UNIVERSITÀ POLITECNICA DELLE MARCHE

Department of Agricultural, Food and Environmental Sciences

PhD Research in Agricultural, Food and Environmental Sciences
17° (XXXI) cycle
(2015-2018)

"Enhancement of Brassica species for innovative products to zero waste"

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SINTESI IN ITALIANO

I tre anni di ricerca hanno avuto come tematica principale l'investigazione della qualità nutrizionale, sensoriale e organolettica di verdure appartenenti alla famiglia delle *Brassicaceae,* commercializzate in prima gamma evoluta con marchio "Zeroscarti®", fornite dall'azienda agricola Valli di Marca, che ha co-finanziato il dottorato Eureka. Lo studio ha riguardato tutti i fattori che influenzano e determinano la qualità del prodotto sia in pre che in post raccolta; la conoscenza acquisita permette di migliorare e incrementare la qualità del prodotto fresco ma anche di trovare nuove strategie di conservazione per sviluppare nuove tipologie di prodotto da proporre al consumatore.

Inizialmente si è partiti dall'esigenza di individuare le componenti della qualità delle brassiche ricercandone le innumerevoli proprietà benefiche a livello salutare, con la caratterizzazione delle specie di brassiche ed erbe spontanee maggiormente commercializzate dall'azienda come prodotto fresco. L'obiettivo è stato quello di individuare e definire la qualità nutrizionale del prodotto finito, evidenziandone le componenti antiossidanti.

Dalla necessità di risolvere delle problematiche aziendali, quali la gestione del surplus, scarti ed eccedenze di produzione, si è sviluppata una linea di ricerca sui metodi di conservazione, quali disidratazione e liofilizzazione, utili ad incrementare la shelf life del prodotto, ponendo particolare attenzione all'impatto di questi sul profilo qualitativo e antiossidante delle brassiche. Infine è stato anche realizzato un panel test per valutare e confrontare la qualità sensoriale dei prodotti trattati. Dai risultati emerge come un prodotto liofilizzato possieda una maggiore ritenzione di componenti fitochimiche rispetto ad un prodotto disidratato, sebbene abbia costi più elevati.

Da questo studio è nata una nuova tipologia di prodotto disidratato dalle caratteristiche nutrizionali ed antiossidanti elevate, utilizzato tal quale per arricchire la dieta dei consumatori.

ENGLISH SUMMARY

The general aim of this PhD course was an investigation about the nutritional and sensorial quality of *Brassicaceae* species provided by the agricoltural company Valli di Marca that produce fresh vegetables in first evolved range “Zeroscarti®” brand; this work wants to deepen the knowledge on the phytochemical compounds content of those vegetables, and to investigate the factors that could affect them.

The research begun with a characterization of the nutritional and sensorial quality of fresh and raw vegetables belonging to *Brassicacee* family; these kinds of vegetables are produced in first evolved class, and represented new products for the consumers. The main aim is to confirm the high nutritional quality of fresh brassica products, highlighting the antioxidant compounds that exert healthy properties in the human diet.

Subsequently, the research concerned the effects of the post-harvest processes, like air-drying and freeze-drying, applied for increase the shelf life of vegetables, and that allowed the uses of the production waste and surplus. An investigation about the impact of process on nutritional quality was made. For evaluating the sensorial quality of final products, fresh, dried and freeze dried samples were submitted to a panel test. The freeze dried process results the best to maintain and preserve the nutritional and sensorial quality of Brassicas, but is the most expensive.

This study aimed to be an instrument for obtaining products with high nutritional quality, optimizing the pre-harvest factors and the post-harvest processes.

This study demonstrated and confirmed the higher nutritional, sensorial and healthy value of *Brassicacee* vegetables, and the possibility to produce and provide to the consumers high quality fresh products in first evolved class. Indeed, a new typology of brassica products was proposed, the air dried product, to use like an additive for enrich consumers’ diet and preserve their health.

Un ringraziamento dovuto e sentito va al personale delle aziende Valli di Marca e Agrinovana che hanno reso possibile questo progetto di ricerca grazie alle loro idee, intuizioni, curiosità ed esigenze di continuo miglioramento del proprio operato. Non è da tutti mettere al primo posto la qualità del prodotto e la salute del consumatore, sintomo di grande passione e lungimiranza, qualità che hanno saputo trasmettermi in questi tre anni di collaborazione.

Azienda agricola Valli di Marca Ss e Agrinovana Srl

L'azienda Agricola Valli di Marca Ss insieme alla Società commerciale Agrinovana Srl hanno richiesto questo progetto di ricerca per indagare sulle qualità nutrizionali ed organolettiche della vasta gamma di prodotti da loro commercializzati. Il desiderio di voler fornire al consumatore un prodotto dalle elevate capacità antiossidanti e nutrizionali li ha spinti ad investire su un dottorato per investigare sui fattori che determinano la qualità del prodotto finale e come questi possano essere controllati per migliorare l'intero processo produttivo.

La ricerca ha portato non solo a confermare le capacità antiossidanti dei prodotti già disponibili, ma ha avuto tra gli obiettivi, quello di svilupparne dei nuovi.

L'azienda Valli di Marca ss si occupa dell'intero processo produttivo, dalla coltivazione alla commercializzazione di verdure appartenenti alla famiglia delle *Brassicaceae* o *Crucifere* quali broccoli, cime di rapa, cavolo nero, kale, verze, cavolfiore e altre erbe spontanee come pimpinella, borraggine, papavero, bietola selvatica, rapa rossa, ravanello selvatico, tarassaco, di elevata qualità mantenuta grazie all'adozione della prima gamma evoluta che permette il mantenimento delle caratteristiche organolettiche e nutrizionali. Le due aziende, insieme, hanno creato e registrato il marchio Zeroscarti® che denota l'interesse verso le problematiche ambientali, alla riduzione dello spreco e degli scarti; il prodotto cioè non necessita di ulteriori lavorazioni o preparazioni da parte del consumatore che deve solo lavare e cuocere l'intero contenuto della confezione. Il marchio Zeroscarti® prevede un protocollo di produzione peculiare che si contraddistingue già dalla raccolta manuale della sola porzione edule della pianta, costituita dai getti ascellari più teneri. Segue un'ulteriore lavorazione e selezione in magazzino dove la materia prima viene mondata, tagliata, confezionata, etichettata e conferita alla GDO nazionale ed internazionale. Il prodotto realizzato ha insito la valorizzazione del territorio, la tutela della biodiversità con la scelta di ecotipi e varietà locali e con la riscoperta delle erbe spontanee marchigiane; affiancando al discorso produttivo anche un discorso di tutela dell'ambiente e di difesa della tradizione.

