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 (North and East areas) on the main bioactive compounds, glucosinolates (GLS), total phenolic compounds (TPC), vitamin C, as well as myrosinase activity and antioxidant capacity in five white cabbage (*Brassica oleracea* L. var*. capitata*) cultivars were investigated. Cabbages with the highest concentration of total GLS presented the highest vitamin C level (r=0.75, P≤0.05) and the lowest antioxidant capacity (r=-0.76, P≤0.05). The cultivars with the highest vitamin C content had the lowest myrosinase activity (r=-0.89, P≤0.05) and antioxidant capacity (r=-0.86, P≤0.05), while those with the largest TPC amount showed the highest antioxidant capacity (r=0.71, P≤0.05). Cabbage cultivars grown in North area of Spain with low temperatures and radiation led to higher mean values of myrosinase activity (29.25 U/g d.m.), TPC (10.0 GAE mg/g d.m.) and antioxidant capacity (81.6 µmol Trolox/g d.m.), while cultivars grown in the East area with high temperature and radiation led to larger mean values of GLS (14.3 µmol/g d.m.) and vitamin C (5.3 mg/g d.m.). 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

Epidemiological 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 by either myrosinase (β-thioglucoside glucohydrolase) within the plant or by enzymatic decomposition within the gastrointestinal tract yield biologically active hydrolysed products.[5] There is a large body of evidence showing the chemopreventive action of GLS hydrolysis products by modulating detoxification enzymes[6], which protects from 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 which may exert their action directly by scavenging free radical species, by metabolyzing peroxides to non-radical products and by chelating metal ions to prevent generation of oxidizing species.[8-10] In addition, some phenolic compounds inhibit pro-oxidant enzymes and modulate proinflammatory gene expression, enzyme activities and proinflammatory molecules such as cytokines,

prostaglandins and reactive oxygen species, thus they could contribute to 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 the 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 glucosinolates (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 the North and East area of Spain.

MATERIALS AND METHODS

Plant material. White cabbage (*Brassica 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: the North (Calahorra, La Rioja) and the East (Alboraya, Valencia) areas of the country. 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 -20ºC for further analysis.

Individual and total glucosinolate (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 were extracted twice in 3 mL of 70% (v/v) methanol at 70 ºC and held at that temperature for 2 min. Samples were homogenised for 1 min using an Ultra-Turrax T25 Digital (IKA-Werkle GmbH & Co, Staufen, Germany) at 20,000 rpm and centrifuged at 1089 *x g* for 10 min at 5 ºC. Desulfatation of GLS and sinigrin (Sigma-Aldrich, Steinheim, Germany) used as standard were carried out in Sephadex A25 columns (Sigma-Aldrich) using 75 μL of purified sulfatase from *Helix pomatia* Type H-1 (E.C 2312.772.1 10kU/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, USA) with a photodiode array detector and a Spherisorb ODS2 column (150 x 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 0-0.25 mM) and response factors relative to desulfated sinigrin.[20] Samples were independently analyzed in triplicate and results were reported as μmol GLS per gram of dry matter (μmol/g d.m.).

Myrosinase activity. Myrosinase activity was determined as described by Travers-Martin et al.[21] with some modifications as it is described below. Crude extracts were prepared by homogenizing 350 mg of a freeze-dried sample in 10 mL 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 23,708 *x g* for 15 min at 4 ºC. To remove endogeneous glucosinolates and glucose, the crude extract was applied onto an Amicon ultrafiltration cell (Millipore, Billireca, MA, USA) of 10,000 Da molecular weight cut off and washed several times at 4 ºC 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 freshly prepared color reagent (pH 7.0) containing 57 U/mL glucose oxidase from *Aspergillus niger* (E.C. 1.1.3.4., Sigma-Aldrich), 5.6 U/mL horseradish peroxidase (E.C. 1.11.1.7, Sigma-Aldrich), 2.8 mM 4-aminoantipyrine (Sigma-Aldrich), 30.7 mM phenol (Sigma-Aldrich) and 0.136 M

