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Variations in the Phytochemical Contents and Antioxidant Capacity of Organically and Conventionally Grown Italian Cauliflower (*Brassica oleracea* L. subsp. *botrytis*): Results from a Three-Year Field Study

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ABSTRACT: A three-year field study (2009−2011) was performed to evaluate phytochemicals and antioxidant capacities of two genotypes (HF1 Emeraude and the local variety, Velox) of green cauliflower grown under organic and conventional management. The conventional system increased yield, but had little effect on the dry matter, whereas the organic system increased the soluble solids. Phytochemicals and antioxidant capacity showed significant year-to-year variability. During the third year, the scarce rainfall determined a significant increase of total glucosinolates and a general decrease of antioxidants in all samples. Interestingly, in the same year organic plants were less affected by the unfavorable climatic conditions, as they increased ascorbic acid, polyphenols, and carotenoids with respect to conventional ones. The overall results for the three years showed that the two genotypes responded differently. Compared to the conventional system, Velox showed 24, 21, 13, 48, and 44% higher content of ascorbic acid, polyphenols, carotenoids, volatiles, and antioxidant capacity, respectively. In contrast, no significant increase in the phytochemicals or the antioxidant potential was found in organic Emeraude, with the exception of total volatiles (+41%). These findings suggest that organic cultivation may be highly effective for particular cauliflower genotypes.

KEYWORDS: *cauliflower, organic and conventional farming, phytochemicals, antioxidant capacity*

INTRODUCTION

In the past few decades, several studies have compared aspects of the quality of organically and conventionally grown plant-derived foods (reviewed by Rembialkowska[1]), and a number of these works indicated the importance of considering the effect of the cultivar as well as the interannual variability due to different climatic growth conditions. Thus, in the organic management of crops, the varietal choice and the agro-ecosystem context must also be taken into consideration.[2] Changing climatic conditions are a relevant topic for the Mediterranean basin, particularly for Italy, where the studies conducted to date indicate that the Italian climate appears to be getting warmer and drier.[3] The use of sustainable agricultural techniques may help to address this problem. Phytochemicals are secondary plant metabolites that have been studied due to their physiological roles in plants, such as the response to biotic or abiotic stress. In addition, phytochemicals may be nutritionally important because of their critical roles in human health. Significant differences in their content exist among varieties within crops, and nutritional quality often depends on specific management practices. Cauliflower (*Brassica oleracea* L. subsp. *botrytis*) is particularly rich in phytochemicals, such as glucosinolates, vitamin C, polyphenols, thiols, and, to a lesser extent, carotenoids. Moreover, upon cellular disruption glucosinolates are hydrolyzed by the action of myrosinase, forming various bioactive breakdown products (isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines).[4] Ascorbic acid, which is known to directly detoxify reactive oxygen species (ROS),[5,6] is the most abundant hydrophilic antioxidant in cauliflower. Phenolic compounds are a large group of secondary metabolites able to neutralize or quench free radicals. Fruits and vegetables are the richest potential sources of these substances, and they are produced by plants under physiologically stressful conditions.[7] Previous studies that evaluated the content of polyphenols in *Brassica* vegetables (reviewed by Podsedek[8]) reported contents ranging from 15 to 337 mg of gallic acid equiv/100 g of edible portion. However, total phenol analysis is generally based on the Folin−Ciocalteu reagent,[9−11] which is known to react with interfering reducing substances, such as ascorbic acid, which is particularly abundant in cauliflower. For this reason, an HPLC-based approach for determining the phenolic content of cauliflower should be chosen. A recent paper indicated that the polyphenol content determined using HPLC is 4.6 mg/100 g fresh weight (fw).[12] Similar results were also obtained by Gratacós-Cubarsí et al.,[13] who found that the main polyphenols in green cauliflower were represented by sinapic acid (2.6−6.2 mg/100 g fw) and, to a lesser extent, flavonoids (0.4−1.5 mg/100 g fw). Thiol-type antioxidants constitute a class of organic sulfur derivatives (mercaptans) with sulfhydryl functional groups (−SH), which play a crucial role in protecting cells from oxidative damage.

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Table 1. Details of Some Farming Practices during the Three Years of Cauliflower Growth in Conventional (Conv) and Organic (Or) Systems[a]

	cultivation year and system					
	2009		2010		2011/2012	
cultivation details	Conv	Or	Conv	Or	Conv	Or
transplanting date	Aug 25, 2009	Aug 24, 2009	Aug 23, 2010	Aug 18, 2010	Sept 3, 2011	Aug 17, 2011
harvesting date	Dec 11–29, 2009	Dec 10–22, 2009	Dec 1–13, 2010	Nov 24–Dec 3, 2010	Jan 13, 2012	Dec 16, 2011–Jan 4, 2012
nutritive elements applied (kg/ha)						
N	180	130	160	135	160	130
P	50	35	40	30	40	20
K	115	105	160	100	160	100

[a]Date of transplanting, harvest, and off-farm applied nutritive elements are presented.

Biologically derived thiols, such as glutathione, cysteine, and homocysteine, are often called biothiols. Few data exist about the content of biothiols in cauliflower. Demirkol et al.[14] reported an amount of approximately 13 nmol/g fw. Carotenoids (carotenes and xanthophylls) are lipid-soluble antioxidants generally found in relatively low concentrations in cauliflower.[8] Due to their conjugated double bonds, carotenoids are both radical scavengers and quenchers of singlet oxygen.[15]

