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Effect of sulphuric fertilization on the shelf life of Broccoli packaged in protective atmosphere

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Abstract: Broccoli (*Brassica rapa* L.) of *Lingua di cane* ecotype were cultivated in presence and in absence of a sulphuric fertilization. After being minimally processed, samples were packaged in protective atmosphere (air and 100 % O_2), using a semi permeable film. Aim of the present work was to verify the effectiveness of the packaging in protective atmosphere on broccoli shelf life, storing the samples at 4±1°C until 10 days. The influence of the presence and the absence of the sulphuric fertilization on leaves samples quality, in terms of nitrate and chlorophyll contents, colour, pH, TSS, and dry matter were assessed. Moreover, the attention was turned on the different fertilization influence on organic volatile compounds that, present in the headspace of the packages, determine the characteristic odour of the product, noticed in particular after the cooking phase.

Key words: chlorophyll, glucosinolates, GC/MS, nitrates, organic volatile compounds, shelf life, sulphuric fertilization

تأثير التسميد الكبريتي على الفترة التخزينية Broccoli المعبأ في وسط جوى وقائي

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الملخص :تم زراعة Broccoli من نوع (*Lingua di cane*) باستخدام التسميد الكبريتي وبدونه. وبعد معالجته معاملة محدودة عبأ في عبوات لها وسط جوي واقي (الهواء و١٠٠ ٪ أوكسجين) باستخدام فيلم شبه نفاذ. الهدف من هذه الدراسة التحقق من فعالية التعبئة والتغليف في وسط جوي واقي على الفترة التخزينية Broccoli. تم تخزين العينات علي درجة حرارة ٤±١م لمدة ١٠ أيام. تم تقييم تأثير التسميد الكبريتي على جودة الأوراق بتقدير محتواها من النترات والكلوروفيل، واللون، ودرجة الحموضة، المواد الصلبة الكلية والمادة الجافة. بالإضافة إلى دراسة تأثير طرق التسميد المختلفة على المركبات العضوية المتطايرة المتواجدة في الفراغ القمى للعبوات، وتحديد الرائحة المميزة للمنتج الملاحظة خاصة بعد مرحلة الطبخ.

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Introduction

In the last years the food tendencies are strongly changed and are evolved towards the demand, from the consumer, of fresh foods with low calorie, healthy, nourishing and with high quality. All of this, together with the reduction of the times dedicated to the meals use, has moved the interest of the consumers toward products already washed, cut and ready to use: the minimally processed products. Processing technique, sanitation, packaging and temperature management during shipping, handling and marketing play important roles in maintenance of fresh cut quality (Brecht et al., 2003). Often, the physiological behaviour of the cut vegetable, such as the physical characteristics of the plastic film employed for the packaging, are not known adequately, with consequent production of commodities with low qualitative level during distribution.

The respiration of the horticultural products fresh or preserved in protective atmosphere, the phenomena of transpiration, responsible of the wrinkling of the product, as well as the alterations due to microbial species, can compete to a reduction of the product shelf life. Moreover, the manipulations to which minimally processed vegetables are submitted to, can provoke stresses. The operations of cut, in particular, induce an increase of the ethylene and carbonic dioxide production, with a consequent reduction of the potential shelf life.

The application of modified atmosphere packaging (M.A.P.) on such products, allows to extend the conservation and therefore their shelf life, by inhibiting or slowing down those chemical and biological trials that determine the deterioration of the product (Piergiovanni, 1997), such as production of off-flavours. Different studies (Powrie et al., 1990; Hansen et al., 2001) tried to find the more suitable atmospheric parameters that allow a prolonged storage of broccoli, with the respect of their qualitative characteristics. In particular a concentration of O_2 between 1-3% and of CO_2 between 5-15% at 1°C, was found to be capable of prolonging the shelf life of broccoli until 21 days (Jacobsson et al., 2004). Broccoli result to be particularly sensitive to M.A.P., creating, in anaerobic conditions, sulphur-containing volatiles, including methanethiol and dimethyl disulfide, which could influence the sensorial characteristics of fresh cut florets (Forney et al., 1991).

Also glucosinolate degradation products (isothiocyanates, nitriles, thiocyanates, indoles and oxazolidinethiones) contribute to the characteristic flavour and taste of *Brassica* vegetables. A significant variation in the level of glucosinolates (Rosa et al., 1994; Rosa 1997) and phenolic compounds biosynthesized is due to variations in environmental and agronomic factors, such as water availability (irrigation), soil composition (mineral and organic nutrients), intensity of sulphur fertilization (mainly due to different effects on phenolic enzymes), harvest time and climate (Zhao, 1994).

The 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.

Storage and processing of the vegetables can also greatly affect the glucosinolate content, in fact processes such as chopping, cooking and freezing influence the extent of hydrolysis of glucosinolates and the composition of the final products (Fahey et al., 2001; Baik et al., 2003; Cieślik et al., 2007; Song andThornalley, 2007).

Although little information are reported on the influence of M.A.P. on total or individual glucosinolate content of *Brassica* vegetables, an increase in total glucosinolate content was registered in broccoli florets when stored in air or in controlled atmosphere (C.A.), while the absence of O_2 with a 20% CO_2 resulted in total loss (Verkek et al., 2001; Jones et al., 2006).

