# Nutritional and nutraceutical potential of rape (*Brassica napus* var napus) and "tronchuda" cabbage (*Brassica oleraceae* var costata) inflorescences

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**Abstract**

Two traditional cultivated vegetables highly consumed among Northern Portuguese regions were tested for their chemical composition, nutritional profile and *in vitro* antioxidant properties using four assays: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity, reducing power, inhibition of β-carotene bleaching and inhibition of lipid peroxidation by thiobarbituric acid reactive substances (TBARS) assay. The studied varieties of two *Brassica* species, locally known as "grelos" (rape) and "espigos" ("tronchuda" cabbage) are nutritionally well-balanced vegetables; particularly "tronchuda" cabbage revealed the highest levels of moisture, proteins, fat, energy, β-carotene and vitamin C; rape gave the highest contents of ash, carbohydrates, sugars (including fructose, glucose, sucrose and raffinose), essential n-3 fatty acid α-linolenic acid, and the best ratios of PUFA/SFA and n-6/n-3 fatty acids, tocopherols, lycopene, chlorophylls, phenolics, flavonoids, and also the highest antioxidant properties. The health benefits associated to the antioxidant properties reinforce their contribution to a healthy and balanced diet, highlight the interest of their consumption, validate the empirical use and add new values to traditional/regional products which have been used for a long time.

*Keywords:* Leafy-vegetables, "tronchuda" cabbage, rape, *Brassica napus* var *napus*, *B. oleracea* var *costata*, nutraceuticals

## 1. Introduction

In the Iberian Peninsula wild and traditionally cultivated greens have been particular important as staple foods since ancient times. Dietary patterns and local food knowledge are part of the cultural heritage of the Mediterranean which is changing and disappearing rapidly, mainly affected by rural exodus and abandonment of traditional/regional farming systems and by global lifestyles recently introduced. At the same time, nutraceuticals and functional foods have become key issues in behavioural nutrition and diets. The nutritional potential of local vegetables (cultivated for centuries and non-cultivated) and their potential health benefits are recognised as important domains of research. Rape (port: couve-nabo, grelos; *Brassica napus* L. var. *napus*) and “tronchuda” cabbage (port: couve-portuguesa, couve-tronchuda, espigos; *Brassica oleracea* L. var. *costata* DC.), both vernacular English names according to USDA plants database, are examples of vegetables traditionally cultivated and widely consumed in rural communities in Northern Portugal. Regional ancient crop varieties, cultivated in fall/winter, present cold resistance and are multipurpose has they provide leaves, taproots and inflorescences used as fodder and as food for humans, seeds, and plant matter that is recycled and incorporated in soil (Carvalho, 2010).

According to ethnobotanical surveys the food products are tastier and more nutritious in the cold months, after the first frost which increase their edibility (Carvalho, 2010). Besides the leaves (consumed in fall and winter) which are common accompaniments of fish and meat dishes and important ingredients for a popular pork and beans stew, the inflorescences, occurring in terminal tender branches in late winter and early spring, are especially appreciated for particular soups and rice and to eat with traditional sausages. These products are nowadays included in many gastronomic menus and national

gourmet events. Together with this renewed interest, the assumption of health benefits and the revival of popular food/habits has contribute to revitalize their use and consumption and to increase the prizes and then farmers and markets interest in these crops (Carvalho, 2010).

It is known that plants possess free sugars, organic acids, amino acids (free and in proteins), lipids and minerals which are natural components of many fruits and vegetables and they play an important role in maintaining fruit and vegetable quality and determining nutritive value in human diet (Ayaz et al., 2006; Belitz and Grosch, 2006). Furthermore, several epidemiological studies have indicated that a high intake of plant products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (Farag and Motaal, 2010; Gosslau and Chen, 2004; Gundgaard et al., 2003; Kris-Etherton et al., 2002). These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids (Podsędek, 2007).

Nutrient antioxidants may act together to reduce reactive oxygen species level more effectively than single dietary antioxidants, because they can function as synergists. In addition, a mixture containing both water-soluble and lipid-soluble antioxidants is capable of quenching free radicals in both aqueous and lipid phases (Podsędek, 2007).

Some literature can be found about phenolic and organic acids composition, and antioxidant properties of inflorescences (Sousa et al., 2008), leaves (Vrchovská et al., 2006) and seeds (Ferreres et al., 2007) of "tronchuda" cabbage (*Brassica oleracea* L. var. *costata* DC.); nevertheless, there are no reports on its nutritional value and detailed characterization in sugars, fatty acids, vitamin C, vitamin E and carotenoids. Moreover,

as far as we know, this is the first report on nutritional and antioxidant properties of rape (*Brassica napus* L. var. *napus*).