The differences between organic and conventional farming systems may affect the phytochemical composition of plants. To date, more than 95% of organic agriculture is based on crop varieties that were bred for the conventional high-input sector, therefore lacking important traits required under organic production condition.[16] Previous findings have not produced definitive conclusions about the effects of production methods on the phytonutrient levels. Some studies have demonstrated a significant difference between organic and conventional food products,[1,17] whereas other studies did not detect significant differences.[18] These apparently different findings may be because most of the studies were conducted using plants from only one year of harvest and without considering the possibly different responses of different cultivars. Moreover, according to the recent review of Lester et al.,[19] it is important to consider nitrogen profiling, as differences in nitrogen availability in organic and conventional production systems may affect the level of phytochemicals such as phenolic compounds or ascorbic acid. In this review the authors stress the importance of conducting experiments under fully known conditions (e.g., previous crop or crop rotation, soil fertility, and nitrogen supply and availability). To date, specific data on Brassicaceae are scarce and sometimes greatly vary, thus causing difficulties in their interpretation.[20−22] To further explore the effects of organic management on cauliflower cultivation and to investigate the possible effects of the year-to-year variations, the aim of the present work was to analyze two genotypes of a green typology "Verde di Macerata" across three harvesting seasons, comparing the common quality parameters and the phytochemical contents of plants grown using conventional (Conv) and organic (Or) farming systems. The changes in the content of glucosinolates and the characteristic cauliflower volatiles as well as the amount of antioxidant compounds (ascorbate, polyphenols, thiols, and carotenoids) were studied. In addition, two assays of antioxidant capacity were performed to evaluate the nutraceutical potential. Given the fact that the most common problem encountered in the application of methods of measuring antioxidant capacity is the so-called "biological relevance", in the present study we measured the antioxidant potential of cauliflower extracts by the use of one of the most widely used assay, that is, the DPPH scavenging method, and a biologically relevant test that involves the use of a naturally produced radical species, that is, the superoxide anion. The electron paramagnetic resonance (EPR) technique was used, which represents an interesting method of free radical detection because it is able to directly detect a molecule with an unpaired electron.

MATERIALS AND METHODS

Plant Materials and Cultivation. The present research was performed using two genotypes of cauliflower of the same green typology "Verde di Macerata": the HF_1 "Emeraude" (Clause Italia S.p.A.) and the local cultivar "Velox" (selected by the Research Unit of Horticulture, National Agricultural Research Council, CRA-ORA, and distributed by the National Consortium for seed valorization, Convase), both of which ripen early. The two varieties of the green typology were chosen for their higher commercial value compared to the white typology as well as for their widespread diffusion in the area of the middle Adriatic side of Italy, where the experiment was performed. Moreover, the Velox variety was tested because it is often chosen by local organic farmers. The plants were grown in conventional and organic farming systems over three consecutive seasons in two bordering fields at the CRA-ORA, located in Monsampolo del Tronto, Italy (42° 53′ 52″ N; 13° 47′ 31″ E). As reported in Table 1, samples were cultivated in 2009, 2010, and across 2011/2012 in the third year of study. For brevity, the samples from each growing season have been indicated in the text as 2009, 2010, and 2011. The fields are situated 36 m above sea level on a flat terrain. They are characterized by loamy soil, pH 7.8, with levels of organic matter at 1.2 and 1.6% in the Conv and Or systems, respectively. Both fields were subjected to the same rotation protocol, with lettuce as the preceding crop. The cultivation details for cauliflower are reported in Table 1. The organic cultivation system has been certified for organic farming since 2001, according to European Council (EC) Regulation No. 834/07 (this followed Regulation EEC No. 2092/91), and is based on a four year crop rotation of six main crops. Additional information about the experimental site, the system management, and its agronomic and environmental performances is reported in Campanelli and Canali.[23] The cauliflower seedlings were transplanted with a spacing of 70 × 60 cm. All of the cauliflower plants (n = 30) harvested from each of the elementary plots (12.6 m^2) were used to determine the marketable yield. The latter was determined by removing all of the leaves and measuring the net curd weight (kg) and the total yield (t/ha). Approximately one-third (n = 10) of the curds harvested from each elementary plot were used to determine the common quality parameters and to conduct the biochemical analysis. Triplicate samples of approximately 1 kg were obtained by hand, to avoid cutting the curds. The samples were rapidly frozen at −50 °C in an air-forced tunnel and then lyophilized. Each sample was then ground (0−2 °C) to a fine powder using a Waring blender and stored at −20 °C until further analysis.

Climate Monitoring. During the three years of study, the daily mean temperature and the measured rainfall from the time of transplantation until the date of harvest (September−January) were registered by a nearby weather station associated with the agro-meteorological national database (http://cma.entecra.it/homePage.htm). The data were processed as the monthly mean temperature and decadal mean rainfall and were compared with the respective historical series of data (1971−2000).

Common Quality Parameters. Dry matter (dm) was determined as the percentage of the lyophilized weight with respect to the fresh weight. The soluble solid residue (SSR) was determined using an aqueous extract of lyophilized cauliflower powder (2 g was added to 20 mL of distilled water) according to the method of Lo Scalzo et al.[24]

Analysis of Glucosinolates. For analysis of the glucosinolate content, 1.5 g of lyophilized powder was extracted with 45 mL of boiling MeOH for 15 min, using a reflux condenser. The mixture was centrifuged at 8000*g* for 10 min, and the solid residue was re-extracted with a boiling MeOH/water solution (70:30). The collected supernatants were concentrated to dryness in a rotavapor under vacuum, and the residue was added to deionized water to a final volume of 25 mL. Desulfoglucosinolates were prepared and quantitatively determined by HPLC using purified *Helix pomatia* sulfatase.[25] The quantification was based on a standard calibration curve using sinigrin as an external standard. The concentration of individual glucosinolates was expressed in micromoles of sinigrin equivalents per gram of dry weight (dw). The results are given as the total amount of identified glucosinolates, the sum of aliphatic glucosinolates (glucoiberin, progoitrin, epiprogoitrin, sinigrin, glucoraphanin, glucoalyssin, and glucobrassicanapin), and the sum of indolic glucosinolates (glucobrassicin, 4-hydroxyglucobrassicin, and neoglucobrassicin).

Analysis of Volatile Substances. The volatile compounds were extracted by microwave heating and concentrated using a resin-solvent as previously reported.[21] The obtained extracts were analyzed by gas chromatography−mass spectrometry (GC-MS), as described in a previous paper.[26] They were divided into three main classes according to their functional groups as follows: (i) isothiocyanates (NCS (allyl-NCS, propyl-3-(methylthio)-NCS, butyl-4-(methylthio)-NCS, 2-phenylethyl-NCS); (ii) nitriles (CN (butanenitrile-4-(methylthio), pentanenitrile-5-(methylthio), benzene propanenitrile); and (iii) sulfides (S (dimethyl trisulfide, dimethyl disulfide, methyl-(methylthio)-methyl disulfide, dimethyl tetrasulfide). The compounds were identified by comparing the spectra of standards and those contained in a commercial library (Wiley 7 n.1 Library, Mass Spectral Data Base, Hewlett-Packard, Vienna, Austria) or by using commercial standards. Quantitative determination of the components was achieved using pure reference compounds for allyl-NCS, propyl-3-(methylthio)-NCS, dimethyl trisulfide, and dimethyl disulfide (Sigma-Aldrich, Milan, Italy). The other components were quantified using methyl palmitate as an internal standard. The data are given as micrograms per gram dw.