Previous study (De Pascale et al., 2007) investigated 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 broccoli. Starting from this study, the aim of the present work was, therefore, to verify the effectiveness of the packaging in protective atmosphere on the shelf life of one of these two ecotype of broccoli, evaluating the possibility to transform this vegetable in a minimally processed product. Particular attention was turned on the evaluation of the volatile compounds that, present in the headspace of the package, determine the characteristic odour of the product. By considering this characteristic (proper of Brassicaceae), the influence of the presence or of the absence of a sulphuric fertilization was then evaluated on the nitrate and chlorophyll contents, colour, pH, TSS, and of dry matter.

Material and Methods

Plant material

The research was performed on broccoli leaves (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) of known cultivation, in particular on a characteristic ecotype widely distributed in the Campania Region (Southern Italy), characterized by a late cycle (> 90-120 days), and named *Lingua di cane* (Figure 1).

Samples of broccoli were obtained from the University of Naples experimental farm, located in Bellizzi (Salerno, Italy). The soil was a deep clay-sandy soil (International Textural Classes), classified as Inceptsoil Hapluset type (USDA soil taxonomy) with 1.65% organic matter, 1.5% total N and 837 ppm total S.

Figure 1. Leaf of *Lingua di cane* ecotype.

Two different samples were analyzed: 1) a control on which neither direct or indirect quantitative sulphur was applied, and on which the nitrogenous fertilization was effected using urea (total N = 200 kg ha^{-1}); 2) a treatment with 10 q ha^{-1} of agricultural sulphur + 5 ammonium sulphate q ha^{-1} (total applied N = 200 kg ha^{-1}, of which 100 from sulphate ammonium and 100 kg ha^{-1} from urea). The sulphur fertilization, the seeding and the four harvests were performed as reported previously (De Pascale et al., 2007).

Sample preparation

After the harvest, the product was manually husked. The stems, the damaged leaves and the yellow inflorescence were rejected. The entire leaves and the selected tops, instead, were carefully washed with tap water, for three times, so to eliminate the particles of ground, and, subsequently, washed for 15 min in a disinfectant solution of sodium hypochlorite 0.012% brought to pH 5 with acetic acid. Then the leaves were put in perforated baskets to drip, dried with air flow at environment temperature and subsequently sizing (50g) in trays, previously disinfected with an ethylic alcohol solution (70% v/v).

The trays (AERPACK® - CCPL S.c.r.l., mod. B5-70), with a volume equal to 1300 cm^3

and P_{O_2} at 23°C – 50% R.H. equal to 0.080 cm^3 atm^{-1} $tray^{-1}$ 24 h^{-1} were welded through a semi permeable polymeric film (mod. F410 ANTIFOG - Coopbox®) with P_{O_2}, at 23°C and 0% R.H., equal to 1.3 cm^3 m^{-2} 24 h^{-1} atm^{-1} and P_{CO_2}, under the same conditions, equal to 4.5 cm^3 m^{-2} 24 h^{-1} atm^{-1}. The permeability to nitrogen, under the reported conditions, resulted inferior to 1 cm^3 m^{-2} 24 h^{-1} atm^{-1} and the permeability to the aqueous vapour, measured at 38°C and in presence of the 98% R.H., was 5.1 g m^{-2} 24 h^{-1}.

After the sizing step, packages were filled with two different atmospheres, in particular air (78% N_2 – 21% O_2 ~ 0.03% CO_2) and 100% O_2 and, then, preserved at 4±1°C for different times (0, 1, 2, 3, 5, 6, 7, 9, 10 days). All the analyses were performed both on the control (no-packaged) and on the packaged product.

Chlorophyll analysis

The procedure for chlorophyll analysis was based on those reported by Jeffrey and Humphrey (1975). Two leaf disks of known leaf area (about 2 cm^2 each) were grinded in about 5 ml of 90% acetone, then transferred to a centrifuge tube and brought to exactly 10 ml with additional 90% acetone. Tubes were shaken and then put in the dark for 10-15 minutes. Samples were centrifuged at 1800-1920 r.p.m., the supernatant were collected for reading in the spectrophotometer (HACH mod. DR/2000) at absorbances of 647, 664 and 750 nm. This last was used to adjust interferences. The total chlorophyll was calculated in according to the followings formulas:

$$A664\ (final) = A664 - A750 \quad [1]$$
$$A647\ (final) = A647 - A750 \quad [2]$$
$$chl\ a = 11.93(A664) - 1.93(A647) \quad [3]$$
$$chl\ b = 20.36(A647) - 5.50(A664) \quad [4]$$
$$chl\ total\ (\mu g\ ml^{-1}) = chl\ a + chl\ b \quad [5]$$

Nitrates analysis

The analysis of the nitrates was affected on samples dried in stove at 60°C. The dry leaves were grinded in a mill (ANALYSENMÜHLE - type A 10) and put in doses of 0.5 g in numbered matrass and filled with 100 ml of distilled water. After agitation for one minute, 50 ml of the content of the matrasses was poured in becker. A spoon of active carbon was added. Subsequently the becker content was filtered twice and picked in graduated cylinders, from which 10 ml were withdrawn and put in cuvettes. A volume of 25 ml of distilled water was added. The reagent Nitraver 5 (Hach, Permachen Reagents) was poured in the cuvettes that were then covered for 5 minutes and, finally, submitted to spectrophotometric analysis (HACH mod. DR/2000) at 500 nm. The results furnished the percentage of nitric nitrogen (N-NO_3) on the dry sample, from which it was possible to calculate the content of nitrates expressed in mg kg^{-1} of fresh product.

