C. Migliori[1],
L.F. Di Cesare[1],
R. Lo Scalzo[1],
V. Ferrari[2]

Aromatic-nutraceutical profiles of stored "Romanesco" and "Violetto di Catania" cauliflowers

Progress in Nutrition
Vol. 13, N. 2, 104-110, 2011

Titolo
Profili aromatici e nutraceutici di cavolfiore "Romanesco" e "Violetto" di Catania" dopo frigoconservazione

Key words
Volatile substances, sulforaphane, GC/MS analysis, vitamin C, total anthocyanins, HPLC analysis, refrigerated storage

Parole chiave
Sostanze volatili, sulforafane, analisi GC/MS, vitamina C, antocianine totali, analisi HPLC, frigoconservazione

[1] CRA-IAA Unità di Ricerca per i Processi dell'Industria Agro-Alimentare, Milano
[2] C.R.A. – ORA Unità di ricerca per l'Orticoltura di Monsampolo del Tronto, Monsampolo del Tronto (AP)

Indirizzo per la corrispondenza:
Dr. Luigi Francesco Di Cesare,
CRA-IAA Unità di Ricerca per i Processi dell'Industria Agro-Alimentare,
Via Venezian, 26 - 20133 Milano
Tel. 02/239557215
E-mail: luigi.dicesare@entecra.it

Summary
Post-harvest aromatic-nutraceutical profiles by the measure of the content of volatile substances, sulforaphane or 4-methylsulphinylbutyl NCS, vitamin C (ascorbic+ dehydroascorbic acid) and total anthocyanins were determined in "Romanesco" and "Violetto di Catania" cauliflowers (*Brassica oleracea L.* var. *botrytis*) stored at +4°C for 7-14 days. Total volatile characteristic substances after 7 days of storage were similar to the starting samples for both cauliflowers, but they decreased after 14 days. In both cauliflowers, sulforaphane was almost constant after 7 days; while then it decreased. Ascorbic acid already decreased after 7 days of storage and was constant after 14 days in both vegetables. In "Violetto di Catania", the decrease of ascorbic acid was balanced by the presence of dehydroascorbic acid, that, despite having an hydroxyl group oxidized, is still biologically active as antioxidant molecule. Anthocyanins in "Violetto di Catania" increased after the first week of storage and then decreased after 14 days.

Riassunto
È stata studiata l'influenza della conservazione a +4°C per 7-15 giorni sul profilo nutraceutico-aromatico del cavolfiore (*Brassica oleracea L.* var. *botrytis*) "Romanesco" e "Violetto di Catania", mediante la determinazione del contenuto in sostanze volatili, sulforafane o 4-metilsulfinilbutil NCS, vitamina C (acido ascorbico + deidroascorbico) ed antociani totali. Le sostanze volatili caratteristiche totali si mantenevano simili al prodotto fresco dopo 7 giorni di conservazione in entrambe le cultivar, mentre diminuivano dopo 14 giorni. Il sulforafane mostrava un andamento simile. L'acido ascorbico diminuiva già dopo una settimana per poi mantenersi costante dopo 14 giorni per entrambe le cultivar. Nel "Violetto di Catania" la perdita in acido ascorbico era bilanciata dalla presenza di acido deidroascorbico che, nonostante abbia un gruppo ossidrilico ossidato, presenta ancora attività biologica come antiossidante. Le antocianine nel "Violetto di Catania" aumentavano nella prima settimana di conservazione per poi diminuire durante la seconda.

Introduction

Cabbage, Brussels sprouts, broccoli, cauliflower etc, are characterized by some high biological activity compounds such as glucosinolates (1). Their content varies among cultivars and plant parts (2-4), cultivation practices (5) and post-harvest storage (6). Isothiocyanates, breakdown products from glucosinolates by myrosinase (β-thioglucoside glucohydrolase; EC 3,2. 3-1), seem to reduce cancer risk in lung, oesophagus, fore stomach, colon, mammary glands and pancreas (7). Sulforaphane, or 4-methylsulfinylbutyl NCS, is the principal and exceedingly potent inhibitor of these diseases (7-10). Isothiocyanates, as well as sulforaphane, opposite to the glucosinolates, are instable at high temperature and their breakdown products are sulphides, nitriles and thiocyanates, that characterize the flavour of all cooked *Brassicaceae* (11).