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1. INTRODUCTION

Brassicaceae family are among the oldest crops cultivated in the world (Snowdon et al., 2007). Brassica vegetables had countless uses thanks its large and wide diversification of crops; they are used for biofuel, edible oil, biofumigants, human food, and animal feed. Their strength is their great adaptability to different environments and climatic conditions, with a temperature optimum between 14 and 21°C and minimum and maximum temperature of 4 and 30°C, depending on the varieties (Wurr et al., 1996). Brassicaceous are cultivated during cold season in temperate regions, or at high altitudes in tropical and sub-tropical regions.

Brassica genus assumed great importance in European context thanks its wide genetic variability provided by countless species and varieties. This wide biodiversity reported in Europe suggests that the centre of origin and diversification of these species resides in the Mediterranean area (Vavilov, 1926). This theory is confirmed also by Branca and co-authors (2013) that observed a wide variability of cultivated types of *B. oleracea* in this area, as well as a wide range of wild species of Brassica (n = 9), suggesting the presence of a centre of diversity of *B. oleracea*. It may also be a center of origin, challenging the earlier theory that domestication was initiated along the Atlantic coast of central and northern Europe.

The high variability found in the species of Brassica genus is linked to their prevalent self-incompatibility; which is why cross pollination and fertilization are almost obligatory for many genotypes of the species. Also the presence of several spontaneous species of the genus Brassica characterized by the same chromosomal set favour the high variability.

1.1. Brassicas as healthy vegetables

In recent years, the increasing incidence of cardiovascular, degenerative diseases and different form of cancers is stimulating the interest of the consumers to healthy and unhealthy foods. One of the main factors of this phenomenon is the change of lifestyle and diet habits, as a consequence of the abandonment of the Mediterranean diet which, in contrast to other eating regimes, has taken as a model of healthy eating for years.

Epidemiological studies carried out all over the world demonstrated the efficiency of several compounds of natural origin in preventing the onset of malignant neoplasms (Kaur and Kapoor, 2001). This fact brought to modifying eating habits to increasing portion of fruits and vegetables consumed in the diets.

Fruits and vegetables, in fact, contain phytochemicals that act as protective factors for human body, as they are able to prevent DNA damage from oxidative degeneration, to inhibit the metabolic activation of carcinogens, to modulate the activities of detoxifying enzymes, to inhibit inflammatory processes, and to direct cancer cells to cell cycle arrest or apoptotic death.

Among vegetables, Brassicas in the last years received a lot of attention for the phytochemical compounds content and antioxidant capacity that confer nutraceutical value to the product. The main healthy benefits given by the habitual intake of Brassica vegetables in the diet are:

- Reduction of the risk of age-related chronic diseases (cardiovascular and other degenerative diseases);
- Reduction and prevention of a lot of types of cancer, as mouth, larynx, esophagus, stomach, intestines and prostate cancers (Cohen, et al., 2000; Cartea & Velasco, 2008; Jahangir et al., 2009; Dinkova-Kostova, 2012; Rodriguez-Mateos et al., 2014; Bjorkman et al. 2011);
- Protection of DNA from oxidation;
- Protection from chronic inflammatory bowel disease or hypothyroidism;
- Alleviation of muscle and joint pain;
- Antioxidant activity: the antioxidant potential of *Brassicaceae*, in particular *Brassica oleracea* species, is higher than other vegetables family (Zhou and You 2006).

The interest for these characteristics allowed to define the product as functional food. This term defines the food products that, in addition to carrying out the traditional food function, perform preventive and/or therapeutic effects against various human diseases, in particular chronic-degenerative diseases (Dauchet et al., 2006; He et al., 2006).

This healthy potential, in Brassicas, is bounded to their phytochemical compounds. The main responsible of the healthy function are phenolic compounds, vitamins (C, B9, K), provitamin A (β-carotene), lutein and different types of glucosinolates (Farnham et al., 2004).

1.2. Consumption and production

Brassica vegetables, mainly cultivated in temperate regions of the Northern hemisphere, are among the top 10 economic crops in the world (FAOSTAT 2011); in table 1, reported below, are indicated the main cultivated and consumed in the world:

Table 1- Main species and varieties of Brassica crops cultivated and produced in the world

Brassica species	*Spp./variety*	Common name/ Italian name
Brassica oleracea L.	*italica*	Broccoli
	capitata	Cabbage
	capitata f. rubra	Red cabbage
	capitata f. alba	White cabbage
	botrytis	Cauliflower
	acephala sabellica	Curly Kale, Red, Green and Russian curly kale
	acephala laciniata	Black Cabbage, Italian or Tuscan cabbage
	acephala	Collards
	gemminifera	Brussels sprouts
	gongylodes	Kohlrabi
	sabauda	Savoy cabbage
Brassica rapa L.	*rapa*	Turnip
	sylvestris	Turnip top, broccoli raab/cima di rapa, friarielli
	peckinensis	Chinese cabbage
	chinensis	Pak-choi
Brassica napus L.		Rapeseed
Raphanus sativus L.		Radish
Raphanus raphanistrum L.		Wild radish
Sinapis alba L.		Mustard/Senape bianca
Nasturtium officinale R. BR.		Watercress/Crescione d'acqua
Eruca sativa Mill.		Rocket/Rucola
Eruca vesicaria L.		Rocket Ruca, ruchetta
Diplotaxis tenuifolia L.		Wild rocket/Rughetta selvatica
Diplotaxis muralis L.		Wall rocket

Francisco et al., 2017; Ciancaleoni 2014; FAOSTAT 2011

Food and Agriculture Organization (FAO) reported a global production of Brassica crops of 92 million tons in 5.4 million hectares of cultivated surface in 2012, while in 2016 the production decreased to 71.2 million tons in 2.5 million ha of cultivated surface (FAOSTAT 2018).

The 70% of total world production of Brassicas is obtained in Asia. China is the main producer, followed by India, Korea and Russia. For what concern broccoli and cauliflower, China is again the main producer followed by Spain, Mexico, Italy, France and USA (FAOSTAT 2011). In Italy, the cultivation and production of cabbage and other brassicas suffered of a strong variation from 2006 to 2016, with a decrease of 24% of the cultivated area. In the same time frame, there was a reduction of 18% of the production quantity, but with an increase of 8% of yield. This yield increase resulted by an improvement of efficiency and effectiveness of cultivation practices, by the creation of new more productive varieties obtained in breeding programs, and by the optimization of fertilizer and pesticides use (FAOSTAT 2018).

In detail, in Marche region, from 2005 to 2015, a decrease of 48% of cultivated area destined to cabbage and other brassicas was registered, with a consequent production decrease of 38%. From 2010 to 2011, only in the Fermo province an increase of 3% in the cultivated area was registered (Istat 2018).