Dry matter

The dry matter was determinated after drying the fresh product and the preserved samples in stove at 100°C until steady weight.

Colour

Colour evaluation was performed by means of a colorimeter MINOLTA (Chroma Meter, model CR-300), equipped with three pulsed xenon lamps and a white calibration plate as a standard. The parameters *L**, *a** and *b**, defined according to CIELAB-system (1976), were measured.

Chemical indexes

The measures of pH and total soluble solids (TSS) were carried out on broccoli juice, obtained by centrifugation of leaves for 12 minutes at 12000 r.p.m. (ALC Multispeed Refrigerated Centrifuge, mod. PK 131-R). The pH were determined by means of a Mettler Toledo pHmeter (mod. MP 220), while TTS were determined by using a digital refractometer (ATAGO Palette, mod. PR-32) and expressed as °Brix.

Headspace analysis

Samples were submitted to qualitative gas-chromatographic analysis with the purpose to evidence the volatile compounds that characterize the aromatic profile of broccoli during the storage period. For this analysis a Gas-Chromatograph/Mass-Spectrometer was used (GC/MS).

Volatile analysis was performed with an headspace auto sampler (Agilent mod. 7694,

USA) tied to gas chromatograph (Agilent Technologies, mod. HP 6890, USA) equipped with a 30 m x 0.25 mm ID, film thickness 0.25 µm capillary column (HP-5MS, Agilent Technologies, USA) and a mass spectrometer (Agilent Technologies, mod. HP 5973). Gas carrier was Helium (flow 1.2 ml min^{-1}) and split injector ratio was 1/5. Broccoli leaves (~ 2 g) were put into headspace vial (50 ml) and equilibrated in auto sampler at a temperature of 80°C, then sampling for 1 min before the injection in column. Transfer line temperature was 100°C. Oven temperature was kept at 40°C for 4 min, then to 120°C for 5 min, at 7°C min^{-1}, then from 120°C to 220°C at 10°C min^{-1}, kept hold for 10 min. Injector temperature was 270°C. Mass spectrometer operated in scan mode over mass range from 35 to 350 amu (2 s $scan^{-1}$) at an ionization potential of 70 eV. Mass spectral matches were made by comparison of mass spectra and retention time with those of MS database (NIST 98 e WILEY 275).

Data processing

Data were analyzed (ANOVA and Least Significant Difference test) in order to individualize the effect of factors time, atmosphere and fertilization on quality indexes and to evidence significant differences among samples ($\alpha = 95\%$).

Results and Discussion

At the end of the growing cycle, the yields registered were 30.8 and 26.2 t ha^{-1} for the samples submitted to sulphuric fertilization (+S) and without it (-S), respectively (Table 1). The presence or the absence of a sulphuric fertilization did not provoke differences in the production during the different harvests. The one that mostly contributed to the total yield was the first, in particular with 46.8% (+S) and 47.7% (-S), even if in the firms the last harvest is often not effected because it is not convenient from an economic point of view, or because qualitatively not satisfactory (because of tissues hardness due to the lignification process beginning).

Table 1. Distribution of the production in the different harvests.

Harvest	Fertilization			
	+ S		- S	
	t ha^{-1}	% tot	t ha^{-1}	% tot
1st	14.4	46.8	12.5	47.7
2nd	8.9	28.9	7.3	27.9
3rd	5.3	17.2	4.6	17.6
4th	2.2	7.1	1.8	6.9
Total	30.8	100	26.2	100

Samples growth in presence of the sulphur fertilization (+S) showed a chlorophyll content on average of 25% higher than that of non-fertilized samples (-S). This result is positively correlated with the greater productivity found for the crops submitted to sulphuric treatment. Both the chlorophyll and the nitrates contents in broccoli were similar if packaged in air or in 100% O_2. During the storage, it was observed a continuous loss of chlorophyll between 25 and 30% after six days (Table 2). As it regards the nitrates content, it appeared rather constant (829-837 mg kg FW^{-1}). This could be probably explained with the greater content of chlorophyll that, promoting the photosynthesis, would facilitate the organication of the nitrates.

The effect of the sulphuric fertilization influenced the chlorophyll content, while it did not have effect on the nitrates content. Moreover, the protective atmospheres showed not to have effect on the parameters of *Lingua di cane* broccoli and during the storage there were variations of the chlorophyll content, but not of that of nitrates. The three variables, finally, in any case didn't interact each other in a significant way, as it is possible to observe in Table 2.