Analysis of Ascorbic Acid. To prepare the samples, 30 mg of lyophilized powder was extracted with 1 mL of 3% metaphosphoric acid, homogenized, centrifuged at 25000*g* for 5 min at 4 °C, and immediately analyzed. The ascorbic acid (AsA) content was determined by HPLC using an Intersil ODS-3 analytical column (250 × 6 mm i.d.) that was maintained at 30 °C. Isocratic elution was performed using a mobile phase of 0.02 M orthophosphoric acid with a flow rate of 0.7 mL/min; 20 μL samples were injected and monitored at 254 nm, and the retention time was 7.8 min. The concentration of ascorbic acid was calculated from the experimental peak area by analytical interpolation using a standard calibration curve and was expressed as milligrams per 100 g of dw.

Analysis of Total Polyphenol Content. The analysis of the polyphenol content was performed according to the method of Soengas et al.,[27] with some modifications. This method was utilized for total polyphenol determination rather than the widely used Folin−Ciocalteu assay because the Folin−Ciocalteu reagent strongly reacts with ascorbic acid, which is particularly abundant in cauliflower. For the extraction, 300 mg of lyophilized powder was dissolved in 10 mL of a 2:1 v/v mixture of 80% EtOH and 3 N HCl. The mixture was vortexed for 1 min and subjected to acid hydrolysis by maintaining it in a stoppered test tube for 1 h in an oven adjusted to 85 °C. The acid hydrolysis product was centrifuged at room temperature (20000*g* for 5 min), and an aliquot (400 μL) was diluted with an equal volume of 80% EtOH plus 50 μL of 50% CH_3COOH prior to HPLC injection. The HPLC analysis was performed using a JASCO system equipped with a diode array detector (MD-910 JASCO). The pump (PU-980 JASCO) was coupled to a ternary gradient unit (LG-1580-02 JASCO). The data were evaluated using a software management system for chromatographic data (ChromNAV, Jasco). The separation was performed by reversed-phase chromatography using an ODS-3 Lichrosorb 250 × 4 mm column. The flow rate was 0.7 mL/min, the injection volume was 30 μL, and the oven temperature was 45 °C. The mobile phase consisted of 5% CH_3COOH in water (solvent A) and MeOH/water/CH_3COOH (90:5:5) (solvent B). The gradients were as follows: (A/B) 95:5 for 0−3 min, from 95:5 to 70:30 in 5 min, 70:30 for 20 min, from 70:30 to 35:65 in 7 min, 35:65 for 5 min, from 35:65 to 95:5 in 10 min, and 95:5 for 12 min. The total analysis time was 59 min. The peaks were identified both by direct comparison with commercial standards and by their spectral and chromatographic properties with respect to relevant data reported in the literature. The main compound observed was sinapic acid, whereas other identified compounds were four caffeoyl derivatives and three sinapoyl derivatives (Figure 1). Quantification was based on the calibration curves of external standards. Solutions of caffeic acid were used to quantify caffeoyl derivatives, and sinapic acid was used for sinapic and sinapoyl derivatives. The levels of sinapic acid and that of the total polyphenols (mg/100 g dw), resulting from the sum of contents of caffeoyl derivatives, sinapic acid, and the sinapoyl derivatives, are reported.

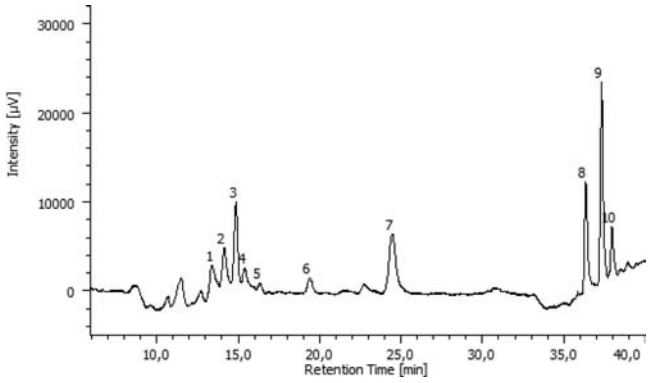

Figure 1. Example of chromatogram acquired by HPLC-DAD (320 nm) from green cauliflower (*B. oleracea* subsp. *botrytis*) extract after acid hydrolysis. See Material and Methods for details. Peaks: 1−6, caffeoyl derivatives; 7, sinapic acid; 8−10, sinapoyl derivatives.

Analysis of Thiols. The total content of free soluble −SH groups was determined according to the method used by Hawrylak and Szymanska,[28] with some modifications. The detailed method was reported in Picchi et al.[21] The number of nonprotein −SH groups was calculated from a standard curve prepared for L-cysteine, and the data are given as milligrams per 100 g of dw.

Analysis of Total Carotenoids. The analysis of the total carotenoids was performed according to the method described in a previous study.[21] The total carotenoid content was determined spectrophotometrically by measuring the maximum absorption in the visible region at 436 nm, and the results are expressed as lutein equivalents (mg/100 g of dw).

Antioxidant Capacity. The antioxidant capacity (AC) was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) radical quenching and superoxide anion ($O_2^{\bullet-}$) radical scavenging methods. The determinations were made by electron paramagnetic resonance (EPR), using a MiniScope MS200 Magnettech (Berlin, Germany)

instrument. The detailed protocol was previously reported by Picchi et al.[21]

Statistical Analysis. All of the analyses were conducted using three biological replicates, and the repeatability was determined from triplicate analyses of each sample. To test the effect of the year of harvest, the system of cultivation (treatment), and the genotype on each measured parameter, the results obtained were evaluated using a three-way factorial analysis of variance (ANOVA). The mean values associated with the main factors (year, genotype, and treatment, as well as their interactions) were evaluated using Tukey's test, and statistically significant differences were accepted at the minimum probability level of $P < 0.05$. The data for the yield and the common quality parameters, phytochemical content and antioxidant capacity, are reported as the mean values ± standard error of the mean (SE), and comparisons among the mean values were evaluated using Tukey's test. All of the statistical analyses were performed using the Statgraphics v.7 (Manugistic Inc., Rockville, MD, USA) software package.

