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# CHEMICAL AND NUTRACEUTICAL PROFILES OF ORGANIC AND CONVENTIONAL VEGETABLES

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*A Marta e Mirko.*

*Just living is not enough....*

*one must have sunshine, freedom*

*and a little flower*

*(H.C. Handersen)*

**ABSTRACT**: The influence of organic (OR) and conventional (CONV) systems on chemical and nutraceutical parameters in different genotypes of vegetables, widely consumed in Italy and important for their high healthy potential, was evaluated.

Some genotypes of cauliflower (*Brassica oleracea* L. var. *Botrytis* ) as Emeraude, Velox and Jesino; tomato (*Solanum lycopersicum* L.), as Giulianova and Perbruzzo; sweet red pepper (*Capsicum annuum* L.), as Alceste and RS08; onion (*Allium cepa* L.), as Rossa di Suasa, Tropea, Density and Precoce di Romagna; watermelon (*Citrullus lanatus* L. ) as Bagnacavallo, Faenza, Monaco and Talete, grown with OR and/or CONV systems, were tested. The research was performed in three years of experimentation (2010-2012). In the first part, the average values for all the genotypes of each species, obtained during the whole period, was considered. In the second one, the average values obtained for each species, collected year by year, were taken into consideration. The collected data were subjected to statistical analysis.

Cauliflower: the chemical quality of the OR and CONV cauliflower involved the characteristic volatile compounds of this species (sulfides, nitriles, etc.). The nutraceutical substances in cauliflower are represented by glucosinolates, vitamin C, polyphenols and carotenoids, that have protective effect against different form of human cancer, cardiovascular diseases etc.

Positive effects of OR method on the chemical quality were ascertained in all the genotypes. For the nutraceutical quality, positive effects of OR method were found in Emeraude, as concern glucosinolates. The other parameters were similar in the OR and CONV samples, for all genotypes.

Tomato: the analysis of OR and CONV tomato, harvested at the unripe and ripe stages, were focused on the determination of the characteristic volatile compounds (hexanal, 2(E)-hexenal, 2-isobutylthiazole), soluble sugars and pH, for the chemical quality; while lycopene and vitamin C for the nutraceutical one. In the ripe OR Giulianova, the flavour compounds increased, and contemporary the lycopene decreased with respect to the CONV samples. An opposite behavior was noted in Perbruzzo. In the unripe samples, the positive influence of the OR system was evident only in Perbruzzo, especially as concern the flavour compounds and lycopene. The content of soluble sugars, vitamin C and pH were similar in all genotypes, for both ripening stages and both cultivation systems.

Sweet red pepper: the analysis of OR and CONV pepper regarded the content of volatile pyrazines (2-methoxy-2-isopropyl pyrazine and 2-

methoxy-2-isobutyl pyrazine) and soluble sugars, for chemical quality. As concern the nutraceutical one, some antioxidant molecules (vitamin C, polyphenols, carotenoids) and the antioxidant capacity (DPPH*) were evaluated. For chemical quality, total pyrazynes were negatively affected by OR system, while soluble sugars were scarcely influenced. A positive effect of OR system for ascorbic acid was noted only in Alceste but the antioxidant capacity was significantly higher in the CONV samples for both genotypes.

In OR Alceste, submitted to cooking (by grilling) pyrazynes and the other volatiles contents were modified. Moreover, other new “Maillard” volatile compounds, such as furan and furanone derivatives, were found. Soluble sugars, carotenoids, polyphenols, vitamin C and, consequently, the antioxidant power decreased .

Onion: the OR onion was submitted to the evaluation of chemical quality (soluble sugars and characteristic volatile substances = saturated and unsaturated sulfides) and nutraceutical one (unsaturated sulfides, polyphenols as anthocyanins and quercetins, and antioxidant capacity). Density, Precoce di Romagna and Tropea genotypes showed a better chemical quality (flavour profiles and soluble sugars content), and contemporary an higher level of unsaturated sulfides (diallyl disulfide and allyl methyl trisulfide), probably linked to a stronger potential antitumor activity. Moreover, Density had the highest antiradical capacity, due to the highest content in polyphenols, in particular quercetins.

Watermelon: the analysis of OR watermelon genotypes involved the chemical quality (soluble sugars and characteristic volatile compounds = saturated and unsaturated aldehydes and alcohols with 9 carbon atoms), and the nutraceutical one (lycopene, L-citrulline, and ascorbic acid). Monaco genotype had the highest chemical profile, thanks to its highest amount of characteristic volatile compounds and soluble sugars. As concern the nutraceutical quality, Faenza, Monaco and Talete genotypes were clearly distinguishable from Bagnacavallo cultivar, for their higher contents of healthy compounds.

The powdered rind of Talete genotype, thanks to its higher content in L- citrulline, has been used for the preparation of functional foods, such as apple-jellies, with good chemical and rheological characteristics and microbiological stability.

In conclusion, regarding chemical aspects, positive effects of the OR cultivation are observed in cauliflower and tomato (Giulianova). For the nutraceutical quality, instead, a positive effect of OR method is found in

some genotypes of cauliflower (Emeraude), sweet red pepper (RS08) and tomato (Perbruzzo). In watermelon and onion, cultivated only with OR system, a deep influence of the genotype was noticed, as concern the quality parameters.

**RIASSUNTO**: Oggetto di studio delle presente tesi di dottorato è stata la valutazione del patrimonio chimico e nutraceutico di alcuni genotipi di diverse specie vegatali largamente consumate in Italia e con un notevole impatto positivo sulla salute umana, coltivate sia in modo tradizionale (CONV) sia secondo il metodo dell'agricoltura biologica (OR).

Sono stati studiati alcuni genotipi di cavolfiore (*Brassica oleracea* L. var. *Botrytis* ), in particolare Emeraude, Velox e Jesino; pomodoro (*Solanum lycopersicum* L.), come Giulianova e Perbruzzo; peperone dolce (*Capsicum annuum* L.), come Alceste ed RS08; cipolla (*Allium cepa* L.), come Rossa di Suasa, Tropea, Density e Precoce di Romagna; cocomero (*Citrullus lanatus* L.) come Bagnacavallo, Faenza, Monaco e Talete, coltivati con il metodo biologico e convenzionele. La ricerca è stata articolata in tre anni di sperimentazione (2010-2012) e sono state prese in considerazione le medie dei valori ottenuti nell'intero periodo di studio per ogni genotipo di tutte le specie studiate. I dati raccolti sono stati sottoposti ad analisi statistica.

<u>Cavolfiore</u>: tra i parametri chimici, sono state prese in considerazione le sostanze volatil caratteristiche (solfuri e nitrili, ecc.), mentre per quelli nutraceutici i glucosinolati, la vitamina C, i polifenoli ed i carotenoidi, tutte molecole aventi potere protettivo e perventivo contro diverse forme di cancro, malattie cardiovascolari, ecc.

L'effetto positivo dell'agricoltura biologica sulla qualità chimica è stata riscontrata in tutti I genotipi studiati. Per la qualità nutraceutica, invece, sono stati notati effetti positivi sui glucosinolati del genotipo Emeraude. Per tutti gli altri parametri, nessuna differenza statisticamente significativa è stata evidenziata in nessun genotipo.

<u>Pomodoro</u>: per i campioni OR e CONV di pomodori raccolti sia allo stato maturo che immaturo (invaiato), sono state quantificate le sostanze volatili caratteristiche (esanale, 2(E)-esenale, 2-isobutiltiazolo), insieme agli zuccheri solubili, pH, per i parametri chimici, e, per quelli nutraceutici, licopene e vitamina C.

Nel Giulianova OR maturo, le sostanze volatili e gli zuccheri erano inferiori rispetto al campione CONV, mentre un comportamento opposto era stato notato per il Perbruzzo. Nei campioni immaturi, l'effetto positivo del sistema

OR è stato notato solo nel Perbruzzo, specialmente per sostanze volatili e licopene. Tutti gli altri parametri sono risultati essere molto simili per tutti i genotipi sia OR che CONV ad entrambi gli stadi di maturazione.
Peperone dolce: tra i parametri chimici sono state studiate le pirazine (2-metossi-2-isopropil pirazina e 2-metossi-2-isobutil pirazina) e gli zuccheri solubili, mentre per quella nutraceutica alcune molecole antiossidanti (vitamina C, polifenoli e carotenoidi) unitamente alla misura del potere antiossidante, effettuata con il metodo del (DPPH*).
Le pirazine totali sono state influenzate negativamente dal sistema OR, mentre una scarsa influenza è stata riscontrata per gli zuccheri solubili, in entrambi i genotipi. Un effetto positivo dell'agricoltura OR è stato notato per l'acido ascorbico nell'Alceste, mentre la capacità antiossidante era più elevata nei campioni CONV di entrambi i genotipi.

Nel campione OR Alceste, sottoposto a cottura (su piastra), le pirazine e le altre sostanze volatili sono risultate essere profpndamente modificate, in particolare per la presenza di sostanze volatili di “Maillard”, come derivati furanici e furanonici. Gli zuccheri solubili, i carotenoidi, polifenoli ed il potere antiossidante sono notevolmente diminuiti.
Cipolla: i campioni di cipolla, coltivata solo in OR, sono stati sottoposti alla valutazione della qualità chimica (zuccheri solubili, sostanze volatili caratteristiche, in particolare solfuri saturi ed insaturi) e nutraceutica (solfuri insaturi, polifenoli, come antocianine e quercitine, e la capacità antiossidante). I genotipi Density, Precoce di Romagna e Tropea hanno mostrato una migliore qualità nutraceutica e, contemporaneamente un più alto livello di solfuri insaturi (diallil disolfuro e allilmetil trisolfuro), molecole con un dimostrato potere antitumorale. Inoltre, la Density ha mostrato una maggiore capacità antiossidante, dovuta al maggiore contenuto in polifenoli, in particolare quercitine.
Cocomero: l'analisi dei genotipi di cocomero ha riguardato, per la qualità chimica, zuccheri solubili e sostanze volatili caratteristiche (aldeidi ed alcoli, saturi ed insaturi, a nove atomi di carbonio) e, per quella nutraceutica, licopene, L-citrullina e vitamina C. Il genotipo Monaco ha mostrato il più alto contenuto in sostanze volatili e zuccheri solubili. Per quanto riguarda il patrimonio nutraceutico, i genotipi Faenza, Monaco e Talete hanno mostrato un profilo notevolmente più elevato rispetto al Bagnacavallo.

La polvere di buccia essiccata del genotipo Talete, per il suo più elevato contenuto in citrullina, è stata utilizzata per la preparazione di gelatine di mela arricchite, alimento funzionale ad elevato contenuto in citrullina (vasodilatatore).

Concludendo, per quanto riguarda il profilo chimico, effetti positivi del sistema OR sono stati riscontrati per il cavolfiore (tutti i genotipi) ed il pomodoro (Giulianova). Perla qualità nutraceutica, invece, effetti positivi sono stati riscontrati per alcuni genotipi di cavolfiore (Emeraude), peperone (RS08) e pomodoro (Perbruzzo). Nel cocomero e cipolla, coltivati solo con il metodo OR, è stata riscontrata una profonda influenza del fattore genetico sui parametri qualitativi.

# Contents

## PREFACE

The subject of this doctoral thesis comes from the project “VAL.OR.BIO” – “Valorizzazione della tipicità orticola attraverso l'agricoltura biologica”, coordinated by Dr. Gabriele Campanelli and carried out, for its agronomic part, at CRA-ORA, Monsampolo del Tronto (AP), directed by Dr. Valentino Ferrari. The aim of this project was to study and promote the spread of some vegetables of Emilia Romagna, Marche and Abruzzo. The agronomic evaluations focused on some typical crops (tomato, sweet pepper, Fratterosa broad bean, Suasa onion, Pedaso bean, Green cauliflower), which had already been submitted to a conservative selection and genetic improvement by the CRA-ORA. The other vegetables (thistle, Romagna bean, watermelon, giant celery, Precoce di Romagna onion) collected from the “Centro di Ricerca per le Produzioni Vegetali” (CRPV), Cesena (FC), were submitted to breeding programs for the recovery of the original varieties.

Within this project, at the “Unità di ricerca per i processi dell’industria agroalimentare di Milano” (CRA- IAA), the biochemical operating unit, whose scientific manager is Dr. Luigi Francesco Di Cesare, investigated the quality traits of some vegetables, from both organic and conventional systems, such as cauliflower, tomato, pepper, onion and watermelon.

# 1 – INTRODUCTION

## 1.1 – BRIEF ON ORGANIC FARMING

The objective of organic farming is to offer products without residues of pesticides or chemical fertilizers, and to reduce the negative impact on the environment in terms of pollution of water, soil and air (IFOAM, February 18, 2009; IFOAM, The Principles of Organic Agriculture). In organic farming, the soil fertility is maintained through the use of organic fertilizers, the practice of crop rotation and the keeping of the soil structure and the percentage of organic matter. The control of plants diseases is only permitted by herbal, mineral, animal and microbiological preparations, not chemically synthesized, except some products considered "traditional" (AIAB. Cos'è il BIO?).

Organic farming in Europe has been regulated for the first time in 1991, with the * Reg (EEC) n ° 2092/91 on organic production. Only in 1999 with the * Reg (EC) n ° 1804/99 animal products were also regulated. In June 2007, a new EC Regulation for organic farming, Regulation (EC) n ° 834/2007 was adopted. It repeals the previous regulations and it concerns the organic production and labeling of organic products (including aquaculture). In Italy, this Regulation was implemented by the D.M. No. 18354 of 27/11/2009.

## 1.2 - Production and consumption of vegetables in Italy

In 2012, the Italian production of fresh vegetables, according to ISTAT data, showed the lowest level respect to the previous years. The harvested fresh vegetables, grown both in organic and in conventional systems in 2012, amounted to 7.5 million tons, that was lower of 10% with respect to 2011, and 13% than the mean values of 2005-2007. Among the vegetables, the processing tomato production (not included in the aggregate “fresh vegetables”), was nearly 4.7 million tons (-13% on 2011), lower than 5.6 million tons of previous three years. The production of salads decreased about 2% with respect to the previous year. A reduction of about 5% was showed by the cabbage group. Also a significant decline in the production of horticultural vegetables was noted for fresh tomatoes (-19%), melons (-17%), zucchini (-15%), watermelons (-15%), strawberries (-12%), eggplants (- 6%), peppers (-5%), carrots (-6%) and onions (-25%) (cso servizi, report 2012).

The data relating to the production of organic vegetables appear to be in sharp contrast. In fact, the data provided by SINAB - National Information System on Organic Agriculture showed an overall increase in the number of operators (+3%). In Italy, the highest concentration of organic farms was highlighted in Sicily, followed by Calabria region, while the highest number of processors is located in Emilia Romagna, followed by Lombardia and Veneto. The cultivated area under organic system amounted to 1˙167˙362 ha, with a total increase of 6.4% respect to the previous year (Sinab, Bio in cifre 2012). Tab. 1 shows the average production, as total (CONV+OR ) and only OR, of the vegetables examined in this thesis, for the years 2012 and 2013 (agri.istat.it ).

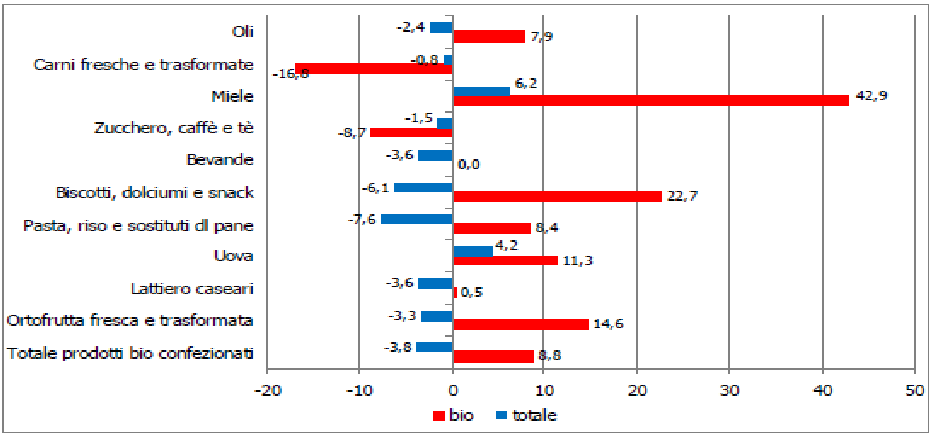
| | TOTAL YIELD 2012/2013 | | ORGANIC PRODUCTION 2012/2013 | |
|---|---|---|---|---|
| | ha | tons | ha | tons |
| **TOMATO** | 16˙323 | 489˙635 | 2087 | 163˙078 |
| **ONION** | 10˙749 | 341˙706 | 270 | 826 |
| **CAULIFLOWER** | 17˙098 | 428˙664 | 914 | 20˙126 |
| **WATERMELON** | 8˙705 | 359˙655 | 254 | 10˙243 |
| **SWEET PEPPER** | 9˙036 | 199˙967 | 300 | 12˙693 |

**Tab˙ 1:** Comparison among the mean agronomical data of some Italian vegetables cultivated in OR and CONV, in 2012 and 2013. (Source= agri.ista.it)

The table highlights how in Italy the agricultural fields for cultivation of organic horticultural crops, are still limited. Among the vegetables, the tomato is the most widely cultivated plant with the OR system, followed by cauliflower, while for other vegetables examined, the cultivated hectares range between 250 and 300.

In December 2012, compared to the same month of 2011, the market for fresh vegetables decreased of about 1.1 %. Among them, it was noted a declining purchase of artichokes (-10.1%), lettuce (-11%) and to a lesser extent also carrots, zucchini and fennel. On the contrary, there was an increased market of potatoes (+1.2), onions (+1.3%) and peppers (+3.3%). (Macfrut, consumi ortofrutticoli 2012).

Despite the economic and financial crisis, the Italian market of organic products continues to grow, as already noted in previous years. In the first half of 2013, domestic purchases of organic packaged products increased by 8.8 % in value, while at the same time, the agri-food expenditure was down (-3.7 %). The 2013 trend depended in particular by the increased consumption of organic fresh and processed fruit and vegetables (+14.6 %), as well as biscuits, sweets, organic snacks (+22.7 % in value), and eggs (+11.3 %) (Panel Data Families - Gfk Eurisko by Ismea). What enhances the good performance of the organic food is the comparison with the whole agri-food sector. Almost all product categories showed a more positive trend for organic sector than that recorded in the whole agri-business (organic + non-organic). This is confirmed by the data shown in Fig. 1, from which it is observed that in the first half of 2013 only in the "fresh and processed meats" and "sugar, coffee and tea" segments the organic production reported less favorable market trends, while the fresh and processed fruits and vegetables showed a favorable one, together with sweets, honey and eggs.




Fonte: Ismea, Panel Famiglie GFK-Eurisko

**Fig. 1:** Trend of domestic purchase, in value, of organic products in comparison with corresponding sector of general market, in the first half of 2013 (Var. % I sem '13/I sem '12).