Previous studies performed about the variation in glucosinolates and their breakdown products in three cabbages cultivars during storage, showed that the concentration of the thiocyanate ions, isothiocyanates and goitrin, decreased during storage and this was associated with the decreasing quality of cabbage (12).

Similar results were found for cabbage stored under controlled atmosphere (CA), but at the early storage period, isothiocyanates and goitrin increased and then decreased towards the end of storage (13).

Some authors have determined the total and individual glucosinolates in "Marathon" broccoli florets stored 7 days at 10°C under air, 0.5% O_2, 0.5% O_2+ 20% CO_2 or 20% CO_2 atmosphere, followed by transfer to air for 2 days. The total glucosinolates increased 42% and 21% under air and 0.5% O_2 + 20% CO_2 respectively, and decreased 15% in broccoli stored under 20% CO_2. As concern the individual glucosinolates, glucobrassicin decreased 36% under 0.5% O_2 + 20% CO_2 or 20% CO_2. On the contrary, 4-methoxyglucobrassicin content increased during storage under low O_2 atmosphere and increased further after transfer to air (6).

Brassicaceae, besides glucosinolates and isothiocyanates, contains vitamin C and some varieties, as "Violetto di Catania" cauliflower, also anthocyanins, whose profile has been definitively elucidated (14).

It's known the importance of vitamin C and anthocyanins in the prevention of cancer and cardiovascular diseases and in slowing down cellular aging, due to the high antiradical and antioxidant power (15-19).

Since post-harvest quality evaluation has not been studied enough for cauliflower, the aim of this research was to determine the volatile characteristic substances, sulforaphane, vitamin C and total anthocyanins in two varieties of cauliflowers ("Romanesco" and "Violetto di Catania") stored at +4 °C for 7 and 14 days, to know and deepen the evolution of aromatic-nutraceutical parameters in these vegetables. "Violetto di Catania" and "Romanesco" have been chosen because the consumption of these cauliflowers constantly increases in the last years in Italy. In this paper the optimal conditions of domestic refrigerated storage of some Brassicaceae, after the market purchase, are established, to preserve for several days the alimentary and nutraceutical qualities if these vegetables are not immediately consumed. The obtained biochemical data can suggest the optimal range within it is possible to eat a product with good nutritional quality after several post harvest weeks.

Materials and methods

Plant source and storage. 9 heads of "Romanesco" and "Violetto di Catania" cauliflowers (*Brassica oleracea L.* var. Botrytis), were grown in the C.R.A.-ORA, Monsampolo del Tronto (AP, Italy). The heads, after three days from harvest, were divided into 3 lots con-

sisting of three samples for each variety. The first lot was immediately decored to florets, freeze-dried and the obtained powder was stored at -80 °C before analysis (t_0). Each head of the other lots were stored in "film wraps" at +4 °C for 7-14 days (t_7, t_{14}).

The experimental scheme was reported in table 1. After storage, the samples were treated as above described. Each analytical determination was repeated three times.

Extraction-concentration of volatile substances. The volatile compounds were extracted and concentrated by microwave- resin- solvent and the obtained extracts were analyzed by GC/MS. The method was reported in a previous note (11).

GC/MS determination of sulforaphane. This isothiocyanate, obtained by enzymatic hydrolysis from corresponding glucoraphanin, was separated from vegetable matrix with dichloromethane and the extracts were quali-quantitatively analyzed by GC/MS. The method, reported in a previous paper (20), is similar to the procedure pointed out by Chang et al. (21) and Omary et al. (22).