In opposition to their substantial constancy during the storage period, the colorimetric parameters showed significant differences in function of the addition of sulphur into the soil. In fact, leaves derived from fertilization without sulphur applied (-S) had low *-a** values (greeness), that indicated a sample more green, and high *+b** values (yellowness), to which corresponded a product more yellow, in comparison to those growth in a soil fertilized with sulphur applied (+S) (Table 3).

Table 2. Chlorophyll and nitrates contents on fresh weight (FW) of broccoli leaves in function of experimental variables.

Variables		Chlorophyll (mg g FW^{-1})	Nitrates (mg kg FW^{-1})
Fertilization (F)	**+ S**	4.73	818
	- S	3.74	781
		**	n.s.
Protective Atmosphere (P.A.)	**100%O_2**	4.15	792
	Air	4.32	807
		n.s.	n.s.
Storage days (D)	**0**	4.89	829
	3	4.09	732
	6	3.74	837
		**	n.s.
Interactions	**F x P.A.**	n.s.	n.s.
	F x D	n.s.	n.s.
	P.A. x D	n.s.	n.s.

+S = sulphur fertilization; -S = control. * Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$; n.s. = not significant.

Evaluating both the storage time and the different suphur treatment on the colorimetric coordinate *a**, it was observed (Figure 2) a differentiation on the behaviour of samples. After the first three days of storage, in fact, broccoli cultivated without added sulphur resulted to be greener. The same behaviour was observed for the colorimetric coordinate *b** (Figure 3). Also in this case, samples differed each other after the third day and the samples (-S) were those that presented a more intense yellow coloration. Values of both colorimetric parameters did not differ significantly if considered in function of the only temporal variable.

The packaging in air, in comparison to that in presence of 100% O_2, determined a greater brightness (*L**) of the broccoli leaves. Moreover, the protective atmosphere resulted to influence the dry matter determining a difference of 0.24% less for samples preserved in air, while during the storage the decrease resulted to be of 1.5%. This parameter, in accord with other works on the brassicaceae and on other minimally processed products (Serrano et al., 2006), increased during the time in all the analyzed samples, from an initial value of 13% for the control, to 14.5% after 9 days of storage (Table 3).

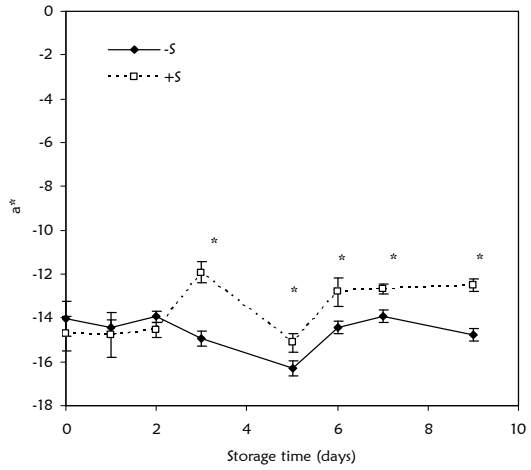

Figure 2. Effects of fertilization on a* values during storage time.
(The asterisks indicate significant differences at p≤0.05*, p≤0.01).**

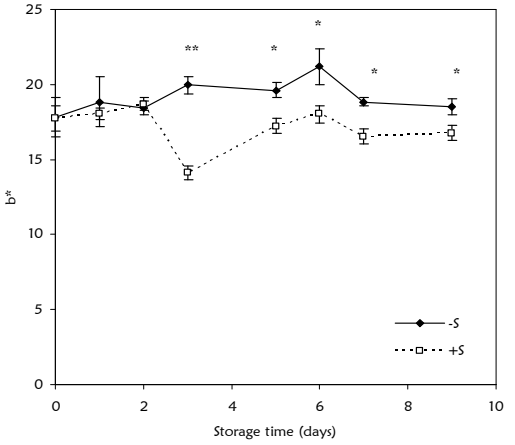

Figure 3. Effects of fertilization on b* values during storage time.
(The asterisks indicate significant differences at p≤0.05*, p≤0.01).**

Table 3. Colour parameters, dry matter (DM) percentage, pH and total soluble solids (TSS) of broccoli leaves in function of the experimental variables.

Variables		Colour			DM (%)	pH	TSS (°Brix)
		L*	a*	b*			
Fertilization (F)	**+ S**	39.63	-13.73	17.15	14.01	5.90	1.57
	- S	40.40	-14.59	19.16	13.90	5.94	1.56
		n.s.	*	*	n.s	n.s.	n.s.
Protective Atmosphere (P.A.)	**100%O_2**	39.47	-14.22	17.86	13.86	5.92	1.57
	Air	40.56	-14.10	18.49	14.10	5.92	1.54
		*	n.s.	n.s.	*	n.s.	n.s.
Storage days (D)	**0**	41.31	-14.37	17.80	13.00	5.67	1.57
	1	40.33	-14.41	18.45	13.57	5.86	1.56
	2	37.96	-13.93	18.59	13.41	6.03	1.51
	3	41.92	-14.93	17.04	14.07	5.95	1.46
	5	37.73	-16.28	18.42	14.24	5.96	1.54
	6	41.96	-14.44	19.61	14.28	6.09	1.55
	7	39.01	-13.91	17.70	14.54	5.93	1.60
	9	39.92	-14.79	17.79	14.54	5.87	1.68
		n.s.	n.s.	n.s.	**	*	*
Interactions	**F x P.A.**	n.s.	n.s.	n.s.	**	*	n.s.
	F x D	n.s.	*	*	**	n.s.	n.s.
	P.A. x D	n.s.	n.s.	n.s.	**	n.s.	n.s.
Coeff. var.		6.52	20.88	22.39	1.48	11.04	20.09

+S = sulphur fertilization; -S = control. * Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$.