1.3. The commercialization classes

Vegetables are classified into four commercialization classes, depending on the types of processes to which they were subjected. Generally, fresh vegetables, commercialized unprocessed, like bench vegetables, belong to the first class; if they, are stabilized (like canned) or frozen (like legumes), they belong to the second and third class, respectively. Finally, if they are commercialised as ready-to-eat vegetables, like fresh salads, they belong to the fourth class (Baldi and Casati 2009).

During the past 10 years, the increasing consumer awareness of the strict correlation between vegetable consumption and their health (Florindo, et al., 2015), together with a frenetic urban lifestyle trends in Western populations, led to constant increase of the demand for products that can be consumed without home labour, like ready-to-eat (RTE) vegetables. In particular, leafy vegetable production was increasing thanks to innovation and new storage technology that increased and extended the quality of RTE foods, delaying their perishing. The increased consumer demand caused companies which produced RTE food to adapt to a dynamic market by optimizing cultivation techniques and focusing on product quality and innovation. The virtuous market competition has decreased production cost, increasing the power of purchase of the consumers (Baldi and Casati, 2009).

1.4. First Evolved Class “Zeroscarti®” (Zero Waste) Brand

The agricultural company that provided all the plant material used in this research choose to produce and commercialize vegetables in the so called First Evolved Class; this products category comprises fresh vegetables, cut, packaged and ready to eat after washing by the consumer: an intermediate solution between the fresh (first class) and the ready to eat products (RTE- fourth class). The characteristic of this kind of packaged product is the maintenance and preservation of nutritional and organoleptic quality thanks the protection of plastic films, but only if the cold chain is respected.

Valli di Marca agricultural company invented and registered its brand called Zeroscarti® that means zero waste. The concept is that all the content of the purchased package is available for consumption without other selection cuts of vegetables from the consumer, so without waste. The product only needs to be washed and cooked by the consumer.

The Zeroscarti® chain starts in the field with the hand harvesting of younger jets and shoots, more tender and tastier. The topic of beneficial effects of the younger plant portion harvest is widely described in following chapter.

The production chain continues with the selection, cutting and cleaning of the harvested vegetables. When fresh vegetables are cut for selection and preparation of a first evolved class product, a multitude of reactions are triggered by enzymes that change the bioavailability of phenol compounds. At the beginning, enzymatic oxidation brought to a phenol's degradation; however, fresh-cut vegetables possess living tissues, physiologically active and capable to synthesize new phenol compounds during shelf life, counteracting the oxidation products. This useful stress response was studied and reported in several fresh-cut vegetables like carrots (Alegria et al., 2016) and broccoli florets, where there was an increase of phenolic content of 53.2 % after 24h of storage; the percentage of the phenolic increase depended by the cutting styles adopted (Torres-Contreras et al., 2017).

After this step, vegetables are packaged, labelled and conferred to the distribution channel (GDO). The final product, of which an example is shown in figure 1, is particularly tender, tasty and rich of phytochemical compounds, as reported by several authors (Guo et al., 2001; Fernandes et al., 2007). The choice of use the plastic film packaging is supported by several studies that reported a retention of 100% of ascorbic acid in packaged broccoli stored at room temperature for 3 days, and a non-significant reduction after 5 days, has been demonstrated (Serrano et al., 2006; Cefola et al., 2010 a).

Figure 1 -"Cime di rapa" or turnip top produced in First Evolved Class by Valli di Marca and Agrinovana companies following Zeroscarti® protocol.

2. FACTORS INFLUENCING THE BRASSICACEE QUALITY

2.1. Brassicaceae quality

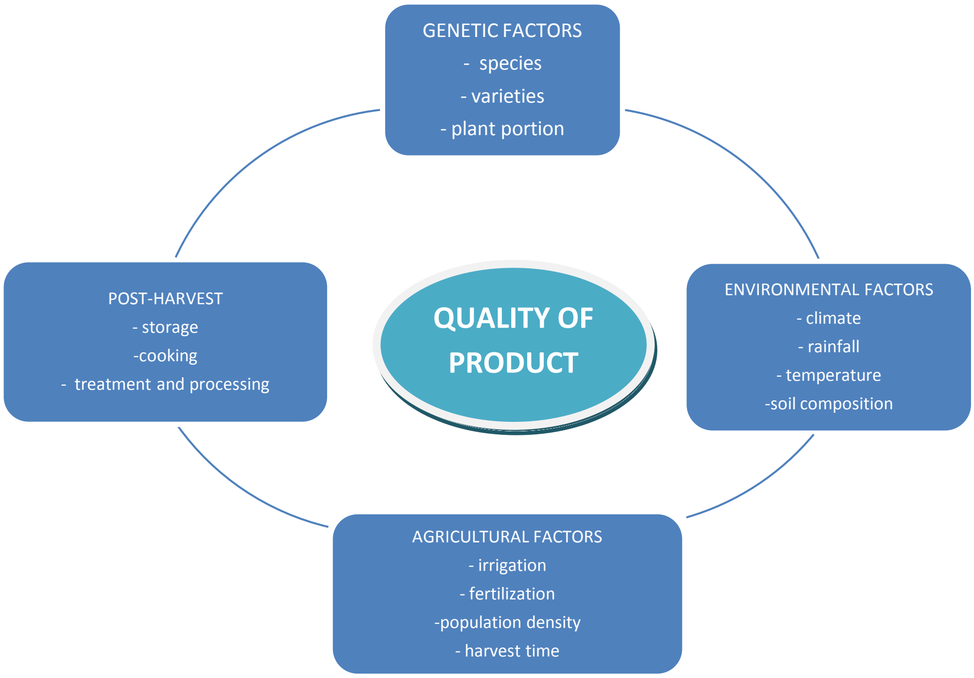


Figure 2- Factors that influences the quality of the final product

The concept of quality of a product derives from a combination of agronomical (plant yield efficiency), organoleptic/sensorial (color, sugar, acidity, pH, aroma) and nutritional (antioxidant, vitamins, etc.) attributes (Di Vittori et al., 2018). In the actual study, when proposing a new Brassica product to the consumers, sensorial and nutritional qualities are the main factors to keep in mind (schematized in figure 2), and strictly related to each other. This aspect could be easily explained by the fact that some particular bioactive compounds, such as phenolic compounds, could contribute not only to the health properties, but also to the sensory attributes of Brassicas.

2.1.1. Nutritional quality

The term nutritional quality, in the strict sense, could be defined as the amount of healthy bioactive compounds present in the fruits and vegetables matrix (Diamanti et al., 2014). Those compounds could belong to the class of antioxidant compounds (e.g. phenols, vitamin C, etc.), exerting their health effects through the ability of scavenge free radicals, or to non-antioxidant compounds (e.g. minerals, glucosinolates, etc.) that exert their function through direct mechanisms in the human metabolism different from the scavenger activity.

