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Bioactive Compounds, Myrosinase Activity, and Antioxidant Capacity of White Cabbages Grown in Different Locations of Spain

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ABSTRACT: The influence of two Spanish growing locations with well-differentiated climatic conditions (northern and eastern areas) on the main bioactive compounds, glucosinolates (GLS), total phenolic compounds (TPC), and vitamin C, as well as myrosinase activity and antioxidant capacity in five white cabbage (*Brassica oleracea* L. var. *capitata*) cultivars was investigated. Cabbages with the highest concentration of total GLS presented the highest vitamin C level ($r = 0.75$, $P \leq 0.05$) and the lowest antioxidant capacity ($r = -0.76$, $P \leq 0.05$). The cultivars with the highest vitamin C content had the lowest myrosinase activity ($r = -0.89$, $P \leq 0.05$) and antioxidant capacity ($r = -0.86$, $P \leq 0.05$), whereas those with the largest TPC amount showed the highest antioxidant capacity ($r = 0.71$, $P \leq 0.05$). Cabbage cultivars grown in the northern area of Spain with low temperatures and radiation led to higher mean values of myrosinase activity (29.25 U/g dm), TPC (10.0 GAE mg/g dm), and antioxidant capacity (81.6 μmol Trolox/g dm), whereas cultivars grown in the eastern area with high temperature and radiation led to larger mean values of GLS (14.3 μmol/g dm) and vitamin C (5.3 mg/g dm). The results of this investigation provide information regarding the most suitable Spanish growing location to produce white cabbage with an optimized content of health-promoting compounds.

KEYWORDS: white cabbage, glucosinolates, myrosinase, vitamin C, total phenolics, antioxidant capacity, growing location, genotype

INTRODUCTION

Studies supported by extensive research in human volunteers, animal models, and cell culture systems have established the protective role of *Brassica* vegetables in several types of cancer.[1−3] This protective effect is associated with the health-promoting phytochemical content in *Brassica* vegetables, which includes glucosinolates (GLS) and their breakdown products (isothiocyanates (ITC) and indoles) as well as antioxidants such as vitamin C and phenolic compounds.[4,5]

GLS are β-thioglycoside *N*-hydroxysulfates that upon degradation either by myrosinase (β-thioglucoside glucohydrolase) within the plant or by enzymatic decomposition within the gastrointestinal tract yield biologically active hydrolyzed products.[5] There is a large body of evidence showing the chemopreventive action of GLS hydrolysis products by modulating detoxification enzymes,[6] which protects against DNA damage and proliferation of cancer cells.[1,2] Ascorbigen is the major GLS breakdown product found in fermented white cabbage (sauerkraut), which results from the hydrolysis of glucobrassicin by myrosinase enzyme and the further reaction with L-ascorbic acid at low pH. The anticarcinogenic, antioxidant, and free radical scavenging properties of ascorbigen have been reviewed recently.[7]

Vitamin C and phenolic compounds are potent antioxidants that may exert their action directly by scavenging free radical species, by metabolizing peroxides to nonradical products, and by chelating metal ions to prevent the generation of oxidizing species.[8−10] In addition, some phenolic compounds inhibit prooxidant enzymes and modulate pro-inflammatory gene expression, enzyme activities, and pro-inflammatory molecules such as cytokines, prostaglandins, and reactive oxygen species; thus, they could contribute to the prevention of cancer and cardiovascular diseases.[11]

Cultivar, location, and growing conditions play important roles in the production of bioactive compounds in *Brassica* vegetables.[12] The concentration and composition of GLS, phenolics, and vitamin C in *Brassica* vegetables is genotype dependent.[13,14] Moreover, climatic factors such as temperature, irradiation, and water supply also have an important influence on the phytochemical content in *Brassica* vegetables.[15,16] GLS breakdown product levels are due to the combination of GLS content in the plant and myrosinase activity.[17] The activity of this enzyme depends also on the genetic variation[18] and on some intrinsic (metal ions, ascorbic acid, pH) and extrinsic (temperature) factors.[17,19] Therefore, cultivar selection should be tailored to specific environmental factors at each location to achieve optimization in phytochemical content of *Brassica* vegetables. In addition, selected white cabbages with an optimized bioactive compound content could be used as raw material for sauerkraut production, enhancing the human dietary intake in health-promoting compounds.

No information has been found about the effect of climatic growing conditions in different Spanish locations on the content of health-promoting compounds of diverse white cabbage cultivars. Therefore, the objective of the present work was to determine the content of individual and total GLS, myrosinase activity, vitamin C, total phenolics compounds (TPC), and antioxidant capacity (ORAC) in five white cabbage (*Brassica oleracea* L. var. *capitata*) cultivars grown in northern and eastern areas of Spain.