Vitamin C. Ascorbic and dehydroascorbic acids were extracted with distilled water + metaphosphoric acid (6%) from freeze-dried tissues, and analyzed by HPLC (20, 23).

Total anthocyanins. Those compounds were extracted from freeze-dried samples by methanol + HCl, and spectrophotometrically analyzed at λ=528 nm, according to Swain e Hills (24).

Dry matter. Was determined using a laboratory oven at 80°C up to a constant weight (25).

Statistical analysis. The Tukey test was used to evaluate the differences between the raw and stored samples. Mean values were considered significantly different when ρ≤0.05.

Results and discussion

Storage. Table 1 shows that the weight loss was different for the two genotypes. In fact at t_7, "Romanesco" cauliflower had a lower weight loss than "Violetto di Catania"; while at t_{14}, the weight losses were similar for both varieties. This behaviour might depend on different vegetable structure.

Volatile substances. At t_0, among volatile substances in two varieties, were identified 8 sulphides, 2 thiocyanates and 1 nitrile (Tab. 2).

Table 1 - Experimental storage scheme (+4°C for 7-14 days) of "Romanesco" (R) and "Violetto di Catania" (VC) cauliflowers and weight losses percentage at t_7 and t_{14}. Different letters indicate significant difference (ρ≤ 0,05), small letters among different genotypes, capital letters between t_7 and t_{14}

Samples	t_7 weight loss %	t_{14} weight loss %
1 R	1,92	
2 R	1,74	
3 R	1,43	
4 R		4,54
5 R		4,03
6 R		3,73
% average	1,70 ± 0.25aB	4,10 ± 0.44aA
1 VC	3,96	
2 VC	3,21	
3 VC	3,96	
4 VC		4,06
5 VC		3,67
6 VC		4,74
% average	3,71 ± 0.43bA	4,16 ± 0.54aA

Table 2 - Influence of storage at +4 °C for 7-14 days on volatile characteristic substances of "Romanesco" and "Violetto di Catania" cauliflowers. Different letters indicate significant difference (ρ≤0,05)

Volatile substances (mg/100 g d.m.)	"Romanesco"			"Violetto di Catania"		
	t_0	t_7	t_{14}	t_0	t_7	t_{14}
Sulphides						
dimethyl disulphide	3,76±0.18c	2,50±0.10b	1,87±0.09a	1,56±0.08a	1,64±0.09a	1,96±0.1b
dimethylethyl disulphide	0,14±0.01a	0,16±0.02b	0,17±0.02b	0,16±0.01a	0,17±0.01a	0,20±0.02b
diethyl disulphide	0,05±0.01a	0,17±0.03c	0,13±0.02b	0,18±0.01a	0,18a±0.01	0,17±0.01a
dimethyl trisulphide	8,36±0.38c	6,29±0.25b	3,76±0.10a	7,83±0.28b	7,65±0.32b	0,38±0.02a
methyl(methylthio) methyl disulphide	0,04±0.01a	0,09±0.02b	0,03±0.01a	0,05±0.02a	0,06±0.02a	0,10±0.02b
dimethyl tetrasulphide	0,41±0.03b	1,23±0.08c	0,34±0.01a	1,42±0.05b	1,38±0.06b	0,88±0.02a
methyl,methylsulphinylmethyl sulphide	-	-	-	0,05±0.01a	0,06±0.02a	0,09±0.01b
dimethyl pentasulphide	-	-	-	0,11±0.02b	0,03±0.01a	0,17±0.03c
Total	12,62±0.063c	10,64±0.53b	6,13±0.31a	11,34±0.57b	11,16±0.56b	8,35±0.42a
Thiocyanates						
4-(methylthio)butanenitrile	0,03±0.01a	0,05±0.008b	0,09±0.03c	0,12±0.006c	0,05±0.01b	0,03±0.01a
5-(methylthio)butanenitrile	0,02±0.01a	1,80±0.08c	0,09±0.02b	0,31±0.02c	0,03±0.01b	0,02±0.01a
Total	0,05±0.01a	1,85±0.08c	0,18±0.03b	0,43±0.09c	0,08±0.02b	0,05±0.01a
Nitriles						
benzenepropanenitrile	0,11±0.02a	0,32±0.06c	0,21±0.04b	0,05±0.01a	0,10±0.02b	0,18±0.03c

Dimethyl trisulphide was the predominant compounds in both varieties, followed by dimethyl disulphide, ranging from 1.56 to 3.76 mg/100 g d.m., while the values of the other compounds were ranged between 0.03-1.40 mg/100 g d.m.