2.1.1.1. ANTIOXIDANT CAPACITY

Total antioxidant capacity (TAC) is the ability of a food to preserve an oxidizable substrate, inactivating the radical species or reducing an antioxidant. The TAC is considered a fundamental parameter for the description of fruits and vegetables nutritional quality; it is an indicator of the presence of bioactive substances belonging to the antioxidants group. Each antioxidant compound performs its protecting activity with different mechanisms and with different efficiency depending on its chemical structure and on the matrix it acts. For this reason, an evaluation of TAC is usually preferred than a measurement of the single concentration of each antioxidant, mainly if the objective of the study is a general screening of the health effects of different vegetables.

In bibliography exists a lot of fast screening methods to investigate the TAC, based on spectrophotometric measurements: the mainly used are TEAC (Trolox Equivalent Antioxidant Capacity) (Miller & Rice-Evans, 1997), FRAP (Ferric Reducing Antioxidant Power) (Benzie and Strain, 1996), DPPH (Radical Scavenging Activity) (Lim, Lim & Tee, 2007) and ORAC (Oxygen Radical Absorbance Capacity) (Huang et al., 2002). In any case, all the methods measure the scavenging capacity of antioxidants against radical solutions, and not their preventive action aimed at preventing their formation (Huang, 2005).

Brassica vegetables, broccoli and kale, showed higher antioxidant potential than other vegetable crops like spinach, carrot, potato, been and onion. In general, among Brassica vegetables, Brussels sprouts, broccoli, and red cabbage belong to the group of the highest antioxidant capacity products. Common cabbage possesses the lowest antioxidant capacity (Cao et al., 1996; Ou et al., 2002; Zhou and You 2006). For what concerns cauliflower, contrasting results were reported by Azuma et al. (1999) and Wu et al. (2004). The analysis of the TAC is influenced by the extraction method and the type of reactive species in the reaction mixture (Azuma et al., 1999).

A lot of scientific authors studied and identified the main antioxidant molecules present in *Brassicaceae.* It is possible to divide those antioxidant compounds in two main groups: water-soluble antioxidants and lipid-soluble antioxidants (Podsedek, 2005; Soengas et al., 2011). Kurlich et al. (2002) and Wu et al. (2004) reported that hydrophilic antioxidants are responsible for 80-95% of the TAC of the *Brassicaceae* using the ORAC method for the measurement, while lipo-soluble antioxidants account only for the 5-20%.

2.1.1.1.1. Water-Soluble Antioxidants

2.1.1.1.1.1. Phenolic compounds

Phenolic compounds are the most widespread antioxidant family present in the vegetables. This large group of compounds is particularly present in the Brassica vegetables and constituted the main source of antioxidants in these plants (Jahangir et al., 2009 a; Podsedek, 2007). The plants produce them as secondary metabolites for protection from pests and insects attack.

Their importance on human health is related to their anti-oxidative properties, donating electrons to scavenge reactive oxygen species (Rice-Evans et al., 1996). Among Brassica species, kale and broccoli resulted with the highest quantity of total polyphenols (Vallejo, 2003a; Moreno, 2006; Heimler et al., 2006).

Flavonoids represent the widespread phenolic compounds in Brassicas; they possess a lot of biological properties, e.g. antioxidant activity, a capillary protective effect, and an inhibitory effect elicited in various stage of tumor (Cartea et al., 2011; Czeczot 2000; Podsedek 2007). They are characterized by numerous subclasses, but the most important in Brassicas are the following:

1) *Flavonols:* together with anthocyanins, they are the main represented flavonoids in Brassica species; they can be found in internal and external part of leaves, seeds, shoots and sprouts leaves (Ferreres et al., 2009; Sousa et al., 2007). The most represented flavonols in Brassica vegetables are quercetin, kaempferol and isorhamnetin. Quercetin, mainly represented in broccoli, is characterized by a strong antioxidant power (higher than vitamin C); it exerts its activity against free radical oxygen and acts on the prevention of cardiovascular diseases and cancer, atherosclerosis and chronic inflammation, and the induction of enzymes that detoxify carcinogens (Ackland et al., 2005; Kim et al., 2004). Kaempferol 3-O-sophoroside is the main represented flavonol in broccoli florets*;* its high intake is linked with a lower risk of coronary heart disease (Calderon-Montano et al., 2011). Kaempferol and quercetin, and in less amount myricetin, are the main represented flavonols in *B. rapa subsp. sylvestris*.

2) *Flavones*: apigenin and luteolin are the only flavones detected in the hydrolysed extracts of different Brassica vegetables, except for broccoli (Bahorun et al., 2004).

3) *Anthocyanins* were detected in Brassica vegetables and described by several authors (Wu et al., 2004; Jahangir et al., 2009b; Moreno et al., 2010; Lo Piero et al., 2013). They are present only in species and varieties with bright colour, red, orange and purple pigmentation, like some kales, purple broccoli, and red and black cabbage. These compounds show an interesting antioxidant activity. The 80% of anthocyanins present in Brassica species are in the acylated form, more stable and easily absorbable by the organism. The main represented anthocyanins in cruciferous are *cyanidin derivatives.* In particular, red cabbage possesses 15 types of anthocyanins, all acylglycosides of cyanidin; cyanidin-3-diglucoside is the most represented (Dyrby et al., 2001). In broccoli, more than 17 anthocyanins were detected (Moreno et al., 2010).

Among phenolic compounds, even if they are not hydro soluble, are to be mentioned the lignans, diphenolic compounds that possess several biological activities, through their antioxidant and oestrogenic properties. Lignans may reduce the risk of certain cancers and cardiovascular diseases (Soengas et al., 2011). Some studies reported that lignans are mainly present in kale family, broccoli and Brussel sprouts with lariciresinol and pinoresinol being the most abundant (Milder et al., 2005; Soengas et al., 2011).

2.1.1.1.1.2. Vitamin C and vitamin B9 (Folic Acid)

Vitamin C is a powerful antioxidant widely present and studied in the fruits, but a lot of recent works were focused on the importance of vitamin C in vegetables, mostly in *Brassicaceae* family. In Brassica vegetables the vitamin C concentration varies a lot among species and subspecies, and it is strictly genotype- and environment-dependent (Kurilich et al., 1999; Vallejo et al., 2002). Vitamin C, or ascorbic acid, performs countless biological activities in human body and represents a nutritional compound fundamental for health. Ascorbic acid is a radical scavenger, an enzyme cofactor and a donator/acceptor in electrons transport at the plasma membrane level; its role is fundamental in the regeneration of α-tocopherol, and in the prevention and treatment of malignant and degenerative diseases (Davey et al., 2000, Kurilich et al., 1999).