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MATERIALS AND METHODS

Plant Material. White cabbages (*B. oleracea* L. var. *capitata*) cv. Hinova, cv. Megaton, cv. Alfredo, cv. Candela, and cv. Bronco were provided by Bejo Iberica S.L. (Madrid, Spain), planted in September 2008, and harvested in December 2008 in the fields of two different geographical Spanish locations: Calahorra, La Rioja (north), and Alboraya, Valencia (east). Three cabbage heads for each cultivar were randomly selected from the field at each location. Cabbage heads were trimmed of their outer leaves, and their central cores were removed. White cabbages were frozen upon reception in liquid nitrogen, freeze-dried, milled, and stored at −20 °C for further analysis.

Individual and Total GLS Content. Total GLS were extracted from freeze-dried samples followed by an enzymatic desulfatation, according to the method reported in the *Official Journal of European Communities*.[20] Briefly, 200 mg of freeze-dried samples was extracted twice in 3 mL of 70% (v/v) methanol at 70 °C and held at that temperature for 2 min. Samples were homogenized for 1 min using an Ultra-Turrax T25 Digital (IKA-Werkle GmbH & Co, Staufen, Germany) at 20000 rpm and centrifuged at 1089*g* for 10 min at 5 °C. Desulfatation of GLS and sinigrin (Sigma-Aldrich, Steinheim, Germany) used as standard was carried out in Sephadex A25 columns (Sigma-Aldrich) using 75 μL of purified sulfatase from *Helix pomatia* type H-1 (EC 2312.772.1 10 kU/g solid from Sigma-Aldrich) purified according to the *Official Journal of European Communities*.[20] For separation of desulfo-GLS, an Alliance Waters 2695 HPLC (Waters, Milford, MA) with a photodiode array detector and a Spherisorb ODS2 column (150 × 4.6 mm, 3 μm) from Waters was used. Separation, detection, and identification were performed as described elsewhere.[15] GLS content was quantified by using desulfated sinigrin as external standard (concentration range of 0−0.25 mM) and response factors relative to desulfated sinigrin.[20] Samples were independently analyzed in triplicate, and results were reported as micromoles of GLS per gram of dry matter (μmol/g dm).

Myrosinase Activity. Myrosinase activity was determined as described by Travers-Martin et al.[21] with some modifications as described below. Crude extracts were prepared by homogenizing 350 mg of a freeze-dried sample in 10 mL of Tris−EDTA buffer (200 mM Tris, 10 mM EDTA, pH 5.5) for 1 min in an ice bath using an Ultra-Turrax homogenizer T25 digital. The homogenate was centrifuged at 23708*g* for 15 min at 4 °C. To remove endogeneous glucosinolates and glucose, the crude extract was applied onto an Amicon ultrafiltration cell (Millipore, Billerica, MA) of 10000 Da molecular weight cutoff and washed several times at 4 °C using Tris−EDTA buffer, pH 5.5. The myrosinase activity was measured as the release of glucose using the GOD−PAP method.[22] Briefly, 100 μL of purified extracts and 25 μL of 2 mM sinigrin in 0.2 M phosphate buffer, pH 6.5, as substrate were mixed with 50 μL of freshly prepared color reagent, pH 7.0, containing 57 U/mL glucose oxidase from *Aspergillus niger* (EC 1.1.3.4, Sigma-Aldrich), 5.6 U/mL horseradish peroxidase (EC 1.11.1.7, Sigma-Aldrich), 2.8 mM 4-aminoantipyrine (Sigma-Aldrich), 30.7 mM phenol (Sigma-Aldrich), and 0.136 M imidazole (Sigma-Aldrich). The release of glucose was determined by measuring the absorbance of the colored product *N*-(4-antipyryl)-*p*-benzoquinone imine at 492 nm at room temperature in a 96-well clear-bottomed polystyrene plate (Sterilin, London, U.K.) using a microplate reader (Biotek Instruments, Winooski, VT). Absorbance was read every minute for 60 min, and the plate was shaken between measurements. A linear part of at least 25 time points of the reaction kinetic was selected to determine myrosinase activity. Means of three replicate absorbance measurements were calculated after subtraction of the means of the background controls (100 μL of extraction buffer, 25 μL of 0.2 M phosphate buffer, pH 6.5, and 50 μL color reagent). Glucose concentrations were calculated using a linear standard curve. Samples were independently analyzed in triplicate, and myrosinase activity was expressed as micromoles of glucose formed per minute and gram of dry matter (U/g dm) as well as per milligram of protein (specific activity, U/mg soluble protein). The soluble protein concentration of cabbage extracts was determined using the DC Protein Assay (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's instructions, and bovine serum albumin was used as the standard.