143 imidazole (Sigma-Aldrich). The release of glucose was determined by
144 measuring the absorbance of the colored product N-(4-antipyryl)-p-
145 benzoquinone imine at 492 nm at room temperature in a 96-well clear-
146 bottomed polystyrene plate (Sterilin, London, UK) using a microplate reader
147 (Biotek Instruments, Winooksi, USA). Absorbance was read every minute for
148 60 min and plate was shaken between measurements. A linear part of at least
149 25 time points of the reaction kinetic was selected to determine myrosinase
150 activity. Means of three replicate absorbance measurements were calculated
151 after subtraction of the means of the background controls (100 μL of extraction
152 buffer, 25 μL of 0.2 M phosphate buffer pH 6.5 and 50 μL color reagent).
153 Glucose concentrations were calculated using a linear standard curve.
154 Samples were independently analyzed in triplicate and myrosinase activity was
155 expressed as μmol of glucose formed per minute and gram of dry matter (U/g
156 d.m.) as well as per mg of protein (specific activity, U/mg soluble protein). The
157 soluble protein concentration of cabbage extracts were determined using the
158 DC Protein Assay (Bio-Rad Laboratories, Hercules, CA) following the
159 manufacturer´s instructions and bovine serum albumin was used as the
160 standard.
161
162 **Vitamin C content.** Determination of vitamin C was performed in freeze-
163 dried samples by capillary electrophoresis (CE) using a P/ACE system 2050
164 (Beckman Instruments, Fullerton, CA, USA) and UV detection at 254 nm as
165 described earlier.[23] Briefly, 300 mg of freeze-dried cabbage were extracted with
166 20 mL of 3% (w/v) metaphosphoric acid (Sigma-Aldrich) and homogenised
167 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 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 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 mg L-ascorbic acid per gram of dry matter (mg/g d.m.).

Total phenolic compounds (TPC) content. The TPC content of white cabbage was determined in methanolic extracts using the Folin-Ciocalteu colorimetric method[24]. Briefly, 1g 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 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 mg of gallic acid equivalents per gram of dry matter (mg GAE/g d.m.).

Antioxidant capacity. Oxygen Radical 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 485 nm and emission 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 AUC of blanks

subtracted. Results were expressed as μmol Trolox equivalents (TE) per gram of dry matter (μmol TE/g d.m.).

Statistical analysis. Data were expressed as means ± standard deviation 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 to determine differences between the same genotype grown in different locations; principal component analysis to examine the relationships among variables; and stepwise discriminant analysis to select the variables most useful in differentiating the groups. The STATISTICA 7.0 (Statsoft Inc. Tulsa, OK, USA) and STATGRAPHICS 5.0 (Statistical Grapahics Corp, Rockville, MD, USA) softwares for Windows were used.

RESULTS

Figure 1 shows the climatic conditions including global radiation ($MJ/m^2/d$), precipitation (L/m^2) and temperature (max, min, and mean, ^oC) during the cabbage growing periods in the North and East 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 North 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 d.m. (cv. Candela) and 10.3 µmol/g d.m. (cv. Megaton) while those harvested in the East contained amounts between 10.7 - 20.0 µmol/g d.m. 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 Estern areas, respectively. Cabbages cultivated in the North contained sinigrin between 2.8-6.0 µmol/g d.m., and glucobeirin between 1.3-5.4 µmol/g d.m. while those cultivated in the East showed values of 3.4-6.6 and 1.6-8.2 µmol/g d.m., respectively. Progroitrin, glucoraphanin and gluconapin were present in lower amounts ($P \leq 0.05$), showing Eastern cabbages higher content than those from the North ($P \leq 0.05$). Regarding 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-Methoxy glucobrassicin 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. Similar tendency was observed in myrosinase activity (Table 2). Cabbages cultivated in the North of Spain shown 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 d.m., respectively), while cv. Bronco and cv. Alfredo showed the lowest values (25

265 and 21 U/g d.m., respectively). The lowest (P≤0.05) specific myrosinase activity
266 was observed in the Northern white cabbages cv. Megaton and cv. Alfredo
267 (0.83 and 0.86 U/mg soluble protein, respectively). Among the white cabbages
268 cultivated in the East, cv. Megaton and cv. Bronco presented significantly
269 (P≤0.05) higher myrosinase activity (12-13 mg/g d.m.) than cv. Hinova, cv.
270 Alfredo and cv. Candela (10-11 mg/g d.m.). Regarding myrosinase specific
271 activity, cv. Candela showed the highest (P≤0.05) value (0.7 U/mg soluble
272 protein) and no significant differences (P≤0.05) among the rest of cultivars were
273 found (0.6 U/mg soluble protein) (Table 2).

