Available online at www.sciencedirect.com
ScienceDirect
Europ. J. Agronomy 26 (2007) 418–424
European Journal of Agronomy
www.elsevier.com/locate/eja

Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*

Stefania De Pascale [a], Albino Maggio [a,*], Rita Pernice [b], Vincenzo Fogliano [b], Giancarlo Barbieri [a]

[a] *Department of Agricultural Engineering and Agronomy, University of Naples Federico II, Via Università 100, Portici 80055 (NA), Italy*
[b] *Department of Food Science, University of Naples Federico II, Via Università 96, Portici 80055 (NA), Italy*

Received 11 September 2006; received in revised form 20 November 2006; accepted 26 December 2006

Abstract

Sulphur is essential in the biosynthesis of secondary metabolites with high nutritional value that typically accumulate in brassica species. Among these, glucosinolates are the most representative. The level of glucosinolates in these plants is highly dependent on genetic factors as well as environmental determinants, such as the available soil sulphur content. There is an increasing need of defining the metabolic profile of brassica species in response to both cultivation practices and environmental factors since a targeted modification of its constituents may significantly affect the functional properties and the commercial value of these vegetables. Here, we report on the effects of sulphur fertilization on flavonols, phenolic acids and glucosinolates contents of two *friariello* (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) ecotypes—*Lingua di Cane* and *Sorrentino. Friariello* quality in terms of sprouts plus inflorescence nitrate and chlorophyll contents was also assessed. We found a significantly higher flavonols content in the ecotype *Sorrentino*, whereas its glucosinolates level was relatively smaller compared to *Lingua di Cane*. Sulphur fertilization significantly improved the antioxidant activity of both ecotypes and was associated with a genotype-dependent significant reduction of leaf nitrate content.

Keywords: Antioxidant activity; *Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.; Flavonol; Glucosinolate; Nitrate; Phenolic acid

1. Introduction

Epidemiological studies have demonstrated that the regular consumption of fruits and vegetables may reduce the risk of developing chronic diseases, including cardio-vascular disease and cancer (Willett, 2002). These beneficial properties have been associated to the presence of bioactive compounds in fresh vegetables, such as phenolic compounds, glucosinolates, α- and β-carotene, α- and β-tocopherols and ascorbic acid (Williamson et al., 1998). Broccoli, white cabbage and cauliflower have a great health potential, mainly attributable to their level of glucosinolates, which are an important group of phytochemicals mostly present in brassica species (Zhang et al., 1994; Fahey and Stephenson, 1999). These metabolites exhibit a minimal anticancer activity per se, but after myrosinase hydrolysis they generate aglycones, which have a high chemopreventive activity (Faulkner et al., 1998). In particular, some of these compounds, such as isothiocyanates, inhibit Phase I and activate Phase II liver enzymes, a combination of events that participates in cancer prevention by simultaneously increasing carcinogens excretion and decreasing DNA damage (Hecht et al., 2004). Sulphorafane, the aglycone derived by hydrolysis of glucoraphanin is considered one of the most potent naturally occurring inducers of Phase II enzymes (Fahey et al., 1997).

Phenolic compounds also contribute to the health properties of these vegetables (Hertog et al., 1993; Garcia-Closas et al., 1999). These molecules are able to inhibit LDL cholesterol oxidation, to chelate redox-active metal ions and to attenuate other processes involving reactive oxygen species, as they are highly effective free radical scavengers (Rice-Evans et al., 1997). It is known that the degree of glycosylation significantly affects the antioxidant properties of certain molecules. For example, aglycones of quercetin and myricetin are more active than their relative glycosides (Hopia and Heinonen, 1999).

The phenolic content in plants depends on: (1) genetic determinants (Vallejo et al., 2003a,b); (2) environmental

* Corresponding author. Tel.: +39 081 2539133; fax: +39 081 2539129.
E-mail address: albino.maggio@unina.it (A. Maggio).

1161-0301/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2006.12.009

conditions (temperature, light, water availability, nutrient availability) (Zhao et al., 1994; Rosa et al., 1996); (3) agricultural practices (date of harvest, etc.); (4) post-harvest conditions (Rodriguez and Rosa, 1999). All these factors, beside the biosynthesis of phenolic antioxidant compounds, also affect polyphenol oxidase (PPO) and peroxidase (PX) activities and, consequently, the final concentration of polyphenols in plant tissues (Tomas-Barberan and Espĩn, 2001).