The determination of the chemical composition of rape and "tronchuda" cabbage inflorescences has become an interest of ours due to its extensive consumption in the north of Portugal. The aims of this work were: i) to contribute to the scarce knowledge about their free sugars, fatty acids and lipid-soluble (vitamin E and carotenoids) and water-soluble (ascorbic acid, phenolics and flavonoids) antioxidants composition; ii) to display the nutritional profile of the plants and their antioxidant properties, in order to know how nutritionally important are rape and "tronchuda" cabbage in the diet of people who consume them; iii) to increase value to local food products which have been used for many generations.

## 2. Materials and methods

### *2.1. Samples*

The plant materials analysed were those usually known in Bragança, North-eastern Portugal, as "grelos", referring to sections with yellow flowers of *Brassica napus* L. var. *napus,* and "espigos", related to portions with white flowers of *Brassica oleracea* L. var. *costata* (Carvalho, 2010).

In early spring of 2009, specimens of the above mentioned plants were bought from small farmers, at the local market in Bragança, considering consumers' recommendations and preferences, as reported in ethnobotanical surveys (Carvalho, 2010). In both cases, pieces of about 30 cm, corresponding to terminal soft leafy branches with inflorescences are picked up in spring from the plants remaining in the

fields, after the main leaves have been cut during winter. For consumption the soft leaves and the inflorescences are detached from the main stems (Carvalho, 2010).

The samples for analysis correspond to detached leaves and inflorescences of the studied materials, "grelos" ( *Brassica napus* L. var. *napus*) and "espigos" (*Brassica oleracea* L. var. *costata*), taking in account users'practices.

Morphological key characters from the Flora Iberica (Castroviejo, 2003) were used for identification and nomenclature. Voucher specimens are deposited in the Escola Superior Agrária de Bragança herbarium (BRESA).

The samples were lyophilized (Ly-8-FM-ULE, Snijders, Holland), reduced to a fine powder (20 mesh), and kept in the best conditions for subsequent use.

*2.2. Standards and Reagents*

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, L-ascorbic acid, tocopherols and sugars standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin. Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

*2.3. Nutritional value*

*Macronutrients.* The samples were analysed for chemical composition (moisture, protein, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations: Energy (kcal) = 4 × (g protein +g carbohydrate) + 9 × (g lipid).

*Sugars.* Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by Barros et al. (2010a). Dried sample powder (1.0 g) was spiked with the melezitose as internal standard (IS, 5 mg/ml), and was extracted with 40 ml of 80% aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged (Centorion K24OR- 2003 refrigerated centrifuge) at 15,000 g for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted three times with 10 ml of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 ml and filtered through 0.2 µm nylon filters from Whatman. Soluble sugars were determined by HPLC. The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 $NH_2$ column (4.6 x 250 mm, 5 mm, Knauer) operating at 30ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 7:3

(v/v) at a flow rate of 1 ml/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Quantification was based on the RI signal response of each standard, using the IS method and by using calibration curves obtained from commercial standards of each compound (fructose, glucose, sucrose, trehalose and raffinose; 0.18-0.24 mg/ml). The results are expressed in g per 100 g of dry sample.

*Fatty Acids.* Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Barros et al., 2010a), and after the following trans-esterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 ml of methanol:sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 ml of deionised water were added, to obtain phase separation; the FAME were recovered with 3 ml of diethyl ether by shaking in vortex , and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Whatman. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30 m x 0.32 mm ID x 0.25 µm $d_f$). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 10ºC/min ramp to 240 ºC and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. For each analysis 1 µl of the sample was injected in GC. Fatty acid identification was made by comparing the relative retention times of

FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

*2.4. Lipo-soluble antioxidants*

*Tocopherols.* Tocopherols content was determined following a procedure previously optimized and described by Barros et al. (2010b). BHT solution in hexane (10 mg/ml; 100 μl) and IS solution in hexane (tocol; 50 μg/ml; 400 μl) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 ml) by vortex mixing (1 min). Subsequently, hexane (4 ml) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 ml) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 ml of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 µm nylon filters from Whatman, transferred into a dark injection vial and analysed by the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 x 4.6 mm) normal-phase column from YMC Waters operating at 30ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min, and the injection volume was 20 µl. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS method and by using calibration curves

obtained from commercial standards of each compound (α-, β-, γ- and δ-tocopherol; 0.25-16.00 μg/ml). Tocopherol contents in the samples were expressed in mg per 100 g of dw.