274 The vitamin C content of cabbages (Table 3) was dependent on the
275 cultivar and Spanish growing location. Among the cabbages cultivated in the
276 North, cv. Alfredo exhibited the highest vitamin C content (3.6 mg/g d.m.)
277 followed in descending order by cv. Hinova and cv. Bronco (2.9 mg/g d.m.), cv.
278 Candela (2.7 mg/g d.m) and cv. Megaton (2.4 mg/g d.m.). A significantly larger
279 (P ≤ 0.05) vitamin C content was found in cabbages grown in the East,
280 irrespective of the cultivar; their values ranged from 4.2 to 6.0 mg/g d.m. In this
281 Spanish geographical region, cv. Candela contained the highest vitamin C
282 content (6.0 mg/g d.m.) whereas cv. Hinova, cv. Megaton, cv. Alfredo and cv.
283 Bronco showed significantly lower (P≤0.05) values (5.0, 4.2, 5.5 and 5.6 mg/g
284 d.m., respectively).

285 Northern white cabbage cv. Alfredo was characterized by the highest
286 TPC content (12.3 mg/g d.m.) followed by cv. Bronco, while cv. Hinova, cv.
287 Megaton and cv. Candela showed significantly (P≤0.05) lower values (9.0 to 9.2
288 mg/g d.m.) (Table 3). TPC of Eastern white cabbage cultivars were significantly
289 (P≤0.05) lower than those from the North and ranged from 7.7 to 8.5 mg/g d.m.,

290 with the exception of cv. Megaton which showed the lowest TPC content (6.1
291 mg/g d.m.).
292 Antioxidant capacity of white cabbage (Table 3) was also influenced by
293 cultivar and growing location. Across growing location, the antioxidant capacity
294 ranged from 45.2 to 92 μmol TE/g d.m.; the cv. Alfredo grown in the North
295 contained the highest level. In general, the influence of geographical growing
296 location on the antioxidant capacity, irrespective of the cultivar, was similar to
297 those observed for TPC content. Thus, cultivars grown in the Northern area of
298 Spain exhibited significantly ($P \leq 0.05$) higher antioxidant capacity (ORAC) levels
299 than those grown in the Eastern area.
300 In order to examine the relationship among the variables (GLS, vitamin
301 C, myrosinase activity, TPC, and antioxidant capacity) of white cabbage
302 cultivars and among cabbages grown in different geographical areas, principal
303 component analysis from the correlation matrix was used in the present work.
304 This analysis suggested that there are two main components which explained
305 87.5% of the total variance. The first principal component, which explained
306 74.1% of the total variance, was highly correlated with vitamin C content
307 ($r=0.94$), myrosinase activity ($r=-0.87$), antioxidant capacity ($r=-0.95$), total GLS
308 content ($r=0.83$) and TPC ($r=-0.70$). The second principal component explained
309 13.4% of the total variance. Figure 2 shows the graphical representation of the
310 contribution of these quantitative variables of cabbages in the plane defined by
311 the first two factors. Figure 3 shows the cabbage samples on the plane defined
312 by the first two principal components and it can be observed that the Northern
313 cabbages had clearly different values from the Eastern ones, and among these,

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 ($r=0.75$, $P\leq0.05$) and the lowest level of antioxidant capacity ($r=-0.76$, $P\leq0.05$). The cultivars with the highest amount of vitamin C had the lowest level of myrosinase activity ($r=-0.89$, $P\leq0.05$) and antioxidant capacity ($r=-0.86$, $P\leq0.05$), while the cabbages with the highest amount of TPC also presented the highest levels in antioxidant capacity ($r=0.71$, $P\leq0.05$).

Table 4 shows the means 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 the North of Spain were characterised by the highest TPC content, myrosinase activity and antioxidant capacity ($P\leq0.05$) and the lowest amounts of total GLS and vitamin C content ($P\leq0.05$).