Significant level: $p \leq 0.05$*; $p \leq 0.01$**; n.s. = not significant

The interaction "fertilization x days", showed a high significance for the water content of the samples. During the first five days of storage, both (+S) and (–S) samples showed a linear decrease of water content until to lose around the 1%. Subsequently the samples (-S) maintained almost constant this loss until the ninth day, while those (+S) continued to lose water, even if with more slowly. The behaviour of the broccoli (-S) resulted to be analogous to that of plants submitted to stresses, that tried to "safeguard" water through modification of the tissues, in this case was probably due to the not immediate availability of sulphur.

Evaluating the effect "protective atmosphere x days", the samples exhibited, instead, the same decrease at the end of the storage (- 1.5%); it is interesting to notice, however, that those packaged in air exhibited an initial loss faster in the time, resulting on average less rich of water (85.90%) in comparison to those packaged in 100 % O_2 (86.14%) (Figure 4 and 5).

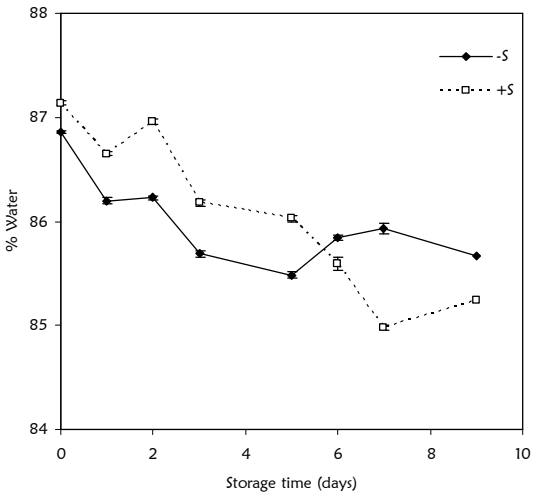

Figure 4. Interaction "fertilization x days" for the water loss.

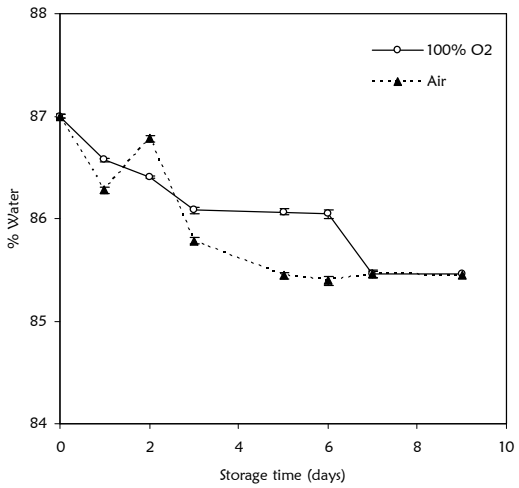

Figure 5. Interaction "protective atmosphere x days" for the water loss.

For all the analyzed samples the variation of pH was only observed as function of the days of storage, showing an increase from the initial value of 5.67 to a maximum peak of 6.09 at the sixth day. In the following days, probably because of the increase of metabolic activity of the contaminating microflora, a light decrease was observed until a pH value of 5.87 at the end of the storage (Figure 6 and Table 3).

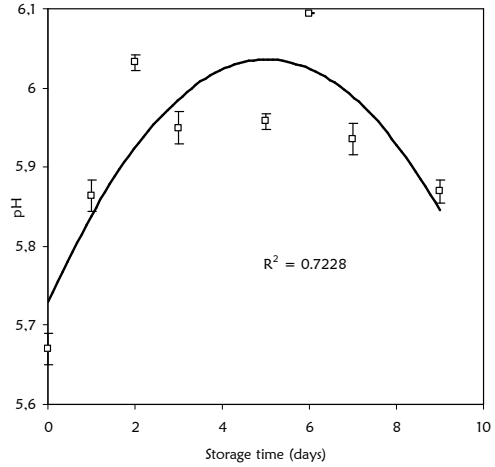

Figure 6. pH values during the storage time.

Also the data related to TSS content revealed significant differences during the storage period. In opposite to what it was found for the pH, the TSS decreased in the first three days from values of around 1.56 to 1.46 and then increased up to 1.68 in the last day of storage (Figure 7 and Table 3). This behaviour could be explained with the respiration of the product in the first days of storage and consequent consumption of sugars, to which a greater microbial proliferation, and therefore their polysaccharidic activity, could follow during the time.