The glucosinolates content may be significantly affected by both sulphur and nitrogen fertilization (Kim et al., 2001). Zhao et al. (1994) have reported that abundant nitrogen applications increase the progoitrin content in *Brassica napus* while they decrease its sinigrin concentration. Other reports also indicate that sulphur fertilization may affect the glucosinolates level more than nitrogen fertilization (Rosa et al., 1996). Moreover, for sulphur-deficient soils, sulphur fertilization increased three-fold the glucosinolates content of rapeseed, while nitrogen fertilization reduced it. In general, it has been observed a 40% glucosinolates reduction in plants cultivated at low sulphur fertilization regime in light soils, respect to heavy soils (Ciska et al., 2000). Aires et al. (2006) found that sulphur fertilization has a detrimental effect on the level of aliphatic glucosinolates of broccoli sprouts, but the opposite effect was observed for indole and aromatic glucosinolates.

For some brassica species, the antioxidants profile has been described. It has been reported that the main phenolic constituents of broccoli are flavonols (quercetin and kaempferol derivates) and phenolic acids (hydroxycinnamic acids derivates) (Vallejo et al., 2003a,b). High levels of kaempferol have been found in kale and broccoli, also (Justesen et al., 1998; Price et al., 1998). Different types of *Brassica rapa* are cultivated in Southern Italy over more than 10,000 ha. The cultivation of these species usually occurs in winter cycles, even though these plants may be easily grown all year round, with a seed-to-harvest cycle ranging between 60 and 150 days. The nutritional quality of many *B. rapa* ecotypes, for which consumers seem to have an increasing interest, has not been described.

The aim of the present study was to investigate the effects of sulphur availability on shoot yield (sprout plus inflorescence), quality (nitrate and chlorophyll contents), and nutritional value (with a particular focus on antioxidant – flavonols and phenolic acids – and glucosinolates contents) of two ecotypes of *B. rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort. widely distributed in the Campania region (Southern Italy) and locally known as *friariello*.

2. Materials and methods

The research was carried at the University of Naples experimental farm (40°31′N; 14°58′E). The soil was a deep clay-sandy soil (International Textural Classes), classified as Inceptisoil Haplustet type (USDA soil taxonomy) with 1.65% organic matter, 1.5‰ total N, 837 ppm total S. The effect of two sulphur fertilizations, 0 kg S ha^{-1} (−S) and 1000 kg S ha^{-1} (+S) was evaluated on two *friariello* (*B. rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) ecotypes, *Lingua di Cane* (LC) and *Sorrentino* (Sr). The average maximum temperature (T_{max}) during the experiment ranged between 25.7 (first decade of October) and 10.4 °C (third decade of January) whereas the average minimum temperature (T_{min}) were 15.7 (first decade of October) and 2.5 °C (third decade of January). The average solar radiation during the same period was 13.7 MJ m^{-2} day^{-1} (first decade of October) and 4.8 °C (third decade of December). After autumn ploughing (40 cm depth), the soil was fertilized with 200 kg N ha^{-1} as urea (46% N) and harrowed prior to sowing. Plants were direct seeded in the field on October 3, 2003 in rows 0.5 m apart. At 2 weeks after emergence, seedlings were thinned by hand to a 0.2-m spacing between plants (for a final planting density of 10 plants per metre square). Sulphur was applied before sowing to the +S plots in the form of elemental agricultural S. The experiment was conducted under rain-fed conditions. Weeds were controlled throughout cropping season by a combination of pre-emergence herbicide (trifluralin) and manual weed management. Diseases and insects were regularly controlled with a range of commercial chemicals based on standard procedures. Harvest begun on January 13 and ended on February 2, 2004. Shoots were harvested three times (102, 111 and 122 days after sowing, DAS) at commercial maturity of the inflorescence by leaving 10–15 cm of the stem above ground to allow new sprouting. The experimental design was a randomized block with four replications. Individual plots were 9 m long and 4 m wide with 2 m of bare soil between replications to reduce plot interactions (total area per plot = 36 m^2).

At the first harvest (102 DAS), number of plants, marketable and non-marketable yield were recorded. The dry matter was determined after drying the plants at 60 °C until steady weight. Colour parameters L^*, a^* and b^* were evaluated on leaves using a portable colorimeter (Minolta Chroma Meter CR-300) equipped with three pulsed xenon lamps and a white calibration plate as a standard. According to the CIELAB-system, the L^* component represents lightness, the a^* component represents values running from green (−) to red (+), and the b^* component represents values running from blue (−) to yellow (+). Nitrate content was measured on sample extracts from fully expanded leaves by spectrophotometric (HACH DR/2000 spectrophotometer) determination after cadmium reduction (Sah, 1994). Total soluble solids (TSS) were determined on homogenized leaf samples using a portable ATAGO-PR32 refractometer, and expressed as °Brix. Chlorophyll content was determined on leaf discs of 2 cm^2 as described by Jeffrey and Humphrey (1975).