Vitamin C Content. Determination of vitamin C was performed in freeze-dried samples by capillary electrophoresis (CE) using a P/ACE system 2050 (Beckman Instruments, Fullerton, CA) and UV detection at 254 nm as described earlier.[23] Briefly, 300 mg of freeze-dried cabbage was extracted with 20 mL of 3% (w/v) metaphosphoric acid (Sigma-Aldrich) and homogenized using an Ultra-Turrax homogenizer T25 digital for 2 min. Final volume was adjusted to 25 mL with 3% metaphosphoric acid. The resultant slurry was filtered through a Whatman no. 1 filter paper. A volume of 100 μL of isoascorbic acid (Fluka, Steinheim, Germany) at a concentration of 0.6 mg/mL containing 0.2% (w/v) D,L-dithiothreitol (Sigma-Aldrich) was added as internal standard into 1.5 mL of filtrate. Final volume was adjusted to 2 mL with 0.2% (w/v) D,L-dithiothreitol, mixed thoroughly, and filtered through a 0.45 μm membrane. D,L-Dithiothreitol was added to prevent the oxidation of ascorbic acid to dehydroascorbic acid. Vitamin C content was quantified by external calibration using L-ascorbic acid (Sigma-Aldrich) (concentration range of 0−50 μg/mL) and using a response factor relative to the internal standard. Samples were independently analyzed in triplicate, and results have been reported as milligrams of L-ascorbic acid per gram of dry matter (mg/g dm).

TPC Content. The TPC content of white cabbage was determined in methanolic extracts using the Folin−Ciocalteu colorimetric method.[24] Briefly, 1 g of freeze-dried sample was suspended in 10 mL of 70% methanol and stirred for 1 h at room temperature. Extracts were filtered using Whatman no. 1 filter paper. An aliquot of 400 μL of a 20-fold dilution of each extract was mixed with 2.5 mL of distilled water, 1 mL of 7.5% Na_2CO_3 (w/v), and 100 μL of 2 N Folin−Ciocalteu reagent (Sigma-Aldrich). Samples were vortexed and incubated for 30 min at room temperature. The absorbance was measured at 736 nm using a microplate reader (Biotek Instruments). Total phenolics were quantified by external calibration using gallic acid (Sigma-Aldrich) as standard. Samples were independently analyzed in triplicate, and results were expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g dm).

Antioxidant Capacity. ORAC was determined in methanolic extracts by suspension of 1 g of freeze-dried sample in 10 mL of 70% methanol, which was stirred for 1 h at room temperature. Extracts were filtered using Whatman no. 1 filter paper. The ORAC value was determined as described by Davalos et al.[25] Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer, pH 7.4, and the final assay mixture (200 μL) contained 70 nM fluorescein (Sigma-Aldrich), 12 mM 2,2′-azobis(2-methylpropionamidine) dihydrochloride (Sigma-Aldrich), and antioxidant [1−8 μM Trolox (Sigma-Aldrich) or sample at different concentrations]. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer, pH 7.4. Fluorescence measurements were carried out on a Polarstar Galaxy microplate reader (BMG Labtechnologies GmbH, Offenburg, Germany) equipped with a fluorescent filter (excitation at 485 nm and emission at 520 nm) using a black 96-F Microwell (Nunc A/S, Roskilde, Denmark). The plate was automatically shaken before the first reading, and the fluorescence was recorded every minute for 98 min. The equipment was controlled by Fluostar Galaxy software version 4.11-0 for fluorescence measurement. All reaction mixtures were prepared in triplicate, and at least two independent analyses were performed for each sample. The areas under the fluorescence decay curve (AUC), based on relative fluorescence values to the initial reading, were recorded and the AUCs of blanks subtracted. Results were expressed as

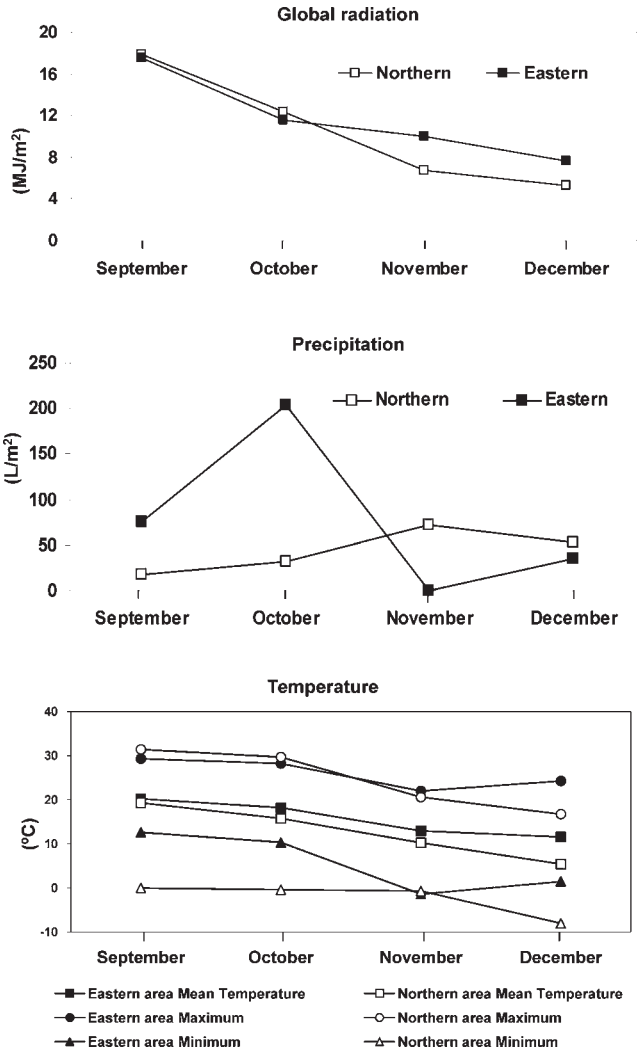

Figure 1. Climatic conditions during cabbage-growing period in the northern and eastern areas of Spain.

micromoles of Trolox equivalents (TE) per gram of dry matter (μmol TE/g dm).