Besides, table 2 shows the influence of storage on total amount of sulphides, thiocyanates and nitriles in both cauliflower. In "Romanesco", the concentration of sulphides at t_7 was similar to t_0; while at t_{14} the values were halved. The same trend was noted in the "Violetto di Catania" cauliflower; but at t_{14} the losses were lower than "Romanesco" cauliflower.

In "Romanesco"; the concentrations of thiocyanates and nitrile increased at t_7 and then decreased at t_{14}. In "Violetto di Catania", the values of thiocyanates decreased during storage, while nitrile increased.

Nutraceutical parameters. Table 3 shows that the content of sulforaphane in "Romanesco" was about 5 times than "Violetto di Catania" at t_0. At t_7, in "Romanesco", the value of sulforaphane was constant; while at t_{14} it decreased of 20% respect of t_0. In the "Violetto di Catania", sulforaphane decreased up to 9% at t_7, and 55% at the end of storage.

For vitamin C, it has been determined both ascorbic and dehydroascorbic acids. At t_0, the level of ascorbic acid in "Romanesco" was 2.5 fold than "Violetto di

Table 3 - Influence of storage at +4 °C for 7 and 14 days on sulforaphane, ascorbic acid, dehydroascorbic acid and total anthocyanins in "Romanesco" and "Violetto di Catania" cauliflowers. Different letters indicate significant differences ($\rho \leq 0.05$)

	"Romanesco"			"Violetto di Catania"		
	t_0	t_7	t_{14}	t_0	t_7	t_{14}
Sulforaphane (μmol/g d.m.)	0,102±0,015b	0,111±0,009b	0,081±0,007a	0,022±0,003b	0,020±0,004b	0,010±0,005a
Ascorbic acid (mg/100 g w.w.)	122,90±15,28b	83,30±6,68a	82,40±7,24a	50,00±3,68b	25,36±2,15a	27,40±3,18a
Dehydroascorbic acid (mg/100 g w.w.)	0,12±0,05a	0,92±0,08b	0,99±0,09b	0,20±0,07a	11,41±1,21b	13,70±0,85c
Total Anthocyanins (mg/100 g w.w.)	–	–	–	20,57±1,60b	24,00±2,21c	11,87±1,05a

Catania". In "Romanesco" (Tab. 3), the content of ascorbic acid decreased about 32% at t_7, and then it was constant at t_{14}. Dehydroacorbic acid had an increase 7.6 fold at t_7 respect to t_0, and then it was constant until the end of the storage. This behaviour is perhaps due to an oxidative stress.

In "Violetto di Catania" (Tab. 3) the decreasing of ascorbic acid was about 50% at t_7 and 45% at t_{14} respect of t_0. Dehydroascorbic acid increased about 58 fold at t_7 and t_{14}, respect of t_0.

In table 3 is also reported the behaviour of anthocyanins during the storage. They increased about 17% at t_7, while at t_{14} there was a loss of 42% to the value ascertained at the starting of the storage.

Our researches pointed out that aromatic and nutraceutical parameters can be influenced both by cauliflower genotypes and operative conditions. As concern the volatile substances, we observe the preservation of the organoleptic characteristic in both cauliflower during the first storage week, but at the end of the storage, they decrease. This trend is more evident for "Romanesco" cauliflower.