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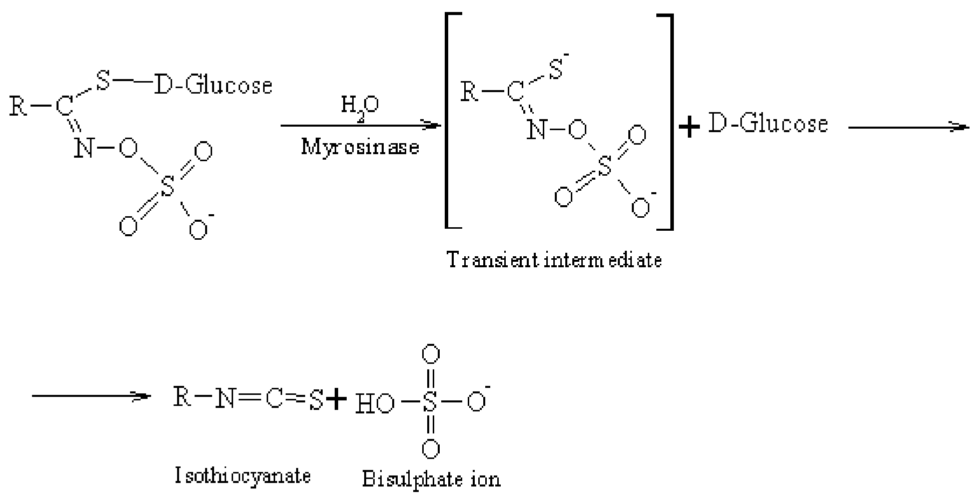
# 2 - STATE OF THE ART

## 2.1 - CHEMICAL AND NUTRACEUTICAL COMPOSITION OF THE STUDIED VEGETABLES

CAULIFLOWER. The cauliflower (*Brassica oleracea* L.), belonging to the *Brassicaceae* family, is appreciated for good supply of minerals (Ca, K, Fe, Mg), dietary fiber, vitamin A and C, and for the low fat content. Another very important aspect of this family is the content in volatile substances, that are interesting not only for the organoleptic aspects but also for its health benefits. The volatile substances (isothiocyanates), in raw and intact vegetables, are linked to glucose to form low volatile compounds called glucosinolates or tioglucosides (sinigrin, glucobrassicin, progoitrina ,etc.) (Fenwick et al., 1983).

The glucosinolates are hydrolyzed to isothiocyanates by endogenous myrosinase (β-thioglucoside glucohydrolase; EC 3,2. 3-1), that is normally segregated from glucosinolates and is released after cellular damage. Myrosynase catalyzes at pH=7 the following reaction (VanEtten et al., 1977; Chung, 1992):




In particular some isothiocyanates (NCS), as 3-metilsulfinilpropil NCS, sulforaphane or 4-metilsulfinilbutil NCS, during cooking will degrade in nitriles, sulfides, and tionitriles, that are responsible of cooked Brassicaceae odour (VanEtten et al., 1977). In this way, it can better appreciate the characteristic flavour of this family and the volatile components can be easily extracted from the vegetable matrix and analyzed. It has long been known that isothiocyanates have different biological effects due to their chemical reactivity (Chung et al.1992). Isothiocyanates, such as 4-methylsulfinylbutyl NCS or sulforaphane, 3-methylsulfinylpropyl NCS, 3(methylthio)propyl NCS, 2-phenylethyl NCS, allyl NCS and methyl NCS (Guo et al., 1992), have been studied for their effects in cancer prevention. These isothiocyanates are potent inhibitors of tumourgenesis in various animal models (Faulkner et al., 1998; Lo Scalzo et al., 2008). The dual action of isothiocyanates is to inhibit phase I enzymes, that are responsible for the bioactivation of carcinogenesis, reducing the production of electrophilic intermediates, and to enhance the activity of phase II enzymes such as glutathione transferases, epoxide hydrolases, NAD(P)H: quinone reductases and glucoronosyl transferases, that increase the detoxification and clearance of carcinogens (Bogaards et al., 1990). The effects on phase I and II enzymes, and consequently a reduced cancer risk, have been demonstrated in humans that consume a large quantity of cruciferous vegetables (Kech et al. 2004).

Cauliflowers, in addition to glucosinolates, also contain vitamin C, polyphenols and carotenoids. It's well-known the importance of vitamin C for healthy benefits due to its strong antioxidant and anti-radical power, that is expressed by slowing down in cellular aging processes and in the prevention of cardiovascular disease and cancer. The antiradical and antioxidant effect of vitamin C seems to be due to its important role played in red-ox reactions catalyzed by oxygenase (hydroxylation, synthesis, catabolism, etc.). Vitamin C can decrease the formation of intestinal nitrosamines and various oxidizing compounds (superoxide radical, hypochlorous acid and hydroxyl radicals ) (Bremus et al. 2006). Polyphenols are recognized as powerful antioxidants (Rice-Evans, et al, 1997), but few studies that evaluated their content in *Brassica* species (Podsedek, 2007), even if cauliflower seems to be particularly rich in polyphenols (Wu et al. 2004) are available. Only recently, there have been some studies that insight the phenol components of Brassicaceae (Sengas et al., 2012). Carotenoids (carotenes and xanthophylls) are lipid-soluble antioxidants that were generally found in relatively low concentration in cauliflower. Due to conjugated double bonds, they are both radical scavengers and quenchers of singlet oxygen (Krinsky, 1998).

TOMATO. Tomato (*Solanum lycopersicum* L. and *Lycopersicum esculentum* L.) belonging to the *Solanaceae* family, is an annual plant whose fruits are red coloured at the ripe stage. The average composition of a ripe tomato is as follows: water 94.5 g/100 g f.w., protein 1 g/100 g f.w., fats 0.29 g/100 g f.w., soluble sugars 3.5 g/100 g f.w., fiber 1 g/100 g f.w.

The mineral salts are represented by Na (6.58 mg/100 g f.w.), K (297.09 mg/100 g f.w.), Ca (15.54 mg/100 g f.w.), P (29.58 mg/100 g f.w.), Mg (19.52 mg / 100 g f.w.) and Cl (46.56 mg/100 g f.w.), and the vitamin by vitamin C (24.50 mg/100 g f.w.), vitamin K (0.60 mg/100 g f.w.), nicotinamide (0.57 mg/100 g f.w.) and provitamin A (1.22 mg/100 g f.w.) (G. Silvestri et al., 1991). However, the parameters normally taken into consideration for the evaluation of nutritional quality in these berries are volatile substances, soluble sugars, dry matter and organic acids; while lycopene and vitamin C for the nutraceutical one.

In fresh tomato many volatile compounds have been identified and, among these, the most common ones are the following: 2(E)-hexenal, hexanal, 3(Z)-hexen-1-ol, β-ionone, 2,4(E,E)-decadienal, 2-isobutylthiazole, 3(Z)-hexenal (Dirinck P. et al, 1976). The linolenic acid is the precursor of the 3(Z)-hexenal, formed during the maceration of the plant tissue. Then, 3(Z) –hexenal is a precursor of 2(E)-hexenal (Kazeniac et al., 1970). Sensory tests carried out on different cultivars of fresh tomato have shown that the volatile components responsible of the characteristic flavour are : hexanal, 2(E)-hexenal, 3(Z)-hexen-1-ol and 2-isobutylthiazole (Dirinck P. et al, 1976).

In addition, it should be also taken into account sugars and organic acids, that affect the tomato taste. Sugars and organic acids represent more than half of the dry matter contained in this berry. Soluble sugars are represented mostly by fructose and glucose, with possible traces of sucrose. The organic acids present in tomato are mainly citric and malic acid, with the predominance of the latter (Davies et al., 1981). The intense red tomato color is due to the lycopene, which represents approximately 83% of total pigments (Gould, 1992). To lycopene it has been recognized having a significant antioxidant and antiradical action, which is expressed in the prevention of some epithelial cancers affecting the esophagus, stomach, intestine, pancreas and spinal cord (Gerster et al., 1997). It was also ascertained a significant inverse correlation between diets rich in tomato and its derivatives, and lower risk of prostate cancer (Giovannucci E. et al., 1995). In fresh tomato, lycopene is more found in the all-trans form (79-91 %) and the remaining percentage is represented by the cis - isomers. After ingestion, the cis- isomers constitute more than 50% of total lycopene in human serum. The cis- isomers, with the cis double bound localized in the 5, 7, 9, 11, 13, 15 carbonium, appear to have an higher bioavailability than the all-trans form (Stahl et al., 1992).

SWEET PEPPER. The main feature of the sweet pepper (*Capsicum Annum* L.) is its high content of vitamin C (110-150 mg/100 g f.w.) and carotenoids (20-50 mg/100 g f.w.). The pepper contains 90 % water, 4.2 % carbohydrate, 0.9 % protein, 0.3 % fat, minerals (Ca , Fe, Na , K, P) and vitamins (B1, B2, PP) (INRAN - Food composition database).

The most representative sweet pepper carotenoids are capsanthin and capsorubin. Capsanthin, commonly esterified with fatty acids such as monoesters or diesters or in free form, may represent 50 % of total carotenoids in the red pepper. Capsanthin is a carotenoid with a high nutraceutical capacity, thanks to its remarkable properties to quench singlet oxygen and prevent the colon cancer. Other pigments, found in yellow or red peppers, are the violaxanthin, capsanthin -5,6- epoxide, zeaxanthin, lutein, β-cryptoxanthin and β -carotene. (Kim et al., 2004).

The first quali-quantitative characterization of green and red pepper flavour was carried out by Buttery et al (1969), using the Likens - Nickerson simultaneous extraction-distillation method, under vacuum, and the GC/MS analysis. They identified 3-methoxy-3-isobutylpyrazine, β(E)–ocimene, D,L-limonene, methyl salicylate, linalool, 2,6(E,Z)- nonadienal, 2,4(E,E)-decadienal and 3(Z)-hexen-1-ol. Other compounds were identified when the procedure of extraction-distillation was carried out at atmospheric pressure and in particular: 1(E)-nonen-4-one, 2(E)-nonen-4-one, 2,5(E,E)-nonadien-4-one, 2-pentylfuran and benzaldehyde. The component responsible for the pepper flavour is a derivative of pyrazine, who had an odour threshold of 2 parts per $10^{12}$ shares of water. Typically, about 70-80% of the panel members indicated that the odour of pyrazines in dilute aqueous solutions was similar to that of the green and red pepper.

Many studies have been focused on the potential antitumor effects of sweet peppers. Pepper extracts, in fact, have inhibited the formation and/or the activity of some carcinogenic substances, such as nitrosamines. Ramirez-Victoria et al. (2001) demonstrated antimutagenic effect of pepper extracts against the nitrosation process using the somatic mutation and recombination test in wing cells of *Drosophila melonogaster*. Gonzales de Meja et al. (1998) evidenced in *Salmolella typhimurium* the antimutagenic action of carotenoids extracts from five different types of pepper against 1,6-dinitropyrene and 1,8-dinitropyrene. Moreover, other studies pointed out that the pepper and other fruits and vegetables (kiwi, garlic, onion, etc.) consumption could reduce the risk of brain tumour, probably due to their action on nitrosamines. In fact, Ikken et al. (1999) observed by the Ames test that the alcoholic extracts of pepper and other vegetables had an antimutagenic effect against different nitrosamines. These clear biological actions are probably due to some antioxidant and antiradical substances contained in pepper, such as vitamin C, carotenoids and polyphenols, that could explain the above cited findings.

ONION. The onion (*Allium cepa* L.) belongs to the Liliaceae or Alliaceae family, including long-day and short-day varieties, with different chemical and nutraceutical characteristics. Among them, there are some cultivars with red coloured bulbs such as “Rossa di Tropea”, “Rossa Acquaviva delle Fonti”, and “Rosa and Rossa di Suasa”. Many therapeutic properties have been attributed to the onions, as confirmed by literature, where the following actions has been detected, such as antibacterial, antiparasitic, antifungal, antiplatelet, hypoglycemic, anti-tumour action and anti-asthmatic effects (Fukushima et al., 1997). The various beneficial actions against certain human diseases can be attributed to its particular composition. In fact, in addition to water (89%), protein (1.5%), vitamin B1, B2 and C, soluble sugars (glucose, fructose, sucrose) (Ensminger et al., 1986), in onion there are anthocyanins (for red onions) and organsulfur compounds, substances with anticancer activity (M. Blumenthal et al. 2000).

Particular attention should be given to the sulphur substances responsible for the odour and the lachrymatory effect, which are released after cutting the bulbous cells. The rupture of cells causes the release of allinase enzymes that hydrolyze the S- alkenyl cysteine sulfoxides in pyruvic acid, ammonia and sulphur volatile substance. The allinase enzymes are contained in the vacuoles, while the alkenyl cysteine sulfoxides are present in the cells cytoplasm (Lancaster et al., 1981). In onions, 3 sulfoxides have been found: methyl, propyl and 1-propenyl -cysteine sulfoxides. The 1-propenyl-sulfoxide is the predominant (Randle et al., 1995). Its enzymatic hydrolysis leads to the formation of thiopropanal S-oxide, also called lachrymatory factor (Lancaster et al., 1981) since releases small quantities of sulfuric acid (Ensminger et al., 1998) in the mucous membranes of the eyes, with copious production of tears. The thiopropanal S-oxide is unstable and it degrades, in the vegetable tissues, to saturated and unsaturated organ-sulphur compounds. The saturated organ-sulphur compounds are dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide, propylbuthyl disulfide, dipropyl trisulfide etc., while the usaturated organ-sulphur compounds are E and Z-propenyl methyl disulfide, E and Z propenyl propyl disulfide, E and Z propenyl propyl trisulfide, diallyl disulfide, allyl methyl trisulfide etc. (Block, 1992). These components seem to have anti-tumour activities. Indeed, Munday et al (2001) have suggested that this effect is evident for the sulphur compounds of the onion and is expressed through the induction of detoxification enzymes of phase II and in particular the quinone reductase and glutathione transferase, in rat tissues. The authors tested the activity of enzyme induction of phase II of the propyl sulfide, dipropyl disulfide, allyl sulfide and allyl disulfide, and found a high inductive capacity of the enzymes of phase II for sulfides containing the allyl group and less for the other groups. In another study, Sparnins et al (1988) have studied 8 sulfides (allyl methyl trisulfide, allyl methyl disulfide, diallyl trisulfide, diallyl sulfide and 4 corresponding sulfides saturated) contained in garlic and onion, in particular for their ability to inhibit neoplastic effect of benzopyrene in the stomach and tongue of mice and the activation of the enzyme glutathione transferase of phase II detoxification. They observed that 4 sulfides with allyl groups induced the increase of glutathione transferases in the stomach, tongue and liver. The saturated sulfides showed a less evident inhibitory effects. Fukushima et al. (1997) demonstrated as these unsaturated organ-sulfur compounds can be considered the potential inhibitors of colon and renal carcinogenesis in rats. Galeone et al. (2006) showed an inverse association between the frequency of use of *Allium* vegetables and risks of common cancers in southern European populations.

Onion contains other nutraceutical substances as vitamin C and polyphenols, in particular anthocyanins and quercetins. The anthocyanins are constituted by a glycon (sugar) and aglycon, called anthocyanidins. In nature there are different types of anthocyanidins, including the six most important: pelargonidin, cyanidin, delphinidin, penidin, petunidin and malvidin. However more than 400 anthocyanins have been identified. Anthocyanins, although not essential for human nutrition, have a positive effect against the human disease, thanks to their strong antioxidant activities, due to their scavenger action vs. free radicals. In addition, they have well-known properties against capillary fragility, anti-inflammatory, anti-platelet and anti-edema action and antitumor effects (Doll, 1990). Nevertheless, anthocyanins have been shown to have some positive therapeutic effects including in the treatment of diabetic retinopathy (Scharrer and Ober, 1981), in fibrocystic disease of the breast in human (Leonardi, 1993), and on vision (Politzer, 1977).

Quercetin is a strong antioxidant because it can chelate metals, scavenge oxygen free radicals (Morel et al., 1993; Hanasaki et al., 1994) and prevent the oxidation of low density lipoprotein (LDL) in vitro (Janisch et al., 2004). Since oxidized LDL is hypothesized to be an intermediate in the formation of atherosclerotic plaques, quercetin might therefore contribute to the prevention of atherosclerosis (Juzwiak et al. 2005). An evaluation of the role of dietary quercetin as a protective antioxidant in human is hampered by uncertainties about its absorption. A part of quercetins is present in foods as flavonoid-sugar compounds, named β-glycosides, that are resistant to hydrolysis by pancreatic enzymes and important for the intestinal absorption.

WATERMELON. The watermelon flesh (*Citrullus lanatus* (thunb.) Mansf.), contains 92 % water, with small amounts of protein (0.4%) and fat (0.2%), carbohydrates (7 g/100 g f.w.), fiber (0.2 g/100 g f.w.), minerals such as Ca (7 mg/100 g f.w.), P (12 mg/100 g f.w.), Fe (0.2 mg/100 g f.w.), Na (3 mg/100 g f.w.), K (280 mg/100 g f.w.), vitamins, such as vitamin A (37 mg/100 g f.w.), thiamine (0.02 mg/100 g f.w.), riboflavin (0.02 mg/100 g f.w.), niacin (0.1 mg/100 g f.w.), and vitamin C (8 mg/100 g f.w.) (INRAN - Food composition database). Moreover, the watermelon is also appreciated for the presence in its flesh of volatile substances, giving a green-floral note characteristic of these fruits, and lycopene, a carotenoid found in red watermelon flesh. The most important watermelon volatile components are saturated and unsaturated aldehydes and alcohols with 9 carbon atoms, deriving from the linoleic acid degradation during the maturation of the fruit. Some authors (Beaulieau et al., 2006) have found that 2,6(E,Z)- nonadienal, 3(E)- nonen-1-ol, 2(E)-nonenal and 6(Z)-nonenal are the most abundant compounds of the characteristic watermelon flavour. Some others (Lewinsohn et al., 2005) have found other volatile compounds as geranial, neral, 6-methyl-5-epten-2-one, 2,6-dimethyl-5-heptenal, 2,3-epoxygeranial, (E, E)pseudoionone, geranyl acetone and farnesyl acetone, seemingly deriving from lycopene and other noncyclic tetraterpenoids. In the evaluation of watermelon quality it should be also taken into account sugars and organic acids, that affect the taste of the fruit.

An high nutraceutical capacity has been recognized to watermelon, for the presence in its flesh of lycopene, which characterizes the colour and seems to make an enormous contribution in the treatment of certain human diseases. Its average content in the red flesh is 4.1 mg/100 g f.w., comparable with that of fresh tomatoes of 3.1 mg/100 g f.w., grapefruit 3.36 mg/100 g f.w., and guava 5.4 mg/100 g f.w. (Leffingwell JC Lycopene - The Ultimate Phytochemical Nutraceutical). Among the known carotenoids, lycopene has the highest capacity for quenching singlet oxygen “in vitro”.

Perkins-Veazie al. (2001) have studied the lycopene content in 13 different varieties of watermelon with red flesh. The researchers used a colorimeter to measure the colour visible in each slice of watermelon and compared the optical values with the amount of lycopene extracted from vegetables. The lycopene content varied greatly among the different cultivars examined, with a mean value of 6.5 mg/100 g f,w, and decreases up to 5.0 mg/100 g f.w. in seedless watermelons. The results also showed that the lycopene in watermelon had a greater or equal quantity than that of the fresh tomato and that the quantities contained in fruits depended both on variety and cultivation techniques. In watermelon flesh, it is also present Vitamin C, whose effects are described for cauliflower and red pepper.