RESULTS

Climate Monitoring. The distributions of monthly rainfall and mean temperature during the period of cauliflower growth (September–January) are shown in Figure 2. Compared to

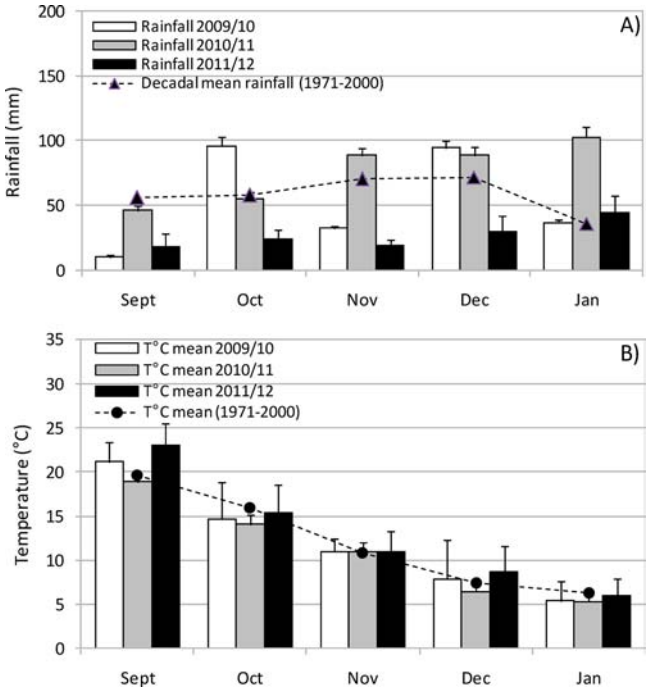

Figure 2. Total rainfall distribution and mean temperatures during the period September–January in Monsampolo del Tronto for the years 2009/2010, 2010/2011, and 2011/2012.

2009/2010 and 2010/2011, the rainfall in 2011/2012 was extremely scarce, particularly from September to December, that is, the period when cauliflower curds are formed. The mean monthly temperatures (T °C mean) in September and December of 2010/2011 were lower than in the other two years (Figure 2), whereas they were generally increased during 2011/2012, when the T °C means were higher with respect to the historical mean temperatures.

Yield and Common Quality Parameters. The results of the multifactorial analysis of variance are shown in Table 2. The net curd weight and yield did not vary with the year of harvest, but they were significantly affected by the genotype. In particular, the values for these two parameters were generally higher in Emeraude (Em) compared with Velox (Vel) cauliflower, with the exception of 2011, when there were not significant differences between the two varieties (Table 3). The yield was also affected by the treatment, with the Conv system generally providing higher yields across the three cultivation years compared to Or management. However, in considering each year of study separately, we did not observe a significant decrease in the yield of the Or samples compared to the Conv ones, with the exception of 2010, when the production of Em Or cauliflower was significantly lower compared to that of Em Conv (−32.4%) (Table 3). Analogously, the net curd weights followed the same trend as the yields, with a slight decrease registered only in 2010 in the Em Or samples.

The SSR and dm values were affected by the three main factors and their interactions (Table 2). As shown in Table 3, the SSR value tended to decrease from 2009 to 2011, and the Or samples presented higher values compared to the Conv samples across the three years of study. This trend was particularly evident in the Vel genotype, which exhibited the greatest increase in 2009 (+45.3%). With regard to the dm, a significant difference was observed in Vel during 2009, when the dm was increased in the Or system (+25.5%).

Phytochemical Content. The treatment and the genotype generally affected the level of phytochemicals, with the exception of the total glucosinolates (GLS), which were not affected by these two factors. Notably, the phytochemical content was also strongly affected by the year, with the exception of the content of total polyphenols (Table 2).

The content of the main classes of glucosinolates and the total glucosinolate levels are shown in Figure 3. The system of cultivation did not significantly affect the glucosinolate levels, except in 2010, when Em Or samples showed a slight decrease in the total GLS content compared to Em Conv (−14.2%). Across the three years of study, we observed a significantly higher amount of total GLS in 2011 compared to 2009 and 2010. With regard to the single classes of GLS, the aliphatic content did not differ according to the type of cultivation in 2009 and 2011, whereas in 2010 the levels were lower in the Or samples. In contrast, the indolic content was significantly increased in the Or samples of both genotypes in 2009, whereas in 2010 it was higher only in the Vel Or samples. An interesting result was observed with regard to the trend for the sum of the aliphatic and indolic GLS content during the three years of analysis. In 2009, the aliphatic contents were higher with respect to the indolic contents, whereas in 2010 the two groups were present in similar amounts. Finally, in 2011, the relationship resembled that of 2009, with a general increase in the aliphatic GLS content with respect to the indolic content.

The level of the total volatiles was influenced by the year, the genotype, and the treatment (Table 2). Organic management generally resulted in a higher content of volatiles compared to the conventional management (Figure 3), reflected mostly in the levels of CN and S compounds. This increase was particularly evident for the Em genotype, in which Or management increased the total volatile production by 60.5, 37.0, and 28.8% in 2009, 2010, and 2011, respectively. Moreover, a significantly higher level was observed in the Em genotype compared to the Vel genotype. Specifically, the S content in the Vel genotype was higher in the Or samples compared to the Conv samples, with the exception of 2010. For that year, we observed a decreased volatile production in the Vel genotype, particularly due to the very low level of CN compounds in both the Conv and Or samples.