Statistical Analysis. Data are expressed as means ± standard deviations of three independent determinations. The statistical methods used were one-way analysis of variance (ANOVA) using the least significant difference test to determine whether there were significant ($P \leq 0.05$) differences between genotypes within growing locations; a second one-way ANOVA was performed to determine differences within the same genotype grown in different locations; principal component analysis (PCA) was used to examine the relationships among variables; and stepwise discriminant analysis was used to select the variables most useful in differentiating the groups. STATISTICA 7.0 software (Statsoft Inc., Tulsa, OK) and STATGRAPHICS 5.0 software (Statistical Graphics Corp., Rockville, MD) for Windows were used.

RESULTS

Figure 1 shows the climatic conditions including global radiation (MJ/m^2/day), precipitation (L/m^2) and temperature (max, min, and mean, °C) during the cabbage-growing periods in northern and eastern areas of Spain. Global radiation was higher in the east than in the North only in November and December. Precipitation was higher in this geographical area during September and October and lower in November and December compared to the northern area. Temperature was higher in the eastern than in the northern area of Spain.

Cabbages grown in the northern region showed a content of total GLS (Table 1) between 7.2 μmol/g dm (cv. Candela) and 10.3 μmol/g dm (cv. Megaton), whereas those harvested in the east contained amounts between 10.7 and 20.0 μmol/g dm. Total GLS content was significantly ($P \leq 0.05$) higher in cabbage cultivars grown in the east than in the north. Aliphatic GLS were predominant and represented 75−90 and 84−89% in those cultivars grown in northern and eastern areas, respectively. Cabbages cultivated in the north contained sinigrin between 2.8 and 6.0 μmol/g dm and glucobeirin between 1.3 and 5.4 μmol/g dm, whereas those cultivated in the east showed values of 3.4−6.6 and 1.6−8.2 μmol/g dm, respectively. Progroitrin, glucoraphanin, and gluconapin were present in lower amounts ($P \leq 0.05$), showing eastern cabbages having a higher content than those from the north ($P \leq 0.05$). With regard to indole GLS, glucobrassicin was predominant, and its ratio within the total GLS content ranged from 8% in cv. Alfredo cultivated in the east to 23% in cv. Megaton cultivated in the north. 4-Methoxyglucobrassicin and neoglucobrassicin were present in negligible amounts, and their contribution to total GLS content was below 3%.

The soluble protein content in cultivars grown in the north (Table 2) tended to be higher than those cultivated in the east. Significant differences ($P \leq 0.05$) were found between growing locations with the exception of cv. Bronco. A similar tendency was observed in myrosinase activity (Table 2). Cabbages cultivated in northern Spain showed significant differences ($P \leq 0.05$) among genotypes. Cultivar Hinova exhibited the highest myrosinase activity, followed by cv. Candela and cv. Megaton (36, 33, and 31 U/g dm, respectively), whereas cv. Bronco and cv. Alfredo showed the lowest values (25 and 21 U/g dm, respectively). The lowest ($P \leq 0.05$) specific myrosinase activity was observed in the northern white cabbages cv. Megaton and cv. Alfredo (0.83 and 0.86 U/mg soluble protein, respectively). Among the white cabbages cultivated in the Eeast, cv. Megaton and cv. Bronco presented significantly ($P \leq 0.05$) higher myrosinase activity (12−13 mg/g dm) than cv. Hinova, cv. Alfredo, and cv. Candela (10−11 mg/g dm). With regard to myrosinase specific activity, cv. Candela showed the highest ($P \leq 0.05$) value (0.7 U/mg soluble protein), and no significant differences ($P \leq 0.05$) among the rest of cultivars were found (0.6 U/mg soluble protein) (Table 2).

The vitamin C content of cabbages (Table 3) was dependent on the cultivar and Spanish growing location. Among the cabbages cultivated in the north, cv. Alfredo exhibited the highest vitamin C content (3.6 mg/g dm) followed in descending order by cv. Hinova and cv. Bronco (2.9 mg/g dm), cv. Candela (2.7 mg/g dm), and cv. Megaton (2.4 mg/g dm). A significantly greater ($P \leq 0.05$) vitamin C content was found in cabbages grown in the east, irrespective of the cultivar; their values ranged from 4.2 to 6.0 mg/g dm. In this Spanish geographical region, cv. Candela contained the highest vitamin C content (6.0 mg/g dm), whereas cv. Hinova, cv. Megaton, cv. Alfredo, and cv. Bronco showed significantly lower ($P \leq 0.05$) values (5.0, 4.2, 5.5, and 5.6 mg/g dm, respectively).