The flavonoids content was determined following the procedure described by Crozier et al. (1997) with some modifications. Briefly, 1 g of lyophilized shoot sample was extracted with 10 ml of 60% aqueous methanol solution containing 0.25 mg of morin as an internal standard. 1.5 ml of this solution was stored while the remaining part was added with 20 mM sodium diethyldithiocarbamate and 5 ml of 6 M HCl, then it was refluxed at 90 °C for 2 h. The extract of 20 μl, taken before and after acid hydrolysis, were analysed by HPLC (Shimadzu LC 10, Shimadzu, Japan) at a flow rate of 1 ml min^{-1}, with diode array detector and a Prodigy column 5μ ODS3 100A, 250 mm × 4.60 mm (Phenomenex, USA). The mobile phase was a mixture of water/formic acid (95:5, v/v) (A) and methanol (B). Flavonoids

elution was achieved using the following linear gradient: starting condition, 70% A, 30% B; 3 min, 50% A, 50% B; 18 min, 40% A, 60% B; 23 min, 20% A, 80% B; 28 min, 10% A, 90% B; 33 min, 70% A, 30% B. Chromatograms were recorded at 256 nm for flavonols and at 325 nm for phenolic acids. The results were expressed as mg 100 g^{-1} of fresh weight. All samples were analysed in duplicate.

The total phenolic content was determined using the Folin-Ciocalteau's reagent (Singleton and Rossi, 1965). One gram of lyophilized shoot sample was extracted with 10 ml of 60% aqueous methanol solution, agitated for 3 min and centrifuged at 4000 rpm for 5 min at 4 °C. The supernatant was collected and opportunely diluted. Subsequently, 125 μl of the extract were mixed with 125 μl of Folin-Ciocalteau and 500 μl of deionized water were added. After 6 min, 1.25 ml of sodium carbonate (7.5%) and 1 ml of water were added. After leaving the mixture for 90 min at room temperature in the dark, the absorbance at 760 nm was measured and compared with a calibration curve obtained with gallic acid. The total phenolic content was expressed as mg of gallic acid equivalents per 100 g of fresh vegetable. The radical scavenging activity was measured following the ABTS method described by Pellegrini et al. (1999). The assay was performed on 100 μl taken from samples extracted for the total phenols measurements, after dilution. The antioxidant activity was expressed as mmol trolox 100 g^{-1} of fresh tissue.

Glucosinolates were analysed after desulphation according with the procedure described by Kiddle et al. (2001) with some modifications. Briefly, 0.2 g of freeze-dried shoot samples were extracted with 3.5 ml methanol–water (70:30, v/v) and heated at 70 °C in a heating bath for 10 min. The extracts were centrifuged at 2000 g for 10 min at 4 °C; the supernatant was refrigerated while the pellet was extracted a second time with 3 ml of methanol–water (70:30, v/v), heated at 70 °C and centrifuged using the previous conditions. The two supernatants were combined and refrigerated. The desulphation reaction was performed with mini-columns prepared with 1 ml Sephadex A25 and 2 M acetic acid (1:1) to have a 0.5 ml bed volume. Columns were washed with 6 M imidazole formiate and with ultra-pure water. Subsequently, 1 ml of the glucosinolate extract was added to the column. The unbound material was removed washing with 0.1 M sodium acetate (pH 4), then 100 μl sulphatase was loaded in the column and desulphation was performed overnight (16 h) at room temperature. The desulphoglucosinolates were eluted with 1.5 ml ultra-pure water and stored −20 °C before analysis. 20 μl of the extract were analysed by HPLC (Shimadzu LC 10, Shimadzu, Japan) at a flow rate of 1 ml min^{-1}, using a Prodigy column 5μ ODS3 100A, 250 mm × 4.60 mm (Phenomenex, USA). The mobile phase was a mixture of ultra-pure water (A) and methanol (B). Desulphoglucosinolates elution was achieved using the following linear gradient: starting condition, 2% B; 5 min, 4% B; 20 min, 20% B; 30 min, 35% B; 35 min, 40% B; 45 min, 30% B; 50 min, 10% B; 52 min, 2% B. Chromatograms were recorded at 227 nm. 2-Propenylglucosinolate (sinigrin) was used as internal standard.

Data were analysed by ANOVA and means, when significant, were compared by least significant difference (LSD) test.