DISCUSSION

The content 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 d.m.)[14] and higher than those presented in cabbages from different European regions (3.3 to 7.7 µmol/g d.m.).[30]

Regarding 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

339 of white cabbages should also be emphasized since this GLS is the precursor
340 of indol-3-carbinol (I3C), a potent chemopreventive agent.[3]

341 Our results showed higher GLS content in the Eastern area of Spain
342 where higher temperature and lower precipitations were registered. This could
343 be due to increased synthesis of GLS precursors such as amino acids and
344 sugars as consequence of higher temperatures.[30] These results are in
345 agreement with previous studies. Martínez-Villaluenga et al.[15] showed that
346 white cabbage cv. Taler cultivated in summer led to larger total GLS content
347 than cabbage cultivated in winter. Similarly, Cartea et al.[29] showed that total
348 GLS concentration in cabbages harvested in the spring season was higher than
349 those in autumn. On the other hand, in the present work glucoraphanin was
350 present in larger amounts in those cabbages cultivated in the Eastern area (0.2-
351 1.6 μmol/g d.m.) where higher temperatures were recorded. A reason for the
352 different GLS profile in cabbages cultivated in different geographical locations
353 could be due to enzymes involved in each GLS synthesis are affected
354 differently by temperature and radiation.[1] Our results agree with those of Cartea
355 et al.,[29] Martínez-Villaluenga et al.[15] and Sarikamis et al.[14] who showed that
356 aliphatic GLS were the most abundant during autumn/winter season cabbages,
357 and glucobrassicin was the predominant indolic GLS.

358 Regarding myrosinase activity determination, methods described by
359 other authors[31,32] were successful to determine myrosinase activity using a
360 commercial enzyme but they did not work in cabbage extracts. These problems
361 could be due to sample turbidity, as reported by Travers-Martin et al.[21] In the
362 present work, a modification to the Travers-Martin method has been introduced
363 to increase myrosinase concentration that consisted of ultrafiltration (10 kDa

364 molecular weight cut off) of cabbage extracts and, hence, allowed the removal
365 of both endogenous glucose and GLS, which could interfere in the analysis.
366 A large intraspecific variation for myrosinase activity among white
367 cabbage cultivars has also been observed by Singh et al.[33] although it was
368 rather lower than those for the Spanish cabbages found in the present work.
369 Furthermore, such activity also seems to depend on the part of the cabbage
370 analyzed, as has been shown by Charron and Sams.[26] Regarding climatic
371 conditions, the growing season and year of cultivation have been reported to
372 affect the myrosinase activity of *Brassica oleracea* cv. Early Round Dutch.[32]
373 As observed in the present study, the vitamin C content in white
374 cabbages was affected by the cultivar, which is in agreement with previous
375 reports.[13,34] Vitamin C contents found in white cabbage cultivars of the present
376 study were comparable to those found by other researchers who obtained
377 values ranging from 0.56 to 4.70 mg/g d.m.[13,15,34,35] Growing location also had
378 an impact on vitamin C content of white cabbages since cultivars grown in the
379 East area of Spain presented higher vitamin C content than those grown in the
380 North. This could be due to the different environmental conditions between both
381 geographical locations. Mean temperature was higher in the East than in the
382 North during the entire growing period (Figure 1). Additionally, global radiation
383 was higher in the East than in the North of Spain during November and
384 December (Figure 1). A higher light quantity and temperature during growth and
385 development of plant tissues promote biosynthesis and accumulation of
386 ascorbate because this antioxidant protects against environmental stress.[36] In
387 particular, high concentrations of ascorbic acid in plant chloroplasts have been
388 reported to protect against damaging oxygen-derived species that are produced

389 in the presence of light.[37] Our results are consistent with those of Vallejo et al.[38]
390 and Martinez-Villaluenga et al.[15] who observed higher vitamin C content in
391 broccoli and white cabbage cv. Taler grown in the spring season than those
392 grown in the winter season.

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

410 In accordance with results observed for antioxidants such as vitamin C
411 and phenolic compounds, antioxidant capacity was also influenced by cultivar
412 and growing location. Zietz et al.[41] showed that antioxidant capacity was
413 strongly positively correlated with total polyphenol content in four kale (*Brassica*

414 *oleracea* var. *sabellica*) cultivars grown in four different autumn/winter months.
415 The antioxidant capacity determined as ORAC values in the white cabbages
416 studied in the present work are within the range reported in 111 white cabbages
417 (23-146 μmoles TE/g d.m.).[42]

418 Taking into consideration the results obtained, it may be concluded that
419 Spanish geographical locations experiencing low temperatures and radiation
420 during the growing season (Northern area) might be more appropriate for the
421 enhanced accumulation of myrosinase activity, total phenolic compounds and
422 antioxidant capacity of white cabbages, while the cabbages cultivated with high
423 temperature and radiation (Eastern area) would present a high amount of total
424 GLS and vitamin C. This knowledge provides information regarding the climatic
425 conditions in which white cabbages with the highest bioactive compounds
426 content can be obtained.