Among Brassica genotypes, broccoli and cauliflower seem to possess the highest content of vitamin C, followed by Brussel sprouts and kale, while white cabbage possesses the lowest amount (Podsedek, 2007).

Vitamin B9 (Folic acid) is an important vitamin present in Brassicas, mainly in raw broccoli, cauliflower and cabbage, that acts as a coenzyme in many single carbon transfer reactions, in the synthesis of DNA and RNA and of protein components. Furthermore, it reduces the level of homocysteine in the blood, a risk factor for cardiovascular diseases. Among the several health activities that folic acid performs, it is strongly important the prevention of megaloblastic anemia, neuropsychiatric disorders and various forms of cancer in the fetus during pregnancy, also reducing the risk of neural tube defects (Jahangir et al., 2009b; Kurilich et al., 1999, Bailey et al., 2003). Furthermore, low folate intake is one of the main causes of the insurgence of anemia.

2.1.1.1.2. Lipid-Soluble Antioxidants

Despite the low incidence of lipo-soluble antioxidants on the TAC of Brassicas, as stated in the chapter 2.1.1.1, several studies confirm the high content of lipid soluble antioxidant in kale and broccoli, moderate in Brussels sprouts, and low amount in cauliflower and cabbage (Kurilich et al., 1999). Among lipo-soluble antioxidants, carotenoids and vitamin E are the most important found in Brassica vegetables.

2.1.1.1.2.1. Carotenoids

Carotenoids are responsible of the orange, yellow and red pigmentation of several fruits and vegetables, mainly carotens and xanthophylls. The most represented carotenoids in Brassica vegetables are β-carotene, that the organism transforms in vitamin A, and lutein and zeaxanthin (Podsedek, 2007). β-carotene prevents from the insurgence of cancer and cardiovascular diseases, and decreases the risk of myocardial infarction among smokers, of immune dysfunction and age-related macular degeneration (Rice-Evans et al., 1997; Kurilich et al., 1999). Muller (1997) analysed the total carotenoid content of several Brassica species and reported them in decreasing order: Brussel sprouts (6.1 mg/100 g), broccoli (1.6 mg/100 g), red cabbage (0.43 mg/100 g) and finally white cabbage (0.26 mg/100 g). In *Brassica oleracea* genus, kale possesses the higher content of carotenoids with over 10 mg/100 g of edible portion (Muller, 1997).

The Brassica vegetable with the highest content of lutein and zeaxanthin is kale (3.04-39.55 mg/100 g); interesting content were found also in broccoli and Brussels sprouts (Podsedek, 2007). In *B. rapa* species, 16 carotenoids were identified by Wills and Rangga (1996); in *B. chinensis, parachinensis* and *pekinensis* lutein and β-carotene are the most abundant carotenoids (Soengas et at., 2011).

2.1.1.1.2.2. Vitamin E

Vitamin E is formed by groups of compounds called tocopherols and tocotrienols; in detail, α-tocopherol is the main represented in Brassica vegetables, with the exception of cauliflower, that contains mainly γ-tocopherol (Podsedek, 2007; Piironen, 1986). Vitamin E performs a protective activity against coronary heart disease through the inhibition of LDL oxidation (Stampfer and Rimm, 1995). A high intake of vitamin E helps in the prevention of cancers, cardiovascular diseases, neurological disorders and inflammatory diseases (Kurilich et al., 1999). The content of vitamin E in Brassica species was studied in literature, and here is reported in decreasing order: broccoli (0.82 mg/100 g), Brussels sprouts (0.40 mg/100 g), cauliflower (0.35 mg/100 g), Chinese cabbage (0.24 mg/100 g), Red cabbage (0.05 mg/100 g), and white cabbage (0.04 mg/100 g) (Piironen et al., 1986)

2.1.1.2. MICRO- AND MACRO-ELEMENTS

Macro-elements, also called macronutrients, are those nutrients that the plants need in greater quantities; essentially play a structural and energetic role. They are indispensable elements for the growth and development of the metabolic functions of plants. The fundamental nutrients are represented by nitrogen (N), phosphorus (P) and potassium (K).

Minerals such as Boron (B), Copper (Cu), Cobalt (Co), Iron (Fe), Manganese (Mn), Zinc (Zn), Selenium (Se), are present in plants in very small quantities and are known as microelements. Their characteristic is to be essential to plants, but only in small quantities. Although trace elements are present in small quantities in plants, they play key roles in plant life; this is also demonstrated by the symptoms associated with deficiency phenomena. Their availability depends on the conditions of the land.

However, micro- and macro-elements exert important roles also in the human body. The elements K, Ca, Mg, Fe, Zn, Se, and Mn are fundamental in the regulation of many metabolic activities, in bones and teeth health, in cancer prevention, in the production of red blood cells, and participating as enzyme co-factors.

Brassicaceae are a good source of calcium, indispensable for teeth and bones health, in particular for vegetarian and vegan people. The calcium shortage brings to a series of disturbances, like insomnia, nervousness, muscle cramps, hypertension, and lower back pains (Moreno et al., 2006; Jahangir et al., 2009a).

2.1.1.3. GLUCOSINOLATES (GLS) AND ISOTHIOCYANATES (ITCs)

Glucosinolates (GLS) are one of the most important *Brassicaceae* secondary metabolites derived from amino acid biosynthesis (Podsedek, 2007). GLS are glucosidic compounds containing sulphur present in Brassica leaves, compartmentalised in the vacuole, at concentration such as to be able to prevent the development of pathogens, diseases and pests (Sisti et al., 2003). Their concentration varies among Brassica species (Branca et al., 2013a) according to the developmental stage, the tissue type, the exposure to salt stress, environmental factors, or plant signalling molecules, including the treatment with salicylic acid (SA), jasmonic acid (JA) and methyl-jasmonic acid (MeJA) (Velasco et al., 2007; Cole, 1997; Burrow et al., 2008; Lopez-Berenguer et al., 2008; Vallejo et al., 2003a; Mikkelsen et al., 2003; Mithen, 2001). GLS can be divided into three chemical classes: aromatic, indole and aliphatic, based on their amino acid precursor (aromatic amino acid, tryptophan and methionine, respectively) like reported in table 2 (Giamoustaris and Mithen, 1995). In Brassica vegetables, the most important GLS belong to the methionine-derived ones (Mithen et al., 2003).