3. Results

3.1. Yield and leaf nitrate content

At the end of the growing cycle, the marketable yield was 35.8 t ha^{-1} in *Sorrentino* and 28.5 t ha^{-1} in *Lingua di Cane* (Table 1). The first harvest (102 DAS) contributed 28 and 47% to the total marketable yield of the two ecotypes, respectively. However, the cumulated marketable yield over four harvest times was 25% higher in *Sorrentino* respect to *Lingua di Cane* (Table 1 and Fig. 1).

Sulphur fertilization resulted in a 18 and 17% increase of the marketable yield and TSS content, respectively, compared to the non-fertilized control (−S) (Table 1) and it had positive effects at each harvest time for both cultivars (Fig. 1). A genotypic variability in terms of leaf colour and chlorophyll contents was also observed with highest $-a^*$ values (greenness), $+b^*$ values

Table 1
Marketable yield (MY), dry matter (DM) percentage, total soluble solids (TSS), chlorophyll and nitrates contents on fresh weight (FW) basis and colour parameters of *friariello* in response to different treatments

Treatment	MY (t ha^{-1})	DM (%)	TSS (°Brix)	pcChlorophyll (mg g FW^{-1})	Nitrates (mg 1000 g FW^{-1})	Colour		
						L^*	a^*	b*
Ecotypes (E)								
Sr	35.8	19.8	1.73	3.97	628.5	36.62	−11.24	15.01
LC	28.5	14.0	1.57	4.24	799.5	40.02	−14.16	18.16
	**	**	*	**	**	**	**	**
Sulphur (S)								
−S	29.6	16.9	1.50	3.55	730.5	38.35	−12.85	17.07
+S	34.8	16.8	1.80	4.66	697.5	38.29	−12.55	16.09
	**	n.s.	*	**	**	n.s.	n.s.	*
Interaction E × S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Sr = *Sorrentino*; LC = *Lingua di Cane*; +S = sulphur fertilization; −S = control; n.s., not significant.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

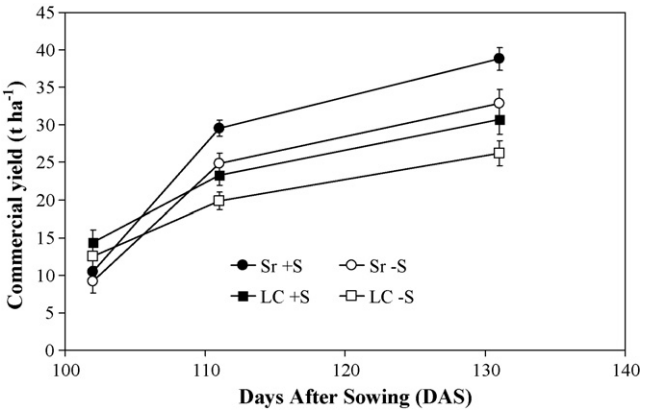

Fig. 1. Cumulated marketable yield of two *friariello* ecotypes (LC = *Lingua di Cane*; Sr = *Sorrentino*) in response to sulphur fertilization (+S = sulphur fertilization; −S = control). Values are means ± standard errors.

(yellowness) and chlorophyll concentration in *Lingua di Cane* leaves (Table 1).

Sulphur fertilization enhanced chlorophyll and TSS contents in both genotypes (Table 1). Moreover, it caused a significant reduction of leaf nitrates, whereas leaf dry matter percentage was not affected by the sulphur treatment (Table 1). Although leaf nitrate contents were much lower than those reported for other leafy vegetables (Santamaria, 2002), we have found a significantly higher nitrate concentration in *Lingua di Cane* respect to *Sorrentino* leaves, indicating a remarkable genetic variability for this trait. In addition, TSS and dry matter contents were significantly lower in *Lingua di Cane* compared to *Sorrentino* leaves (Table 1). Overall, for these parameters, the ecotype *Lingua di Cane* revealed a poorer quality profile respect to *Sorrentino*.

3.2. Polyphenols, flavonols and glucosinolates

The total phenols content was 118 mg gallic acid 100 g^{-1} of fresh weight in *Sorrentino*, whereas it was slightly reduced in *Lingua di Cane* (90 mg gallic acid 100 g^{-1} of fresh weight) (Table 2). Sulphur fertilization increased the average phenols content from 96 to 111 mg gallic acid 100 g^{-1} of fresh weight (Table 2). Consistently, the antioxidant activity of the water–methanol extract was higher in *Sorrentino* (0.72 mmol trolox 100 g^{-1} of fresh weight) relative to *Lingua di Cane* (0.61 mmol trolox 100 g^{-1} of fresh weight). In addition, the antioxidant activity increased of 20% when sulphur was supplied to the plants (Table 2).