*Carotenoids and chlorophylls.* The compounds were determined according to the method of Nagata and Yamashita (1992). A fine dried powder (500 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (AnalytikJena 200-2004 spectrophotometer). Content of β-carotene was calculated according to the following equation: β-carotene (mg/100 ml) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Lycopene (mg/100 ml) = $-0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Chlorophyll a (mg/100 ml) = $0.999 \times A_{663} - 0.0989 \times A_{645}$; Chlorophyll b (mg/100 ml) = $-0.328 \times A_{663} + 1.77 \times A_{645}$, and further expressed in mg per 100 g of dry weight.

### *2.5. Water-soluble antioxidants*

*Vitamin C.* Vitamin C was determined according to the method of Klein and Perry (1982). A fine powder (20 mesh) of sample (500 mg) was extracted with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through Whatman Nº 4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichloroindophenol (9 ml) and the absorbance was measured within 30 min at 515 nm against a blank. Content of vitamin C was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.034-5.68 mM), and the results were expressed as mg per 100 g of dry weight.

*Phenolics and flavonoids.* The extractions were performed using a fine dried powder (20 mesh; ~1g) stirring with 50 ml of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 50 ml portion of methanol. The combined methanolic extracts were evaporated at 35ºC under reduced pressure (rotary evaporator Büchi R-210) and re-dissolved in methanol at a known concentration.

Phenolics were estimated based on procedures described by Wolfe at al. (2003) with some modifications. An aliquot of the extract solution (1 ml) was mixed with *Folin-Ciocalteu* reagent (5 ml, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/l, 4 ml). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to calculate the standard curve (0.050-0.80 mM), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Flavonoids were determined using the method of Jia et al. (1999), with some modifications. An aliquot (0.5 ml) of the extract solution was mixed with distilled water (2 ml) and subsequently with $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution (10%, 0.15 ml) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 ml) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.016-1.0 mM) and the results were expressed as mg of (+)-chatequin equivalents (CE) per g of extract.

*2.6. In vitro antioxidant properties*

*General.* Four in vitro assays were applied to evaluate the antioxidant activity of the samples (Martins et al., 2010). Different concentrations of the extracts prepared in the section 2.5.2. (0.63–20.00 mg/ml) were used to find $EC_{50}$ values.

*DPPH radical-scavenging activity.* This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µl) and aqueous methanolic solution (80:20 v/v, 270 µl) containing DPPH radicals ($6\times10^{-5}$ mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\% \ RSA = [(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where $A_S$ is the absorbance of the solution when the sample extract has been added at a particular level, and $A_{DPPH}$ is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity ($EC_{50}$) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

*Reducing power.* This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 ml) were mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The mixture (0.8 ml) was poured in the 48-wells, as also deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance

was measured at 690 nm. The extract concentration providing 0.5 of absorbance ($EC_{50}$) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

*Inhibition of β-carotene bleaching.* A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 ml). The tubes were shaken and incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity ($EC_{50}$) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

*Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS).* Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 ml) of the supernatant was incubated with the different concentrations of the

extracts (0.2 ml) in the presence of $FeSO_4$ (10 μM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition ($EC_{50}$) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

### *2.7. Statistical analysis*

For each plant, three samples were analysed and the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

## 3. Results and discussion

### *3.1. Nutritional value*

The results of the nutrients composition and energetic value (expressed on dry weight basis) obtained for the studied *Brassica* inflorescences are shown in Table 1. "Grelos" (rape, *Brassica napus* var *napus*) revealed higher moisture content (87.34 g/100 g) than

"espigos" ("tronchuda" cabbage, *Brassica oleraceae* var *costata*) (85.96 g/100 g). Carbohydrates, calculated by difference, were the most abundant macronutrients and were higher for "espigos" (84.82 g/100 g). In fact, "espigos" besides inflorescences, include also small fleshy and highly veined leaves that are in general more fibrous than the leaves of "grelos". Ash content was higher in "tronchuda" cabbage material (7.98 g/100 g), while protein (4.40 g/100 g), fat (3.92 g/100 g) and energy (388.48 kcal/100 g) values were higher in rape material, which is consistent with the presence of greater numbers of flower buds than leaves in "grelos". According to users, the particular texture of each material is well adapted to the regional recipes; "grelos" are mainly consumed sautéed or made in juicy rice, while "espigos" are mostly stewed or cooked and seasoned with olive oil (Carvalho, 2010).

The fat content found in "tronchuda" cabbage was higher than the concentration found in collards (*Brassica oleraceae*, Acephala group) (2.3 g/100 g of dry weight; Almazan and Adeyeye, 1998), a group of *Brassica oleracea* that does not have the usual close-knit core of leaves, and which leaves, inflorescences and local uses are more closer to "tronchuda" ones than the other types of cabbages are.