GLS have not direct functions of human health: the health effects are exerted by their hydrolysis breakdown products, the isothiocyanates (ITCs). Those are aromatic volatile compounds containing sulphur, derived from the hydrolytic action of the enzyme myrosinase on GLS. The plant myrosinase acts in the human gut and hydrolyse GLS in ITCs during human ingestion. However, during the cooking of the vegetables, the exposure to heat treatment can inactivate the plant myrosinase, so the ITCs are obtained thanks to action of myrosinase producted by the human gut flora. Unfortunately, its activity and efficiency is lower than plant myrosinase (Angelino et al., 2015). It is possible to obtain many ITCs, and their production strictly depends by the original GLS, the substrate, the pH conditions, the availability of ferrous ions, and the level of activity of the ESP (epithiospecifier protein), a specific protein factor (Grubb and Abel, 2006; Mithen, 2001).

ITCs are the main responsible of the bitterness, spicy and typical flavour and smell of Brassica vegetables (Padilla et al., 2007). ITCs possess protective and preventing effects against several kinds of cancer like prostate, intestinal, liver, lung, breast, and bladder, chronic inflammation and neurodegeneration, acting on the apoptotic phase of cell developmental cycle; they are effective also in the cholesterol reduction (Keum et al., 2004; Moreno et al., 2006; Cartea and Velasco, 2008; Clarke et al., 2011; Angelino and Jeffery, 2014).

The most studied ITCs in medical research is the sulforaphane (Fahey et al., 2002; Rose et al., 2005; Dinkova-Kostova and Kostov, 2012), mainly represented in broccoli and Brussel sprout. It is the most important ITCs considering its healthy value, and derived from the glucoraphanin (Matusheski and Jeffrey, 2001). The sulforaphane is an indirect antioxidant, because it acts as a catalyst in the stimulation of the cellular antioxidant system. In particular, sulforaphane stimulates some enzymes active against tumoral cell proliferation (Padilla et al., 2007; Kim et al., 2003; Rosa, 1997).

Table 2- Principal glucosinolates identified in leaves of Brassica vegetable crops

Crop	Aliphatic glucosinolates										Indole glucosinolates				Aromatic
	GIB	PRO	SIN	GAL	GRA	GNA	GBN	GIV	GER	GNL	GBS	NGBS	4HGBS	4MGBS	GST
Brassica oleracea															
White cabbage[a,b,c,d]	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Savoy cabbage[a,b,c,d]	+	+	+	–	+	+	+	+	–	–	+	+	–	+	+
Red cabbage[a,c,d]	+	+	+	–	+	+	–	+	–	–	+	+	–	–	–
Kale[a,c,d,e]	+	+	+	–	+	+	–	+	–	–	+	+	+	+	+
Collard[e]	+	+	+	–	–	–	–	+	+	–	+	–	–	–	–
Tronchuda cabbage[c,f]	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+
Broccoli[e,g]	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Brussels sprouts[d,e,g]	+	+	+	–	+	+	–	+	–	–	+	+	–	–	–
Cauliflower[d,g]	+	+	+	–	+	–	–	+	–	–	+	+	–	–	–
Kohlrabi[d]	+	+	+	+	+	+	–	+	–	–	+	+	+	+	–
Brassica rapa															
Turnip[b]	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+
Turnip greens[h]	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+
Turnip tops[i]	+	+	–	–	–	+	+	+	–	–	+	+	+	–	+
Chinese cabbage[b]	+	+	–	–	–	+	+	–	–	+	+	+	–	+	+
Brassica napus															
Swede[b]	–	+	–	–	+	–	+	–	–	+	+	+	+	+	+
Leaf rape[j]	–	+	–	+	–	+	+	+	–	+	+	+	–	+	+

Major glucosinolates found in each crop are shown in bold

GIB: glucoiberin (3-methylsulfinylpropyl); PRO: progoitrin (2-hydroxy-3-butenyl); SIN: sinigrin (2-propenyl); GAL: glucoalysiin (5-methylsulphinylpentyl); GRA: glucoraphanin (4-methylsulphinylbutyl); GNA: gluconapin (3-butenyl); GBN: glucobrassicanapin (4-pentenyl); GIV: Glucoiberverin (3-methylthiopropyl); GER: glucoerucin (4-methylthiobutyl); GNL: gluconapoleiferin (2-hydroxy-4-pentenyl); GBS: glucobrassicin (3-indolylmethyl); NGBS: neoglucobrassicin (1-methoxy-3-indolylmethyl); 4HGBS: 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl); 4MGBS: 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl), GST: Gluconasturtiin (2-phenylethyl)

Sources

[a] VanEtten et al. (1980)

[b] Sones et al. (1984)

[c] Cartea et al. (2007b)

[d] Ciska et al. (2000)

[e] Carlson et al. (1987)

[f] Rosa et al. (1996)

[g] Kushad et al. (1999)

[h] Padilla et al. (2007)

[i] Rosa (1997)

[j] Cartea et al. (2007a)

2.1.1.3.1. Anti-nutritional effects

Beyond to be considered positive for the health, some GLS produces also breakdown products considered damaging for the human and animal health; their presence in seeds of cruciferous for oil (*B. napus, B. rapa, B. juncea*) greatly reduces the quality of flour and of flour produced by the residues of oil extraction; indeed these product are used as feed (Griffiths et al., 1998.). However, some ITCs can cause goitrogenic and growth retardation activities also in animals. The most damaging ITCs is the degradation product of progoitrin, the oxazolidine-2-thione (Rosa et al., 1997). It causes goiter, harmfull effects on nutrition, depressed growth, poor egg production and liver damage in animals. These effects were not found in humans (Mithen, 2001). Plant breeders find a solution to this problem, selecting and improving breeding lines without (or with a low amount of) GLS (progoitrin) and erucic acid, e.g oilseed crop “Canola 00” (Anilakumar et al., 2006; Cartea and Velasco, 2008). Terzo and co-authors (2018), studying Sicilian black broccoli extract, reported that not all breakdown GLS products show more toxicity than their precursors; in fact, GRA (glucoraphanin) and GNT (gluconasturtiin) showed less toxicity than their breakdown products whereas opposite results were obtained with GBS (glucobrassicin) and GBN (glucobrassicanapin). The GLS covertion into ITCs is necessary to exert anticancer activity.

2.1.2. Sensorial and organoleptic quality

The quality of vegetables for the consumer not concerns only the nutritional aspects, but includes also the organoleptic and sensorial parameters that can be measured with different methods.

The principal sensorial parameters are:

- *firmness*, that indicates the resistance of vegetables to mechanical damages; it assumes a great importance during the post-harvest management;
- *colour*, that indicates the freshness of the product and the quality of the storage conditions; it attracts visually the consumers (Bonasia et al., 2013);
- *sweetness*, that is linked to the presence of glucose, fructose and sucrose, and indicated the sweet sensation to the consumer;
- *acidity,* that indicates the acid sensation that the product stimulates in the consumer, it is usually expressed as % of citric acid.