Total flavonols contents for the two ecotypes were 43.6 mg 100 g^{-1} of fresh weight and 21.2 mg 100 g^{-1} of fresh weight in *Sorrentino* and in *Lingua di Cane*, respectively. The most representative flavonols in *friariello* extracts were kaempferol and quercetin derivates, while myricetin was present only in trace amount (Table 3). Flavonols were much higher in *Sorrentino* plants than in *Lingua di Cane*, but unlike the total phenols, they were reduced rather than increased by sulphur fertilization in both ecotypes (Table 3). The phenolic acids were mostly represented by chlorogenic, ferulic and sinapic

Table 2
Total phenolic content [expressed as mg of gallic acid per 100 g of fresh weight (FW)] and antioxidant activity of phenolic compounds [expressed as mmol of trolox 100 g^{-1} of fresh weight basis (FW)] of *friariello* in response to different treatments

Treatment	Total phenolic content	Antioxidant activity
Ecotypes (E)		
Sr	118.3	0.72
LC	89.6	0.61
	**	**
Sulphur (S)		
−S	96.5	0.61
+S	111.4	0.72
	*	**
Interaction E × S	n.s.	

Sr = *Sorrentino*; LC = *Lingua di Cane*; +S = sulphur fertilization; −S = control; n.s., not significant.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

acids (Table 3). The phenolic acids content confirmed that *Sorrentino* was richer in these compounds respect to *Lingua di Cane* (Tables 2 and 3). For this parameter, we have found a significant interaction between ecotype and sulphur fertilization (Fig. 2). In absence of sulphur fertilization (−S), the amount of total phenolic acids in *Sorrentino* was 150% higher than *Lingua di Cane* plants. Nevertheless, this difference was reduced to only 20% upon sulphur fertilization, which increased the phenolic acids content much more in *Lingua di Cane* than *Sorrentino* (Fig. 2). This was mainly due to an increase of ferulic and sinapic acid levels (Table 2).

The concentration of total glucosinolates in *Lingua di Cane* was 31 μmol g^{-1} of dry weight (Table 3), while in *Sorrentino* we measured a significantly lower amount (20 μmol g^{-1} of dry weight). In our experimental conditions, we did not observe a significant effect of sulphur on glucosinolates accumulation (Table 3). This result was confirmed also in terms of single glucosinolates (aliphatic, aromatic and indole) (data not shown).

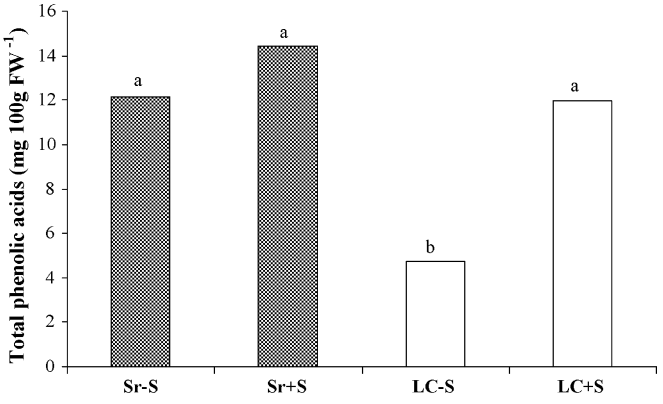

Fig. 2. Total phenolic acids content on fresh weight basis (FW) of *friariello* in response to sulphur fertilization. Within each ecotype, different letters indicate significant difference at $P \leq 0.05$ (Sr = *Sorrentino*; LC = *Lingua di Cane*; +S = sulphur fertilization; −S = control).

Table 3
Flavonols (splitted into quercetin, kaempferol and total) and phenolic acids (splitted into chlorogenic, ferulic, sinapic and total) contents on fresh weight (FW) basis and total glucosinolates content on dry weight (DW) basis of *friariello* in response to different treatments

Treatment	Flavonols			Phenolic acids				Total (mg 100 g FW^{-1})	Total glucosinolates (μmol g DW^{-1})
	Quercetin (mg 100 g FW^{-1})	Kaempferol (mg 100 g FW^{-1})	Total (mg 100 g FW^{-1})	Chlorogenic (mg 100 g FW^{-1})	Ferulic (mg 100 g FW^{-1})	Sinapic (mg 100 g FW^{-1})			
Ecotypes (E)									
Sr	10.2	33.4	43.6	8.4	1.3	3.6		13.3	20.9
LC	3.0	18.2	21.2	3.6	2.6	2.1		8.3	31.4
	**	**	**	**	*	*		**	**
Sulphur (S)									
−S	8.1	28.9	36.9	4.6	1.4	2.4		8.4	25.6
+S	5.1	22.7	27.9	7.4	2.5	3.3		13.2	26.6
	*	*	**	**	*	*		**	n.s.
Interaction E × S	n.s.	n.s.	n.s.	n.s.	*	*		*	n.s.