Table 2. Significance of ANOVA for Each Measured Parameter, Metabolite, and Antioxidant Capacity, Relating to Their Value in the Two Cauliflower Varieties Harvested across Three Cultivation Years and Two Cultivation Systems (Conventional and Organic)[a]

	year (Y)	genotype (G)	treatment (T)	Y × G	Y × T	G × T	Y × G × T
yield and common quality parameters							
net curd wt	ns	***	ns	ns	ns	ns	ns
yield	ns	***	**	ns	ns	ns	ns
SSR (°Brix)	***	***	***	***	***	***	***
dm (%)	***	***	***	***	***	**	***
phytochemicals							
ascorbic acid	***	***	***	**	*	*	ns
total polyphenols	ns	ns	***	*	*	ns	**
sinapic acid	***	**	*	**	*	ns	**
thiols	***	***	ns	ns	ns	ns	ns
carotenoids	***	***	*	***	*	ns	*
total glucosinolates	***	ns	ns	ns	ns	ns	*
total volatiles	**	***	***	ns	ns	*	ns
antioxidant capacity							
DPPH• scavenging	***	ns	**	***	***	***	ns
superoxide scavenging	***	ns	*	**	*	*	ns

[a]ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3. Yield and Common Quality Parameters of Emeraude and Velox Cauliflower Grown with Conventional (Conv) and Organic (Or) Method[a]

		Emeraude					Velox					
		Conv		Or		% variation	Conv		Or		% variation	*P*
net curd wt (kg)	2009	1.0 ± 0.02	b	0.9 ± 0.05	b	−13.1	0.5 ± 0.03	a	0.6 ± 0.05	a	+33.3	***
	2010	1.0 ± 0.07	b	0.8 ± 0.03	ab	−20.8	0.8 ± 0.04	a	0.8 ± 0.05	a	+4.1	*
	2011	0.8 ± 0.12	a	0.8 ± 0.04	a	+3.1	0.8 ± 0.15	a	0.6 ± 0.01	a	−28.6	ns
yield (t/ha)	2009	22.0 ± 0.47	b	19.2 ± 1.18	b	−12.7	10.4 ± 0.64	a	13.8 ± 0.96	a	+32.7	***
	2010	24.8 ± 1.69	b	16.8 ± 1.28	a	−32.4	18.0 ± 0.83	a	14.6 ± 0.95	a	−19.1	**
	2011	19.0 ± 2.96	a	17.0 ± 1.36	a	−10.2	19.3 ± 3.66	a	11.6 ± 0.56	a	−39.9	ns
SSR (°Brix)	2009	6.7 ± 0.08	b	6.8 ± 0.04	b	+2.3	5.1 ± 0.04	a	7.4 ± 0.03	c	+45.3	***
	2010	5.3 ± 0.00	a	5.5 ± 0.00	ab	+3.9	6.0 ± 0.10	b	6.9 ± 0.22	c	+15.8	***
	2011	4.5 ± 0.06	a	5.6 ± 0.03	c	+24.0	5.3 ± 0.09	b	5.8 ± 0.07	c	+10.1	***
dm (%)	2009	11.4 ± 0.07	b	11.5 ± 0.07	b	+0.9	9.2 ± 0.12	a	11.5 ± 0.04	b	+25.5	***
	2010	9.7 ± 0.08	a	10.0 ± 0.16	a	+2.9	10.2 ± 0.06	a	10.1 ± 0.10	a	−0.3	ns
	2011	10.1 ± 0.46	a	10.0 ± 0.03	a	−1.5	9.8 ± 0.09	a	9.6 ± 0.20	a	−2.1	ns

[a]Each value represents the mean ± SE of three different samples. Means followed by different letters in the same row are significantly different according to Tukey's test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant). The percent variations indicate the change in favor of the Or system when positive and in favor of the Conv system when negative.

The statistical analysis of the AsA contents revealed that this metabolite was significantly affected by the year, the genotype, and the treatment (Table 2). The Or system of cultivation induced a general increase in the AsA content in the samples. This increase was detected in both of the varieties, with the exception of 2010, when the AsA levels did not significantly differ among the samples (Table 4). The larger amount of AsA in the Or samples was particularly evident in the Vel genotype, with percent variations of 25.3 and 48.7 in 2009 and 2011, respectively. With regard to the year-to-year variation, we observed a significantly higher AsA content in 2009 (ranging from 541 to 831 mg/100 g dw) compared to 2010 and 2011, with the lowest levels (ranging from 228 to 380 mg/100 g dw) occurring in 2011.

The general mean content of total polyphenols was not significantly influenced by the year, but it was dependent on the genotype and the treatment (Table 2). The TPC was higher in the Vel genotype than in the Em genotype, and a trend toward an increase in the TPC content of Or samples with respect to Conv samples was found, in accordance with the results obtained for the AsA contents. The effect of Or management was particularly evident in the Vel 2009 (+ 40.0%) and the Em 2011 (+75.0%) samples (Table 4), with the latter being the most different. This result is related to the increase of the main polyphenol detected, that is, sinapic acid, which was markedly increased in the Em Or harvested in 2011 (+62.6% compared to Em Conv) (Table 4). Furthermore, the sinapic acid level in the Vel samples significantly varied only in the cauliflower harvested in 2009 (+32.4% in Or samples compared to Conv), whereas the differences were not statistically significant in 2010 and 2011.