427

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430

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FIGURE CAPTIONS

Figure 1. Climatic conditions during cabbage growing period in the Northern and Eastern areas of Spain.

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

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 1. Individual and total GLS content (μmol/g d.m.) in five white cabbage (*Brassica oleracea* var. capitata) cultivars grown in two geographical regions of Spain*.

Growing location	Cabbage cultivars	Aliphatic GLS					Indole			Total GLS
		IB	PROG	SIN	GRAP	NAP	GB	4-met-GB	neo-GB	
Northern area	Hinova	2.06±0.07^{a}	0.22±0.07	6.02±0.17	ND ^{a}	0.13± 0.05	1.00±0.12$^{a}_{A}$	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$^{a}_{B}$	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±0.18^{a}	0.11±0.04^{b}	0.03± 0.01$_{A}$	8.44
Eastern area	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$^{b}_{A}$	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$^{b}_{A}$	20.00

* Mean values ± standard deviation of three independent experiments.

The same subscripts in the same column mean not significant differences between growing locations for each genotype (P≤0.05).

The same superscripts in the same column mean not significant differences among genotypes for each growing location (P≤0.05).

IB=Glucoiberin; PROG=Progoitrin; SIN=Sinigrin; GRAP=Glucoraphanin; NAP=Gluconapin; GB=Glucobrassicin, 4-met-GB=4-Methoxy-glucobrassicin, neo-GB=Neo-glucobrassicin; Total GLS=total glucosinolates. ND = not detected.

Table 2. Myrosinase activity and soluble protein content in five white cabbage (*Brassica oleracea* var. capitata) cultivars grown in two geographical regions of Spain*.

Growing location	Cabbage cultivars	Soluble protein (mg /g d.m.)	Myrosinase activity (U/g d.m.)	Myrosinase activity (U/mg soluble protein)
Northern area	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[a]$_A$	25.20±0.66	1.22±0.06
Eastern area	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]

* Mean values ± standard deviation of three independent experiments.

The same subscripts in the same column mean not significant differences between growing locations for each genotype (P≤0.05).

The same superscripts in the same column mean not significant differences among genotypes for each growing location (P≤0.05).

Table 3. Vitamin C, total phenolic compounds (TPC), antioxidant capacity (ORAC) and water content in five white cabbage (*Brassica oleracea* var. *capitata*) cultivars grown in two geographical regions of Spain*.

Growing location	Cabbage cultivars	Vitamin C (mg/g d.m.)	TPC (mg GAE/g) d.m.)	ORAC (μmoles TE/g d.m.)	Water (%)
Northern Area	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
Eastern area	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

* Mean values ± standard deviation of three independent experiments.

The same subscripts in the same column mean not significant differences between growing locations for each genotype (P≤0.05).

The same superscripts in the same column mean not significant differences among genotypes for each growing location (P≤0.05).

Table 4. Mean value ± SD of discriminant variables of growing location of cabbage cultivars*

Growing location	Total Glucosinolates (µmol/g d.m.)	Vitamin C (mg/g d.m.)	Total Polyphenols (mg GAE/g d.m.)	Myrosinase activity (U/g d.m.)	Antioxidant capacity (µmoles TE/g d.m.)
Northern area	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]
Eastern area	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]

* The same superscripts in the same column mean not significant difference (P≤0.05)

Figure 1.