Sr = *Sorrentino*; LC = *Lingua di Cane*; +S = sulphur fertilization; −S = control; n.s., not significant.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.

4. Discussion

4.1. Beneficial effects of sulphur on yield and leaf nitrate levels

Broccoli represents a rich source of bioactive compounds with a high health potential. In this study, we assessed how sulphur fertilization may affect the quality of two local Italian ecotypes of *friariello* (*B. rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) in terms of flavonols, phenolic acids, glucosinolates, antioxidant activity and nitrate contents. Sulphur fertilization improved the marketable yield of *friariello* (+18%). It is known that sulphur is critical for many physiological functions, including photosynthesis (Marschner, 1995). Sulphur deficiency may negatively affect chloroplast formation and it may eventually lead to chloroplasts degradation (Von Uexküll, 1986). Consistent with this possibility, we measured in −S plants a general reduction in chlorophyll content, sugar concentration and growth rate, all of which reflect an impaired photosynthetic activity (Table 1). In spite of genotypic differences associated with nitrate accumulation (Table 1), the leaf nitrate contents found in this experiment were well below the limits imposed by EU regulations for other leafy vegetables (Santamaria, 2002). In this respect, sulphur fertilization had a positive effect by counteracting leaf nitrate accumulation, also (Table 1). As suggested by other authors (Marschner, 1995), sulphur enhances the incorporation of N into organic compounds and consequently it reduces the leaf nitrate concentration. Alternatively, S-containing fertilizers may either promote nitrate reduction or increase nitrogen use efficiency (Srivastava, 1980). Similar results have previously been reported for cabbage by He et al. (1994), who showed that sulphur fertilization may decrease the leaf nitrate content while increasing, at the same time, the concentration of specific aminoacids. Consistently, S deficiency has been reported to enhance the concentration of non-S-containing soluble amino acids (e.g. asparagine, glutamine and arginine), which in turn may promote N-nitrate accumulation in plant tissues (Hilal et al., 1990). In contrast, sulphur availability prevents the accumulation of non-S amino acids. This generally depresses the activity of nitrate reductases and simultaneously increases the content of the S-rich protein ferredoxin, which is involved in nitrate reduction (Srivastava, 1980).

4.2. Sulphur fertilization and accumulation of functional metabolites

Regarding the polyphenol contents, our results (104 mg gallic acid 100 g^{-1} of fresh weight as an average of the two ecotypes) are in agreement with those reported for broccoli by Chu et al. (2002), who found approximately 100 mg gallic acid 100 g^{-1} of fresh weight. Interestingly, the values of antioxidant activity measured in our *friariello* ecotypes were much higher compared to those reported by Pellegrini et al. (2003) for broccoli (0.30 mmol trolox 100 g^{-1} of fresh weight), suggesting that *friariello* may have better nutritional qualities than the conventional broccoli (Fahey and Stephenson, 1999).

Flavonols, which represent the most important fraction of the phenolic moiety, were mostly represented by kaempferol and quercetin derivates, as also observed in broccoli by Price et al. (1998), and they were reduced by sulphur fertilization (Table 3). This response indicates that the total phenol pool may be shifted towards the accumulation of different compounds based on precursors availability, enzymes activators (or co-factors) and/or other effectors, such as sulphur availability. This aspect should be considered when agricultural practices are specifically targeted to the accumulation of functional metabolites in different vegetables. The total flavonols content found in both ecotypes was higher (43.6 mg 100 g^{-1} of fresh weight in *Sorrentino* and 21.2 mg 100 g^{-1} of fresh weight in *Lingua di Cane*) than those reported for broccoli by Hertog et al. (1993) and Price et al. (1998), who indicated flavonols contents of 13.7 mg 100 g^{-1} of fresh weight (quercetin and kaempferol aglycones) and 10.2 mg 100 g^{-1} of fresh weight, respectively. Concentrations of 3.6 mg 100 g^{-1} of fresh weight have also been documented by Hermann (1976) in broccoli. Interestingly, a wide range of variability with respect to both harvest date and sulphur availability were reported by Vallejo et al. (2003a) for eight different broccoli cultivars, suggesting that multiple genetic and environmental determinants may affect this parameter.