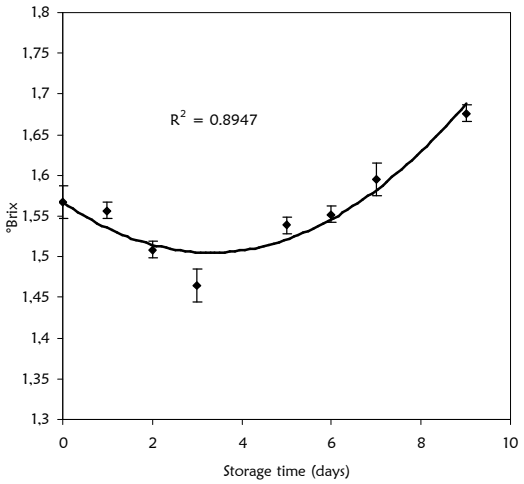

Figure 7. °Brix values during the storage time.

The *Lingua di cane* leaves were, then, submitted to qualitative gas-chromatographic analysis with the purpose to evidence the volatile compounds that characterize the aroma and the odour of broccoli during the storage period. This analysis was carried out only on the samples packaged in air. The volatile organic compounds (COV) extracted by the static headspace of the packages are expressed in concentration % different both during the storage that in the samples fertilized or not with the added of sulphur. The Table 4 reported for each compound the relative percentage area of its own peak in comparison to the total area of all the extract compounds.

Table 4. Evolution of volatile compounds (%) of broccoli packaged in air during the storage.

	Compounds	Storage time (days)							
		1		3		6		10	
		(+ S)	(- S)	(+ S)	(- S)	(+ S)	(- S)	(+ S)	(- S)
1	Metanthiol	100.00	55.66	5.76	3.46	3.30	4.13	2.28	1.44
2	Allyl isothiocyanate	-	-	16.11	1.28	32.89	37.25	2.66	71.49
3	Dimethyl sulfide	-	12.97	7.09	11.15	11.27	9.36	1.85	-
4	1-ciano 4 methyl pentan	-	-	-	-	-	-	2.07	-
5	1-ciano-4-(methylthio)butan O erucin nitrile	-	-	-	10.56	-	2.59	-	-
6	1-ciano-5-(methylthio)pentan	-	1.73	-	66.90	-	17.38	0.61	-
7	terz-butylisothiocyianate	-	14.70	62.95	3.81	51.16	29.29	87.99	26.61
8	1-butyl,4-isothiocyanate	-	14.21	8.09	2.84	1.38	-	2.54	-
9	Dimethyl trisulfide	-	0.73	-	-	-	-	-	0.46

The (-S) samples always showed a greater number of volatile organic compounds characteristic of Brassicaceae in comparison to (+S) samples, with the exception of the tenth day of observation. In particular, in the first day of storage it was possible to note the most greater difference among the two theses: the metanthiol (1) was the only COV quantitatively extracted by the headspace of the broccoli (+S) (Figure 8a), while in the headspace of the samples (-S) appeared also the dimethyl sulfide (3), the 1-ciano-5-(methylthio)pentan (6), the terz-butylisothiocyianate (7), the 1-butyl,4-isothiocyanate (8) and the trimethyl sulfide (9). Among these, the COV mainly present was the metanthiol, while the isothiocyanates (7, 8) resulted to be present in the same percentage (around 14 %); the trimethyl sulfide (9) was present only in traces (Figure 9a). At the third day of storage the headspace of both the typologies of samples became wealthy in characteristic aromatic compounds. In particular the samples (+S) of allyl isothiocyanate (2), dimethyl sulfide (3), terz-butylisothiocyianate (7) and 1-butyl, 4-isothiocyanate (8); while the samples (-S) of the COV 3 (dimethyl sulphide) and of the 1-ciano-4-(methylthio)butan (5). In the samples (+S) the characteristic volatile compound mostly present in the headspace of the packages was the terz-butylisothiocyianate (63%), while in the samples (-S) it was possible to evidence the notable presence of the compound 6 (67%). In both cases the reduction of the metanthiol (1) was noticed. It was interesting to note that from the third to the sixth day of storage for both the theses the qualitative profiles were unchanged, with the only exception of the 1-butyl,4-isothiocyanate (8) that was not found in the sample (-S) anymore. There were, besides, principally for the sample (-S), an enrichment, in the headspace, of dimethyl sulphide (3).

At last day, there was the appearance in the headspace of the samples (+S) of the compound 6 and of the 1-ciano 4 methyl pentan (4): this last was found only in such typology of sample (Figure 8b). In the headspace of the samples (-S), instead, a drastic change of the percentage composition of the COV was observed (Figure 9b). Only four compounds were present (1, 2, 7, 9), among which the principal resulted to be the allyl isothiocyanate (71,49%). The terz-butylisothiocyianate (7) resulted constant in comparison to the sixth day, while the metanthiol (1) and trimethyl sulfide (9) were found only in traces. This last compound (9) was exclusively found in the samples (-S), as the 1-ciano 4 methyl pentan (4).