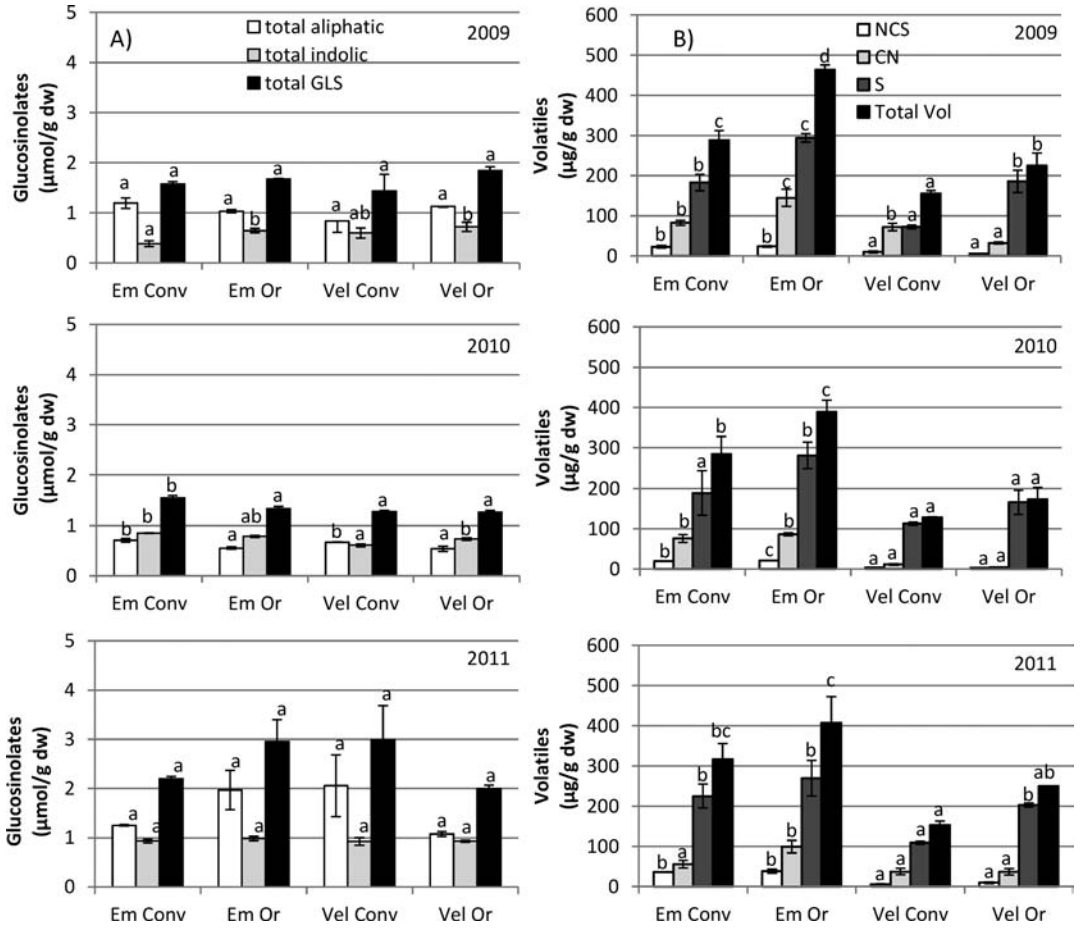

Figure 3. Aliphatic, indolic, and total glucosinolate content (A) and volatile substances (B) of Emeraude and Velox cauliflower grown with conventional (Conv) and organic (Or) methods. Values are the mean of three different samples. For each compound or class of compound, different letters indicate means significantly different for each year of analysis according to Tukey's test ($P < 0.05$). GLS, total glucosinolates; NCS, isothiocyanates; CN, nitriles; S, sulfides; Total Vol, total volatiles.

The thiol content was not affected by the treatments (Table 2), but it was affected by the year of study, with higher contents in 2009 compared to 2010 and 2011, following the trend for the AsA contents (Table 4). In addition, the effect of the genotype was significant, with greater thiol levels in Vel compared to Em. The amount of thiols in Vel averaged 74.7, 57.1, and 57.7 mg/100 g dw in 2009, 2010, and 2011, respectively, whereas those in Em were 49.0, 36.2, and 43.2 mg/100 g dw, respectively (Table 4). It must be noted that in the present cauliflower samples, the amount of total thiols was higher than the TPC (an average of 3.3-fold higher), suggesting that these reducing metabolites should be considered when evaluating the phytochemical contents of cauliflower.

The total carotenoid (CAR) content was affected by the year, the genotype, and the treatment (Table 2). As shown in Table 4, significantly higher levels were found in 2009 with respect to 2010 and 2011, and a greater amount was found in the Vel samples than in the Em samples. The Or method increased the CAR content, particularly in the Vel genotype in 2010 (+20.0%) and 2011 (+38.0%). Interestingly, the amounts of CAR ranged from 2.3 to 9.7 mg/100 g dw, which are considerably smaller amounts compared to the AsA and thiol contents. This result suggests that those metabolites have a minor effect on the total antioxidant profile of the cauliflower.

Antioxidant Capacity. The AC was significantly affected by the year and the treatment but not by the genotype (Table 2). A detailed report of the AC is shown in Figure 4. In 2009, we observed the highest AC, both in terms of DPPH-quenching capacity (ranging from 5.2 to 6.7 mmol AsA equiv/100 g dw) and in terms of superoxide-scavenging capacity (ranging from 258 to 379 mmol Trolox equiv/100 g dw). This result is in accordance with the highest content of AsA occurring in 2009. In 2010 and 2011, we observed a general decrease in the AC, particularly for the superoxide-scavenging capacity, whereas the DPPH-quenching potential was markedly reduced in 2011. With regard to the relationship between the amount of phytochemicals and the antioxidant indices, the linear regression analysis conducted using the 3 year data showed that AsA was primarily responsible for the AC, as suggested by the strong correlation between the content of this metabolite and the values for the DPPH ($r = 0.78$, $P < 0.001$) and superoxide anion quenching ($r = 0.89$, $P < 0.001$). Moreover, the results obtained using the two methods for AC determination were positively related ($r = 0.60$, $P < 0.001$). In addition, the content of carotenoids, although present in low amounts, were significantly related to the AC of the cauliflower extracts ($r = 0.69$ and $r = 0.44$, $P < 0.01$, for DPPH and superoxide anion quenching, respectively). Interestingly, a significant correlation was also found between the content of glucobrassicin and the AC determined by DPPH ($r = 0.42$, $P < 0.05$) and by superoxide anion quenching ($r = 0.46$, $P < 0.01$). In contrast, the 3 year values of TPC were not related to the

Table 4. Concentration of Phytochemicals (Milligrams per 100 g dw) of Emeraude and Velox Cauliflower Grown with Conventional (Conv) and Organic (Or) Methods[a]

		Emeraude					Velox					
		Conv		Or		% variation	Conv		Or		% variation	*P*
AsA	2009	541 ± 4.7	a	636 ± 43.7	ab	+17.7	663 ± 21.8	b	831 ± 12.9	c	+25.3	***
	2010	430 ± 2.0	a	415 ± 46.7	a	−3.5	395 ± 2.9	a	422 ± 25.5	a	+7.0	ns
	2011	228 ± 24.7	a	255 ± 25.3	b	+11.9	255 ± 7.5	ab	380 ± 66.5	b	+48.7	*
TPC	2009	13.1 ± 0.19	a	14.4 ± 0.08	a	+10.0	14.0 ± 0.89	a	19.2 ± 0.01	b	+40.0	***
	2010	18.0 ± 0.31	a	14.9 ± 2.18	a	−17.1	13.7 ± 1.13	a	16.9 ± 0.54	a	+23.8	ns
	2011	11.3 ± 0.37	a	19.7 ± 0.05	b	+75.0	18.6 ± 0.24	ab	19.9 ± 3.21	b	+7.1	*
sinapic	2009	4.9 ± 0.08	c	4.7 ± 0.05	c	−4.5	2.6 ± 0.26	a	3.4 ± 0.09	b	+32.4	***
	2010	2.5 ± 0.01	a	2.1 ± 0.29	a	−15.8	2.7 ± 0.39	a	3.1 ± 0.07	a	+13.7	ns
	2011	5.7 ± 0.13	a	9.2 ± 0.08	b	+62.6	5.7 ± 0.54	a	5.8 ± 1.40	a	+1.0	*
−SH	2009	48.4 ± 4.36	a	49.5 ± 0.79	a	+2.2	70.6 ± 2.54	b	78.7 ± 5.15	b	+11.5	***
	2010	36.6 ± 0.61	a	35.7 ± 0.17	a	−2.6	56.1 ± 0.89	b	58.0 ± 4.06	b	+3.4	***
	2011	46.2 ± 8.57	ab	40.1 ± 1.20	a	−13.2	60.6 ± 1.30	b	54.7 ± 3.68	ab	−9.8	*
CAR	2009	4.9 ± 0.27	a	5.5 ± 0.02	a	+12.4	9.7 ± 0.77	b	9.4 ± 0.61	b	−3.2	***
	2010	6.9 ± 0.14	ab	6.1 ± 0.33	a	−10.7	6.1 ± 0.28	a	7.3 ± 0.09	b	+20.0	*
	2011	2.3 ± 0.31	a	3.4 ± 0.55	ab	+47.2	4.7 ± 0.13	b	6.5 ± 0.40	c	+38.0	***