In watermelon is also present L-citrulline, that is a non-essential amino acid firstly identified from the juice and rind of watermelon, *Citrullus vulgaris* Schrad. and in other cucurbitaceous fruits, including bitter melon, cucumber, muskmelon, pumpkin, bottle gourd, dishrag gourd and wax gourd (Rimando and Perkins-Veazie, 2005). Citrulline is a co-product of nitric oxide generated from the oxidation of arginine catalyzed by nitric oxide synthase. Nitric oxide (NO) functions as a cellular messenger in the cardiovascular system and is a pivotal vasoprotection molecule. However, a study found that citrulline significantly attenuates cardiac contractile dysfunction in the isolated perfused rat heart subjected to ischemia/reperfusion via non-NO-mediated mechanism (Ikeda et al., 2000). Citrulline is also an efficient hydroxyl radical scavenger and a strong antioxidant (Akashi et al. 2001). Dietary supplements containing citrulline have been used to improve sexual stamina and erectile function; however, the mode of action for this activity is still unknown (Drewes et al., 2003). Watermelon is the richest known source of citrulline, and it is thought that this amino acid plays an important role in drought tolerance (Kawasaki et al, 2000). As concern the content of L-citrulline in watermelon, Rimando et al (2005) found that in red, yellow and orange watermelon this aminoacid ranged between 7.9 and 28.5 mg/g d.m., while in the rind the values ranged between 15.6 and 28.2 mg/g d.m.

In the food industry the use of watermelon is relegated only to the flesh preparation, as well as the fresh-cut products (slices and cubes packed in modified atmosphere or chilled), including concentrated and frozen juices, concentrated puree, etc. For the rind, instead, no technological applications were observed. It would be interesting to prepare fruits-products added with watermelon rind as functional foods.

## 2.2 - INFLUENCE OF ORGANIC FARMING ON VEGETABLES COMPOSITION

At the beginning of the soil conversion, the organic farming, since chemical fertilizers and pesticides are not utilized, apparently reduces the production yields. On the contrary, it seems to bring out some macro and micronutrient of vegetables, important for their benefits on the plant and human health, as reported in paragraph 2.1. In this paragraph, a brief summary of the available literature about this topic will be reported.

As reported in the critical review, carried out by Lairon (2009), the number of studies dedicated to vitamin contents is limited to some vegetables. Regarding water soluble vitamins, the most studied one has been Vitamin C (ascorbic acid). Studies performed on potato, tomato, celeriac and kale, showed higher vitamin C levels in organically-grown products. In contrast, no difference was found during studies in leek, carrot or beetroot and apple. As concern the fat-soluble vitamin and carotenoid contents, an higher vitamin E level in organic olive oil has been found. A positive relationship between N-fertilization in organic system and β-carotene levels in carrots has been found. A study on organic vs. conventional tomatoes, instead, showed higher contents of β-carotene in organic ones. Besides, vegetables contain a large variety of microcompounds which are secondary metabolites in plants, such as polyphenols, resveratrol and some non-pro-vitaminic carotenoids. These compounds seems to have drastic regulatory effects at cellular level and are, thus, involved in prevention of certain diseases such as cancers, chronic inflammation and other pathologies. Some of them are phytochemicals which are produced in plants as a response to external stress such as fungal disease. Since several factors can modulate their plant levels such as cultivar, maturity, light or temperature, some studies compared the levels of some of these phyto-microcompounds in vegetables depending on the cropping system. For phenols and polyphenols, many studies showed higher levels in organic foodstuffs such as apple, peach, pear, potatoes, onion, tomato, pepper, orange and olive oil, while some others did not show any difference. Moreover, organic plant foods overall contain double the amount of phenolic compounds. Organically grown tomatoes have an higher salicylic acid content than conventional ones. It is noteworthy that salicylic acid is the active anti-inflammatory compound of aspirin. This critical literature review indicated that organic agriculture has the potential to produce high quality products with some relevant improvements in terms of contents of anti-oxidant phyto-micronutrients, but higher nitrate accumulation in vegetables and toxic phytochemical residue levels.

Similarly, a second review (Zhao et al. 2006), reported the results of papers concerning the enhancement of phytochemicals content in organic vegetables. Some papers reported that organically grown onion (*Allium cepa*), green pepper, and leafy greens showed higher quercetin concentration, antimutagenic and antioxidant activities, compared with conventional crops from a nearby farm. Another study reported that, on a fresh weight basis, organic tomato contained higher lycopene, β-carotene, rutin, and naringenin than conventional tomato; whereas on a dry weight basis, only β-carotene and rutin were significantly higher in organic tomato. When tomato purees were analyzed, production method did not affect the carotenoid content, but polyphenols were increased by organic cultivation. Organic tronchuda cabbage (*B. oleracea* Tronchuda group) tended to have higher total phenolic content in leaves than conventional, while no differences between organic and conventional lettuce, collards (*B. oleracea* Acephala group), and pac choi (*B. rapa* Chinensis group) for most polyphenols and phenolic acids evaluated were found.

More recently, Halmann (2012) compared the effects of organic and conventional production systems, in two growing seasons, on chemical properties and phenolic compounds of two tomato types (standard and cherry). The results obtained have shown that, in both years, the two types of organic tomatoes presented an higher ratio of reducing sugars/organic acids, and contained significantly more total sugars, vitamin C and total flavonoids, in comparison with the conventional fruits. Hallmann and Rembialkowska (2012) found that organic growing increased the level of antioxidant compounds such as carotenoids, phenolic compounds and vitamin C in sweet bell pepper. There are limited information on how watermelon varieties perform when grown with organic practices. Davis et al. (2006) found that high-input production methods (black plastic and mechanical cultivation for weed control) almost doubled the number of fruit produced, increasing the yields, and heavier average fruit weights, but lower °Brix and lycopene content compared to the low-input production method (no-till).

Even at CRA-IAA (Milano) some experiences about the comparison of quality parameters between organic and conventional vegetables were carried out. Two genotypes (Emeraude and Magnifico) of a green typology cauliflower under conventional or organic management were compared. In addition, three fertilization regimes were considered within the framework of organic agriculture. The two genotypes showed a contrasting response to organic practices: the phytochemicals content of Emeraude was generally reduced, while in Magnifico, most of the quality parameters were unaffected or slightly increased. Moreover, under organic management, the use of higher fertilization levels significantly increased the phytochemicals production of Magnifico, in particular ascorbic acid and polyphenols. However, the same fertigation treatments decreased the phytochemicals production of Emeraude, particularly glucosinolates and ascorbic acid. The genotype was identified as a key factor in the determination of cauliflower quality, under different management practices. Furthermore, our results indicated that the addition of fertilizers to organic soil might be effective only with a cauliflower genotype suited to organic agriculture (Picchi et al., 2012).

In another study (Migliori et al. 2010) carried out at CRA-IAA, the effects of organic and conventional agriculture on the chemical (volatile substances, soluble sugars, organic acids, etc.) and nutraceutical (glucosinolates, vitamin C and lycopene) quality in some vegetables were investigated. “Verde di Macerata” cauliflower (F1 hybrid Emeraude and Magnifico), winter melon (F1 hybrid Helios and Purceddu variety) and tomato (unripe and ripe Giulianova variety, F1 hybrid Bigred and Aurea) were utilized. For cauliflower, only in the Magnifico genotype, the organic method increased the content of sulphur volatile substances, glucosinolates and vitamin C, confirming the previous results (Picchi et al. 2012). For winter melon, the organic agriculture had a positive effect on the characteristic volatile compounds in Purceddu variety, while on the content of soluble sugars in both genotypes. The nutraceutical properties, expressed as vitamin C, were higher for Helios genotype. For tomato, in the unripe and ripe Giulianova variety, the organic cultivation increased the content of volatile characteristic compounds; while in the unripe tomato, both treatments enhanced the content of soluble sugars and organic acids. The increase of volatile substances was observed only in the conventional unripe Bigred and Aurea hybrids. The soluble sugars, in the Bigred hybrid, increased in the organic system and organic acids in the conventional system. The soluble sugars presented the same values in organic and conventional Aurea hybrid; while the organic acids were higher in the conventional agriculture. Lycopene increased only in the ripe organic Giulianova.

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## 3- AIM OF THE THESIS

The aim of the present doctoral thesis, in the context of the project VAL.OR.BIO., was to evaluate the influence of organic (OR) and conventional (CONV) cultivation on chemical and nutraceutical parameters in different genotypes of vegetables, pointing out the evaluations on local genotypes, particularly linked to a determined territory. The research was performed in the three years of experimentation (2010-2012), analyzing commercial hybrids and local varieties of vegetable, widely consumed in Italy and important for their high healthy potential. Moreover, during the third year of experimentation, some technological trials as cooking (grilling on plate in a gas powered domestic kitchen) and preparation of functional food (apple jellies added with powdered watermelon rind) were carried out.

# 4 - MATERIALS AND METHODS

## 4.1 - PLANT SOURCE

The genotypes of cauliflower, tomato, onion and sweet red pepper, cultivated both with conventional and organic methods, were grown in the experimental fields of the CRA -ORA Monsampolo del Tronto (AP), while watermelons were grown in organic fields of CRPV, sited in Cesena (FC), as reported in tab.2.

| VEGETABLES | GENOTYPES | | CULTURAL METHODS | | TECHNOLOGICAL TREATMENTS |
|---|---|---|---|---|---|
| | Local varieties | Commercial hybrids | OR | CONV | |
| **Cauliflower** | Velox, Jesino | Emeraude | + | + | |
| **Tomato** | Giulianova (Unripe and Ripe) | Perbruzzo (Unripe and Ripe) | + | + | |
| **Sweet red pepper** | RS08 | Alceste | + | + | Cooking (grilling) |
| **Onion** | Suasa, Precoce di Romagna, Tropea | Density | + | | |
| **Watermelon** | Bagnacavallo, Faenza | Monaco, Talete | + | | Preparation of apple jellies with watermelon rind (L-citrulline dietary supplements). |

**Tab. 2:** List of genotypes, cultural methods and technological treatments of each species tested in the experiment.

## 4.2 - CULTURAL PRACTICES

The CONV system was performed according to the best available agricultural techniques and the OR method according to the current regulation (EU Council Regulation 2007/834; EU Commission Regulation 2008/889).

The two systems were allocate in neighbouring fields of about 2.200 $m^2$ each and opportunely separated to avoid any cross contamination effect between conventional and organic management. Each field (system) was split in four rotational areas of equal size that, according to the length of the cropping cycle of the crops, were able to host the whole rotation (each crop is present every year).

A 4-year crop rotation based on 6 main crops was established in the conventional and in the organic system, as concern tomato and cauliflower, while sweet pepper, onion and watermelon were not included in any crop rotations. The rotation was: tomato (*Lycopersicon esculentum* Mill.), melon (*Cucumis melo* L.), fennel (*Foeniculum vulgare* M. var. *azoricum*), lettuce (*Lactuca sativa* L.), cauliflower (*Brassica oleracea* L. var. *botrytis*) and bean (*Phaseolus vulgaris* L.). In the organic system, three different green manures were included in the rotation: hairy vetch (*Vicia villosa* R.), cropped before the tomato, barley (*Hordeum vulgare* L.), cropped before the melon, and radish (*Raphanus sativus* L.), cropped before the lettuce.

In fig. 2 and 3 an examples of rotational crops, involving cauliflower and tomato, were respectively reported.

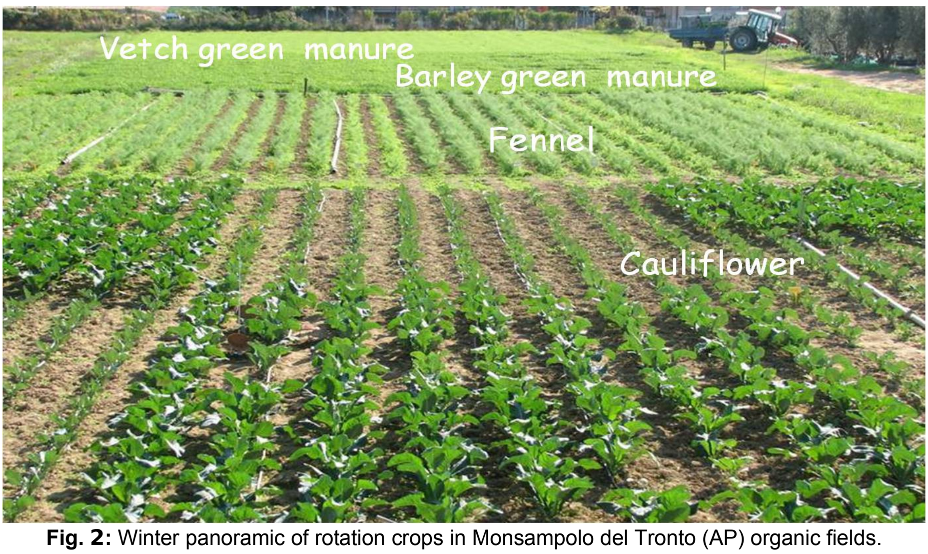


**Fig. 2:** Winter panoramic of rotation crops in Monsampolo del Tronto (AP) organic fields.

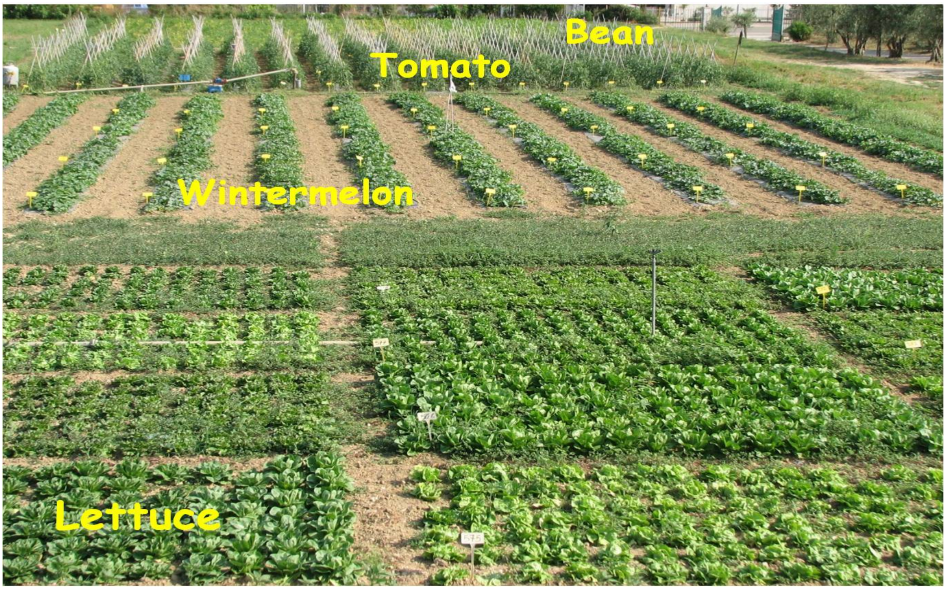


**Fig. 3:** Summer panoramic of rotation crops in Monsampolo del Tronto (AP) organic fields.

All the samples, harvested at commercial maturity, were immediately refrigerated (4-6 °C) and send to the CRA –IAA labs in Milano. The samples were immediately randomized, to create an homogeneous and representative sample for each genotype and cultivation method. They were quickly frozen at -80 °C and, then, freeze-dried, by a CHRIST lyophilizer (Germany). The samples subjected to the evaluation of the volatile components were analyzed as freeze materials.

## 4.3 - TECNOLOGICAL TREATMENTS

### 4.3.1. - PREPARATION OF APPLE-JELLIES WITH WATERMELON RIND

To 890 g of sliced apples, 600 g of water, lemon juice and half a lemon were added. The mixture was boiled for 13-14 min and then homogenized. In the obtained puree, approximately 52 g of powdered Talete rind and 700 g of sucrose were dispersed. After mixing, the product was concentrated up to 55-60 °Brix, by cooking. The concentrate was put into an aluminum container covered with baking paper, forming a film thickness of about 1 cm layer, and then dried for 2-3 days at 70°C in a air-dryer. The dried product was cut into cubes and covered with sugar to eliminate stickiness. The flow sheet is reported in Fig. 4.

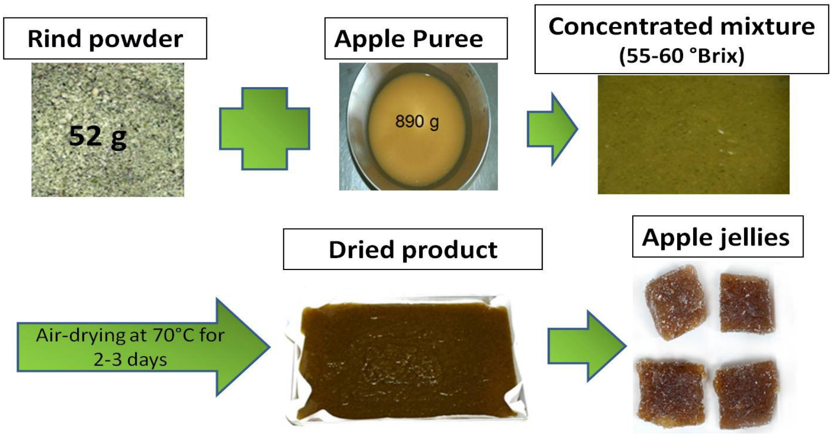


**Fig. 4:** Preparation of apple jellies with powdered watermelon rind formulation.

## 4.3.2 - PREPARATION OF GRILLED SWEET RED PEPPER

The OR Alceste genotype was submitted to an home cooking on plate, in a gas powered domestic kitchen for 25 min (Fig. 5).

**Fig. 5:** Raw and grilled OR Alceste sweet pepper.

## 4.4 – DETERMINATION OF QUALITY PARAMETERS IN THE EXAMINED VEGETABLES

### 4.4.1 - CAULIFLOWER

Characteristic volatile compounds: the volatile compounds were extracted and concentrated by microwave/resin/solvent and the obtained extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) according to Di Cesare et al. (1994). To 300 g of frozen sample, 300 ml of distilled water were added and the dispersion was homogenized by an an Ultra-Turrax (IKA Laboratories, Staufen, Germany) for 3–5 min. Each preparation was placed in a 1 L glass container equipped with two small tubes: one connected to an $N_2$ cylinder and the other to a glass column packed with 25 ml of Kastel S-112 apolar resin (Montedison, Mantova, Italy). The container was placed in a microwave oven operating at 440 W for 40 min. The volatile compounds extracted were continuously removed by an $N_2$ flux and adsorbed in the resin. The volatile compounds were eluted from the resin with 150 ml of pure ethyl ether, which was then dehydrated with anhydrous $Na_2SO_4$ and finally removed in a Kuderna-Danish (Steroglass, Perugia, Italy) evaporator to recover the pure essential oil. Volatiles were analyzed using an Agilent (Agilent Technologies, Palo Alto, CA, USA) GC-MS system (GC 6890N, MS 5973N) with a DB-1 capillary column (60 m ×0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The GC operating conditions were as follows: column temperature 50 °C isothermal for 5 min, then increased to 240 °C at a rate of 2 °C $min^{-1}$ and finally 240 °C isothermal for 20 min; carrier gas helium at a flow rate 0.9 ml $min^{-1}$; injector temperature 200 °C; injected volume 1 μl; split ratio 20 ml $min^{-1}$. The temperature of the transfer line was 240 °C.

The MS operating conditions were: ionization voltage 70 eV; ion source temperature 230 °C; mass range 10–400 amu. Components were identified by retention times of chromatographic peaks, by comparing their mass spectra with those in a commercial library (Wiley 7 n. 1 Library, Mass Spectral Data Base, Hewlett-Packard, Vienna, Austria) and by using commercial standards, when available.