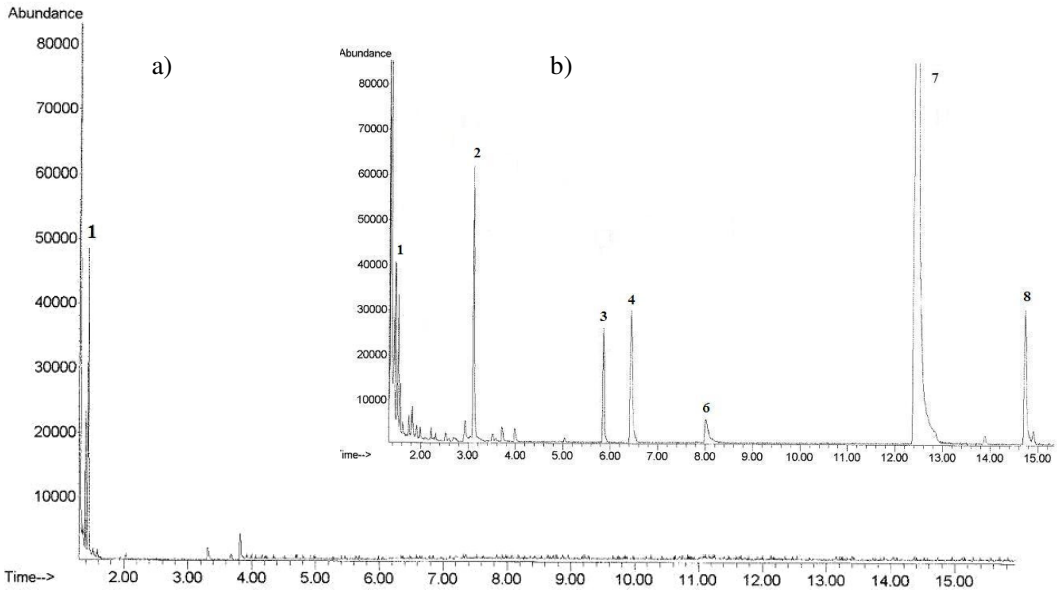

Figure 8 (a-b). (a-b). Chromatogramm of (+S) broccoli after one (a) and ten (b) days of storage.

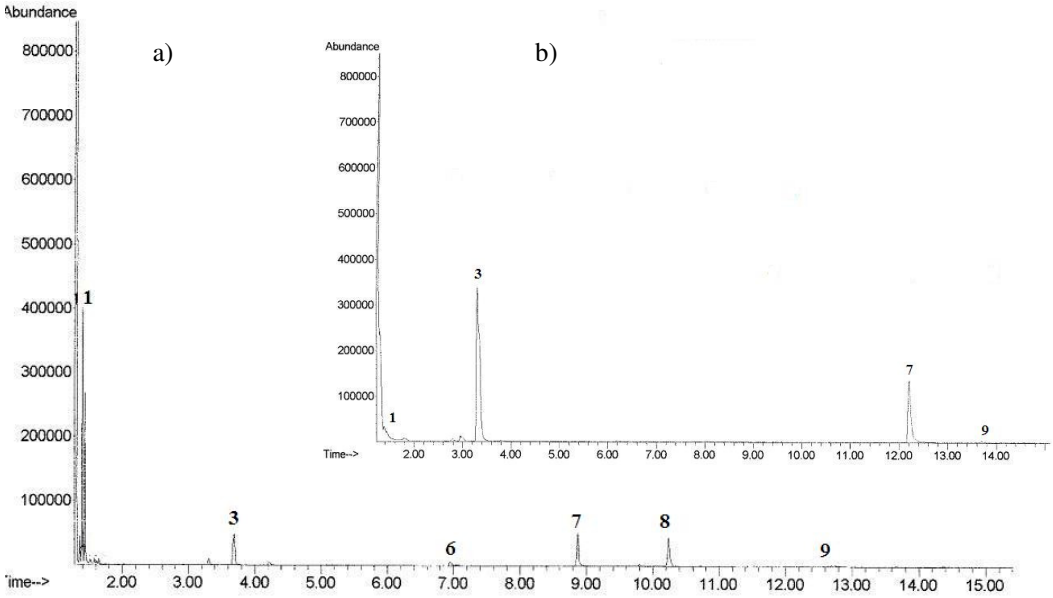

Figure 9 (a-b). (a-b). Chromatogramm of (-S) broccoli after one (a) and ten (b) days of storage.

With regard to total glucosinolates, Vallejo et al. (2003) reported that there were no significant differences between the highest value with poor fertilization (66 µmol g^{-1} in the second development stage) and the highest value with the rich one (64 µmol g^{-1} in the third development stage), both of them found in the cultivar of broccoli Monterrey. During the normal development of a cruciferous plant, volatile hydrolysis products are constantly released at low concentrations, probably as a result of damage and cell death (Cole, 1980). This could explain the decrease of glucosinolate concentration found in work of Vallejo et al. (2003) in proportion to broccoli inflorescence development. On the other hand, total glucosinolate concentrations showed no significant differences between poor (15 kg ha^{-1}) and rich (150 kg ha^{-1}) sulphur fertilization, in contrast to the results reported in Brassicaceae (Marschner, 1995). Our research resulted to be in accord with this last author, in fact, at the end of storage, in headspace of samples growth in soil fertilized with sulphur, there was a

greater percentage concentration of isothiocyanates, products of the hydrolysis of glucosinolates. This result could be explained because of the sulphur enters in the biosynthesis of sulphured aminoacids, that are precursors of glucosinolates.