Northern white cabbage cv. Alfredo was characterized by the highest TPC content (12.3 mg/g dm) followed by cv. Bronco, whereas cv. Hinova, cv. Megaton, and cv. Candela showed significantly ($P \leq 0.05$) lower values (9.0−9.2 mg/g dm)

Table 1. Individual and Total GLS Contents (μmol/g dm) in Five White Cabbage (*Brassica oleracea* L. var. *capitata*) Cultivars Grown in Two Geographical Regions of Spain[a]

		aliphatic GLS					indolic GLS			total GLS
growing location	cabbage cultivar	IB	PROG	SIN	GRAP	NAP	GB	4-met-GB	neo-GB	
north	Hinova	2.06 ± 0.07 a	0.22 ± 0.07	6.02 ± 0.17	ND a	0.13 ± 0.05	1.00 ± 0.12 aA	0.08 ± 0.02	0.08 ± 0.01 a	9.61 a
	Megaton	1.33 ± 0.33	2.25 ± 0.03	4.06 ± 0.01 b	0.08 ± 0.02	ND a	2.41 ± 0.34	0.12 ± 0.03 b	0.09 ± 0.02 a	10.33 b
	Alfredo	5.42 ± 0.21	0.14 ± 0.03 a	3.44 ± 0.43 a	0.11 ± 0.03	ND a	0.89 ± 0.09 aB	0.05 ± 0.00 a	0.09 ± 0.00 a	10.13 ab
	Candela	1.91 ± 0.05 a	0.85 ± 0.04	2.77 ± 0.06 a	ND a	ND a	1.39 ± 0.05	0.03 ± 0.00 a	0.25 ± 0.01	7.20
	Bronco	2.67 ± 0.24	0.15 ± 0.02 a	4.46 ± 0.25 b	ND a	ND a	1.02 ± 0.18a	0.11 ± 0.04 b	0.03 ± 0.01 A	8.44
east	Hinova	4.18 ± 0.37	0.31 ± 0.03	4.18 ± 0.33	0.17 ± 0.01	0.57 ± 0.06	1.11 ± 0.11 bA	0.16 ± 0.01	0.05 ± 0.01	10.68 c
	Megaton	1.65 ± 0.03	3.19 ± 0.03	3.40 ± 0.03	1.61 ± 0.07	0.28 ± 0.07	1.12 ± 0.16 b	0.26 ± 0.05	0.02 ± 0.01 b	11.53 cd
	Alfredo	2.88 ± 0.73	1.10 ± 0.27 b	5.82 ± 0.55 c	0.74 ± 0.10	0.24 ± 0.07	0.87 ± 0.07 B	0.36 ± 0.03	0.03 ± 0.03 b	12.05 d
	Candela	4.19 ± 0.42	2.29 ± 0.17	6.25 ± 0.40 cd	0.50 ± 0.05	0.68 ± 0.07	1.53 ± 0.11	0.13 ± 0.01	0.07 ± 0.01	15.64
	Bronco	8.19 ± 0.71	1.23 ± 0.08 b	6.62 ± 0.45 d	0.25 ± 0.02	0.41 ± 0.03	3.06 ± 0.21	0.21 ± 0.02	0.03 ± 0.00 bA	20.00

[a] Mean value ± standard deviation of three independent experiments. The same upper case letters in the same column indicate no significant differences between growing locations for each genotype ($P \leq 0.05$). The same lower case letters in the same column indicate no significant differences among genotypes for each growing location ($P \leq 0.05$). IB, glucoiberin; PROG, progoitrin; SIN, sinigrin; GRAP, glucoraphanin; NAP, gluconapin; GB, glucobrassicin, 4-met-GB, 4-methoxyglucobrassicin; neo-GB, neoglucobrassicin; total GLS, total glucosinolates. ND, not detected.

Table 2. Myrosinase Activity and Soluble Protein Content in Five White Cabbage (*Brassica oleracea* L. var. *capitata*) Cultivars Grown in Two Geographical Regions of Spain[a]

growing location	cabbage cultivar	soluble protein (mg/g dm)	myrosinase activity (U/g dm)	myrosinase activity (U/mg soluble protein)
north	Hinova	20.00 ± 1.29 a	36.28 ± 1.25	1.82 ± 0.06
	Megaton	37.20 ± 2.69	30.61 ± 1.60	0.83 ± 0.02 a
	Alfredo	24.45 ± 0.72 b	21.06 ± 0.50	0.86 ± 0.03 a
	Candela	23.85 ± 2.21 b	33.10 ± 1.49	1.39 ± 0.07
	Bronco	20.70 ± 0.93 aA	25.20 ± 0.66	1.22 ± 0.06
east	Hinova	17.13 ± 0.50	10.72 ± 1.20 a	0.63 ± 0.08 a
	Megaton	19.69 ± 0.53	12.30 ± 0.38 b	0.63 ± 0.03 a
	Alfredo	15.73 ± 0.44	9.89 ± 0.29 a	0.63 ± 0.01 a
	Candela	13.68 ± 0.92	10.00 ± 0.97 a	0.73 ± 0.03
	Bronco	20.95 ± 0.61 A	12.89 ± 0.56 b	0.62 ± 0.04 a

[a] Mean value ± standard deviation of three independent experiments. The same upper case letters in the same column indicate no significant differences between growing locations for each genotype ($P \leq 0.05$). The same lower case letters in the same column indicate no significant differences among genotypes for each growing location ($P \leq 0.05$).