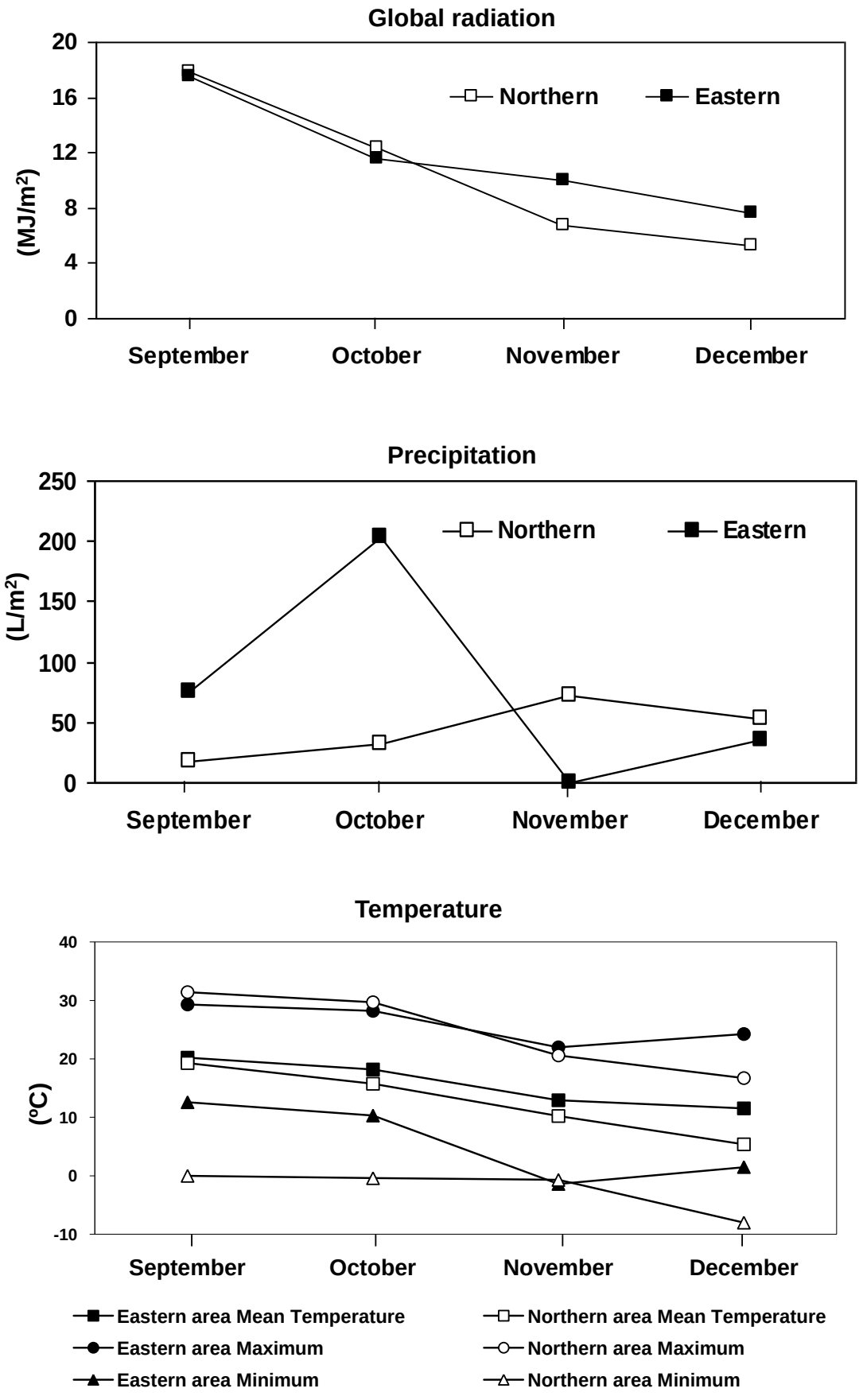

Global radiation
20
16
12
8
4
0
(MJ/m²)
Northern
Eastern
September
October
November
December
Precipitation
250
200
150
100
50
0
(L/m²)
Northern
Eastern
September
October
November
December
Temperature
40
30
20
10
0
-10
(ºC)
September
October
November
December
Eastern area Mean Temperature
Northern area Mean Temperature
Eastern area Maximum
Northern area Maximum
Eastern area Minimum
Northern area Minimum

Figure 2.