Both inflorescences presented fructose, glucose, sucrose, trehalose and raffinose (Table 1). Sugars are only a small part of carbohydrates (Table 1) that also include polysaccharides such as amide and cellulose. The monosaccharides fructose and glucose were the most abundant sugars in "tronchuda" cabbage and rape, respectively. The latter revealed the highest total sugars content (9.35 g/100 g), with the highest levels of all the sugars unless the disaccharide trehalose. The value of total sugars found in "tronchuda" cabbage inflorescences (7.13 g/100 g) was higher than the concentration found in leaves of kale (*Brassica oleraceae* L., var. *acephala* DC.) (3.96 g/100 g; Ayaz et al., 2006), for

instance. Furthermore, the oligosaccharides trehalose and raffinose were not found in that sample.

Lipids are partly responsible for the physical and chemical properties of food and those that are of major nutritional interest are the fatty acid esters. Many lipid properties in food are explained in terms of their fatty acid composition (Ayaz et al., 2006). The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 of the studied *Brassica* inflorescences are shown in Table 2. A significant prevalence of α-linolenic acid (C18:3n3) was observed in "tronchuda" cabbage and rape (61 and 70%, respectively). Linoleic acid (C18:2n6) was also abundant in both samples and, therefore, PUFA predominated over MUFA and SFA contents. The nutritional value of n-3 and n-6 fatty acids is widely known for its health beneficial effects (Guil et al., 1996). The profile obtained for "tronchuda" cabbage inflorescences is similar to the one observed in leaves of kale (*Brassica oleraceae* L., var. *acephala* DC.) (Ayaz et al., 2006). Palmitic acid was the SFA most abundant in the samples (~10%). Twenty two fatty acids were identified and quantified.

Both samples showed PUFA/SFA ratios higher than 0.45 and n-6/n-3 ratios lower than 4.0 (Table 2) as recommended for "good nutritional quality" (Guil et al., 1996); the best values were revealed by rape inflorescences (5.13 and 0.15, respectively).

### *3.2. Lipo-soluble and water-soluble antioxidants*

Lipo-soluble (tocopherols, carotenoids and chlorophylls) and water-soluble (vitamin C, phenolics and flavonoids) antioxidants contents were determined and the results are given in Table 3. The four vitamers of tocopherols were detected, being α-tocopherol

the major compound in both samples. Rape presented the highest content of tocopherols (59.49 mg/ 100 g of dry weight) with the highest levels of all the isoforms unless β-tocopherol. β-carotene, lycopene and chlorophylls a and b were quantified in both samples being the first pigment higher in "tronchuda" cabbage (23.00 mg/100 g dry weight; Table 3), while the others predominated in rape (3.53, 43.09 and 16.84 mg/100 g, respectively; Table 3). In a study with *Brassica* vegetables (broccoli, Brussels sprouts, white cabbage, red cabbage, kale and cauliflower), Podsędek (2007) reported kale (*Brassica oleraceae* L., var. *acephala* DC.) as the best source of lipo-soluble antioxidants including carotenoids (β-carotene- 9.23 mg/100 g of fresh weight and lutein+zeaxanthin- 39.55 mg/100 g of fresh weight) and tocopherols (α and γ tocopherols- 2.15 mg/100 g of fresh weight). The samples analysed in the present study revealed higher contents of tocopherols (2.31 and 7.17 mg/100 g of fresh weight for "tronchuda" cabbage and rape, respectively) but lower contents of β-carotene (3.23 and 1.69 mg/100 g fresh weight for "tronchuda" cabbage and rape, respectively).

Vitamin C, which includes ascorbic acid and its oxidation product- dehydroascorbic acid, is an important nutrient in vegetables, and it was quantified in both samples, being more abundant in "tronchuda" cabbage inflorescences (126.39 mg/100 g of dry weight; Table 3). Other authors reported ascorbic acid contents in "tronchuda" cabbage leaves (1734.3 mg/100 g of lyophilised extract; Vrchovská et al., 2006) and seeds (854.6 mg/100 g of dry weight; Ferreres et al., 2007), but not in inflorescences that are herein reported for the fist time. Another *Brassica oleracea* variety, kale, showed an ascorbic acid concentration of 92.6 mg/100 g of fresh weight (Podsędek, 2007). Nevertheless, not only cooking process of *Brassica* vegetables but also their manipulation (eg. freezing) affects the retention of ascorbic acid in the tissues, due to its high degree of

water solubility and low stability, resulting in a great lost of vitamin C (Francisco et al., 2010).