(Table 3). TPC of eastern white cabbage cultivars were significantly ($P \leq 0.05$) lower than those from the north and ranged from 7.7 to 8.5 mg/g dm, with the exception of cv. Megaton, which showed the lowest TPC content (6.1 mg/g dm).

The antioxidant capacity of white cabbage (Table 3) was also influenced by cultivar and growing location. Across growing location, the antioxidant capacity ranged from 45.2 to 92 μmol TE/g dm; cv. Alfredo grown in the north contained the highest level. In general, the influence of geographical growing location on the antioxidant capacity, irrespective of the cultivar, was similar to that observed for TPC content. Thus, cultivars grown in the northern area of Spain exhibited significantly ($P \leq 0.05$) higher antioxidant capacity (ORAC) levels than those grown in the eastern area.

To examine the relationship among the variables (GLS, vitamin C, myrosinase activity, TPC, and antioxidant capacity) of white cabbage cultivars and among cabbages grown in different geographical areas, PCA from the correlation matrix was used in the present work. This analysis suggested that there are two main components, which explained 87.5% of the total variance. The first principal component, which explained 74.1% of the total variance, was highly correlated with vitamin C content (r = 0.94), myrosinase activity (r = −0.87), antioxidant capacity (r = −0.95), total GLS content (r = 0.83), and TPC (r = −0.70). The second principal component explained 13.4% of the total variance. Figure 2 shows the graphical representation of the contribution of these quantitative variables of cabbages in the plane defined by the first two factors. Figure 3 shows the cabbage samples on the plane defined by the first two principal components, and it can be observed that the northern cabbages had clearly different values from the eastern ones, and among these, the Bronco and Candela cultivars were completely separated from the rest of the eastern cabbages.

From the matrix correlation carried out with the data, it was observed that the cabbages with the highest level of total GLS presented the highest level of vitamin C (r = 0.75, $P \leq 0.05$)

Table 3. Vitamin C, Total Phenolic Compounds (TPC), Antioxidant Capacity (ORAC), and Water Contents in Five White Cabbage (*Brassica oleracea* L. var. *capitata*) Cultivars Grown in Two Geographical Regions of Spain[a]

growing location	cabbage cultivar	vitamin C (mg/g dm)	TPC (mg GAE/g dm)	ORAC (μmol TE/g dm)	water (%)
north	Hinova	2.92 ± 0.19 a	9.21 ± 0.16 a	83.80 ± 3.29 a	92.1
	Megaton	2.41 ± 0.17	9.15 ± 1.43 a	70.48 ± 1.85	91.7
	Alfredo	3.58 ± 0.12	12.26 ± 1.53	91.97 ± 3.60	91.9
	Candela	2.68 ± 0.12	9.06 ± 0.44 a	77.37 ± 5.63	89.9
	Bronco	2.89 ± 0.11 a	10.41 ± 0.02	84.44 ± 3.86 a	92.2
east	Hinova	5.04 ± 0.13	8.48 ± 0.19 b	57.39 ± 3.29	91.7
	Megaton	4.20 ± 0.22	6.09 ± 1.12	63.80 ± 2.02	91.7
	Alfredo	5.52 ± 0.09 a	8.56 ± 0.58 b	51.32 ± 0.64	91.3
	Candela	5.99 ± 0.08	7.76 ± 0.05 a	47.81 ± 0.59	88.9
	Bronco	5.62 ± 0.23 a	7.73 ± 0.13 a	45.17 ± 1.53	91.2

[a] Mean value ± standard deviation of three independent experiments. The same lower case letters in the same column indicate no significant differences between growing locations for each genotype ($P \leq 0.05$).

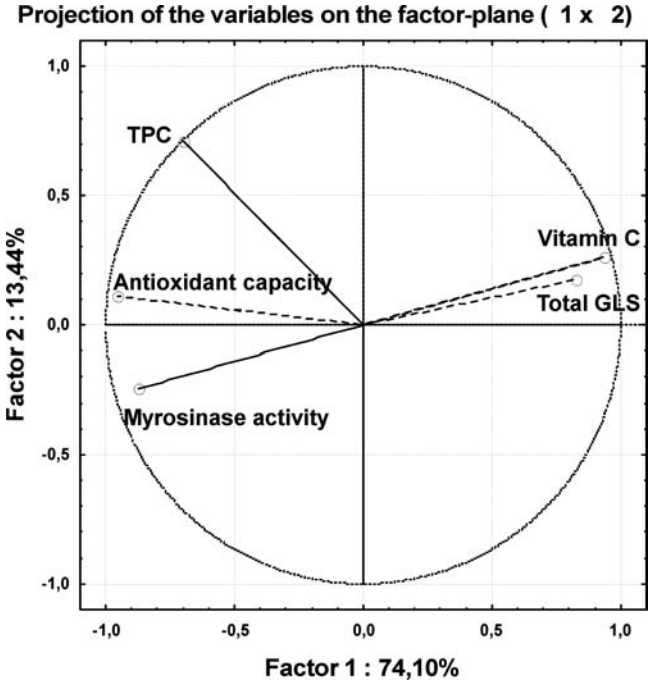

Figure 2. Plot of the contribution of variables (total GLS, vitamin C, TPC, antioxidant capacity, and mirosinase activity) of cabbages in the plane defined by the first two factors.