As concern nutraceutical substances, the content of sulforaphane, is a little lower respect of the raw samples during the first week of storage, while it strongly decreases during the second one, for both vegetables. The decrease of sulforaphane in the second week, depends on the corresponding glucosinolate. This decline probably reflects membrane damage and cell rupture, conditions favourable for hydrolytic breakdown of glucoraphanin by myrosinases, that catalyze hydrolysis or autolysis (3, 26).

In the intact cell, myrosinases are well separated from glucosinolates (27). When glucosinolates and myrosinases are brought in contact, a number of isothiocyanates are formed, depending on the structure of glucosinolates and myrosinases and the actual condition for hydrolysis (26, 28).

As concern ascorbic acid, it seems to depend more than the other parameters on the genotypes considered, particularly for his oxidation to dehydroascorbic acid. In fact, in "Romanesco", even if the loss in ascorbic acid is less evident than in "Violetto di Catania", there is not any compensation with the oxidation in dehydroascorbic acid. In "Violetto di Catania", instead, to the loss in ascorbic acid corresponds a bigger production of dehydroascorbic acid. This molecule still has a biological activity, because in the human body, this is

reduced again in ascorbic acid (29).

The best retention of vitamin C (ascorbic + dehydroascorbic acid) could be related to the highest phenolic content of "Violetto di Catania" variety than "Romanesco". The antioxidant activity of polyphenols could slow the degradation of dehydroascorbic acid (30). The content in anthocyanins, during the first week of storage, increases. This behaviour is due to new synthesis of these compounds after physiological stress for exposition of vegetables to low temperature. Then the cell rupture may cause their degradation, no more compensated by synthesis ex novo (30).

Conclusions

The evolution of aromatic-nutraceutical parameters in two stored cauliflowers, shows that the storage can prolong for 7 days because the content of the volatile characteristic substances and sulforaphane is almost constant, anthocyanins increase and only vitamin C decreases his biological activity. This loss is partially balanced by dehydroascorbic acid, particularly in the "Violetto di Catania".

After 14 days, even if the values of vitamin C are constant, all the other parameters drastically decay, lacking the alimentary and nutraceutical quality in both cauliflower genotypes.

References

1. Bjerg B, Sorensen H. Isolation of intact glucosinolates by column chromatography and determination of their purity. In Wathelet JP (ed): World crops: production, utilization, description. Glucosinolates in rapeseed: Analytical aspects. Martinus Nijhoff Publ, Boston, 1987a: 59-75.
2. Lewis J, Fenwick GR. Glucosinolates content of Brassica vegetables: analysis of twenty-four cultivars of calabrese (green sprouting broccoli, Brassica oleracea L. var botrytis subvar. Cymosa Lam.). Food Chem 1987; 25: 259-68.
3. Olsen O, Sorensen H. Recent advances in the analysys of glucosinolates. J Amer Oil Chem Soc 1981; 58: 857-65.
4. Vanetten CH, Daxenbichler ME, Williams PH, Kwolek WF. Glucosinolates and derived products in cruciferus vegetables. Analysis of their edible part from twenty-two varieties of cabbage. J Agr Food Chem 1976; 25: 259-68.
5. Heaney RK, Spinks EA, Fenwick GR. The glucosinolates content of Brussels sprouts: Factors affecting their relative abundance. Z Pflanzenzüchtg 1983; 91: 219-26.
6. Hansen M, Moller P, Sorensen H. Glucosinolates in broccoli stored under controlled atmosphere. J Amer Soc Hort Sci 1995; 120 (6): 1069-74.
7. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci USA 1997; 94 (19): 10367-72.
8. Zhang Y, Talalay P, Cho CG, Posner GH. Proc Natl Acad Sci USA 1992; 89: 2399-403.
9. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P. Proc Natl Acad Sci USA 1994; 91: 2147-50.
10. Gerhäuser C, You M, Liu J, et al. Cancer Re 1997; 57: 272-8.
11. Di Cesare LF, Viscardi D, Genna A, Ferrari V. Influenza della cottura e dell'acido citrico sui componenti volatili ed antocianine nel cavolfiore. Industrie Alimentari 2005; XLIV: 26-33.
12. Chong C, Berard LS. Changes in glucosinolates, during refrigerate storage of cabbage. J Amer Soc Hort Sci 1983; 108: 688-91.
13. Berard LS, Chong C. Influence of storage on glucosinolate fluctuations in cabbage. Acta Hort 1985; 157: 203-10.
14. Lo Scalzo R, Genna A, Branca F, Chedin M, Chassaigne H. Anthocyanin composition of cauliflower (Brassica oleracea L. var. bothrytis) and cabbage (B. oleracea L. var. capitata) and its stability in relation to thermal treatments. Food Chemistry 2008; 107: 136-44.
15. Harris JR. Ascorbic acid: Biochemistry and Biochemical cell Biology. Subcellular Biochemistry. Plenum Press, New York, USA 1996; vol. 25: 464.
16. Wada L, Ou B. Antioxidant activity and phenolic content of Oregon caneberries. J Agric Food Chem Jun 2002; 50 (12): 3495-500.
17. Hou DX. Potential mechanism of cancer chemopreventions of anthocyanins. Curr Mol Med Mar 2003; 3 (2): 149-59.
18. Karlsen A, Retterstøl L, Laake P, et al. Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy-adults. J Nutr 2007; 137 (8): 1951-4.
19. Neto CC. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. Mol Nutr. Food Res 2007; 51 (6): 652-4.
20. Di Cesare LF, Viscardi D, Forni E, Migliori C, Ferrari V. Influenza della cottura su alcune sostanze nutraceu-