Phenolics were the major antioxidant components and rape inflorescences revealed the highest content in phenolics (1745.74 mg /100 g of dry weight), a value slightly lower than the concentration described by Sousa et al. (2008) (1957 mg/100g). The studied samples showed similar contents of flavonoids (~220 mg/100 g of dry weight; Table 3). Sousa et al. (2008) reported that "tronchuda" cabbage (*B. oleracea* var. costata) and kale (*B. oleracea* L. var. *acephala*) inflorescences presented a similar qualitative phenolic composition, exhibiting several complex kaempferol derivatives and 3-*p*-coumaroylquinic acid, while turnip (*B. rapa* var. *rapa*) was characterized by kaempferol and isorhamnetin glycosides and several phenolic acids derivatives.

The major antioxidants identified (phenolics, flavonoids, vitamins C and E, and carotenoids) scavenge radicals and inhibit the chain initiation or break the chain propagation (the second defence line). Vitamin E and carotenoids also contribute to the first defence line against oxidative stress, because they quench singlet oxygen. Flavonoids as well as vitamin C showed a protective activity to α-tocopherol in human LDL, and they can also regenerate vitamin E, from the α-chromanoxy radical (Podsedek, 2007).

*3.3. In vitro antioxidant properties*

To evaluate the antioxidant activity of the *Brassica* inflorescences, four different *in vitro* assays were performed. The first one was the evaluation of scavenging effects on DPPH radicals and the results expressed the decrease in DPPH radical absorption, at 515 nm, after exposure to radical scavengers. The extracts scavenging effects on DPPH

radicals increase with the concentration (Figure 1a). The second assay evaluated the reducing power of the samples measuring the conversion of a $Fe^{3+}$/ferricyanide complex to $Fe^{2+}$. Reducing power of the samples increased with the increase of concentration (Figure 1b). A high value of absorbance at 700 nm is related to a high reducing power. The inhibition of β-carotene bleaching was another assay that measured the capacity of the samples to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models. The results are given in Figure 1c. The last assay evaluated the inhibition of lipid peroxidation in brain cells homogenates (Figure 1d) by measuring the colour intensity of the complex formed between thiobarbituric acid and products of lipid peroxidation such as malondialdehyde (MDA-TBA complex).

Rape inflorescences showed higher antioxidant activity than "tronchuda" cabbage inflorescences, revealing the lowest $EC_{50}$ values in all the assays (Table 4). These results are in agreement with the highest content of phenolics and flavonoids found in rape sample. Particularly, DPPH scavenging activity of both samples was higher than the one reported by Sousa et al. (2008) for "tronchuda" cabbage (0.754 mg/ml), kale (0.565 mg/ml) and turnip (0.774 mg/ml) inflorescences, and than DPPH scavenging effects reported by Farag and Motaal (2010) for turnip leaves (1 mg/ml).

Overall, the nutritional composition of the studied *Brassica* material ("grelos" and "espigos") confirms the benefits of its inclusion in the typical regional gastronomy and validates the extended consumption since a long time ago. They are nutritionally well-balanced vegetables; particularly "tronchuda" cabbage revealed the highest levels of moisture, proteins, fat, energy, β-carotene and vitamin C; rape gave the highest contents

of ash, carbohydrates, total sugars (including fructose, glucose, sucrose and raffinose), essential n-3 fatty acid α-linolenic acid, and the best ratios of PUFA/SFA and n-6/n-3 fatty acids, tocopherols (including -α, -γ and δ- isoforms), lycopene, chlorophylls, phenolics, flavonoids, and also the highest antioxidant properties. The present study reports a nutritional characterization of "tronchuda" cabbage (the available studies were focused on phytochemical characterization) and rape (as far as we know there are no such reports), but also their composition in lipo-soluble and water-soluble antioxidants. Furthermore, the health benefits associated to their antioxidant properties reinforce their importance in local diets and add value to regional food products consumed for many generations. These results also emphasize the role of wild and cultivated food plants in regional cuisine and as part of cultural heritage of a region.

Our findings stress that interdisciplinary studies regarding traditional food culture are required, due to dramatic loss of plant knowledge, uses and practices, global development and changing lifestyle. Moreover, systematic research may contribute to maintain the use of non-cultivated and long time regionally cultivated food plants, as well as to the search for new nutraceuticals which are of potential interest in the prevention of aging related diseases as recommended by The Local Food-Nutraceuticals Consortium (Heinrich et al., 2005).