The two cultivars also presented significant differences in terms of constitutive phenolic acids content (higher in *Sorrentino* compared to *Lingua di Cane*) and induced phenolic acids accumulation in response to sulphur fertilization (Table 3 and Fig. 2). A great variability of phenolic acids levels respect to cultivars and different agronomic techniques has also been reported by Vallejo et al. (2003a), who indicated a range of 23–151 mg kg^{-1} of fresh weight for caffeoyl-quinic acid derivates and of 57–173 mg kg^{-1} of fresh weight for bulked sinapic and ferulic derivates. Clifford (1999) also reported for broccoli values of 60 mg 1000 g^{-1} of fresh weight of chlorogenic acid plus 20 mg kg^{-1} sugar esters. This indicates a quite strong diversity, within the same species, in terms of metabolic responses to environmental factors and cultural practices.

The amount of glucosinolates found in both cultivars were similar to those reported for broccoli (20 μmol g^{-1} of dry weight) by Rodriguez and Rosa (1999) and Vallejo et al. (2003b), who found in three broccoli varieties (Marathon, Monterrey and Vencedor) total glucosinolates contents between 20 and 56 μmol g^{-1} of dry weight. The pattern of glucosinolates accumulation in response to sulphur fertilization was consistent with the results of Vallejo et al. (2003a), who compared the response of three broccoli cultivars to 15 and 150 kg ha^{-1} of calcium sulphate (13% S) fertilization. An increase of the total glucosinolates level in response to sulphur availability has also been documented in turnip rape (*B. rapa* L.) (Kim et al., 2001) and kale (*Brassica oleracea* L. Acephala Group) (Kopsell and Kopsell, 2003). Clearly, the natural/constitutive soil sulphur content should be considered in order to compare different experiments since this may be sufficiently high to saturate plant requirements for glucosinolates biosynthesis and to hidden any effect of additional sulphur fertilization.

Overall, the response to sulphur fertilization in terms of leaf nitrates, phenolic compounds accumulation and antioxidant activity suggests that there is a good margin for improving the nutritional value of *friariello* using proper agronomic practices. However, these practices should be associated to the selection of suitable genotypes since genetic variability may play a primary role in determining the amount of functional metabolites, such as flavonols, phenolic acids and glucosinolates.

References

Aires, A., Rosa, E., Carvalho, R., 2006. Effect of nitrogen and sulfur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). J. Sci. Food Agric. 86, 1512–1516.

Chu, Y.F., Sun, J., Wu, X., Liu, R.H., 2002. Antioxidant and antiproliferative activities of common vegetables. J. Agric. Food Chem. 50, 6910–6916.

Ciska, E., Martyniak-Przbyszewska, B., Kozlowsja, H., 2000. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J. Agric. Food Chem. 48, 2862–2867.

Clifford, M.N., 1999. Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. J. Sci. Food Agric. 79, 362–372.

Crozier, A., Lean, M.E.J., Mc Donald, M.S., Black, C., 1997. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. J. Agric. Food Chem. 45, 590–595.

Fahey, J.W., Zhang, Y., Talalay, P., 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Nat. Acad. Sci. U.S.A. 94, 10367–10372.

Fahey, J.W., Stephenson, K.K., 1999. Cancer chemoprotective effects of cruciferous vegetables. Hort. Sci. 34, 1159–1163.

Faulkner, K., Mithen, R., Williamson, G., 1998. Selective increase of the potential anticarcinogen 4-methylsulfphinylbutyl glucosinolate in broccoli. Carcinogenesis 19, 605–609.

Garcia-Closas, R., Gonzales, C., Aguda, A., Riboli, E., 1999. Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. Cancer Causes Control 10, 71–75.

He, T., He, C., Härdter, R., 1994. The effects of K and Mg fertilizers applied to cabbage on yield, quality and economic returns. Potash Review, Subject 9, 7th Suite, No. 2.

Hecht, S.S., Carmella, S.G., Kenney, P.M.J., Low, S.H., Arakawa, K., Yu, M.C., 2004. Effects of cruciferous vegetable consumption on urinary metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in Singapore Chinese. Cancer Epidemiol. Biomarkers Prev. 13, 997–1004.

Hermann, K., 1976. Flavonols and navones in food plants: a review. J. Food Technol. 11, 433–448.

Hertog, M.G.L., Feskens, E.J.M., Hollman, P.C.H., Katan, M.B., Kromhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Eldery Study. Lancet 342, 1007–1011.