[a]Each value represents the mean ± SE of three different samples. Means followed by different letters in the same row are significantly different according to Tukey's test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant). The percent variations indicate the change in favor of the Or system when positive and in favor of the Conv system when negative.

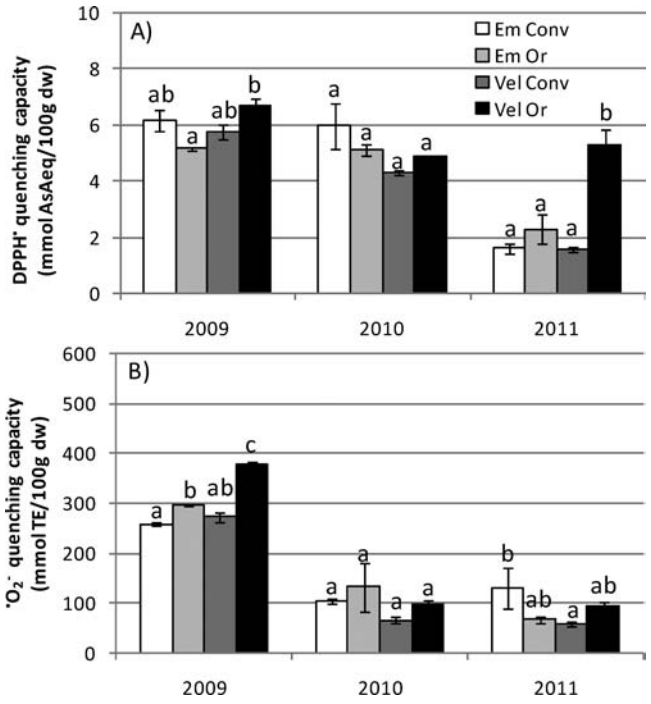

Figure 4. Antioxidant activity measured as DPPH• (A) and superoxide (B) scavenging capacity of Emeraude and Velox cauliflower grown with conventional (Conv) and organic (Or) methods. Values are the mean of three different samples. Different letters indicate means significantly different for each year of analysis according to Tukey's test ($P < 0.05$). Data are expressed as millimoles of ascorbic acid (AsA) and in millimoles of trolox (TE) equivalents.

AC values. Despite this fact, in analyzing each years' data separately, we found a significant correlation between the TPC and superoxide-scavenging capacity ($r = 0.98$, $P < 0.001$, and $r = 0.70$, $P < 0.01$) or between the TPC and the DPPH-quenching capacity ($r = 0.92$, $P < 0.001$, and $r = 0.61$, $P < 0.05$) in 2009 and 2010, whereas the TPC data for 2011 were not correlated with the AC values. In fact, in 2011, the increased polyphenols in Or-cultivated cauliflower did not correspond to a similar increase in the AC. This result could be related to the marked decrease in AsA content registered in the same year, which strongly affected the AC of the plants.

DISCUSSION

The results obtained for the net curd weights and the total yields indicated that organic farming had a minor effect on these parameters. However, the conventional system generally produced higher yields across the three cultivation years compared to the organic system. Our study showed that the different response of the yield to organic management was mainly due to the genotype and that only Emeraude was negatively affected by organic management in 2010. In a previous study, the commercial yield of differently fertilized cauliflowers was evaluated, and the life cycle assessment clearly showed a higher yield from plants cultivated using mineral fertilizers rather than compost manure.[29]

The growing system had little effect on the dry matter values, whereas the soluble solid residue values of the organic samples generally were higher than those of the conventional samples across the three years of study. This was particularly evident for the soluble solid residue of the Velox genotype, which significantly increased with organic management compared to those of the conventional samples. Analogous results of increased soluble solid residue were also observed in other organically cultivated crops, as reviewed by Rembialkowska.[1]

Most of the phytochemical contents, as well as the antioxidant capacity, were significantly affected by the year and the year × genotype interaction (Table 2). Although only two genotypes were used, this study confirmed that the levels of

these metabolites were affected not only by the cultivation system but also by the environmental conditions and the variety. In our survey, the climatic variations had a strong effect on the phytochemical concentrations and the antioxidant capacity of the cauliflowers, particularly in the third year of analysis, due to the exceptional drought caused by a great decrease in rainfall. This condition induced a higher total glucosinolate content in 2011, consistent with the results of Ciska et al.,[30] in which sparse rainfall and a high temperature induced an increase in the glucosinolate content in different *Brassica* species, including cauliflower. In the present study, the relative proportion of the aliphatic and indolic glucosinolates also varied from year to year. The importance of climatic factors in determining the levels of different alkyl and alkenyl glucosinolates, as well as the indolic glucosinolates levels, was noted by Mithen et al.[31] and Schonhof et al.[32] We can conclude that the distinct differences in the proportions of individual glucosinolates compounds among the groups, as well as their levels of total glucosinolate, were mostly affected by the climatic conditions.

