·l^{-1}, $p < 0.05$). No significant differences between antiproliferative activity of conventionally grown cauliflower and broccoli and organically grown kohlrabi against MCF 7 cells were observed (IC_{50} values of 1.17 ml·l^{-1}, 1.50 ml·l^{-1} and 1.55 ml·l^{-1}, respectively, $p > 0.05$; Tab. 10). Antiproliferative activity of these samples was significantly higher than that of their counterparts (organically grown cauliflower and broccoli and conventionally grown kohlrabi). Interestingly, among conventionally grown vegetables, cauliflower showed the highest and kohlrabi the lowest antiproliferative activity against MCF 7 cells, whereas kohlrabi showed the highest and cauliflower the lowest inhibitory activity among organically grown vegetables (Tab. 10). Juice of conventionally grown broccoli showed significantly higher inhibition of proliferation of HT-29 cells (IC_{50} of 1.55 ml·l^{-1}) than organically grown broccoli and organic and conventional kohlrabi (4.69 ml·l^{-1}, 3.18 ml·l^{-1} and 4.60 ml·l^{-1}, $p < 0.001$, $p < 0.01$ and $p < 0.001$, respectively; Tab. 10). High cancer chemopreventive activity of juices of broccoli and cauliflower was already described by Boivin et al. [33], while in the study conducted by Bachiega et al. [34], only non-polar extracts of broccoli showed considerable inhibition of proliferation of various cancer cells except of HT-29 cells. Interestingly, juice of conventionally grown

Tab. 9. Statistical significance of main effects on antiproliferative activity.

	Statistical significance		
	HeLa	MCF 7	HT-29
Vegetable variety	ns	***	*
Cropping system	ns	***	**
Interaction effect	***	***	***

HeLa – cervical cancer; MCF 7 – breast adenocarcinoma, HT-29 – colon adenocarcinoma-.
ns – not significant; *, **, *** – significant at $p < 0.05$, $p < 0.01$ or $p < 0.001$, respectively (two-way ANOVA).

Tab. 10. Antiproliferative activity of vegetable juices.

	IC_{50} [ml·l^{-1}]			
	HeLa	MCF 7	HT-29	MRC-5
Organic cropping				
Broccoli	1.98 ^{aA}	2.08 ^{aAB}	4.69 ^{aC}	(2.54) ^{B}
Cauliflower	nd	4.69 ^{c}	nd	nd
Kohlrabi	1.41 ^{abA}	1.55 ^{bA}	3.18 ^{bB}	(2.30) ^{C}
Conventional cropping				
Broccoli	1.04 ^{bA}	1.50 ^{bAB}	1.55 ^{cB}	(1.59) ^{B}
Cauliflower	(0.74) ^{A}	1.17 ^{bB}	(2.00) ^{C}	(1.70) ^{D}
Kohlrabi	1.90 ^{aA}	3.29 ^{dB}	4.60 ^{dC}	nd

Mean values of 4 replicates are presented. Means followed by different lowercase superscript letters in a column are significantly different (Tukey's HSD test). Means followed by different uppercase superscript letters in a row are significantly different (one-way ANOVA with post hoc Tukey's HSD test). Means in brackets were not subjected to two-way ANOVA, IC_{50} of the counterpart was not determined.
IC_{50} – inhibitory concentration of vegetable juice that causes 50% of cell growth inhibition, HeLa – cervical cancer, MCF 7 – breast adenocarcinoma, HT-29 – colon adenocarcinoma, MRC-5 – fetal human fibroblasts, nd – not determined (> 5 ml·l^{-1}).

broccoli showed high effectiveness against these cells in our study.

Cancer chemopreventive role of cruciferous vegetables is usually related to the content of glucosinolates and products of their degradation. Asc provides an extra source of ascorbic acid to a cell upon cleavage [35]. Indole moiety induces apoptosis and can inhibit invasion and expansion and adapts cellular signalling pathways [36]. Therefore, suppression of proliferation was probably associated with the presence of Asc in juices of the examined vegetables. Also, plant polyphenols alone or in combination can show pro-apoptotic effect and selectively target cancer cells at concentrations higher than those found in foods [37]. One-way ANOVA followed by Tukey's HSD test showed significantly higher antiproliferative activity of conventionally grown cauliflower and organically grown kohlrabi against HeLa and MCF 7 cells in comparison to cells of healthy tissue (Tab. 10). HT-29 and Raji cells appeared to be more resistant than healthy cells to the cytotoxic effect of juices at the applied concentrations (data for Raji cells are not shown because IC_{50} was determined only for organically grown kohlrabi and it was 2.59 ml·l^{-1}).

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