Hilal, M.H., Abdel-Fattah, A., Korkor, S.A., 1990. Effect of fine and granular sulphur application on root depth and yield of Lupinus in sandy soils. In: Proceedings of the Middle East Sulphur Symposium, Cairo, 12–16 February, pp. 207–216.

Hopia, A.I., Heinonen, M., 1999. Comparison of antioxidant activity of flavonoid aglycones and their glycosides in methyl linoleate. J. Am. Oil Chem. Soc. 76, 139–144.

Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1, c2. Biochem. Phisiol. Pflanzen. 18, 167–172.

Justesen, U., Knuthsen, P., Leth, T., 1998. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high performance liquid chromatography with photodiode array and mass spectrometric detection. J. Chromatogr. 799, 101–110.

Kiddle, G., Bennett, R.N., Botting, N.P., Davidson, N.E., Robertson, A.A.B., Wallsgrove, R.M., 2001. High-performance liquid chromatographic separation of natural and synthetic desulphoglucosinolates and their chemical

validation by UV, NMR and chemical ionisation-MS methods. Phytochem. Anal. 12, 226–242.

Kim, S.J., Matsuo, T., Watanabe, M., Watanabe, Y., 2001. Effect of nitrogen and sulphur application on the glucosinolate content in vegetable turnip rape (*Brassica rapa* L.). J. Plant Nutr. Soil Sci. 48, 43–49.

Kopsell, D.E., Kopsell, D.A., 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. J. Agric. Food Chem. 51, 5319–5325.

Marschner, H., 1995. Functions of mineral nutrients: Macronutrients: sulphur. In: Mineral Nutrition of Higher Plant, second ed. Academic Press, Cambridge, UK, pp. 255–265.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C., 1999. Screening of dietary carotenoids and carotenoid-rich-fruit extracts for antioxidant activities applying 2,2′-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid radical cation decoloration assay. Methods Enzymol. 299, 379–384.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., Brighenti, F., 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. Nutr. Requir., 2812–2819.

Price, K.R., Casuscelli, F., Colquhoun, I.J., Rhodes, M.J.C., 1998. Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. J. Sci. Food Agric. 77, 468–472.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152–159.

Rodriguez, A.S., Rosa, E.A.S., 1999. Effect of post-harvest treatments on the level of glucosinolates in broccoli. J. Sci. Food Agric. 79, 1028–1032.

Rosa, E.A., Heaney, R.K., Portas, C.A., Roger, G., 1996. Changes in glucosinolate concentrations in Brassica crops (*B. Oleracea* and *B. napus*) throughout growing seasons. J. Sci. Food Agric. 71, 237–244.

Sah, R.N., 1994. Nitrate-nitrogen determination—a critical review. Comm. Soil Sci. Plant Anal. 25, 2841–2869.

Santamaria, P., 2002. Breve storia della pericolosità del nitrato. Colture Protette 31, 4–6.

Singleton, V.L., Rossi, V.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Srivastava, H.S., 1980. Regulation of nitrate reductase activity in higher plants. Phytochemistry 19, 725–733.

Tomas-Barberan, F.A., Espīn, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric. 81, 853–876.

Vallejo, F., Tomas-Barberan, F.A., Garcia-Viguera, C., 2003a. Effect of climatic and sulphur fertilisation conditions, on phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars. Eur. Food Res. Technol. 216, 395–401.

Vallejo, F., Garcia-Viguera, C., Tomas-Barberan, F.A., 2003b. Changes in broccoli (*Brassica oleracea* L. var. *italica*) health-promoting compounds with inflorescence development. J. Agric. Food Chem. 51, 3776–3782.

Von Uexküll, H.R., 1986. Sulphur interactions with other plant nutrients. In: Sulphur in Agricultural Soils. Proceedings of the International Symposium, Dhaka, April 20–22, pp. 212–242.

Willett, W.C., 2002. Balancing life-style and genomics research for disease prevention. Science 296, 695–698.

Williamson, G., Faulkner, K., Plumb, G.W., 1998. Glucosinolates and phenolics as antioxidants from plant foods. Eur. J. Cancer Prev. 7, 17–21.

Zhang, Y., Kenski, T.W., Cho, C.G., Posner, G.S., Talalay, P., 1994. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc. Nat. Acad. Sci. U.S.A. 91, 2147–2150.

Zhao, F., Evans, E., Bilsborrow, P., Syers, J.K., 1994. Influence of nitrogen and sulphur on the glucosinolate profile of rapeseed (*Brassica napus* L.). J. Sci. Food Agric. 64, 295–304.