Our data suggest that organic farming improved the total volatile content of both varieties during the three years of analysis, particularly the levels of nitriles and sulfides. Similar findings have been reported for a green cauliflower genotype (Magnifico)[21] and three white cauliflower genotypes (Aviron, Escale, and Triomphant).[33] A positive effect of organic farming on the content of volatile organic compounds was also observed in garlic, where the diallyl trisulfide content was increased by up to 20% compared to that of conventional crops.[34]

In addition to the glucosinolate content, an obvious effect of the different growing conditions during the three years of harvest was observed for the ascorbic acid content, which was markedly decreased in 2011. Similar findings were reported in the study of Hanson et al.[35] using two *Brassica* vegetables, in which the ascorbic acid content during a dry season was significantly lower compared to the amount detected in a wet season. The influence of the climatic conditions on the quality of green cauliflower was reported in a previous study,[24] wherein adverse environmental conditions in the period close to the harvest (scarce rainfall and very low temperatures) strongly affected the phytochemical content, particularly by decreasing the ascorbic acid content and increasing the total polyphenols compared to those of the plants cultivated during a favorable season. Our results are in accordance with these findings; in fact, during the third year of analysis, the amounts of ascorbic acid and carotenoids were greatly reduced, whereas the total polyphenols were generally increased. Interestingly, during the same year, we found higher levels of phytochemicals (particularly ascorbic acid and polyphenols) and a higher antioxidant capacity in the organic samples compared to the conventional samples. This result may be related to the requirement of the organic plants to devote greater resources to the synthesis of their own chemical defense compounds. Indeed, increases in antioxidant contents have been attributed to their production for plant defense. Consequently, it can be argued that plants cultivated in organic systems may be less affected in terms of phytochemical content in the case of unfavorable climatic conditions. Nevertheless, the result that the organic system of cultivation induced a general increase in ascorbic acid and total polyphenols content may have been influenced also by the level and type of fertilization. In fact, it seems that in conventional system, in which nitrogen is readily available, the metabolism of plants changes toward the production of nitrogen-containing compounds such as free amino acids, proteins, and alkaloids, which adversely affect the synthesis of bioactive compounds.[36] Additionally, it was reported that the use of poorly available nitrogen fertilizer, that is, the case of organic management, contributes to increase the ascorbic acid and polyphenols content in tomatoes and peppers.[36,37]

Our data indicate a general reduction of the antioxidant capacity, particularly in the third year of harvest. However, the organic samples exhibited higher antioxidant indices compared to the conventional samples, particularly those of the Velox genotype. This result is in accordance with the trend in the ascorbic acid content and confirms that this metabolite is the main phytochemical compound positively affected by organic management.[1,38] Moreover, the antioxidant indices were highly correlated with the ascorbic acid content, confirming the primary role of ascorbic acid in determining the antioxidant potential of *Brassica* extracts.[9] Additionally, significant correlations were found between the antioxidant indices and the content of other substances not immediately recognized as antioxidants, specifically glucobrassicin. Cabello-Hurtado et al.[4] recently found that some glucosinolates play a role in the overall antioxidant capacity of cauliflower and that the main glucosinolate indole of cauliflower, glucobrassicin, is the most potent antioxidant, as determined by both ORAC and superoxide radical-scavenging activity assays.

The effect of organic farming on the average phytochemical contents and antioxidant capacities of the two genotypes of green cauliflower during the three year period is shown in Figure 5. The volatiles were the most influenced by the organic

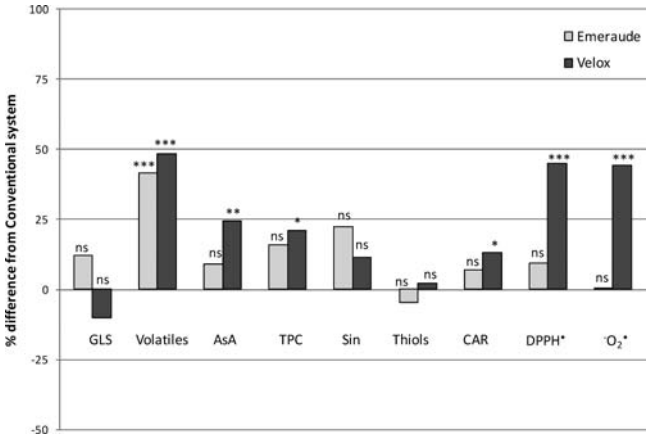

Figure 5. Comparison across the three years of study of the phytochemical content and the antioxidant capacity of Emeraude and Velox cauliflower cultivated with organic farming. Percent changes with respect to conventional samples (assumed equal to 100%) are reported. Asterisks indicate significant differences according to Tukey's test (∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$; ns, $P > 0.05$).

system in both varieties, being strongly increased, whereas the thiols, the sinapic acid, and the glucosinolates were the less affected by the two farming methods. Another important result that appears from that figure is that the two genotypes responded differently to the system of management. Notably, the Velox genotype exhibited ascorbic acid, total polyphenols, carotenoids, and total volatile contents that were 24, 21, 13, and 48% higher, respectively, under organic management than under conventional management. Furthermore, the antioxidant

capacities of Velox organic samples determined using the DPPH and superoxide anion quenching methods were significantly higher, by 44%, compared to those of the Velox conventional samples. The level of thiols appeared unaffected, whereas a tendency toward a decrease in the total glucosinolate content was observed. In contrast, in the Emeraude genotype, all of the measured parameters, with the exception of the total volatiles, were not significantly affected or were increased by organic management. These findings are consistent with the conclusion that organic cultivation requires a particular varietal choice, as recently stated by other authors concerning the breeding of cereals[39] and as specifically confirmed by some Brassicaceae breeders participating directly with farmers in programs in which the local organic farmers make specific varietal selections.[2] This type of participatory plant breeding originated in the agro-ecosystems of developing countries and is now being applied in more industrialized countries, concurrent with the spread of organic crop management.[40]

This study contributes to existing efforts to clarify the effect of organic cultivation on the phytochemical content of cauliflower. Our findings demonstrated some differences between two genotypes of conventionally and organically produced green cauliflower and confirmed that the interannual variability of environmental factors, such as climatic conditions, deeply affected the nutraceutical quality of cauliflowers; in some cases, this interannual variability may be the main factor that regulates their phytochemical content. Finally, our results suggest that organically cultivated cauliflowers may be less affected in terms of phytochemical content in the case of unfavorable climatic conditions compared to their conventionally farmed equivalents. Therefore, it would be interesting to encourage organic agriculture in an Italian and Mediterranean context in an attempt to counteract the ongoing negative effects of climate change.

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Notes

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