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**Table 1.** Moisture (g/100 g of fresh weight), nutrients (g/100 g of dry weight) and energetic value (Kcal/100 g of dry weight) of *Brassica* inflorescences (mean ± SD; n=9). In each row, different letters mean significant differences ($p<0.05$).

| | *Brassica oleraceae* var *costata* | *Brassica napus* var *napus* |
|---|---|---|
| Moisture | 85.96 ± 0.14 b | 87.34 ± 0.34 a |
| Ash | 7.98 ± 0.01 a | 7.79 ± 0.07 b |
| Proteins | 4.19 ± 0.00 b | 4.40 ± 0.08 a |
| Fat | 3.01 ± 0.32 b | 3.92 ± 0.60 a |
| Carbohydrates | 84.82 ± 0.22 a | 83.88 ± 0.52 b |
| Energy | 383.14 ± 1.15 b | 388.48 ± 1.93 a |
| Fructose | 3.26 ± 0.11 b | 4.02 ± 0.18 a |
| Glucose | 2.97 ± 0.10 b | 4.75 ± 0.35 a |
| Sucrose | 0.41 ± 0.09 a | 0.33 ± 0.05 a |
| Trehalose | 0.20 ± 0.02 a | 0.05 ± 0.00 b |
| Raffinose | 0.29 ± 0.02 b | 0.20 ± 0.01 a |
| Total Sugars | 7.13 ± 0.30 b | 9.35 ± 0.61 a |

**Table 2.** Composition of *Brassica* inflorescences in fatty acids (mean ± SD; n=9). In each row different letters mean significant differences ($p<0.05$).

| | *Brassica oleraceae* var *costata* | *Brassica napus* var *napus* |
|---|---|---|
| C6:0 | 0.71 ± 0.10 | 0.25 ± 0.05 |
| C8:0 | 0.04 ± 0.00 | 0.07 ± 0.01 |
| C10:0 | 0.11 ± 0.01 | 0.11 ± 0.02 |
| C12:0 | 0.30 ± 0.04 | 0.15 ± 0.04 |
| C14:0 | 0.68 ± 0.14 | 0.43 ± 0.02 |
| C14:1 | 0.16 ± 0.01 | 0.11 ± 0.00 |
| C15:0 | 0.36 ± 0.06 | 0.24 ± 0.03 |
| C16:0 | 10.87 ± 1.02 | 10.41 ± 0.03 |
| C16:1 | 0.24 ± 0.02 | 0.26 ± 0.01 |
| C17:0 | 0.20 ± 0.01 | 0.16 ± 0.02 |
| C18:0 | 2.17 ± 0.23 | 2.71 ± 0.15 |
| C18:1n9 | 1.48 ± 0.14 | 2.26 ± 0.01 |
| C18:2n6 | 19.60 ± 0.86 | 10.30 ± 0.14 |
| C18:3n3 | 60.56 ± 2.76 | 70.02 ± 0.13 |
| C20:0 | 0.49 ± 0.06 | 0.43 ± 0.00 |
| C20:2 | 0.21 ± 0.01 | 0.25 ± 0.02 |
| C20:4n6 | 0.32 ± 0.03 | 0.24 ± 0.03 |
| C20:3n3+C21:0 | 0.10 ± 0.00 | 0.16 ± 0.00 |
| C22:0 | 0.18 ± 0.03 | 0.65 ± 0.05 |
| C22:1n9 | 0.14 ± 0.04 | 0.53 ± 0.02 |
| C24:0 | 1.02 ± 0.00 | 0.19 ± 0.01 |
| C24:1 | 0.07 ± 0.01 | 0.08 ± 0.00 |
| SFA | 17.13 ± 1.70 a | 15.79 ± 0.03 a |
| MUFA | 2.09 ± 0.22 b | 3.24 ± 0.00 a |
| PUFA | 80.78 ± 1.91 a | 80.97 ± 0.03 a |
| PUFA/SFA | 4.75 ± 0.58 a | 5.13 ± 0.01 a |
| n-6/n-3 | 0.33 ± 0.03 a | 0.15 ± 0.00 b |