For what concern Brassica vegetables quality, fundamental sensorial parameters are those related to taste and flavor, and that can be investigated through the implementation of a panel test. In particular, they are of considerable importance the after-taste and the off-odors, because through these parameters is possible to evaluate the typical sulphurous and pungent flavor given by GLS, ITCs and sulphured amino acids. Also, green note is a particularly important characteristic to recognize in Brassicas and is conferred by alcohols and aldehydes formed by the enzymatic degradation of free fatty acids.

Also, bitterness is particularly accentuated in Brassicas; this sensation is caused by ITCs sinigrin, gluconapin, progoitrin, glucobrassicin, neoglucobrassicin in different intensities (Rosa et al., 1997; Schonhof et al., 2004; Francisco et al., 2011). Several studies affirm that the GLS and their breakdown products aren’t the only responsible of the bitter taste and

Brassica flavour, but these resulted from a synergic activity of various phytochemicals (indole hydrolysis products, flavonoids, etc.), (Drewnowski et al., 2001; Baik et al., 2003; Padilla et al., 2007).

2.2. Genetic factors influencing Brassica quality

As previously stated in chapter 2.1, Brassica quality is influenced by several factors, but the principal is represented by the genotype characteristics. Many breeding programs are working for the creation and selection of new productive and qualitative genotypes. Those processes are particularly implemented in the Mediterranean Area, where many spontaneous and wild Brassicas provide genetic diversity and variability, allowing to develop new breeding programs. In particular, in Italy and Spain is it possible to find countless ecotypes and populations of *Brassica oleracea* and *Brassica rapa* species, handed down by generations of farmers (Branca et al., 2013a).

2.2.1. Influence of the genotype

The antioxidant potential of *Brassica oleracea* and *Brassica rapa* crops have been widely studied and showed a wide diversity among species.

For *Brassica oleracea* group, a lot of studies reported the nutritional quality and the high phytochemicals content, in particular in broccoli, Brussels sprouts, kale, tronchuda and red cabbage; lower amounts were detected in white cabbage, cauliflower, and Savoy cabbage (Wu et al., 2004). Kale seems to be the ancestor of several *B. oleracea* vegetable crops because it is very similar to *B. oleracea* wild type and to several wild Brassica species (n=9) (Branca et al., 2013).

Brassica rapa included turnip tops and leaves (cima di rapa and friariello), turnip, pak choi, Chinese cabbage, choy-sum and mizuna. From the countless scientific works in this topic, emerges the wide variability among close species (Bahorun et al., 2004; Singh et al., 2007), and varieties of the same species (Francisco et al., 2009). The problem sometimes is the comparison of data, because exist a lot of different analytic methodologies for extraction and measurement of nutritional parameters.

Phenolic compounds are mainly affected by the effect of environment and genotype-environment interaction; this means that their variability strictly depends by the environmental conditions; and they possess low heritability.

The indolic GLS are most influenced by environment, while aliphatic GLS are mainly influenced by genotype (Francisco et al., 2011).

2.2.1.1. BRASSICA OLERACEA SPECIES

Inside this species, several differences among varieties were reported, e.g., the highest content of total phenolic was found in curly kale that showed a concentration 10 times higher than cauliflower and white cabbage (Nilsson et al., 2006; Jagdish et al., 2009; Sikora et al., 2008). Although the methodologies of analysis used in many studies were different, all of them agree about the lower content of phytochemicals in white cabbage, in respect to broccoli, Brussel sprouts, curly kale and red cabbage. About cauliflower, there are controversial results because it showed a high activity in liposomal phospholipid suspension system, but low activity in oxygen radical absorption capacity (ORAC method) (Podsedek, 2007; Soengas et al., 2011).

As mentioned above, the variability it is also expressed among genotypes of the same varieties in broccoli (Kaur et al., 2006; Pérez-Balibrea et al., 2011), cauliflower (Volden et al., 2009a), cabbage (Kim et al., 2004); in general, the higher content of antioxidants is detected in the varieties with red or purple pigmentation. Broccoli are important for their cancer-protective compounds, in particular for their content of glucoraphanin, and its active form sulforaphane. Broccoli had the highest levels of phenolics (63.4 mg /100g fw), vitamin C (52.9 mg/100g fw), β-carotene (0.81 mg/100 g/fw), lutein (0.68 mg/100 g fw), and α-tocopherol (0.47 mg/100 g fw), followed by Brussels sprouts (Singh et al., 2007).

Sicilian landraces of violet cauliflower, characterized by high plant rusticity and adaptability to Mediterranean climatic condition, could be considered environmental friendly crops, by containing the use of pesticides and fertilizers (Branca et al., 2013b).

2.2.1.2. BRASSICA RAPA SPECIES AND OTHER CRUCIFEROUS CROPS

Choy sum, a *Brassica rapa* variety, showed the highest antioxidant potential compared with broccoli, cabbage, and cauliflower (Wachtel-Galor et al., 2008). Some studies described the higher antioxidant potential of watercress than salad rocket; however, those 2 varieties, together with wild rocket and mizuna, are good sources of antioxidants (Martìnez Sànchez et al., 2008; Martìnez-Villaluenga et al., 2010). In *B. rapa,* the aliphatic GLS is the predominant form, with the gluconapin as the most abundant, followed by glucobrassicanapin (Francisco et al., 2011). *B. rapa* varieties shown a high concentration of isorhamnetin, irrespective of the plant organs considered (Jeffery et al., 2003).

2.2.2. *Influence of plant portion and plant developmental stage*

The variation of nutritional and phytochemical content does not differ only among species and varieties of the same species, but can change during growth period (Francisco et al., 2017) and based on plant portion (Velasco et al., 2007), as reported below.

2.2.2.1. PLANT PORTION

Sometimes, the more interesting parts of the plant from the point of view of the nutritional quality are not consumed. For example, seeds seem to possess the highest content of phytochemical compounds but are not usually consumed and appreciated by consumers; this aspect is confirmed in kale, where seeds possess higher antioxidant content than leaves (Ferreres et al., 2009). In turnip, flower buds registered the highest antioxidant content, in respect to leaves, stems and roots (Fernandes et al., 2007).

Also, GLS content differs based on plant portion. Seeds possess the highest concentration of GLS, followed by inflorescences, siliques, leaves, roots, stems and petioles (Rosa et al., 1997; Brown et al., 2003). Indeed, the concentration of aliphatic GLS in kale (B. oleracea acephala) leaves increases during time, from seedling to early flowering stages; At that stage, the aliphatic GLS content in leaves of B. oleracea declined drastically over time as the content in the flower buds increased. The highest contents of indolyl glucosinolate (glucobrassicin) and of the aromatic glucosinolate occurred in leaves harvested at the optimum consumption stage while flower buds contained the highest concentration of aliphatic glucosinolates, especially sinigrin (Velasco et al., 2007).