Conclusions

From an agronomic point of view, the applied sulphur in the suitable quantity provoked a productive advantage on average of 18%. Nevertheless, it necessary to appraise the productive answer to inferior doses and the economic convenience of such treatment. However a positive "physiological" effect must be signalled tied up to the chlorophyll increase of 35% with the sulphuric fertilization. From a technological point of view, comparing two different way of packaging adopted for the ecotype *Lingua di cane* (air and 100% O_2), the results of the instrumental analyses did not shown significant differences during the storage.

Gas-chromatographic analysis, effected on the static headspace of the broccoli, revealed the presence of isothiocyanates, nitriles, thiocyanates and sulphurs. The evolution of the organic volatiles compounds over the time suggests differences among the samples due to the raw material. In fact, the fertilization with the added of sulphur provoked, at the end of storage, the presence of a great percentage of isothiocyanates, a product of the hydrolysis of glucosinolates, characteristics compounds of broccoli.

References

Aires, A., E. Rosa and R. Carvalho. 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.

Baik, H. Y., J. A. Juvik, E. H. Jeffery, M. A. Wallig, M. Kushad and B. P. Klein. 2003. Relating glucosinolate content and flavour of broccoli cultivars. J. Food Sci. 68:1043-1050.

Brecht, J. K., K. V. Chau, S. C. Fonseca, F. A. R. Oliveira, F. M. Silva, M. C. N. Nunes and R. J. Bender. 2003. Maintaining optimal atmosphere conditions for fruits and vegetables throughout the post harvest handling chain. Post. Bio. Tech. 27:87-101.

Cieślik, E., T. Leszczyńska, A. Filipiak-Florkiewicz, E. Sikora and P. M. Pisulewski. 2007. Effects of some technological processes on glucosinolate contents in cruciferous vegetables. Food Chem. 105:976–981.

Ciska, E., B. Martyniak-Przbyszewska and H. Kozlowsja. 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.

Cole, R. A. 1980. Volatile components produced during ontogeny of some cultivated crucifers. J. Sci. Food Agric. 31:549-557.

De Pascale, S., A. Maggio, R. Pernice, V. Fogliano and G. Barbieri. 2007. Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. Eur. J. Agron. 26:418-424.

Fahey, J. W., A. T. Zalcmann and P. Talalay. 2001. Review: The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5-51.

Forney, C. F., J. P. Mattheis and R. K. Austin. 1991. Volatile compounds produced by broccoli under anaerobic conditions. J. Agric. Food Chem. 39:2257–2259.

Hansen, M. E., H. Sørensen and M. Cantwell. 2001. Changes in acetaldehyde and aminoacid concentrations in broccoli florets during air and CA storage. Post. Biol. Tech. 22:227-237.

Jacobsson, A., T. Nielsen and I. Sjoholm. 2004. Influence of temperature, modified atmosphere packaging, and heat treatment

on aroma compounds in broccoli. J. Agric. Food Chem. 52:1607–1614.

Jeffrey, S. W. and G. R. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen Bd. 18:167-172.

Jones, R. B., J. D. Faragher and S. Winkler. 2006. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. Post. Bio. Tech. 41:1–8.

Marschner, H. 1995. Functions of mineral nutrients: Macronutrients: Sulphur. In Mineral nutrition of higher plant. 2nd ed.; Academic Press: Cambridge pp.255-265.

Piergiovanni, L. 1997. Shelf-life dei prodotti alimentari confezionati con l'impiego di gas. Rassegna dell'Imballaggio, XVIII (12):6-13.

Powrie, W. D., R. Chiu and H. Wu. 1990. Preservation of cut and segmented fresh fruit pieces. U.S. Patent Number 4:729-895.

Rosa, E. A. S. R. K. Heaney, F. C. Rego and G. R. Fenwick. 1994. The variation of glucosinolate concentration during a single day in young plants of *Brassica oleracea* var. acephala and capitata. J. Sci. Food Agric. 66:457-463.

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

Rosa, E. A. S. 1997. Daily variation in glucosinolate concentrations in the leaves and roots of cabbage seedlings in two constant temperature regimes. J. Sci. Food Agric. 73:364-368.

Serrano, M., D. Martinez-Romero, F. Guillèn, S. Castello and D. Valero. 2006. Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Post. Bio. and Tech. 39:61–68.

Song, L. and P. J. Thornalley. 2007. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. Food and Chem. Toxicol. 45:216–224.

Vallejo, F., C. Garciäa-Viguera and F. A. Tomaäs-Barberaän, 2003. Changes in Broccoli (*Brassica oleracea* L. Var. italica) Health-promoting compounds with inflorescence development. J. Agric. Food Chem. 51:3776-3782.

Verkerk, R., M. Dekker and W. M. F. Jongen. 2001. Postharvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. J. Sci. Food Agric. 81:953-958.

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