and the lowest level of antioxidant capacity ($r = -0.76, P \leq 0.05$). The cultivars with the highest amount of vitamin C had the lowest level of myrosinase activity ($r = -0.89$, $P \leq 0.05$) and antioxidant capacity ($r = -0.86$, $P \leq 0.05$), whereas the cabbages with the highest amount of TPC also presented the highest levels in antioxidant capacity ($r = 0.71$, $P \leq 0.05$).

Table 4 shows the mean and standard deviation of selected quantitative variables (GLS, vitamin C, TPC, myrosinase, and antioxidant capacity) taking into account the cabbages cultivated in different areas of Spain. Cabbages grown in northern Spain were characterized by the highest TPC content, myrosinase activity, and antioxidant capacity ($P \leq 0.05$) and the lowest amounts of total GLS and vitamin C contents ($P \leq 0.05$).

DISCUSSION

The contents of total GLS found in the present work for cabbages are within the range reported in the literature.[15,26−29] However, GLS values found in the present work are rather lower than those observed in Turkish cabbages (50 and 70 μmol/g dm)[14] and higher than those presented in cabbages from different European regions (3.3−7.7 μmol/g dm).[30]

With regard to the profile of individual GLS observed in the current work, our results agree with several studies[15,26,30] showing sinigrin as the predominant GLS in cabbage. The presence of the indolic glucobrassicin in the edible parts of white cabbages should also be emphasized because this GLS is the precursor of indol-3-carbinol (I3C), a potent chemopreventive agent.[3]

Our results showed higher GLS content in the eastern area of Spain where higher temperature and lower precipitation were registered. This could be due to increased synthesis of GLS precursors such as amino acids and sugars as a consequence of higher temperatures.[30] These results are in agreement with previous studies. Martínez-Villaluenga et al.[15] showed that white cabbage cv. Taler cultivated in summer led to larger total GLS content than cabbage cultivated in winter. Similarly, Cartea et al.[29] showed that total GLS concentration in cabbages harvested in the spring season was higher than that of those harvested in autumn. On the other hand, in this work glucoraphanin was present in larger amounts in those cabbages cultivated in the eastern area (0.2−1.6 μmol/g dm) where higher temperatures were recorded. A reason for the different GLS profiles in cabbages cultivated in different geographical locations could be because enzymes involved in each GLS synthesis are affected differently by temperature and radiation.[1] Our results agree with those of Cartea et al.,[29] Martínez-Villaluenga et al.,[15] and Sarikamis et al.,[14] who showed that aliphatic GLS were the most abundant during autumn/winter season cabbages and that glucobrassicin was the predominant indolic GLS.

With regard to myrosinase activity determination, methods described by other authors[31,32] were successful in determining myrosinase activity using a commercial enzyme, but they did not work in cabbage extracts. These problems could be due to sample turbidity, as reported by Travers-Martin et al.[21] In the present work, a modification to the Travers-Martin method to increase myrosinase concentration has been introduced, which consisted

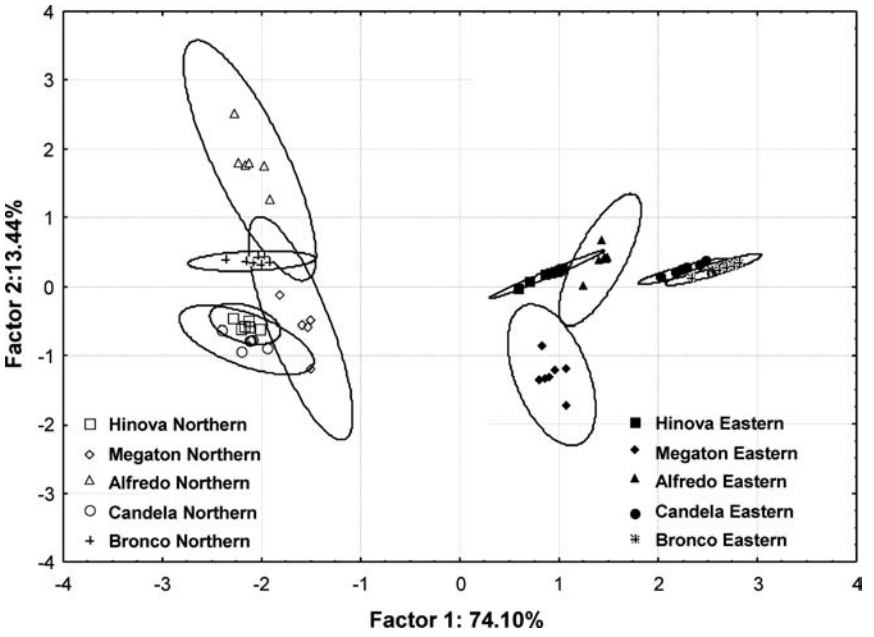

Figure 3. Plot of cabbage cultivars growing in northern and eastern areas of Spain in the plane defined by the first two principal components and ellipses for 95% confidence.