The cauliflower volatile compounds were divided into three main classes, according to their functional groups: (i) isothiocyanates (allyl-NCS, propyl-3-(methylthio)-NCS, butyl-4-(methylthio)-NCS, 2-phenylethyl-NCS); (ii) nitriles (butanenitrile-4-(methylthio), pentanenitrile-5-(methylthio), benzenepropanenitrile); and (iii) sulfides (dimethyl trisulfide, dimethyl disulfide, methyl-(methylthio)-methyl disulfide, dimethyl tetrasulfide).

Quantitative determination of the components was achieved using pure reference compounds for allyl-NCS, propyl-3-(methylthio)-NCS, dimethyl trisulfide, and dimethyl disulfide (Sigma-Aldrich, Milan, Italy). For quantitative analysis, 20, 40 and 80 μl of each commercial standard were dispersed in 10 ml of pure ethyl ether. The other components were quantified using methyl palmitate as an external standard (20, 40 and 80 μl in 10 ml of pure ethyl ether). The data are given as μg/g dry matter (d.m.).

<u>Glucosinolates</u>: For analysis of the glucosinolate content, 1.5 g of lyophilized powder was extracted with 45 ml of boiling MeOH for 15 min, using a reflux condenser. (ISO. 1992. Rapeseed- Determination of

glucosinolate content- Part 1: Method using High Performance Liquid Chromatography. ISO 9167-1:1-9). The mixture was centrifuged at 8000g for 10 min, and the solid residue was re-extracted with 30 ml of a boiling MeOH/water solution (70:30). The collected supernatants were concentrated to dryness in a rotavapor under vacuum, and the residue was added to deionized water up to a final volume of 25 ml. Desulfoglucosinolates were prepared and quantitatively determined by HPLC using purified Helix pomatia sulfatase (Wathelet et al., 1999). The quantification was based on a standard calibration curve using sinigrin as an external standard. The concentration of individual glucosinolates was expressed in micromole of sinigrin equivalents per gram of dry matter (μmol/g d.m.). The results are given as the total amount of identified glucosinolates, the sum of aliphatic glucosinolates (glucoiberin, progoitrin, epiprogoitrin, sinigrin, glucoraphanin, glucoalyssin, and glucobrassicanapin), and the sum of indolic glucosinolates (glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin).
Ascorbic Acid: 30 mg of lyophilized powder were extracted with 1 ml of 3% metaphosphoric acid, homogenized, centrifuged at 25000 rpm for 5 min at 4 °C, and immediately analyzed. The ascorbic acid content was determined by HPLC using an Intersil ODS-3 analytical column (250 × 6 mm i.d.) that was maintained at 30 °C. Isocratic elution was performed using a mobile phase of 0.02 M orthophosphoric acid with a flow rate of 0.7 ml/min; 20 μl samples were injected and monitored at 254 nm, and the retention time was 7.8 min. The concentration of ascorbic acid was calculated from the experimental peak area by analytical interpolation, using a standard calibration curve and was expressed as mg/100 g d.m. (Lo Scalzo et al. 2007).
Total polyphenols: Total polyphenols were spectrophotometrically determined by the Folin-Ciocalteu assay (Slinkard and Singleton, 1977) at 730 nm, according to Heimler et al. (2006), starting from 30 mg of lyophilized powder. Total phenols were estimated by comparison with the standard curve obtained with gallic acid. Results were expressed as gallic acid equivalents on mg of fresh weight (GAE/100g f.w.).
Total carotenoids: their content was spectrophotometrically determined at 436 nm, starting from 30 mg of lyophilized powder and estimated using the molar extinction coefficient of lutein (141000) (Goodwin, 1955). Results were expressed as lutein equivalents (LE/100 g f.w.).
Antioxidant capacity: The antioxidant capacity was measured using 1,1-diphenyl-2- picrylhydrazil (DPPH·) quenching radical and scavenging, according to Brand-Williams, et al. (1995) and Valavanidis et al. (2004),

respectively, introducing some modifications. All measurements were made by Electronic Paramagnetic Resonance (EPR) using a MiniScope MS200 Magnettech (Berlin, Germany). For sample preparation, 30 mg of lyophilized powder were extracted with 1 ml of a 1:1 mixture of EtOH and 0.02 N HCl, homogenized and centrifuged at 25000 rpm for 5 min at 4 °C. DPPH·is a commercially available free radical compound, which is soluble and stable in ethanol. The blank solution contained 0.3 ml methanol, 0.1 ml extracting solution and 0.1 ml of a 0.5 mM DPPH ethanolic solution. The test solution was prepared as above, substituting the 0.1 ml of extracting solution with the equivalent volume of a 10-fold diluted cauliflower extract. These solutions were accurately mixed in a glass tube assay and successively placed in a 50 μl capillary for EPR measurements. The experimental settings of the spectrometer were as follows: field set, 3350 G; scan range, 50 G; scan time, 120 s; modulation amplitude, 2000 mG; microwave attenuation, 7 dB; and receiver gain, 7 x 10. EPR spectra were recorded after 1 min of reaction at 25 °C.
Dry matter: Dry matter content was determined by heating 5 g of sample material in a laboratory oven at 85 °C to a constant weight (AOAC, 1980. AOAC. In: (13th edn. ed.),Official Methods of Analysis, Association of Official Analytical Chemists, Washington DC (1980) No. 22.018-46.024-22.061).

**4.4.2 - TOMATO**

Characteristic volatile compounds: they were extracted, concentrated and analyzed as described for cauliflower. For quantitative analysis, three standard solutions (20, 40 and 80 μl in 10 ml of diethyl ether) of the characteristic volatile compounds, responsible of the fresh tomato flavour (hexanal, 2(*E*)-hexenal and 2-isobutylthiazole), whose standards were commercially available, were prepared and used to calculate the response factors.
Soluble sugars: Soluble sugars were extracted from 10 g of fresh homogenized sample with 100 ml of distilled water and separated by centrifugation at 10000 rpm for 10 min. The supernatant was filtered (0.45 μm nylon filter) and analyzed by high-performance liquid chromatography (HPLC), under the conditions described by Forni et al. (1992).
Lycopene: it was extracted under controlled conditions (in darkness at 0–1 °C to avoid sample decomposition) from 10 g of frozen homogenized sample using 100 ml of hexane/acetone/ethanol (2:1:1 v/v/v) solution, supplemented with 1 mg $ml^{-1}$ butylated hidroxytoluene (BHT), as described

by Shi et al. (1999). The mixture was separated by centrifugation at 5000 rpm for 10 min at low temperature. As the method followed made only one extraction, we validated it by some laboratory trials with subsequent extractions, also checking the colour of the pellet, which remained uncoloured after the first solvent treatment. Chromatograms of the second and subsequent extracts gave no significant peaks. The clean organic layer was filtered (0.45 μm regenerated cellulose filter) and analyzed by HPLC using the method carried out in our laboratory as described by Migliori et al. (2008). The all-*trans* and *cis* (sum of 3, 5, 9, 13 and 15-*cis* forms) isomers of lycopene were identified and quantified on the basis of both a commercial lycopene standard (Sigma-Aldrich,www.sigmaaldrich. com) and the elution profile analysis used in a previous research (Shi et al., 1999).

Ascorbic acid: it was extracted and determined as described for cauliflower.

pH: the measure was performed using a pH-meter.

Dry matter: it was determined as described for cauliflower.

### 4.4.3 - SWEET PEPPER

Characteristic volatile compounds: they were extracted, concentrated and analyzed as described for cauliflower. For quantitative analysis, three standard solutions (20, 40 and 80 μl in 10ml of diethyl ether) of the characteristic volatile compounds (2-methoxy-2-isopropylpirazine and 2-methoxy-2-isobutylpirazine), whose standards were commercially available, were prepared and used to calculate the two response factors.

Ascorbic acid: it was extracted and determined as described for cauliflower.

Total polyphenols and carotenoids: they were determined as described for cauliflower, using gallic acid and β-carotene, respectively, for building up the calibration curves.

Antioxidant capacity: it was determined as described in cauliflower.

Dry matter: it was determined as described for cauliflower.

Soluble sugars: They were determined as for tomato.

### 4.4.4 - ONION

Characteristic volatile compounds: they were extracted, concentrated and analyzed as described for cauliflower. The quantitative analysis was performed using three standard solutions at various degrees of dilution containing dimethyl disulfide, dimethyl trisulfide, methylpropyl disulfide and propylallyl disulfide (20, 40 and 80 μl of each standard in 10 ml of diethyl ether). The volatile components, whose commercial standards were not available, were quantified by the external standard procedure (10, 30 and 60 mg of methyl palmitate adjusted to 10 ml with diethyl ether).

Soluble sugars: They were determined as for tomato.

Ascorbic acid: it was extracted and determined as described for cauliflower.

Total polyphenols: Total polyphenols were determined as described for cauliflower, using gallic acid for building up the calibration curves.

Individual classes of polyphenols: in onion polyphenols are represented by quercetin, anthocyanines (in red types) and other phenolic substances belonging to this family.

Quercetin: 300 mg of freeze-dried samples and 10 ml of MeOH/HCl 0.02N (1:1 v / v), were stirred at room temperature for 2 hours. The mixture was centrifuged a 20.000 rpm for 10 min and the supernatants, filtered through 0.45 um filter, were used for HPLC analysis. The HPLC separation, with DAD detection, was performed on a column Hypersil ODS-3, 250 × 4 mm, flow 0.7 ml/min at 45 °C, eluent A sol 5% acetic acid in $H_2O$, B sol 5% acetic acid in MeOH. The gradient elution was performed from 95% A up to 10 min, then decreasing up to 50% after 30 min, 10 min at 50% and then regenerating the initial conditions in 10 min.

The results, expressed as total quercetin, were obtained by the dosage of the three main quercetin eluted (quercetin-4'-glucoside, quercetin-3-4'-diglucoside, quercetin-7-glucoside) and identified by comparison with both literature data (Slimestad et al., 2007) and with those of a commercial quercetin standard.

Total anthocyanins. These compounds were extracted from freeze-dried samples by MeOH+ HCl, and spectrophotometrically analyzed at λ=528 nm, according to Swain e Hills (1959).

Piruvic acid (pungenzy): this assay was carried out by HPLC on an aqueous extract of fresh onion. The extract was left 5 min at room temperature and then acidified with 4 mN $H_2SO_4$, according to Yoo and Pike (2001). The HPLC isocratic separation was performed on column Alltech IOA-1000 300×7.8 mm, flow 0.65 ml/min at 80 °C, using 4 mN $H_2SO_4$ as the eluent. Pyruvic acid was determined by the external standard method, using a calibration curve with a commercial standard. The evaluation of pungency was performed according to the scale proposed by Dhumal et al (2007).

Antioxidant capacity: it was determined as described for cauliflower.

Dry matter: it was determined as described for cauliflower.

#### 4.4.5 - WATERMELON

Characteristic volatile compounds: they were extracted, concentrated and determined as described for cauliflower. For the volatile components, whose commercial standard were available, have been set up three standard solutions containing respectively 20, 40 and 80 µl of 2(E)-nonenal, 2,6(E,Z)-nonadienal, 2,6(E,Z)-nonadien-1-ol, 3(E)-nonen-1-ol, 2(E)-nonen-1-ol and 1-nonanol, diluted to 10 ml with ethyl ether.

Soluble sugars: They were determined as for tomato.

Lycopene: it was extracted and determined as described for tomato.

Ascorbic acid: it was extracted and determined as described for cauliflower.

Microbiological analysis methods: these analyses were performed in CRA-FLC (Lodi) laboratory, by dr. Domenico Carminati.

10 g of sterile weighted sample, were added with 90 ml of sterile diluents Buffered Peptone Water ($10^{-1}$ dilution) (tab. 3) and homogenized with Stomacher homogenizer. Decimal dilutions up to $10^{-5}$ were set up (ISO 6887/1: 1999 - Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions).

| BUFFERED PEPTONE WATER | |
|---|---|
| **Composition** | **Amount** (g/l) |
| **Peptone** | 10.0 g |
| **Sodium chloride** | 5.0 g |
| **Sodium phosphate** | 3.5 g |
| **Potassium phosphate** | 1.5 g |
| **Distilled water** | Up to 1000 ml |

**Tab. 3**: Preparation of Buffered Peptone Water (OXOID product, cod. CM 509).

Total Aerobic spore-forming: sample homogenates (all decimal dilution) were treated at 80 °C for 15 min and 0.1 ml of homogenate samples were inoculate on the surface of TSA medium (Tryptone Soya Agar) (Tab. 4), previously solidified and dried under a laminar flow hood. The plates were aerobically incubated at 37 °C for 48 hours (Galli and Franzetti 2003).

| TRYPTONE SOYA AGAR (TSA) | |
|---|---|
| **Composition** | **Amount** (g/l) |
| **Pancreatic digest of casein** | 15.0 |
| **Enzymatic digest of soya bean** | 5.0 |
| **Sodium chloride** | 5.0 |
| **Agar** | 15.0 |
| **Distilled water** | Up to 1000 ml |

**Tab. 4:** Preparation of TRYPTONE SOYA AGAR (TSA). (Commercial medium – OXOID, cod. CM0131).

Total anaerobes spore-forming: sample homogenates (all decimal dilution) were treated at 80 °C for 15 min and 0.1 ml of homogenate samples were inoculate on the surface of the RCM (Reinforced Clostridial Medium) (Tab. 5) medium previously solidified and dried under a laminar flow hood. The plates were incubate in anaerobic jars at 37 °C for 72 hours (Galli and

Franzetti 2003).

| REINFORCED CLOSTRIDIAL MEDIUM AGAR (RCM) | |
|---|---|
| **COMPOSITION** | **Amount** (g/l) |
| **Yeast extract** | 3.0 |
| **`Lab-Lemco' powder** | 10.0 |
| **Peptone** | 10.0 |
| **Glucose** | 5.0 |
| **Soluble starch** | 1.0 |
| **Sodium chloride** | 5.0 |
| **Sodium acetate** | 3.0 |
| **Cysteine hydrochloride** | 0.5 |
| **Agar** | 15.0 |
| **Distilled water** | Up to 1000 ml |

**Tab. 5:** Preparation of Reinforced Clostridial Medium Agar (RCM) (Commercial Medium – OXOID, cod. CM0151).

Total bacterial count: 1 ml of homogenate samples (all decimal dilution) were pipetted in two Petri plate. In each plate, approximately 15 ml of agar medium (PCA - Plate Count Agar) (Tab. 6), previously melted and brought to 45 °C, were added. The upside down plates were aerobically incubated at 30 ± 1 °C for 72 hours (ISO 4833: 1991 - General guidance for the enumeration of micro-organisms - Colony count technique at 30 °C).

| PLATE COUNT AGAR (PCA) | |
|---|---|
| **COMPOSITION** | **Amount (g/l)** |
| Tryptone | 5.0 g |
| Yeast extract | 2.5 g |
| Glucose | 1.0 g |
| Agar | 10.0 g |
| Distilled water | Up to 1000 ml |

**Tab. 6:** Preparation of Plate Count Agar (PCA) Commercial Medium – OXOID, cod. CM325).

Eumycota (yeasts and molds): 1 ml of homogenate samples (all decimal dilution) were pipetted in two Petri plate. In each plate, approximately 15 ml of agar medium (YGC Agar) (Tab. 7), previously melted and brought to 45 ° C, were added. The upside down plates were aerobically incubated at 25 ± 1 °C for 5 days. A separate count of yeast and molds colonies was carried out (ISO 7954:1987 - General guidance for enumeration of yeasts and moulds -- Colony count technique at 25°C).

| YEAST EXTRACT GLUCOSE CHLORAMPHENICOL AGAR (YGC) | |
|---|---|
| **COMPOSITION** | **Amount (g/l)** |
| Yeast extract | 5.0 g |
| Glucose | 20.0 g |
| Chloramphenicol | 0.1 g |
| Agar | 15.0 g |
| Distilled water | Up to 1000 ml |

**Tab. 7**: Yeast Extract Glucose Chloramphenicol Agar (YGC) (Commercial Medium – MERCK, cod. 16000).

Alcohol insoluble solids (AIS): The AIS was gravimetrically measured after extraction and removal of water from the matrix, by treatment with boiling EtOH for 3 times and acetone. The residue was dried at room temperature and then weighted to determine the pectin percentage in the starting vegetable sample. This method was performed according to Lo Scalzo et al. (2005).

Texture: it was performed by the cut strength test, using a Stable Micro System TA XT II plus (England), fitted with a 5 kg cell and a Warner - Blatzer blade. The maximum force peaks were directly compared. Results are the means of 10 determinations.

Water activity ($a_w$): it was performed by an electronic hygrometer (Aqua Lab. CX-2 – Decagon Devices, Pullman, USA). Results are the means of 6 determinations.

L-citrulline: the citrulline content was determined, according to Rimando et al. (2005). 500 mg of freeze-dried sample were weighted in a 20 ml screw capped vial and 5 ml of 6 M HCl were added. The vials were placed in an sonicating bath for 20 min and $N_2$ gas was then bubbled through samples for 10 min and the samples were heated at 145 °C for 4 hours. Samples were cooled to room temperature, filtered and washed twice with 5 ml of 6M HCl. The filtrates were combined and 250 µl were dried in a speedvac. Dried extracts were then treated with 100 µl of DMF (N,N-dimethylformamide) and 100 µl MTBSTFA (*N*-Methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide), heated at 80 °C for 40 min and allowed to cool to room temperature. The derivatized samples were analyzed by GC/MS at the operative condition described for volatile compounds of all the examined vegetables. For quantitative analysis, 50 mg of L-citrulline were dispersed in 10 ml of 6 M HCl. 30, 60, 120 and 240 µl of the standard solution were dried in a speedvac. The residues were derivatized and analyzed as reported for the samples.

Dry matter: it was determined as described for cauliflower.

## 4.5 - STATISTICAL ANALYSIS

All determinations were performed in triplicate for each year, on sample divided in two uniform aliquot. So for each theses 18 values/determinations were obtained To evaluate the influence of two types of cultivation on nutraceutical and chemical parameters, in the first part of the thesis, the average values of the three years of experimentation for each genotypes of all species were considered. In the second part the average values for each species, year by year, were considered, for all the determined parameters.

The data were subjected to analysis of variance (ANOVA) and the comparison of the averages was determined by the Tukey test. Differences for $\rho \leq 0.05$ were considered significant. All statistical analyses were performed using Statistica v.6 (StatSoft Inc.). the analysis of the standard deviation was also carried out.

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# PART 1

# 5 - RESULTS AND DISCUSSION

## 5.1 – CAULIFLOWER

The biochemical evaluation of CONV and OR cauliflowers was focused, as concern chemical properties, on the characteristic volatile compounds, containing sulfur and nitrogen, that are responsible of the organoleptic impact. The nutraceutical quality, instead, was represented by glucosinolates, having a protective effect against various forms of human cancer, while vitamin C, polyphenols and carotenoids, are molecules with a significant antioxidant activity.