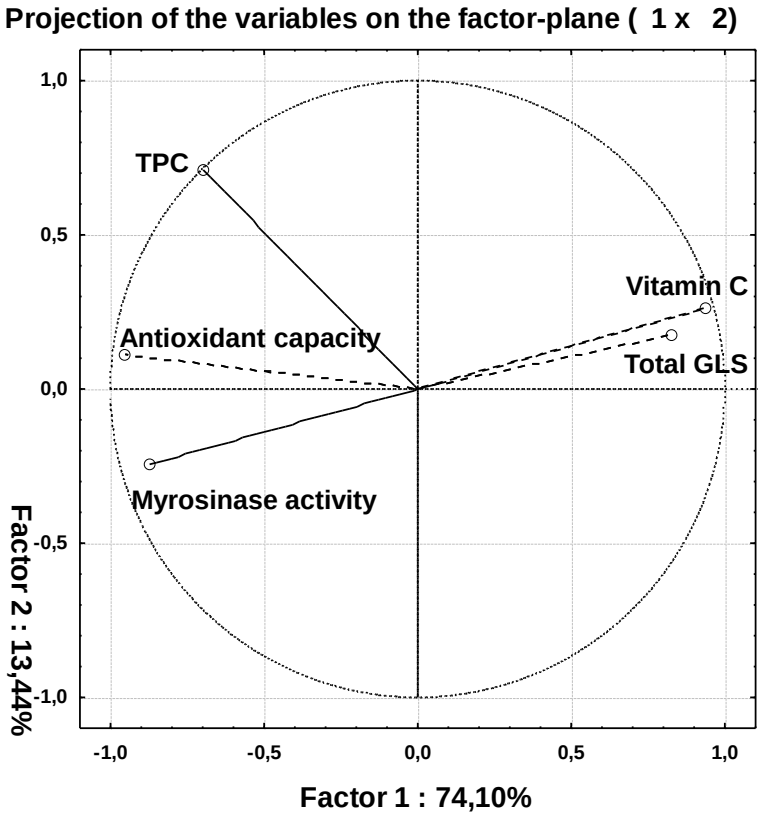
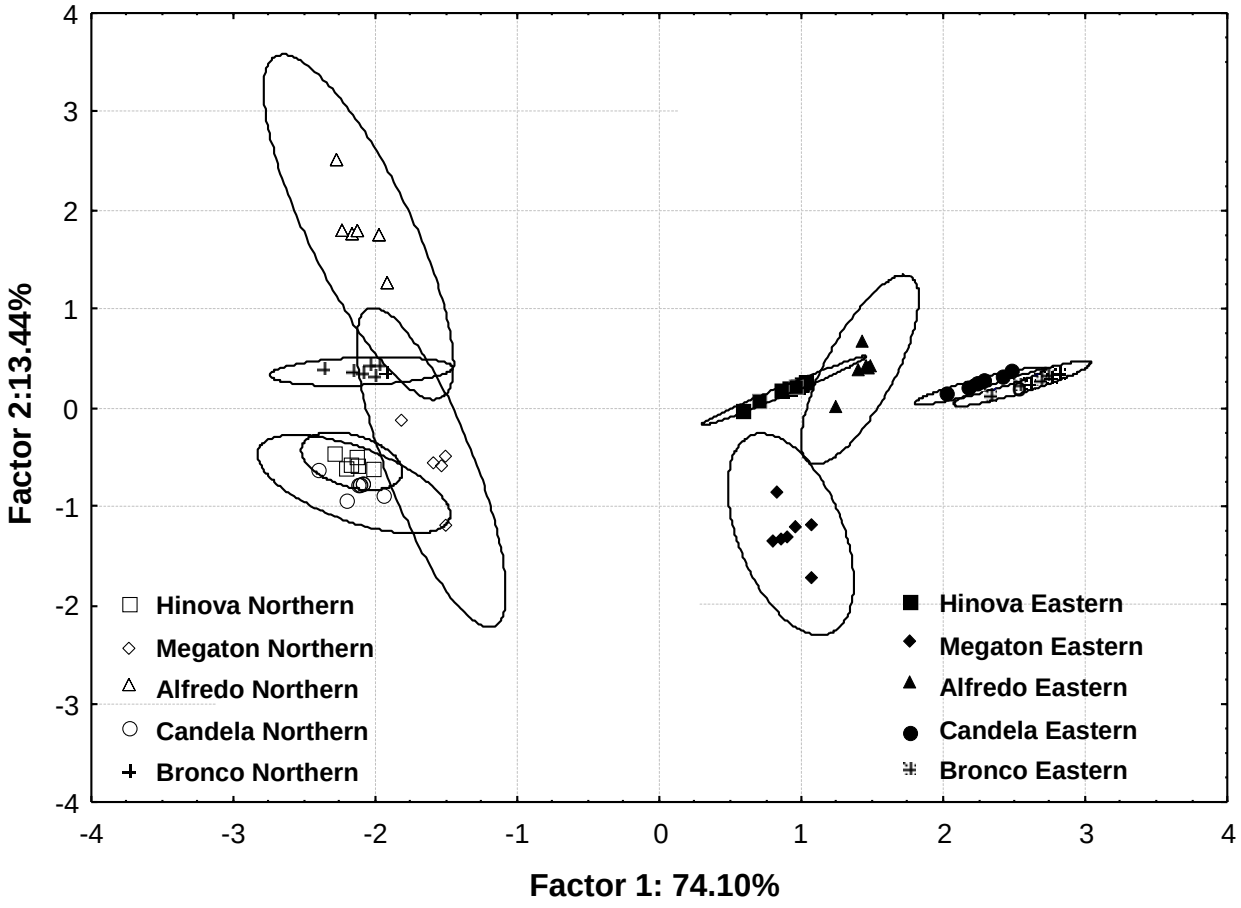
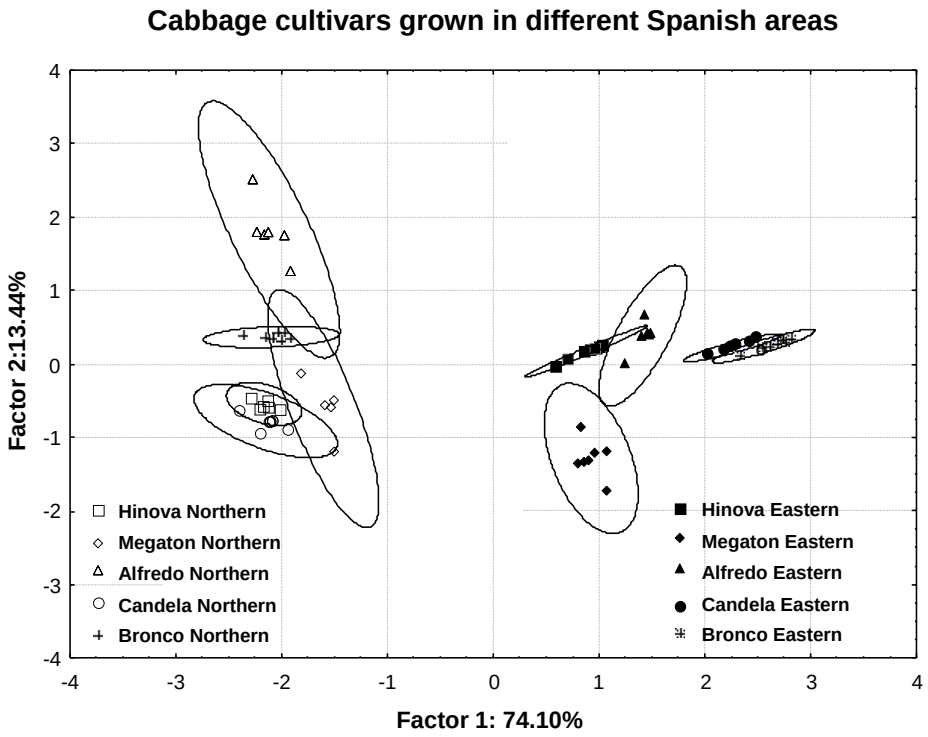

Projection of the variables on the factor-plane (1 x 2)
TPC
Vitamin C
Antioxidant capacity
Total GLS
Myrosinase activity
1,0
0,5
0,0
-0,5
-1,0
-1,0
-0,5
0,0
0,5
1,0
Factor 2 : 13,44%
Factor 1 : 74,10%

Figure 3.


Factor 2:13.44%
Factor 1: 74.10%
Hinova Northern
Megaton Northern
Alfredo Northern
Candela Northern
Bronco Northern
Hinova Eastern
Megaton Eastern
Alfredo Eastern
Candela Eastern
Bronco Eastern

TOC


Cabbage cultivars grown in different Spanish areas
Factor 2:13.44%
Factor 1: 74.10%
Hinova Northern
Megaton Northern
Alfredo Northern
Candela Northern
Bronco Northern
Hinova Eastern
Megaton Eastern
Alfredo Eastern
Candela Eastern
Bronco Eastern