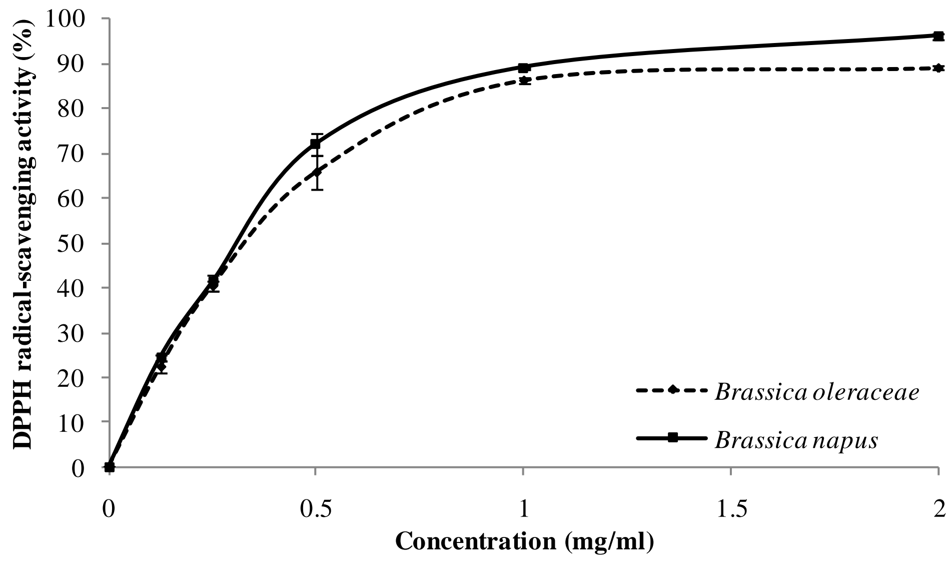
Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); *cis*-11,14-Eicosadienoic acid (C20:2c); Arachidonic acid (C20:4n6); *cis*-11, 14, 17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); Behenic acid (C22:0); Erucic acid (C22:1n9); Lignoceric acid (C24:0); Nervonic acid (C24:1). SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids.

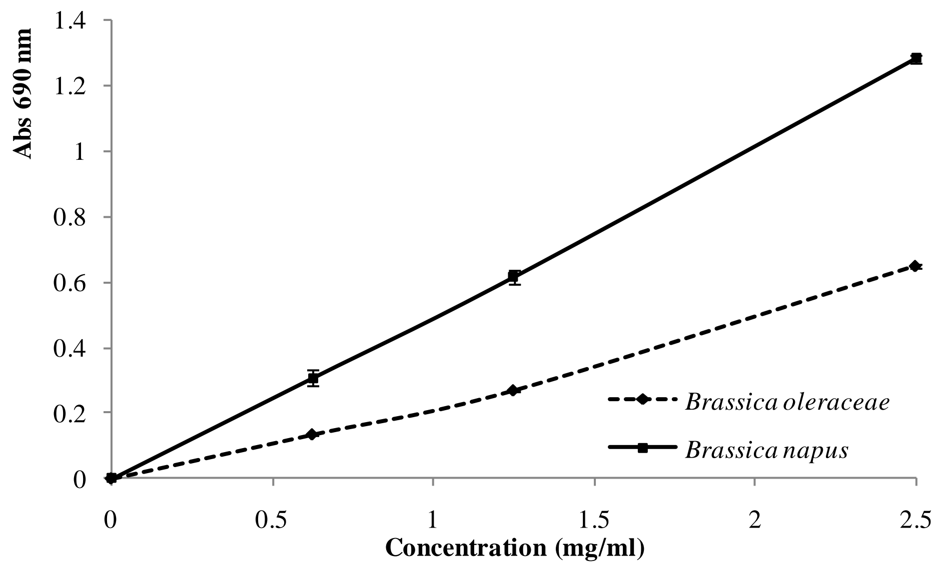
**Table 3.** Composition of *Brassica* inflorescences in lipo-soluble and water-soluble antioxidants (mg/100 g dry weight) (mean ± SD; n=9). In each row different letters mean significant differences ($p$<0.05).