In Tab. 8 the characteristic volatile compounds identified in three cauliflower genotypes were reported. The characteristic volatile compounds were subdivided into three classes, according to the functional groups, and in particularly in isothiocyanates, sulfides and nitriles. The values of most of isothiocyanates in all the genotypes were not affected by the cultivation system, while the positive effect of the OR cultivation for allyl isothiocyanate (NCS) in Jesino e 4-methylthiobutyl NCS in Emeraude. As concern the nitriles compounds, the values of 4-(methylthio) butanenitrile were similar in OR and CONV samples in all examined genotypes. The positive effect of OR cultivation can be observed for the other nitriles in all the genotypes, except for Jesino, where the values of benzene propanenitrile were quite similar in both cultivation systems. The sulfides increased in the OR samples of the Emeraude and Velox genotypes. In the OR Jesino sample, only dimethyl disulfide increased.

| | CAULIFLOWER CHARACTERISTIC VOLATILE SUBSTANCES (µg/g d.m.) | | | | | |
|---|---|---|---|---|---|---|
| | EMERAUDE | | VELOX | | JESINO | |
| ISOTHIOCYANATES | CONV | OR | CONV | OR | CONV | OR |
| allyl NCS | 10.23±2.96 a | 10.54±1.26 a | - | - | 94.94±10.15 a | 127.38±30.16 b |
| 3-(methylthio) propyl NCS | 12.08±0.62 a | 12.82±2.59 a | 2.57±0.78 a | 2.07±0.14 a | 5.19±1.06 a | 5.23±0.78 a |
| 4-(methylthio) butyl NCS | 2.18±0.88 a | 3.34±0.56 b | 2.01±0.47 a | 2.63±0.23 a | 1.63±0.25 b | 0.64±0.09 a |
| 2-phenylethyl NCS | 1.61±0.15 a | 1.52±0.33 a | 2.58±0.40 b | 1.77±0.50 a | 5.18±1.08 a | 5.11±0.77 a |
| NITRILES | | | | | | |
| 4-(methylthio) butanenitrile | 58.55±5.36 a | 93.65±3.76 a | 26.84±5.45 a | 24.18±2.96 a | 32.25±5.26 a | 33.04± 3.88 a |
| 5-(metylthio) pentanenitrile | 11.90±2.56 a | 15.42±1.36 b | 4.23±1.53 a | 6.56±0.79 b | 0.94±0.18 a | 2.79±0.68 b |
| benzene propanenitrile | 0.97±0.48 a | 1.22±0.19 b | 1.47±0.53 a | 2.21±1.04 b | 1.70±0.88 a | 1.60±0.25 a |
| SULFIDES | | | | | | |
| dimethyl disulfide | 47.94±5.76 a | 87.54±11.39 b | 25.82±8.29 a | 44.52 ±13.17b | 33.33±6.28 b | 20.09±3.07 a |
| dimethyl trisulfide | 131.43±9.36 a | 168.09±27.96 b | 69.19±11.98 a | 111.97±19.37 b | 96.52±18.26 a | 106.21±25.68 b |
| methyl-(methylthio)-methyl disulfide | 3.97±1.12 a | 7.57±1.35 b | 3.81±0.26 a | 7.61±1.88 b | 6.68±1.78 a | 6.34±1.55 a |
| dimethyl tetrasulfide | 16.14±1.09 a | 22.68±5.77 b | 7.23±1.80 a | 13.83±2.21 b | 9.48±1.85 b | 8.42±1.73 a |

Tab. 8: Characteristic volatile compounds identified in CONV and OR cauliflower genotypes. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

Fig. 6, where the histograms of the three volatiles classes were reported, shows that the OR cultivation caused an average increase of the concentration of total characteristic volatile compounds, for all the tested genotypes, and, consequently, of the flavour intensity. As concern the subdivision classes of volatiles, in the same figure, the content of nitriles and sulfides increased in OR Emeraude, on the contrary no effects were noted for isothiocyanates. In the OR Velox, sulfides increased, while the other classes were similar to the CONV samples. Finally, the OR cultivation, in Jesino genotype, increased the isothiocyanates content, while the other classes were similar between CONV and OR samples.

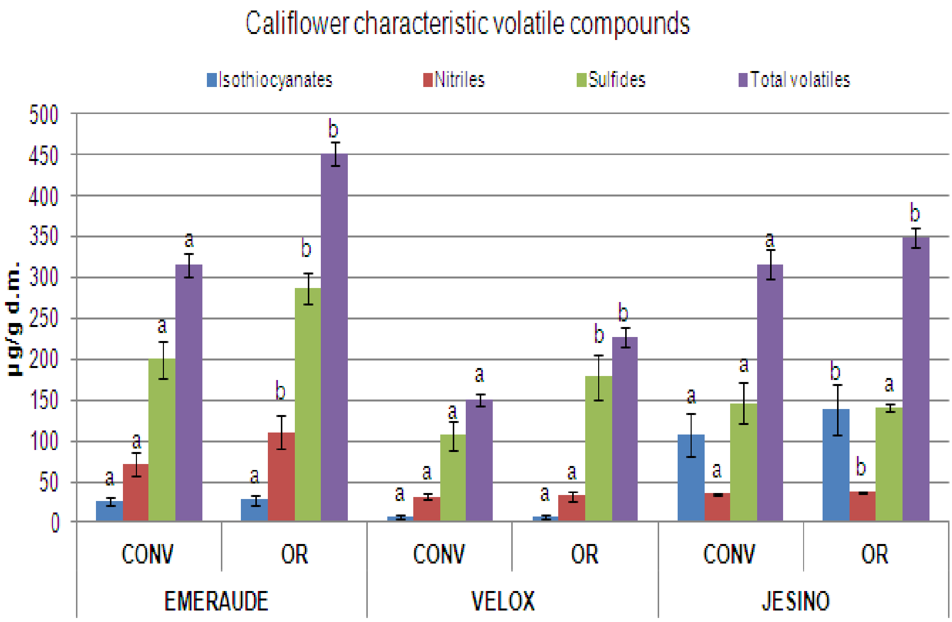


**Fig. 6:** Effects of the cultivation on the total and subdivision classes of cauliflower characteristic volatile compounds. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

In Tab. 9 the influence of OR system on the individual glucosinolates identified in the cauliflower samples were reported. In all the genotypes the individual glucosinolates decreased in the OR samples, except for 4-OH glucobrassicin and glucobrassicanapin in Emeraude, glucobrassicin and 4-methoxyglucobrassicin in Velox, sinigrin and neoglucobrassicin in Jesino, that increased in the OR samples.

| | GLUCOSINOLATES (μmol/g d.m.) | | | | | |
|---|---|---|---|---|---|---|
| | EMERAUDE | | VELOX | | JESINO | |
| | CONV | OR | CONV | OR | CONV | OR |
| **Glucoiberin** | 0.089±0.04 b | 0.075±0.02 a | 0.068±0.01 a | 0.072±0.03 a | 0.056±0.02 a | 0.055±0.00 a |
| **Progoitrin** | 0.026±0.01 b | 0.019±0.00 a | 0.025±0.01 b | 0.022±0.00 a | 0.018±0.00 a | 0.018±0.00 a |
| **Epiprogoitrin** | 0.046±0.10 b | 0.031±0.01 a | 0.047±0.01 a | 0.049±0.01 a | 0.101±0.04 b | 0.057±0.02 a |
| **Sinigrin** | 0.094±0.13 b | 0.074±0.02 a | 0.094±1.14 a | 0.088±0.02 a | 0.397±0.08 a | 0.495±0.09b |
| **Glucoraphanin** | 0.031±0.01 b | 0.015±0.00 a | 0.028±0.01 b | 0.019±0.01 a | 0.022±0.01 b | 0.019±0.00 a |
| **Glucoalyssin** | 0.011±0.00 b | 0.005±0.00 a | 0.012±0.00 a | 0.013±0.01 a | 0.046±0.01 b | 0.018±0.00 a |
| **4-OH glucobrassicin** | 0.121±0.05 a | 0.148±0.09 b | 0.095±0.02 a | 0.091±0.03 a | 0.153±0.03 b | 0.109±0.05 a |
| **Glucobrassicanapin** | 0.748±0.16 a | 0.959±0.06 b | 0.851±0.23 b | 0.663±0.15 a | 1.133±0.33 b | 0.400±0.11 a |
| **Glucobrassicin** | 0.590±0.01 a | 0.662±0.01 a | 0.600±0.12 a | 0.743±0.07 b | 0.424±0.03 a | 0.418±0.17 a |
| **4-methoxyglucobrassicin** | 0.001±0.00 b | 0.000±0.00 a | 0.000±0.00 a | 0.003±0.00 b | 0.006±0.00 b | 0.001±0.00 a |
| **Neoglucobrassicin** | 0.054±0.01 a | 0.051±0.01 a | 0.061±0.02 a | 0.063±0.03 a | 0.048±0.01 a | 0.059±0.01 b |

**Tab. 9:** Individual glucosinolates found in CONV and OR cauliflower genotypes. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

In Fig. 7 the total amount of identified glucosinolates, the sum of aliphatic glucosinolates and the sum of indolyc glucosinolates are reported. In the OR Emeraude, the increase of aliphatic, indolyc and total glucosinolates was noted with respect to the CONV samples. In the Velox genotype, the positive effects of OR cultivation were only observed for the indolyc glucosinolates; while the aliphatic glucosinolates were more evident in CONV samples and the total ones had similar values in both theses. In the Jesino cultivar, the aliphatic and total glucosinolates were higher in the CONV samples, while the indolyc ones were quite similar both in OR and CONV samples.

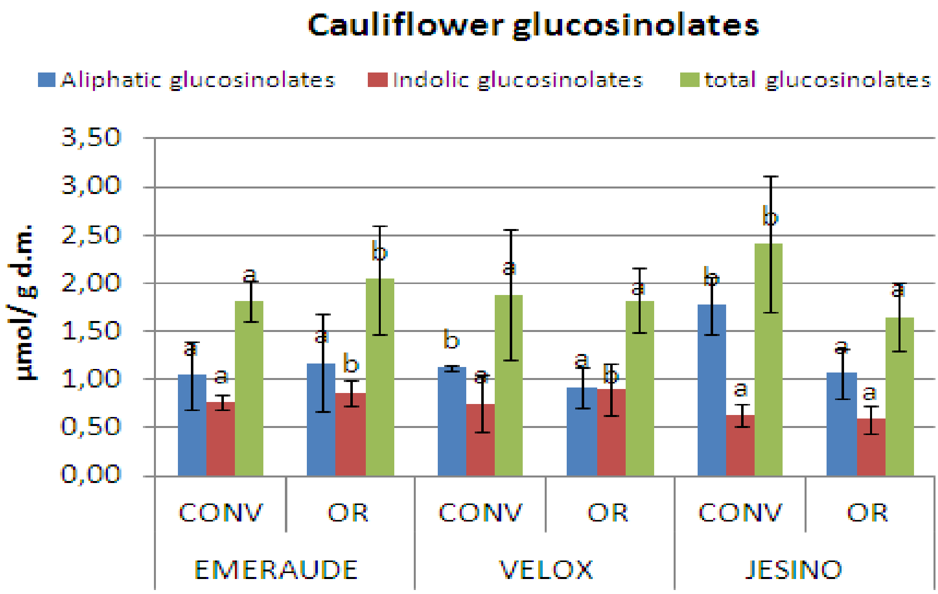


**Fig. 7:** Influence of cultivation on total, aliphatic and indolic glucosinolates in CONV and OR cauliflower genotypes . Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

Tab. 10 shows the values of other nutraceutical parameters in the examined samples. The content of vitamin C and total carotenoids were similar in OR and CONV samples for all the tested genotypes. The content of polyphenols were quite similar in OR and CONV samples for Emeraude and Velox genotypes; on the contrary, they significantly decreased in the OR Jesino samples.

| | **Ascorbic acid** (mg/100 g f.w.) | **Total Carotenoids** (mg/100 g f.w.) | **Total Polyphenols** (mg/100 g f.w.) | **DPPH** (mmoli AsA/100 g d.m.) |
|---|---|---|---|---|
| **EMERAUDE CONV** | 40.67±16.80 a | 0.47±0.14 a | 46.82±12.55 a | 3.56±1.19 a |
| **EMERAUDE OR** | 43.83±19.88 a | 0.50±0.14 a | 48.77±13.47 a | 3.31±1.55 a |
| **VELOX CONV** | 48.78±13.76 a | 0.61±0.20 a | 52.06±15.99 a | 3.95±1.41 a |
| **VELOX OR** | 53.99±21.00 a | 0.69±0.11 a | 54.48±16.60 a | 4.39±1.00 b |
| **JESINO CONV** | 38.96±10.81 a | 0.20±0.07 a | 59.06±4.86 b | 4.23±1.11 a |
| **JESINO OR** | 39.65±7.64 a | 0.20±0.07 a | 45.93±4.77 a | 3.99±1.40 a |

**Tab. 10:** Nutraceutical parameters in CONV and OR cauliflowers genotypes. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

So the influence of OR cultivation on vitamin C, carotenoids and polyphenols was few evident and consequently the values of the antioxidant power were similar in the two set of samples for Emeraude and Jesino, except for Velox, where the increase of the DPPH* index was ascertained in the OR samples.

An opposite behaviour was described by Lo Scalzo et al. (2008), who studied 16 genotypes of cauliflower, grown in OR and CONV systems. In fact, the vitamin C was higher (18%) in OR respect to CONV. Total polyphenol and antioxidant indexes were slightly higher in OR compared to CONV. With respect to characteristic volatile compounds, the total average difference between OR and CONV was not significant, with differences for single sampling. The difference with our results may be due to the different tested genotypes and enviromental conditionts.

Our data, as concern glucosinolates and the antioxidant activity, are in partial accordance with those of Vicas et al. (2013). They have determined and compared the profile of glucosinolates and the antioxidant capacity of five Brassica species, as broccoli, cauliflower, Kohlrabi, white and red cabbage, grown by organic and conventional practices. The highest total glucosinolates levels were found in broccoli and white cabbage grown under conventional practices; while an opposite behavior was noted in the cauliflower, Kohlrabi and red cabbage, where the highest level were found in Or samples. The antioxidant capacity was higher in organic crops, without significant differences among different Brassica varieties.

The obtained data pointed out that the positive effects of OR method on the chemical quality, were noted in all the cauliflower genotypes as concern the increase of characteristic volatile compounds and consequently the flavour intensity. For the nutraceutical quality, the effects of OR method were only found in some genotypes (Emeraude), as concern glucosinolates; while, almost all the other parameters, were similar in the OR and CONV samples for all the examined genotypes.

## 5.2 -TOMATO

Two tomato genotypes, named Giulianova and Perbruzzo, cultivated both in OR and CONV methods, and harvested at the unripe (U) and ripe (R) stage, were submitted to the following analysis: characteristic volatile substances (hexanal, 2(E)-hexenal, 2-isobutylthiazole), pH, soluble sugars, vitamin C and lycopene. Tab. 11, where the concentration of the three characteristic tomato volatile compounds in all the samples were reported, shows that in the unripe Giulianova the content of hexanal and 2(E)-

hexenal were lower in the OR sample, while 2-isobutylthiazole was quite similar in OR and CONV samples. On the contrary, in the ripe Giulianova, all three characteristic volatile compounds of tomato flavour were clearly superior in the OR samples, with respect to the CONV one. Moreover, the values of the characteristic volatile compounds were higher in the ripe samples, with respect to the unripe ones, both in OR and CONV samples. In the unripe OR Perbruzzo, the content of hexanal, 2(E)-hexenal and 2-isobutylthiazole was higher than the corresponding CONV samples. An opposite behavior was found in the ripe Perbruzzo, where the values of the three compounds in the CONV samples were superior than OR samples, except 2-isobutylthiazole.

| | | TOMATO CHARACTERISTIC VOLATILE COMPOUNDS (µg/g d.m.) | | |
|---|---|---|---|---|
| | | **hexanal** | **2(E)-hexenal** | **2-isobutylthiazole** |
| **GIULIANOVA (U)** | CONV | 57.21±15.00 b | 12.79±5.10 b | 71.76±6.96 |
| | OR | 42.63±8.58 a | 8.16±3.37 a | 77.25±18.29 a |
| **GIULIANOVA (R)** | CONV | 95.98±14.32 a | 41.45±8.28 a | 111.58±31.39 a |
| | OR | 164.46±39.38 b | 62.15±17.64 b | 191.28±22.37 b |
| **PERBRUZZO (U)** | CONV | 25.26±5.86 a | 5.92±1.25 a | 42.68±8.60 a |
| | OR | 35.95±2.90 b | 46.21±10.99 b | 66.85±9.27 b |
| **PERBRUZZO (R)** | CONV | 411.30±33.78 b | 44.01±9.90 b | 53.56±8.16 a |
| | OR | 207.04±21.10 a | 28.30±1.29 a | 182.40±30.99 b |

**Tab. 11:** Characteristic volatile compounds in unripe (U) and ripe (R) tomato genotypes, grown with CONV and OR systems. Different letters indicate significant differences among OR and CONV samples within the same genotype ($\rho \leq 0,05$).

Fig. 8, where the total characteristic volatile compounds were plotted, shows that they slightly decrease in the unripe OR Giulianova; an opposite behavior was noted in the ripe samples. In the Perbruzzo exactly the contrary happened, because in the unripe samples, the content of flavour substance was higher in the OR samples, while in the ripe samples the CONV system showed higher concentration.

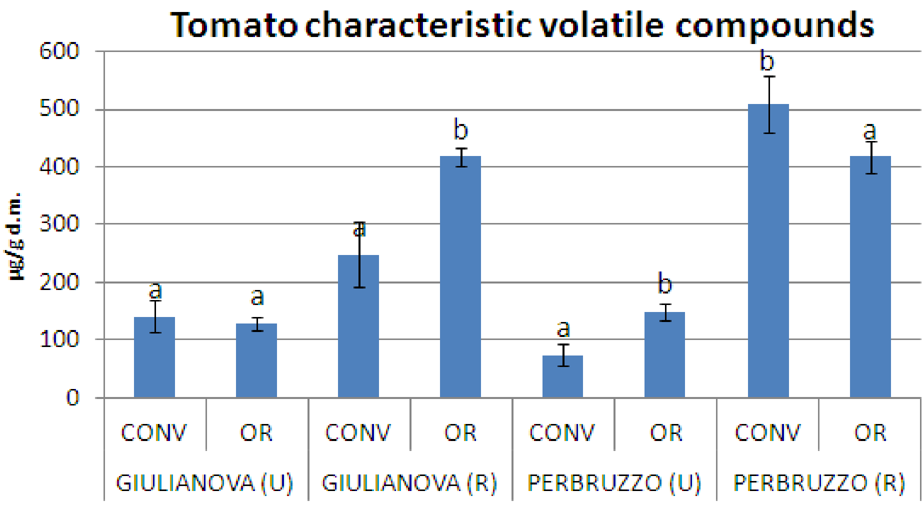


**Fig. 8:** Total characteristic volatile compounds in all the tomato samples. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

In literature, few researches on the aroma composition of organic and conventional tomatoes are available, and most of them concern sensory analysis; no quantitative data on aroma composition have been reported. Previous data, carried out at C.R.A.-I.A.A., demonstrated that OR growing methods had no statistically significant influence on quali-quantitative parameters, but that these parameters were influenced only by cultivar, in partial accordance with other data (Migliori et al., 2012).

As concern pH and soluble sugars, fig. 9 shows that these two chemical parameters remained constant in the samples collected at different ripeness degrees and grown with different methods. The evaluation of pH in tomato appears to be important for the choice of heat-processing, which may be more or less intense for the microbiological stability overtime. This depend on the pH that is higher or lower than 4.3 (Silvestri and Siviero 1991). In our case, the pH values of the examined samples were around 4.3. So it would be advisable, if you want to process these tomatoes, in domestic puree or sauce, using a more intense heat treatment.