tiche del cavolfiore. Ricerche e innovazioni nell'industria alimentare. Chiriotti Editori vol. VII, 2005: 177-83.
21. Chang CKW, Pusateri JD, Leitz AER. Gas Chromatography/Mass Spectrometry method for the determination of sulforaphane and sulforaphane nitrile in Broccoli. J Agric Food Chem 1998; 46: 1018-21.
22. Omary BM, Brovelli AG, Pusateri JD, David P. Sulforaphane potential and Vitamin C concentration in developing heads and leaves of broccoli (Brassica oleracea, var. italica). J of Food Quality 2003; 26: 523-30.
23. Davey MW, Deckempeneer E, Keulemans J. Rocket-powered high-performance liquid chromatographic analysis of plant ascorbate and glutathione. Analytical Biochemistry 2003; 316 (1): 74-81.
24. Swain T, Hills WE. The phenolic constituent of Prunus domestica. 1: the quantitative analysis of phenolic constituents. J Sci Food Agric 1959; 10: 63-8.
25. AOAC, No 22018. In Official methods of analysis, 13th ed.; Association of Official Analitical Chemists. Washington DC, 1975.
26. Sorensen H. Glucosinolate: Structure-Properties-Function, p. 149-172. In Shahidi F (ed): Rapeseed/canola: Production, chemistry, nutrition and processing technology. Van Nostrand Reinhold Publ., New York, 1990.
27. Lüthy B, Matile P. The mustard oil bomb: rectified analysis of the subcellular organisation of the myrosinase system. Biochem. Physiol Pflanzen 1984; 179: 5-12.
28. Vanetten CH, Tookey HL. Glucosinolates. In: M. Rechcigl (ed.). CRC handbook of naturally occurring food toxicants. CRC Press, Boca Raton, Fla. 1983: 15-30
29. Lee SK, Kader AA. Preharvest and postharvest factors influencing vitamin C content in horticultural crops. Postharvest Biol Technol 2000; 20: 207-20.
30. Pizzocaro F, Ferrari V, Acciarri N, Morelli R, Russo-Volpe S, Prinzivalli C. Antioxidant and antiradical acitvities in green and violet cauliflower ecotypes with different maturity stages. Work-shop of IV giornate scientifiche SOI, Sirmione (BS)- Italy. 2000: 34-35.