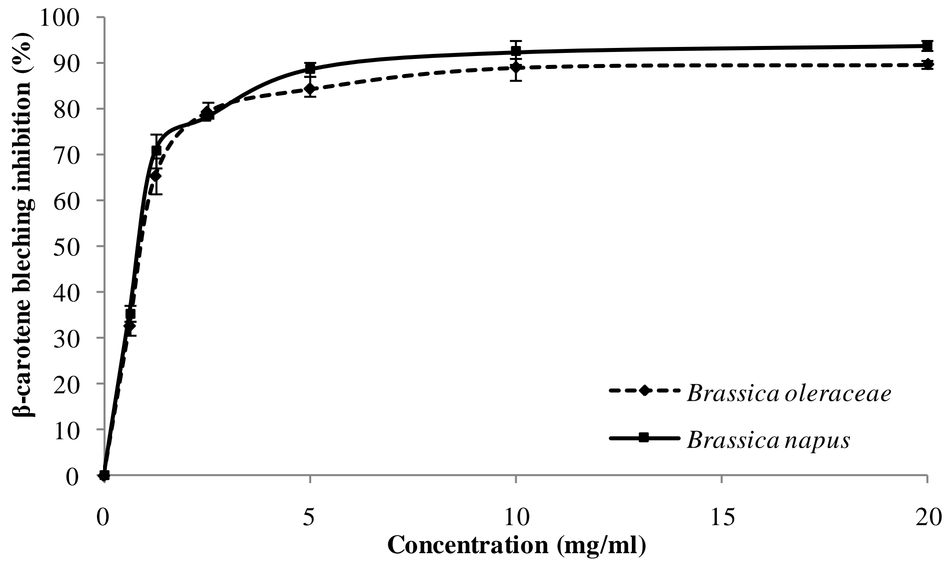
| | *Brassica oleraceae* var *costata* | *Brassica napus* var *napus* |
|---|---|---|
| α-tocopherol | 12.43 ± 1.94 b | 48.22 ± 1.02 a |
| β-tocopherol | 0.92 ± 0.15 a | 0.68 ± 0.05 b |
| γ-tocopherol | 2.86 ± 0.53 b | 6.96 ± 0.04 a |
| δ-tocopherol | 0.26 ± 0.03 b | 0.63 ± 0.06 a |
| Total tocopherols | 16.47 ± 2.65 b | 56.49 ± 1.20 a |
| β-carotene | 23.0 ± 0.41 a | 13.34 ± 1.13 b |
| Lycopene | 1.06 ± 0.07 b | 3.53 ± 0.20 a |
| Chlorophyll a | 28.26 ± 0.01 b | 43.09 ± 0.00 a |
| Chlorophyll b | 16.50 ± 0.00 b | 16.84 ± 0.02 a |
| Vitamin C | 126.39 ± 12.92 a | 63.73 ± 3.82 b |
| Phenolics | 1585.29 ±170.22 b | 1745.74 ± 52.14 a |
| Flavonoids | 222.57 ± 23.90 a | 221.01 ± 6.60 a |

**Table 4.** Extraction yields and antioxidant activity ($EC_{50}$ values, mg/ml) of the *Brassica* inflorescences (mean ± SD; n=9). In each row different letters mean significant differences ($p$<0.05).

| | *Brassica oleraceae* var *costata* | *Brassica napus* var *napus* |
|---|---|---|
| η (%) | 47.19 ± 3.78 | 41.56 ± 5.33 |
| DPPH scavenging activity | 3.46 ± 0.23 a | 3.22 ± 0.15 b |
| Reducing power | 2.11 ± 0.01 a | 1.02 ± 0.04 b |
| β-carotene bleaching inhibition | 0.96 ± 0.07 a | 0.88 ± 0.04 b |
| TBARS inhibition | 1.93 ± 0.12 a | 1.59 ± 0.06 b |


DPPH radical-scavenging activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
Concentration (mg/ml)
Brassica oleraceae
Brassica napus


Abs 690 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
Concentration (mg/ml)
Brassica oleraceae
Brassica napus



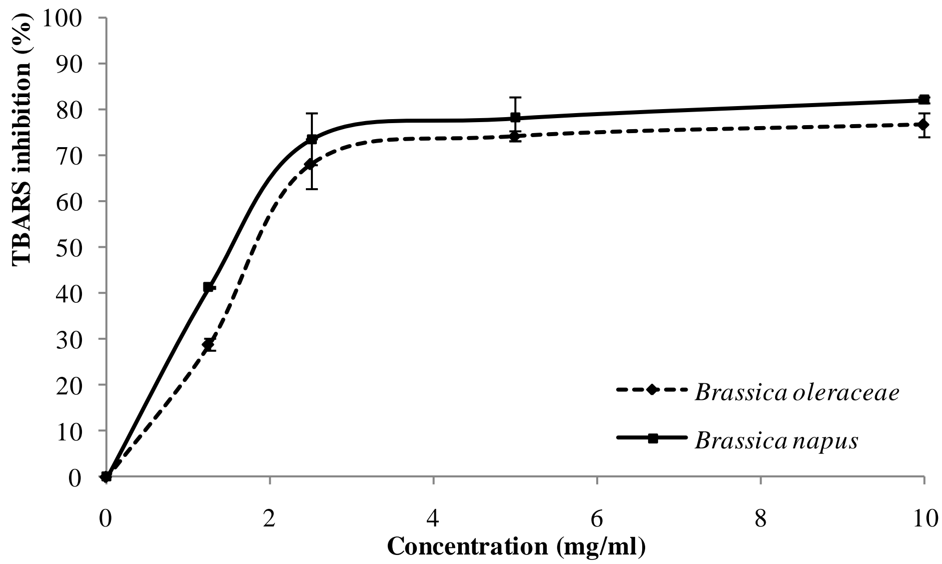




**Figure. 1.** DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation ihibition through TBARS assay of the *Brassica* inflorescences.