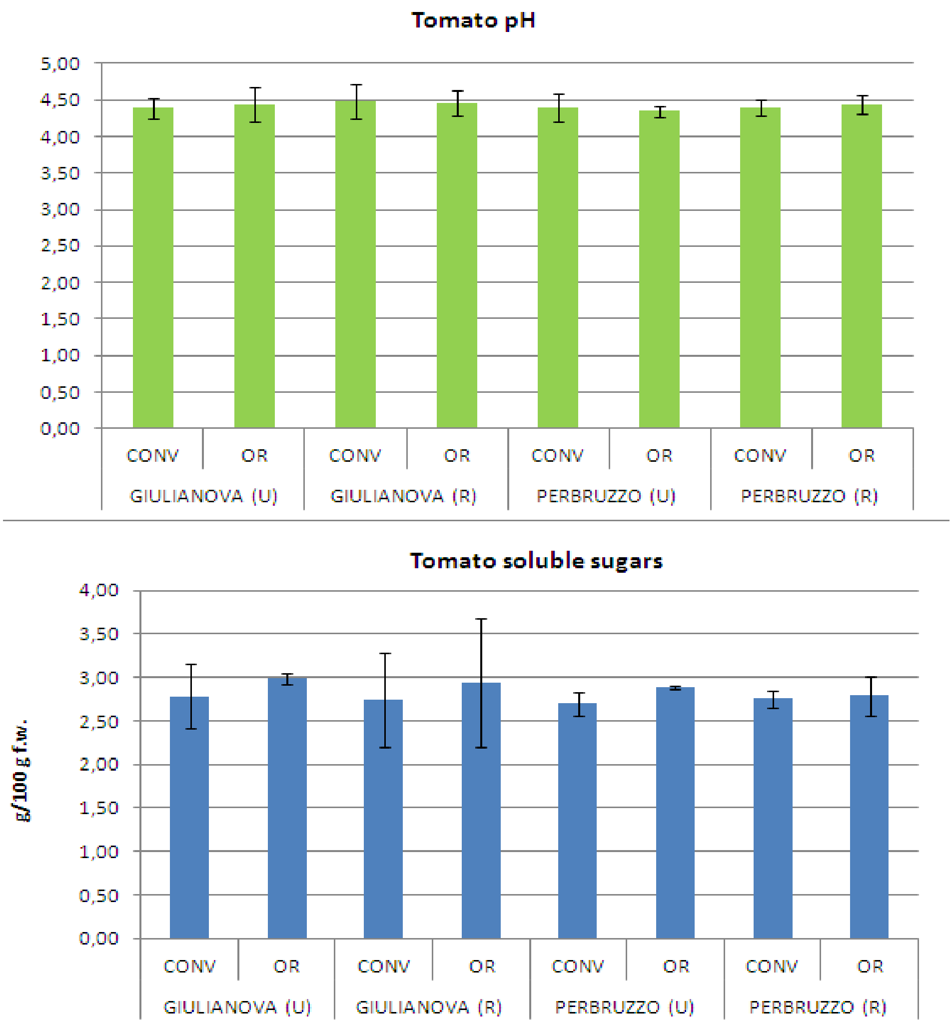


**Fig. 9:** pH and soluble sugars trend in OR and CONV tomato samples. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

Fig. 10 shows the values in lycopene and vitamin C in all the samples. In the unripe Giulianova, the content of lycopene was slightly higher in OR samples, while an opposite behavior was detected in the ripe samples. As expected, the ripe samples of OR and CONV Giulianova and Perbruzzo genotypes had higher levels of lycopene with respect to unripe OR and CONV ones. In Perbruzzo genotype, the positive effects of OR system were observed in unripe and ripe samples. These results are in good accordance with another study (Caryis-Veyrat, 2004), where higher level of lycopene in organic tomatoes, when the values were expressed on fresh matter were found. Moreover, in the same paper it is confirmed that variety significantly affects the lycopene content. On the contrary, in other papers (Lucarini et al., 1999; Kopp et al. 1989; Clarke and Merrow, 1979), any significant differences in lycopene content between organic and conventional tomatoes were found.

As concern vitamin C, it was noted an increase of this vitamin in the unripe OR Giulianova, with respect to the CONV one; there were no differences between OR and CONV samples of the ripe tomatoes. In unripe Perbruzzo it was observed that both OR and CONV samples had the same concentration in vitamin C, but a slight decrease was ascertained in ripe OR Perbruzzo, with respect to the CONV one. Moreover, the content of ascorbic acid in the unripe and ripe Giulianova samples were quite similar among them, while the concentration of vitamin C was more evident in the ripe Perbruzzo genotype. These results are in contrast with those of Hallmann (2012), that found higher ascorbic acid content in organic tomatoes, compared with conventional samples, in a two year study. However, the results available in literature, show no clear difference between the contents of vitamin C in organic and conventional vegetables; although a tendency toward higher vitamin C content was found for leafy vegetables grown by organic mode compared to the conventional way (Caris-Veyrat, 2004).

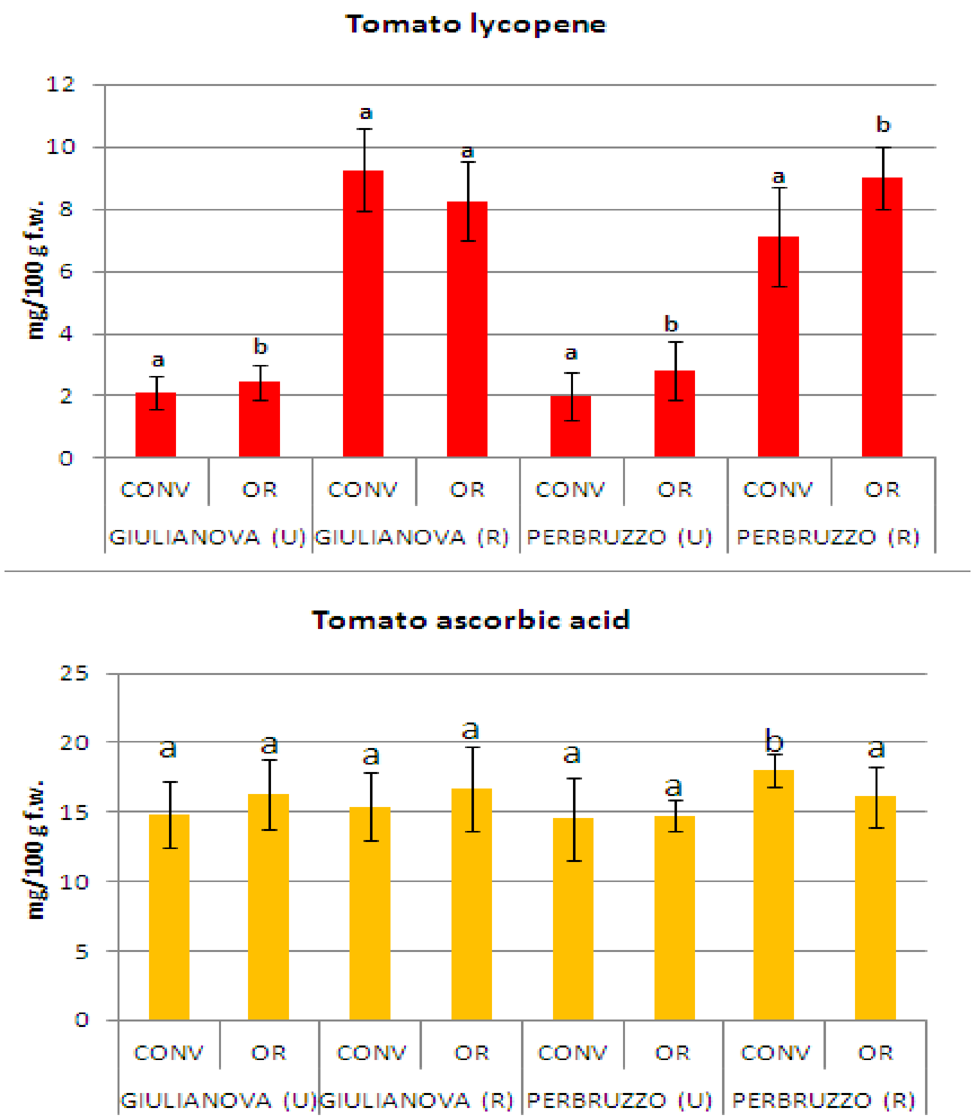


**Fig. 10:** Influence of OR system on lycopene and acorbic acid in tomato samples. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).

Summarizing, the obtained results pointed out a different behavior of two tomato genotypes as concern the concentration of characteristic volatile compounds and lycopene. In fact, in the ripe OR Giulianova the content of the flavour compounds increased, and contemporary the lycopene content decreased with respect to the CONV samples. In the unripe samples, the positive influence of the OR system, was more evident in Perbruzzo, especially as concern the flavour compounds and the lycopene content.

## 5.3 - SWEET RED PEPPER

Two sweet red pepper genotypes, named Alceste and RS08, cultivated with the OR and CONV systems, were submitted to the following analyses: characteristic volatile pyrazines (2-methoxy-2-isopropylpyrazine and 2-methoxy-2-isobutylpyrazine) and soluble sugars to evaluate the chemical composition; vitamin C, polyphenols, carotenoids and antioxidant power, measured with the DPPH* method, to evaluate the nutraceutical quality.

Tab. 12 shows that the two characteristic volatile compounds of pepper flavour were lower in OR Alceste sample, with respect to the CONV one. As concern the soluble sugars, it was observed an increase of glucose in OR samples, and a decrease of fructose in the same sample. In RS08 cultivar, the value of 2-methoxy-2-isopropyl pyrazine decreased in the OR sample, but no differences between the two samples of the same cultivar were noted for 2-methoxy-2-isobutylpyrazine. The soluble sugars had similar values both in OR and CONV samples.

| | ALCESTE CONV | ALCESTE OR | RS08 CONV | RS08 OR |
|---|---|---|---|---|
| **2-methoxy-3-isopropyl pyrazine** (µg/100 g d.m.) | 16.50±1.68 b | 9.68±2.36 a | 18.84±0.58 a | 12.23±1.74 a |
| 2**-methoxy-3-isobutyl pyrazine** (µg/100 g d.m.) | 35.56±4.81b | 16.83±1.35 a | 30.80±2.56 a | 28.36±3.67 a |
| **Glucose** (g/100 g f.w.) | 2.19±0.50 a | 2.46±0.51 a | 2.25±0.13 a | 2.42±0.44 a |
| **Fructose** (g/100 g f.w.) | 2.91±0.41 b | 2.51±0.37 a | 2.29±0.20 a | 2.39±0.21 a |

**Tab. 12**: Chemical parameters of OR and CONV sweet red pepper. Different letters indicate significant differences among OR and CONV samples, within the same genotype (ρ≤0,05).

Tab. 13 shows that, in the Alceste genotype, the positive effects of the OR method was noted on the content of ascorbic acid. The content of total carotenoids, instead, was quite similar, in two treated samples. The polyphenols and, consequently, the antioxidant activity, were lower in the OR samples. For RS08 cultivar, no influence was ascertained on ascorbic acid and polyphenols, while a significant increase was found for carotenoids in OR samples. Nevertheless, the antioxidant power had a higher value in the CONV sample. These data disagree with the data of the sum of measured antioxidants, where a slight increase was found especially in OR Alceste.

| | ALCESTE CONV | ALCESTE OR | RS08 CONV | RS08 OR |
|---|---|---|---|---|
| **Ascorbic acid** (mg/ g f.w.) | 113.98±15.16 a | 137.33±19.95 b | 162.51±4.35 a | 157.35±19.95 a |
| **Total Carotenoids** (mg /100 g f.w.) | 11.30±2.15 a | 10.61±2.90 a | 10.93±3.18 a | 13.22±2.55 b |
| **Polyphenols*** (mg /100 g f.w.) | 61.01±8.85 b | 52.78±7.23 a | 45.91±8.31 a | 50.78±9.14 a |
| **Sum of measure antioxidant molecules** (mg/100 g f.w.) | 186.29±15.85 a | 200.72±10.02 b | 219.35±5.28 a | 221.35±9.69 a |
| **Antioxidant activity** (mmol ASA/100 g d.m.) | 3.35±0.81 b | 2.64±0.80 a | 3.46±0.22 b | 2.79±0.35 a |

**Tab. 13:** - Nutraceutical parameters of OR and CONV sweet red pepper. Different letters indicate significant differences among OR and CONV samples within the same genotype (ρ≤0,05).
*The Values of polyphenols were obtained after subtracting ascorbic acid content.

From reported data, it can be deduced that the tested sweet red pepper cultivars appeared to be little affected by OR cultivation, both for nutraceutical and chemical aspects.

In a 3-year study (Chassy et al., 2006), total phenolics, percent soluble solids and ascorbic acid, have been measured in two varieties of bell peppers, grown by certified organic and conventional practices in a model system. Bell peppers were scarcely influenced by environment and did not display cropping system differences, as noted in our studies. On the contrary, in disagree with our results, Szafirowska and Elkner (2008) studied the effect of cultivar and growing system (organic vs. conventional) on sweet pepper yielding, fruit morphology and antioxidants content. They found that pepper fruits obtained from organic cultivation system comprised higher amount of vitamin C and polyphenols than those from the conventional system.

### 5.3.1. INFLUENCE OF GRILLING ON SWEET PEPPER NUTRACEUTICAL QUALITY

The OR Alceste was submitted to an home roasting on plate. In tab. 14 the identified volatile compounds of the raw and grilled sweet pepper were compared. In the raw vegetable, in addition to the pirazynes (characteristic pepper volatiles), saturated and unsaturated alcohols and aldehydes, terpenes, benzene derivatives (aromatic compounds) and sulfur compounds were found. In the roasted peppers, a part of the characteristic volatile compounds of raw sample disappeared, while other volatile compounds, containing the furan ring, as 3(2H)-furanone-dihydro-2-methyl, named “coffee furanone”, 2-furan-methanol, 2-acetyl-furan, named “pop-corn furan”, 5-methyl-2-furfural, and a furanone derivative, as 2-(3H)furanone dihydro or butyro lactone, were identified. All these compounds come from sugars thermal degradation, in particularly from glucose and fructose, after reaction with protein or amino acids, by Maillard condensation.

| (µg/100 g d.m.) | OR ALCESTE Raw | OR ALCESTE Grilled |
|---|---|---|
| **Acohols** | | |
| 1-penten-3-ol | 1.81±0.45 | - |
| 3-pentanol | 2.02±0.88 b | 1.16±0.15 a |
| 3-methyl-3-buten-1-ol | 61.29±10.21 | - |
| 3(Z)-penten-1-ol | tr. | 64.26±7.67 |
| 2-ethyl-1-hexanol | 3.15±0.91 | - |
| **Carbonyl compounds** | | |
| 2,4-pentanedione | 44.79±6.28 | - |
| hexanal | 35.49±2.87 a | 49.60±5.61 b |
| 4-hydroxy-4-methyl-2-pentanone | 25.77±2.15 | - |
| 3(E)-hepten-2-one | tr. | 41.04±8.85 |
| benzaldehyde | 68.58±7.65 b | 3.16±1.05 a |
| benzeneacetaldehyde | 25.30 ±1.12 a | 77.99±10.17 b |
| **Sulphur compounds** | | |
| 3-(methylthio)-pentanal | 13.60±1.55 | - |
| dimethyltrisulfide | 2.16±0.57 | - |
| **Terpenes** | | |
| D,L-limonene | 288.02±3011 b | 43.10±2.18 a |
| **Aromatic compounds** | | |
| methylsalicylate | 2.27±0.75 | - |
| ethyl-2-methylbenzoate | 51.32±6.21 a | 51.38±8.53 a |
| trans-anethole | 69.85±9.18 b | 32.50±6.16 a |
| **Pirazynes** | | |
| 2-methoxy-3-isopropyl-pirazyne | 9.68±1.78b | 6.83±0.85 a |
| 2-methoxy-3-isobutyl-pirazyne | 16.83±4.55 b | 14.99±3.67 a |
| **Maillard reaction products** | | |
| 3-(2H)-furanone dihydro-2-methyl | - | 0.88±0.21 |
| 2-furan-methanol | - | 1.25±0.88 |

| | | |
|---|---|---|
| 2(3H)-furanone dihydro | - | 2.08±0.98 |
| 2-acethyl-furan | - | 41.52±5.66 |
| 5-methyl-2-furfural | - | 275.90±51.24 |

**Tab. 14:** Volatile compounds identified in raw and grilled Alceste sweet red peper genotype.

In Tab. 15 the percentage variations of the chemical and nutraceutical parameters in the raw and treated samples were reported. The total pyrazines had a 18% decrease, while the soluble sugars loss was lower (-5%). As concern the nutraceutical quality, the same table shows that ascorbic acid and carotenoids had a strong reduction, equal to 51% and 40% respectively, while the loss of total polyphenols was around 12%. Since the antioxidant activity is proportional to the value of these parameters, their loss caused a decrease of this activity too.

| | **Alceste OR** | | |
|---|---|---|---|
| | **Raw** | **Grilled** | **% Loss** |
| **Soluble sugars** (g/100 g d.m.) | 72.67±5.21 a | 68.75±4.88 a | -5.39 |
| **Total pirazynes** (μg/100 g d.m.) | 26.51±3.21 b | 21.82±2.77 a | -17.71 |
| **Ascorbic acid** (mg/100 g d.m.) | 1319.70±138.15 b | 647.85±88.16 a | -50.91 |
| **Total carotenoids** (mg/100 g d.m.) | 110.37±15.66 b | 66.06±10.88 a | -40.15 |
| **Total polyphenols** (mg/100 g d.m.) | 586.07±7016 b | 516.80±90.15 a | -11.82 |
| **Sum of antioxidant molecules** (mg/100 g d.m.) | 2016.14±251.61 b | 1230.71±189.22 a | -38.95 |
| **Antioxidant activity** (mmol AsA/100 g d.m.) | 29.39±5.21 b | 7.36±2.15 a | -74.96 |

**Tab. 15:** Percentage variation of parameters in grilled sweet red pepper.

In the grilled sweet red pepper the chemical parameters, as pirazynes and soluble sugars, slightly decreased. A loss of the antioxidant activity was noted in the same sample, due to the strong decrease of ascorbic acid and carotenoids.

## 5.4 – ONION

The onion samples (Suasa, Precoce di Romagna, Density and Tropea) grown with the OR system, were submitted to the chemical quality evaluation, as characteristic volatile substances and soluble sugars. Moreover, it was performed the determination of the antioxidant power, across the quantification of polyphenols, as well as quercetins and anthocyanins, and the antioxidant power, measured by the DPPH* quenching assay. Finally, the onion pungency was objectively measured by the pyruvic acid concentration (Kondiram et al, 2007).