Table 4. Discriminant Variables of Growing Location of Cabbage Cultivars[a]

growing location	total glucosinolates (μmol/g dm)	vitamin C (mg/g dm)	total polyphenols (mg GAE/g dm)	myrosinase activity (U/g dm)	antioxidant capacity (μmol TE/g dm)
north	9.14 ± 1.23 a	2.91 ± 0.42 a	10.02 ± 1.30 b	29.25 ± 5.69 b	81.61 ± 7.86 b
east	14.27 ± 3.87 b	5.28 ± 0.64 b	7.72 ± 0.90 a	11.28 ± 1.43 a	52.83 ± 6.98 a

[a] Mean value ± standard deviation. The same lower case letters in the same column indicate no significant difference ($P \leq 0.05$).

of ultrafiltration (10 kDa molecular weight cutoff) of cabbage extracts; in addition, the modified method allowed the removal of both endogenous glucose and GLS, which could interfere in the analysis.

A large intraspecific variation for myrosinase activity among white cabbage cultivars has also been observed by Singh et al.,[33] although it was rather lower than those for the Spanish cabbages found in the present work. Furthermore, such activity also seems to depend on the part of the cabbage analyzed, as has been shown by Charron and Sams.[26] With regard to climatic conditions, the growing season and year of cultivation have been reported to affect the myrosinase activity of *B. oleracea* cv. Early Round Dutch.[32]

As observed in the present study, the vitamin C content in white cabbages was affected by the cultivar, which is in agreement with previous papers.[13,34] Vitamin C contents found in white cabbage cultivars of the present study were comparable to those found by other researchers, who obtained values ranging from 0.56 to 4.70 mg/g dm.[13,15,34,35] Growing location also had an impact on vitamin C content of white cabbages because cultivars grown in the eastern area of Spain presented higher vitamin C content than those grown in the north. This could be due to the different environmental conditions between the geographical locations. Mean temperature was higher in the east than in the north during the entire growing period (Figure 1). Additionally, global radiation was higher in eastern than in northern Spain during November and December (Figure 1). A higher light quantity and temperature during the growth and development of plant tissues promote biosynthesis and accumulation of ascorbate because this antioxidant protects against environmental stress.[36] In particular, high concentrations of ascorbic acid in plant chloroplasts have been reported to protect against damaging oxygen-derived species that are produced in the presence of light.[37] Our results are consistent with those of Vallejo et al.[38] and Martinez-Villaluenga et al.,[15] who observed higher vitamin C content in broccoli and white cabbage cv. Taler grown in the spring season than in those grown in the winter season.

TPC in white cabbage was influenced also by cultivar. These results are in accordance with those previously reported by other researchers.[13,34,35] In addition, climatic factors such as temperature and radiation may have an impact in the biosynthesis of phenolic compounds. Our results showed that northern white cabbage cultivars contained higher concentration of TPC than eastern ones. Lower temperatures and lower global radiation (registered during November and December) in northern Spain could be responsible for this effect. Low temperature enhances the formation of reactive oxygen species (ROS), which leads to gene expression of enzymes involved in the biosynthesis of phenolic compounds such as phenylalanine ammonia-lyase and chalcone synthase as has been found in maize and *Arabidopsis thaliana*.[39] Besides low temperature, global radiation also induces the synthesis and accumulation of phenolic compounds such as flavonoids and hydroxycinnamic acids that function as shielding components against UV radiation.[40] A significant increase in flavonoid content during the cultivation of kale (*B. oleracea* var. *sabellica*) at low temperatures parallel with low radiation has been also reported.[16]

In accordance with results observed for antioxidants such as vitamin C and phenolic compounds, antioxidant capacity was also influenced by cultivar and growing location. Zietz et al.[41] showed that antioxidant capacity was positively correlated with total polyphenol content in four kale (*B. oleracea* var. *sabellica*) cultivars grown in four different autumn/winter months. The antioxidant capacity determined as ORAC values in the white cabbages studied in the present work are within the range reported in 111 white cabbages (23−146 μmol TE/g dm).[42]

Taking into consideration the results obtained, it is concluded that Spanish geographical locations experiencing low temperatures and radiation during the growing season (northern area) might be more appropriate for the enhanced accumulation of myrosinase activity, total phenolic compounds, and antioxidant capacity of white cabbages, whereas cabbages cultivated under high temperature and radiation (eastern area) would present high amounts of total GLS and vitamin C. This knowledge provides information regarding the climatic conditions in which white cabbages with the highest bioactive compounds content can be obtained.

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