The onion characteristic volatile substances are represented by saturated (SS) (dimethyl, trimethyl and tetramethyl disulfide, propylbutyl disulfide and dipropyl disulfide) and unsaturated (US) (E or Z propenyl methyl disulfide, E or Z propenyl propyl disulfide and trisulfide, diallyl disulfide and diallyl methyl trisulfide) sulfides. The US, according to current researches, seem to have anticancer effects (Munday et al., 2009). Tab.16 shows the onion volatile sulfur compounds divided into saturated and unsaturated sulfides. Both classes are responsible of the characteristic onion flavour, but a potential anticancer effect is ascertained to unsaturated ones, as reported above. In particular, the diallyl disulfide and allyl methyl trisulfide, considered the most biologically active organosulphur compounds, had the highest values in Tropea and the lowest in Suasa, as concern the allyl methyl trisulfide, and Density, as concern the diallyl disulfide. Moreover, unsaturated sulfides were numerically and quantitatively (partial amount) superior with respect to saturated ones, for all genotypes.

| | ONION CHARACTERISTIC VOLATILE SUBSTANCES (µg/ 100 g d.m.) | | | |
|---|---|---|---|---|
| **SATURATED SULFIDES** | **SUASA** | **PRECOCE DI ROMAGNA** | **DENSITY** | **TROPEA** |
| dimethyl disulfide | 264.21±63.54 b | 286.26±1.38 b | 561.13±22.14 c | 218.03±14.29 a |
| dimethyl trisulfide | 351.19±38.02 a | 465.06±61.65 b | 878.73±55.16 d | 767.15±79.73 c |
| dimethyl tetrasulfide | 237.56±58.45 a | 368.19±13.90 b | 244.02±20.21 a | 248.83±82.10 a |
| propyl butyl disulfide | 61.63±4.66 d | 54.89±6.90 c | 10.16±0.89 a | 19.35±2.66 b |
| dipropyl trisulfide | 22.50±5.99 b | 155.43±42.39 d | 11.50±1.07 a | 40.53±9.48 c |
| **UNSATURATED SULFIDES** | | | | |
| (E) propenyl methyl disulfide | 350.06±55.04 a | 539.74±48.42 b | 855.09±60.15 c | 839.90±55.20 c |
| (Z) propenyl methyl disulfide | 461.48±96.27 b | 556.29±36.25 c | 343.64±40.61 a | 1036.93±86.36 d |
| (E) propenyl propyl disulfide | 223.92±52.49 b | 456.79±69.57 c | 175.37±16.21 a | 449.46±64.88 c |
| (Z) propenyl propyl disulfide | 290.27±52.92 a | 520.98±68.41 c | 262.63±30.78 a | 461.31±57.03 b |
| (E) propenyl propyl trisulfide | 376.46±18.63 a | 561.10±95.74 c | 578.48±25.38 c | 498.10±69.73 b |
| (Z) propenyl propyl trisulfide | 380.89±19.52 a | 826.25±58.28 c | 584.03±61.45 b | 546.30±35.51 b |
| diallyl disulfide | 111.44±10.62 b | 148.24±28.83 c | 91.18±10.64 a | 213.31±13.15 d |
| allyl methyl trisulfide | 52.11±4.60 a | 64.99±0.07 b | 298.19±30.33 c | 310.53±59.67 c |

**Tab. 16:** Organsulfur volatile compounds identified in four OR onion genotypes. Different letters indicate significant differences among genotypes (ρ≤0,05)

In Fig. 11 the distribution of two classes of sulphur compounds are reported. Suasa genotype had the lowest content in saturated and unsaturated sulfides; while, in the other genotypes the values were similar and ranged between 1281 – 1349 µg/100 g d.m. for the saturated sulfides, and 3188 – 3716 µg/100 g d.m. for the unsaturated ones.

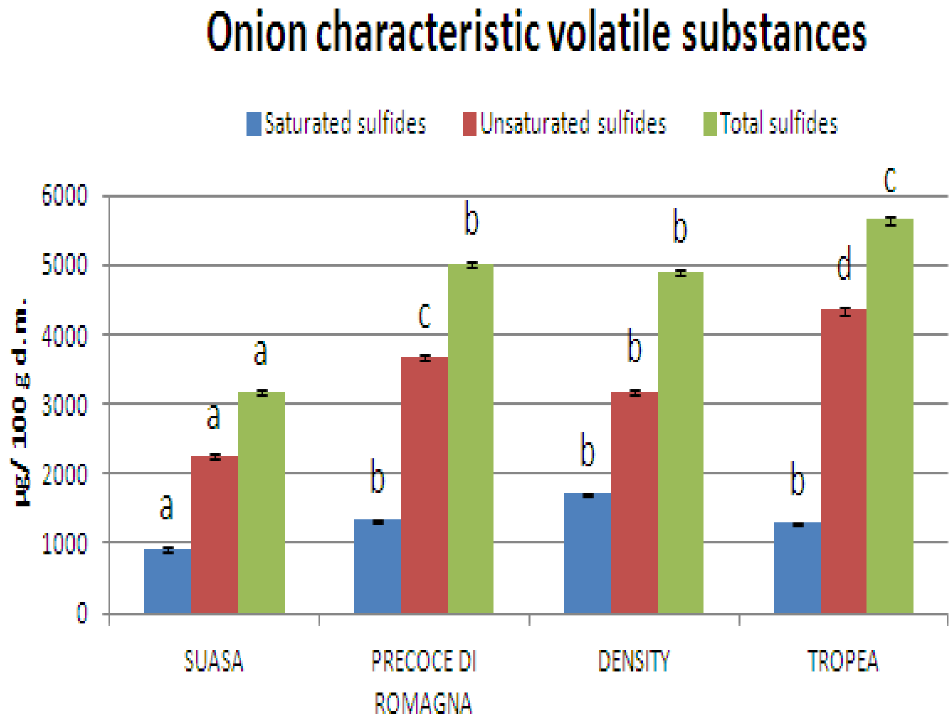


**Fig. 11:** Distribution of unsaturated, saturated and total sulfides in OR onion genotypes. Different letters indicate significant differences (ρ≤0,05) among genotypes.

Tab. 17 shows the content of other parameters related to the chemical (soluble sugars) and nutraceutical (all the other parameters) quality. The highest content of soluble sugars (sucrose, glucose and fructose) was noted in Density genotype, while for the other genotypes, the soluble sugars content ranged between 5,16 and 5,56 g/100 g f.w.

| | Suasa | Precoce di Romagna | Density | Tropea |
|---|---|---|---|---|
| **Sucrose** (g/100 g f.w.) | 0.83±0.19 b | 0.63±0.28 a | 1.43±0.88 c | 0.83±0.10 b |
| **Glucose** (g/100 g f.w.) | 2.38±0.12 a | 2.70±0.03 ab | 2.940.77 b | 2.23±0.68 a |
| **Fructose** (g/100 g f.w.) | 1.95±0.16 a | 2.19±0.34 c | 1.84±0.32 a | 2.49±0.93 d |
| **Ascorbic acid** (mg/100 g f.w.) | 11.04±0.97 c | 4.16±0.65 a | 3.93±0.18 a | 4.90±0.12 b |
| **Polyphenols** (mg/100 g f.w.) | 22.86±2.92 a | 21.17±0.97 a | 43.96±7.24 c | 31.35±7.08 b |
| **Anthocyanins** (mg/100 g f.w.) | 5.72±0.13 a | - | - | 16.26±0.50 b |
| **Quercetin** (mg/100 g f.w.) | 14.79±5.14 a | 14.49±2.02 a | 43.34±3.67 c | 29.50±1.56 b |
| **Antioxidant capacity** (mmoli AsA/100 g d.m.) | 13.03±2.04 a | 11.72±0.90 a | 27.70±1.15 b | 20.67±0.09 ab |
| **Piruvic acid** (mmol/Kg f.w.) | 2.57±0.65 a | 2.60±0.85 a | 6.00±0.97 c | 4.95±0.92 b |

**Tab. 17:** Chemical and nutraceutical parameters monitored in four organic onion genotypes. Different letters indicate significant differeces (ρ≤0,05) among genotypes.

As concern nutraceutical parameters, ascorbic acid, even if its content in all the genotypes was quite low, showed the highest concentration in Suasa. Polyphenols in onions were mainly represented by quercetins and anthocyanins, in the red pigmented genotypes. In Density the highest level of total polyphenols was found. In this genotype the solely identified polyphenol was quercetin. In Tropea, total polyphenols were little lower than in Density and they were represented by quercetin and anthocyanins. Much lower contents, but similar among them, were determined in Precoce di Romagna (quercetin) and Suasa (quercetin and anthocyanins). The anthocyanins, contained only in the red onions, showed the highest value in Tropea with respect to Suasa.

The content in pyruvic acid, that reflected the pungency of onion, was clearly lower in Suasa and Precoce di Romagna, while Tropea and Density had the highest levels, in partial relationship with the presence of sulphur volatiles. According to the ranking of pungency, determined by Dhumal et al (2007), low pungency onions were <3 mmol/kg f.w. and medium (3-7 mmol/kg f.w.): the assayed genotypes resulted of low pungency for Suasa and Precoce di Romagna, while Tropea and Density resulted of medium pungency.

The highest antiradical capacity was showed by Density, followed by Tropea, about two-folds higher than Suasa and Precoce di Romagna, in full accordance with the data of total polyphenols index and quercetins (Tab. 17). The DPPH* *in vitro* test highlighted that there were high correlations between the antiradical capacity and the total polyphenols and quercetins content (rxy=0.92 and 0.94, respectively), with a negative correlation index with ascorbic acid content, still confirming the interesting role of quercetins as powerful free radical scavengers in the present case of onion (Rice-Evans et al., 1996).

Ren et al (2001), have compared the antioxidant activity and total polyphenols content in different organic and conventional vegetables, such as onion. They used water-soluble chitosan as a soil modifier and leaf surface spray (as an alternative natural insecticide). They found that the antioxidant activity in organic onion raised up to 20-50 folds than conventional samples, while polyphenols increased 1.3-10.4 folds in the organic samples with respect to conventional ones. Contrasting results, as concern polyphenols in organic onions, were reported by Søltoft et al. (2010). They found that in onions no statistically significant differences between organic and conventional systems were found for any of the analyzed polyphenols. These results were confirmed by Faller and Fialho (2010), who analyzed six vegetables (potato, broccoli, onion, carrot, tomato and white cabbage) and found that polyphenol content and antioxidant capacity varied among organic and conventional vegetables, with no prevalence from either agricultural type. Even if, the comparison between OR and CONV onions was not carried out in this experiment, the literature supported the high levels of polyphenols found in our OR onions, especially in some genotypes.

The evaluation of chemical and nutraceutical parameters of four organic onion genotypes (Fig. 12) pointed out that Density, Precoce di Romagna and Tropea genotypes showed not only a better chemical quality (flavour profiles and soluble sugars content), but contemporary can have a stronger potential antitumor activity due to higher level of unsaturated sulfides (diallyl disulfide and allyl methyl trisulfide) with respect to Suasa. Moreover, Density showed the highest antiradical capacity due to the highest content in polyphenols, in particular quercetins. Suasa genotype, instead, differed from the other genotypes for ascorbic acid content. In this context, ascorbic acid, obviously, seemed to have scarce influence in the index of antioxidant capacity with respect to polyphenols, due to the low concentration in the onion genotypes.

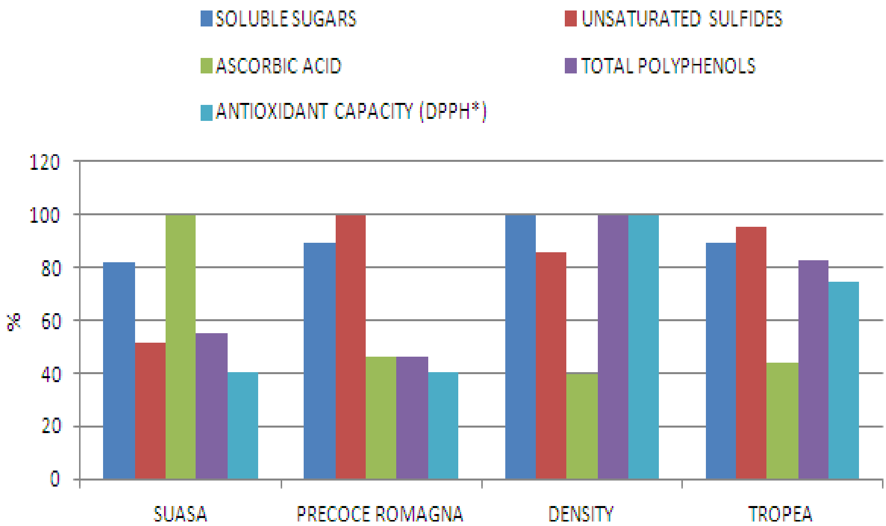


**Fig. 12:** Relative comparison of chemical and nutraceutical parameters of four organic onion genotypes, where the highest values of each parameter is = 100%.

## 5.5 – WATERMELON

Four watermelon genotypes Bagnacavallo, Faenza, Monaco and Talete were grown with the OR system, harvested at commercial ripening and then submitted to qualitative evaluation, such as the concentration of the characteristic volatile compounds, soluble sugars (chemical quality), vitamin C, lycopene and L-citrulline (nutraceutical quality).

The characteristic volatile substances of watermelon are represented by saturated and unsaturated aldehydes and alcohols with 9 carbon atoms (2,6(E,Z)-nonadienal, 2(E)-nonenal, 3(Z)-nonen-1-ol, 2,6(E,Z)-nonadien-1-ol, 2(E)-nonen-1-ol, 1-nonanol). Fig. 13 shows that Monaco genotype had the highest concentration of characteristic volatile substances, followed by Faenza, Bagnacavallo and Talete. As concern the soluble sugars, the highest value was noted in Monaco, followed by Bagnacavallo, Faenza and Talete.

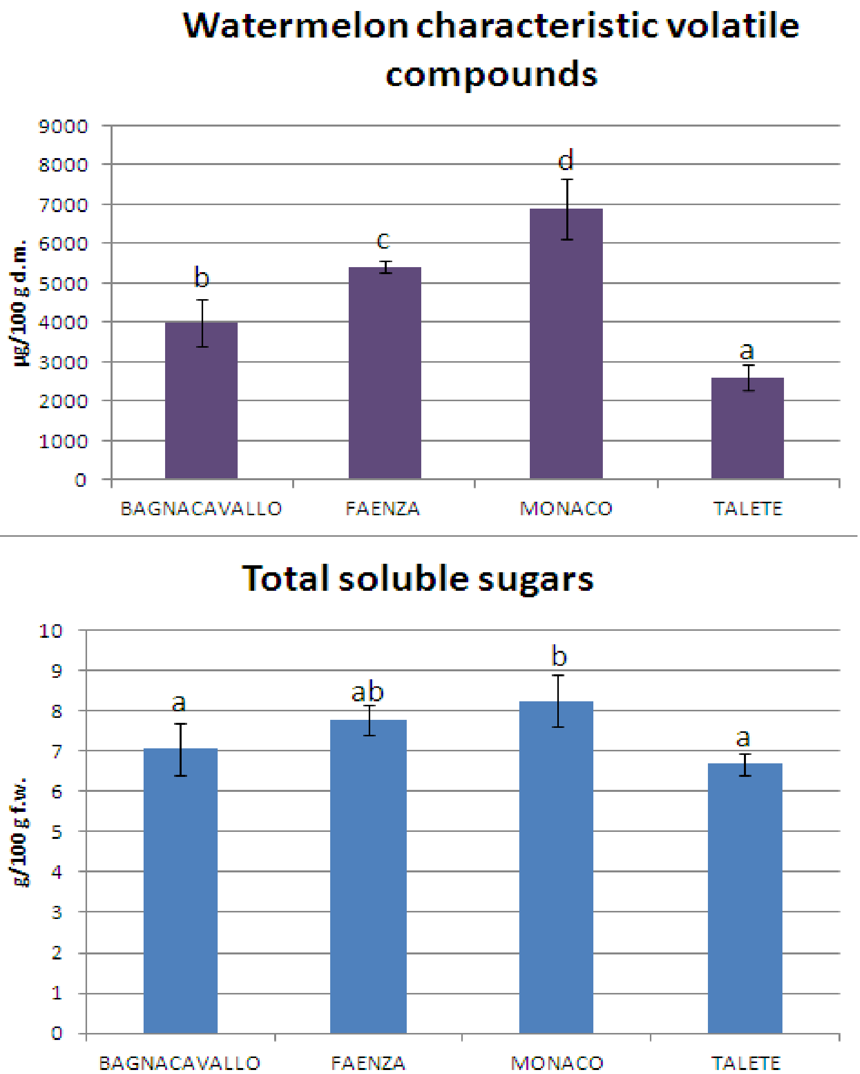


**Fig. 13:** Chemical and nutraceutical parameters of four organic watermelon genotypes. Different letters indicate significant differeces (ρ≤0,05) among genotypes.

In Fig. 14 and Tab. 18 some nutraceutical parameters of this vegetable are reported. Fig. 14 shows that the highest lycopene content was ascertained in Faenza, Talete and Monaco, with almost similar values among them, while in Bagnacavallo was about an half, with respect to the other genotypes. As concern vitamin C, the highest content was observed in Monaco and Faenza; while the lowest ones in Talete e Bagnacavallo. In addition to lycopene and vitamin C, contained in the flesh, in watermelon was also found L-citrulline, predominantly localized in the rind. In the human organism, L-citrulline, although it is a non-essential amino acid, is involved in the production of nitric oxide, that is a potent vasodilator, as previous reported (Rimando et al. 2005).

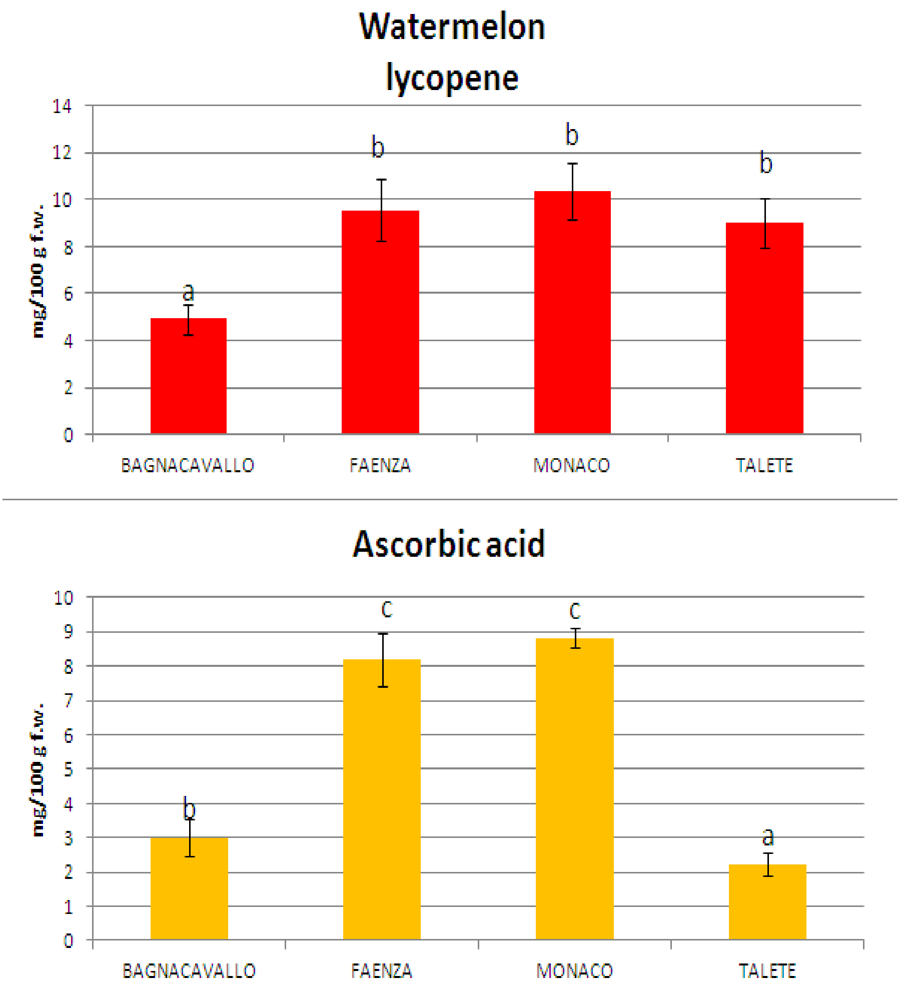


**Fig. 14: -** Nutraceutical parameters in four organic watermelon genotypes. Different letters indicate significant differeces (ρ≤0,05) among genotypes.

Tab. 18 shows that the content of L-citrulline was significantly higher in the rind with respect to the flesh, for each examined genotypes; while in the Monaco flesh L-citrulline was not detected. As concern the watermelon rind, the highest content of L-citrulline was ascertained in Talete cultivar (32.77 mg/ g d.m.). Values around 23 mg/g d.m. were noted in Monaco genotype; while in the other two cultivars the values of L-citrulline ranged between 15 and 16 mg/ g d.m. The same behavior was also observed in the flesh, even if the values were lower.

| | **L-CITRULLINE** (mg/g d.m.) | |
|---|---|---|
| | **Rind** | **Flesh** |
| **BAGNACAVALLO** | 14.79±1.67 a | 5.07±0.85 a |
| **FAENZA** | 16.22±2.16 a | 8.05±1.54 b |
| **MONACO** | 23.17±5.12 b | n.d. |
| **TALETE** | 32.77±6.51 c | 10.57±1.51 c |

**Tab. 18:** Content of L-citrulline in flesh and rind of organic watermelon genotypes. Different letters indicate significant differeces (ρ≤0,05) among genotypes.

On the basis of chemical-nutraceutical parameters, it can be deduced that the Monaco genotype showed the highest organoleptic profile, because it has the highest amount of characteristic volatile compounds and soluble sugars, with respect to the other genotypes. As concern the nutraceutical quality, Faenza, Monaco and Talete genotypes were clearly distinguishable from Bagnacavallo cultivar for higher contents of healthy compounds. However, Monaco cultivar should be preferred to the other genotypes for a fair balance between chemical and nutraceutical parameters. Talete genotypes, thanks to its higher content in L-citrulline in the rind respect to the other genotypes, was used for the preparation of functional foods, such as apple-jellies containing the powdered watermelon rind.

### 5.5.1 – CHARACTERIZATION OF APPLE JELLIES ADDED WITH WATERMELON RIND

Tab. 19 shows that the fruit jellies maintained an high content in L-citrulline, in fact the 10% decrease compared to the theoretical value, was almost exclusively linked to losses due to the applied experimental protocol. The low water activity seemed to guarantee a product stability overtime, according to the high solids content and a good Alcohol Insoluble Solids (AIS). This product had a good chewiness, as demonstrated by cut-strength test. The apple jellies were also submitted to microbiological analyses. Tab. 19 also shows that the apple jellies + L-citrulline were microbiologically stable, because the total bacterial count, eumycota and aerobic-anaerobic spore-formers denoted very low values and near to the sensibility limits of the choice methods (10 UFC/g for total bacterial count and eumycota, 100 UFC/ g for aerobic and anaerobic spore-formers).

| **CHEMICAL AND RHEOLOGICAL CHARACTERISTIC** | |
|---|---|
| **L-Citrulline** (mg/g product) | 1.88±0.52 (2.09) |
| $a_W$ | 0.595±0.05 |
| **d.m.** (%) | 84.87±5.15 |
| **Cut Strenght Test** (g/cm) | 490±80.66 |
| **AIS** (%) | 5.25±0.88 |
| **MICROBIOLOGICAL ANALYSIS (UFC/g)** | |
| **Aerobic spore-formers** | 1.5E+02 |
| **Anaerobic spore-formers** | <1.0E+02 |
| **Total Bacterial Count** (TBC) | 3.0E+02 |
| **Eumycota** | <1.0E+01 |

**Tab. 19:** Chemical-rheological characteristic and microbiological analysis of apple jellies with watermelon powdered rind. The number in brackets indicates the theoretical value.

This results pointed out the possibility of using watermelon ring (unutilized in food factory) for the preparation of products with high nutraceutical content, thanks to the presence of L-citrulline. Moreover, the other parameters showed good technological characteristics that are important for the acceptance of this product.

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## 5.6 – CONCLUSIONS

In most of the examined vegetables, the OR cultivation highlighted during the three years research a certain variability in the chemical and nutraceutical quality. Positive effects of the OR cultivation were noted in cauliflower and tomato regarding chemical aspects. Besides, for the nutraceutical quality, a phytochemicals profile quite similar to that of the CONV samples was found and, only in some cases, a profile superior to that of CONV samples, as in cauliflower, was observed. Local varieties, compared to the commercial ones, were well adapted to the OR cultivation, as in the case of sweet pepper, tomato and cauliflower. In vegetables cultivated only with OR system, such as watermelon and onion, the superiority of the local genotype was not evident both for the chemical and nutraceutical aspects.

In addition, studies carried out in the last year, on grilled pepper and on the preparation of fruit jellies from apples added with watermelon rind, lead to the following general considerations:

1 - heat treatments negatively affect the nutraceutical profile of sweet pepper, in particular the content of ascorbic acid and total carotenoids;

2 – innovative products, enriched with food factory waste, can be studied in order to obtain dietary supplements or pharmaceutical products, with high positive health-impact.

# PART 2

## 6.1 – RESULTS, DISCUSSION AND CONCLUSION

In order to evaluate the influence of two methods of cultivation on chemical and nutraceutical parameters of various vegetables, the average values of the three years of research have been taken into consideration. These statistical calculations were necessary because the differences found among the treated samples appeared to be influenced not only by the agronomic factors but also by environmental ones. For this reason, it will be reported a series of tables, where the average data of the different species of plants, in the experimental period 2010-2012, are discussed. In tab. 20, that shows the nutraceutical and chemical parameters of cauliflower in the studied period, it was noted an increase in the characteristic volatile compounds in the OR samples compared to CONV ones, but in 2011 the volatiles content of the OR samples was significantly lower than those of the OR samples of 2010 and 2012. With regard to glucosinolates, the influence of OR cultivation was low, as it was showed by the different content in glucosinolates, characterized by low values in 2011 compared to 2010 and values more than doubled in 2012. Another different behavior was represented by the evolution of antioxidant molecules, such as vitamin C, carotenoids and polyphenols. For vitamin C, the influence of OR cultivation seemed to be evident only in the first year of the trial, while it was noticed a gradual and significant decrease in vitamin C in both OR and CONV samples during the remaining period of the experimentation. Carotenoids and polyphenols, as well as vitamin C, were little affected by the cultivation mode, while an halving of the carotenoid content and an increase of polyphenols in 2012 in treated samples occurred.

| | CAULIFLOWER | | | | | |
|---|---|---|---|---|---|---|
| | 2010 | | 2011 | | 2012 | |
| | CONV | OR | CONV | OR | CONV | OR |
| **Characteristic volatile Substances** (μg/g d.m.) | 229.97±70.91 aA | 347.95±34.06 bB | 237.99±87.76 aAB | 270.74±55.13 bA | 262.45±98.89 aB | 340.68±78.10 bB |
| **Total Glucosinolates** (μmol/g d.m.) | 1.79±0.61 aB | 1.81±0.46 aB | 1.35±0.66 aA | 1.42±0.52 aA | 2.83±0.28 bC | 2.19±0.71 aC |
| **Vitamin C** (mg/100 g f.w.) | 56.80±7.80 aC | 64.23±14.27 bC | 44.11±3.37 aB | 46.57±7.42 aB | 27.50±5.27 aA | 36.67±4.99 bA |
| **Carotenoids** (mg/100 g f.w.) | 0.48±0.04 aB | 0.53±0.08 aC | 0.52±0.12 aB | 0.49±0.13 aB | 0.28±0.04 aA | 0.36±0.04 bA |
| **Polyphenols** (mg/100 g f.w.) | 48.44±13.87 bA | 41.60±3.36 aA | 45.64±6.48 aA | 44.42±0.72 aA | 63.87±5.77 aB | 63.17±10.95 aB |
| **Antioxidant power** (mmol ASA/100 g d.m.) | 2.94±0.12 aA | 3.02±0.48 aA | 5.32±0.51 aB | 4.94±0.13 aC | 2.57±0.89 aA | 3.86±0.46 bB |

**Tab. 20:** Chemical and nutraceutical parameters of OR and CONV cauliflower, observed in the three years of experimentation.
Different small letters indicate significant differences (ρ≤0,05) between OR and CONV samples within the same year (ρ≤0,05), capital letters among CONV or OR samples in different years.

In tab. 21, the chemical and nutraceutical parameters of unripe and ripe tomatoes were reported. Even in this case, in the unripe tomato an anomalous trend in 2011 was observed, as concern the characteristics volatile substances. In fact, their content was lower in both treated samples compared to those of 2010 and 2012. However, this aspect was not evidenced for the volatile characteristic substances in the ripe tomato. For nutraceutical substances, as lycopene and vitamin C, discordant values among ripe and unripe, CONV and OR samples throughout the entire trial were not found. Therefore, the different content of nutraceutical parameters in the treated samples, might be related to different agronomic treatments.

| | UNRIPE TOMATO | | | | | |
|---|---|---|---|---|---|---|
| | 2010 | | 2011 | | 2012 | |
| | CONV | OR | CONV | OR | CONV | OR |
| **Characteristic volatile substances** (μg/g d.m.) | 137.50±43.54 aC | 205.40±25.75 bC | 83.65±12.16 aA | 99.90±2.15 bA | 102.28±38.33 aB | 110.28±16.57 aB |
| **Lycopene** (mg/100 g f.w.) | 2.70±0.02 aC | 3.29±0.35 bC | 1.43±0.30 aA | 2.03±0.50 bA | 2.02±0.04 aB | 2.56±0.81 bB |
| **Ascorbic acid** (mg/100 g f.w.) | 18.47±2.47 aB | 17.46±2.35 aB | 13.40±3.13 aA | 14.05±2.35 aA | 12.13±0.04 aA | 14.97±0.14 bA |
| | **RIPE TOMATO** | | | | | |
| | **2010** | | **2011** | | **2012** | |
| | **CONV** | **OR** | **CONV** | **OR** | **CONV** | **OR** |
| **Characteristic volatile substances** (μg/g d.m.) | 147.14±8.03 aA | 441.97±9.38 Ba | 156.93±52.32 aA | 402.81±10.63 bA | 180.25±30.89 aB | 408.68±50.93 bA |
| **Lycopene** (mg/100 g f.w.) | 6.88±1.19 aA | 8.77±1.10 bA | 9.18±2.26 aB | 8.73±1.27 aA | 8.52±0.09 aB | 8.40±1.73 aA |
| **Ascorbic acid** (mg/100 g f.w.) | 17.50±1.05 aA | 19.11±1.55 aC | 16.13±1.63 bA | 14.25±0.76 aA | 16.45±3.83 aA | 15.89±1.10 aB |

**Tab. 21:** Chemical and nutraceutical parameters of OR and CONV, unripe and ripe tomatoes, observed in the three years of experimentation.
Different small letters indicate significant differences (ρ≤0,05) between OR and CONV samples within the same year (ρ≤0,05), capital letters among CONV or OR samples in different years.

The same considerations may be made for most of the biochemical parameters of sweet pepper, except for polyphenols, which showed the highest content in both treated samples, in 2010 (Table 22).

| | SWEET RED PEPPER | | | | | |
|---|---|---|---|---|---|---|
| | 2010 | | 2011 | | 2012 | |
| | CONV | OR | CONV | OR | CONV | OR |
| **Total pyrazines** (µg/100 g d.m.) | 44.58±6.61 bB | 27.33±6.01 aA | 39.30±5.66 aA | 39.21±8.27 aB | 40.42±4.85 bB | 29.06±5.10 aA |
| **Soluble sugars** (g/100 g f.w.) | 4.76±0.66 aA | 5.69±0.19 bB | 4.84±0.58 aA | 4.34±0.17 aA | 4.88±0.43 aA | 4.63±0.39 aA |
| **Ascorbic acid** (mg/100 g f.w.) | 136.53±38.28 aA | 165.67±19.64 bC | 137.52±25.66 aA | 148.60±4.19 aB | 140.69±20.84 bA | 127.76±18.65 aA |
| **Total Carotenoids** (mg/100 g f.w.) | 9.28±0.95 aA | 14.93±1.39 bB | 11.94±1.66 bB | 10.77±1.03 aA | 12.12±2.00 bB | 10.05±1.10 aA |
| **Polyphenols** (mg/100 g f.w.) | 60.79±10.15 aB | 62.40±14.26 aB | 47.79±5.25 bA | 35.73±6.82 aA | 51.81±6.22 aA | 57.20±8.83 aB |
| **Antioxidant activity** (mmol AsA/100 g d.m.) | 3.45±0.23 aA | 3.31±0.37 aC | 3.28±0.22 bA | 2.28±0.16 aA | 3.49±0.67 bA | 2.57±0.52 aB |

**Tab. 22**: Chemical and nutraceutical parameters of OR and CONV sweet red pepper, observed in the three years of experimentation.
Different small letters indicate significant differences (ρ≤0,05) between OR and CONV samples within the same year (ρ≤0,05), capital letters among CONV or OR samples in different years.

Tab. 23 shows the biochemical parameters of onion grown only in OR. Regarding the organsulfur compounds, a decrease of the saturated sulfides and an increase of unsaturated sulfides were noted during the three years. Even in this case a decrease of the polyphenols content was noted, while quercetine had the lowest value in 2011.

| | OR ONION | | |
|---|---|---|---|
| | 2010 | 2011 | 2012 |
| **Saturated sulfides** (µg/ 100 g d.m.) | 1331.01±384.04 C | 1198.47±169.65 B | 1047.40±238.22 A |
| **Unsaturated sulfides** (µg/ 100 g d.m.) | 2940.83±924.70 A | 3051.36±28.45 A | 4006.14±646.61 B |
| **Soluble sugars** (g/100 g f.w.) | 5.53±0.65 A | 5.39±0.24 A | 5.55±0.15 A |
| **Ascorbic acid** (mg/100 g f.w.) | 6.18±1.31 A | 6.80±1.44 A | 8.12±1.43 B |
| **Polyphenols** (mg/100 g f.w.) | 29.52±2.64 C | 27.72±4.64 B | 22.94±4.82 A |
| **Quercetin** (mg/100 g f.w.) | 22.60±7.98 B | 19.60±6.70 A | 24.55±5.44 B |
| **Antioxidant capacity** (mmol AsA/100 g d.m.) | 16.68±1.55 A | 16.07±2.19 A | 16.66±2.76 A |
| **Piruvic acid** (mmol/Kg f.w.) | 3.53±0.16 B | 3.13±0.20 A | 4.40±0.70 C |

Tabella 23: Chemical and nutraceutical parameters of OR onions, observed in the three years of experimentation.
Different capital letters indicate significant differences (ρ≤0,05) among OR samples in different years.

Finally, in watermelon grown only in OR, tab. 24 shows a decrease of volatile characteristic compounds in 2011, while the other parameters seemed to be more stable during the whole period.

| | OR WATERMELON | | |
|---|---|---|---|
| | 2010 | 2011 | 2012 |
| **Characteristic volatile substances** (µg/100 g d.m.) | 5403.37±187.44 B | 4650.91±206.93 A | 5326.84±249.38 B |
| **Soluble sugars** (g/100 g f.w.) | 7.14±0.64 A | 7.70±0.86 A | 7.61±0.88 A |
| **Lycopene** (mg/100 g f.w.) | 7.72± 2.96 A | 8.96±2.61 B | 8.38±2.63 AB |
| **Ascorbic acid** (mg/100 g f.w.) | 6.89±0.93 B | 5.55±0.83 A | 5.44±0.17 A |

**Tab. 24:** Chemical and nutraceutical parameters of OR watermelon, observed in the three years of experimentation.
Different capital letters indicate significant differences (ρ≤0,05) among OR samples in different years.

For cauliflowers, the decrease in vitamin C and contemporary the increase of polyphenols in the same period of time, could depend on environmental stress. This can be highlighted by comparing the rainfall data and temperature during the period from sowing to harvest during the three years of observation. The average rainfall was about 340 mm in 2010, 250 mm in 2011 and 271 mm in 2012. The last two values were significantly lower than that recorded in 2010. On the contrary, the differences of temperature, during the whole trial, were not very significant. In fact, average temperatures of 11,20 – 10,41 and 11,30 °C respectively in 2010, 2011 and 2012, were very similar. The hypothesis of environmental stress is in agreement with that made by previous works (Hnilikova, 2004; Lo Scalzo et al. 2007) that showed a significant decrease in the cauliflower ascorbic acid and an increase in polyphenols. In literature, the influence of climatic conditions on the biochemical parameters of the other examined vegetables has been scarcely investigated. However, for the other vegetables, as sweet pepper, onion and tomato, the influence of environmental stress could be assumed. In fact, in the period from sowing to harvest (May-August), while the temperature were almost constant and ranged between 21-23 °C, the rainfall deeply decreased in 2011 (1936 mm) and 2012 (130.4 mm), with respect to 2010 (275.6 mm).

The same consideration can be made for watermelon, cultivated in a different area, respect to the other considered species. Also in this case, while the temperature were constant during the three year of experimentation (21-23 °C), the rainfall values in 2011 (201.6 mm) and 2012 (189.8 mm) were significantly lower than 2010 (403.4 mm). The data concerning the temperature and rainfall for each species, in the period from sowing to harvest, are reported in tab. 25.

| | | **T** (°C) | | | **Rainfall** (mm) | | |
|---|---|---|---|---|---|---|---|
| | | **2010** | **2011** | **2012** | **2010** | **2011** | **2012** |
| C.R.A.-O.R.A. Monsampolo del Tronto (A.P.) | Cauliflower (September-Febraury) | 11.2 a | 10.4 a | 11.3 a | 340.0 b | 250.0 a | 271.0 a |
| | Tomato, sweet pepper, onion (May-September) | 21.0 a | 22.0 a | 23.0 a | 273.6 c | 193.6 b | 130.4 a |
| CRPV - Cesena (FC) | Watermelon (May-September) | 21.0 a | 22.0 a | 23.0 a | 403.4 b | 201.6 a | 189.8 a |

**Tab. 25:** Temperature and rainfall recorded in the period from sowing to harvest for all the tested species, in the whole period of experimentation. Different letters indicate significant differences (ρ≤0,05).

It should also be noted that the polyphenols are the most sensitive to climatic conditions, especially in the case of the pepper and onion. As polyphenols, also the characteristic volatile substances seemed to be more sensitive to environmental stresses. In fact, in almost all the tested vegetables, different behavior for biochemical parameters were observed during the years of experimentation. In the onion species, the nutraceutical quality increased due to the increase of unsaturated sulfides, as well as cauliflower, tomato and watermelon, whose characteristic volatile substances tended to decrease in 2011 and 2012, contemporary with the maximum environmental stress.

***References***

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