Björkman et al. (2008) found a 2.6-time higher total GLS level in white cabbage foliage compared to roots. In contrast to these results, Van Dam et al. (2009) gathered and comparison a multitude of studies of GLU in roots and shoots of 29 plant species concluded that roots have higher concentration and a greater diversity of GLS than shoots.

Variation in plant portion is reported also in Kohlrabi (*Brassica oleracea L. var. gongylodes*), where the GLS profile of the stems and leaves were similar, though stems showed a lower GLS amount and the presence of glucoerucin. The total polyphenols and ascorbic acid content were slightly higher in the leaves than in the stems. The leaves generally showed higher antiradical activity in terms of DPPH quenching compared to skins and stems (Branca et al., 2013c).

A comparison study about turnip tops and turnip greens also reported several and appreciated differences in phytochemicals compounds. Turnips top measured a higher value of GLS (26.02 μmol/g dw) than turnip greens (17.78 μmol/g dw). The opposite trend was reported for total phenolic, where turnip greens showed a higher content (43.81 μmol/g dw) than turnip tops (37.53 μmol/g dw). The content varies also according to the type of GLS, as reported in table 3 (Francisco et al., 2011).

Table 3- Principal GLS content in *B. rapa* varying in based on plant organ (Francisco et al., 2011)

GLS	turnip greens	turnip tops
Gluconapin (84%)	10.21 μmol/ g dw (68%)	17.39 μmol/g dw (78%)
Glucobrassicanapin (7.2%)	1.90 μmol/g dw (5-15%)	2.38 μmol/g dw (5+15%)

Sometimes it is possible to appreciate and detect differences into the same portion of plant, and several studies confirm this thesis. In tronchuda cabbage, the mainly consumed portion is the internal leaves, used for salad or cooked, but these have an antioxidant capacity lower than the external ones, normally thrown away (Ferreres et al., 2009). The same results were found in Chinese cabbage, where the variation in bioactive compounds is evident also among different layers of the same head cabbage; phenolic acids and flavonoids were higher in the outer leaves, followed by the mid- and inner leaves. This result could be explained by the higher exposure of outer leaves to sunlight, which stimulates the production of antioxidants (Seong et al., 2016).

2.2.2.2. PLANT DEVELOPMENTAL STAGE

The growth stage can influence the content and concentration of phytochemical compounds in Brassicas, and the knowledge of this aspect is fundamental to choose the proper harvest moment for obtaining products with the highest quality. Seeds possess the highest antioxidant content, but during the germination process a decrease of this content occurs, because the antioxidant compounds are used for the growth and for the activation of the metabolism of the new plant. Indeed, the juvenile cabbage possesses more flavonols than the mature one (Kim et al., 2004). More precisely, a study about the harvesting date of Chinese cabbage showed that the higher antioxidant content was found within the 8^{th} and 12^{th} week from germination, followed by a gradual decrease (Samec et al., 2011); this is probably due to a greater metabolic activity in the first few months.

Also, total GLS content varies in function of growth stage, and increase from vegetative to reproductive stages and maturity (Fieldsend and Milford, 1994). Consequently, the highest content is found in flower buds, or in leaves harvested at the optimum consumption stage, 180 days after sowing in kale (Velasco et al., 2007). In broccoli heads, the highest glucoraphanin content was also observed 180 days after sowing, with a following decline during flowering (Rybarczyk et al., 2014). In Chinese cabbage sprouts, the maximum of GLS concentrations was registered during the growth stage (Guo et al., 2013). In turnip, the eight days-old sprouts were ideal for the maximum amount of GLS, in respect to their mature stage (Baenas et al., 2014). Fahey et al. (1997) found that young broccoli and cauliflower sprouts (3 days-old) contained 10-100 times higher levels of glucoraphanin per gram compared to the corresponding mature plants. Studying broccoli, cauliflower and white, red and Savoy cabbages, it was found also that the concentration of total aliphatic GLS decreased, whereas that of glucobrassicin increased, during a seven-days sprouting period (Bellostas et al., 2007).

Vallejo et al. (2003 b) found an increase of ascorbic acid and phenol compounds during the development of the inflorescence in three broccoli cultivars.

Carotenoids are also affected by plant developmental stage. In kale, the highest content of lutein was registered in 1 to 2 weeks-old leaves, and the highest content of β-carotene was found in 2 to 3 weeks-old leaves (Lefsrud et al., 2007).

Francisco et al. (2009) reported that total levels of phenolic compounds was higher in turnip leaves (*B. rapa var. rapa*), harvested during the vegetative period (51.71 µmol/g dw), compared to the fructiferous stems with flowers, buds and surrounding leaves (38.99 µmol/g dw), and that flavonoids were the main phenolic compounds. On the contrary, harvest date had a minimal effect on flavonoid concentration in kale, with total flavonoid levels unchanged in 6 out of 8 harvested kale varieties (Schmidt et al., 2010).

The amount of waxes in *A. thaliana* was 25-fold higher in flowering stems compared to leaves; their content varied during developmental stage and differed in plant organs (Jenks et al., 1995, 1996).

Reassuming, some of the health-promoting factors may be present 10 times higher in sprouts than in mature vegetables. Sprouting resulted in an overall increase in the total phenolic content and antioxidant capacity and although germination time was not a discriminating factor, longer germination times resulted in lower antioxidant capacity of the sprouts (Vale, 2014).

All this information is useful for developing new products according to the final users' needs and the final purpose of consumption, considering the different content of phytochemicals in plant portion and developmental stage.

2.3. Environmental factors

Seasonal variation, light exposure, temperature, water availability, soil fertility (De Pascale et al., 2007), phytosanitary measures, sowing date and harvest period (Renaud et al., 2014) are all factors linked to environmental conditions that can influence the quality, in particular the nutritional content and profile, of Brassica vegetables (Francisco et al., 2017). Different responses to seasonal variations were reported in several Brassica plants like broccoli, kale and turnip (Rosa and Rodrigues, 2001); this effect is determined mainly by temperatures and day length during period before harvest.

Countless studies agree that spring season plants, grown at intermediate temperatures, high light intensity, longer days and dry conditions (or low average of rainfall) during their vegetation period, contain an increased total GLS and phytochemicals concentration (Engelen-Eigles et al., 2006; Velasco et al., 2007; Francisco et al., 2011; Lo Scalzo et al., 2013; Renaud et al., 2014). For examples, in canola (Brassica napus) was found that GLS concentration increased with a temperature of 40°C maintained for 4 h on 5 successive days, giving a total of 15 degree-days of stress (15 DD/40°C) (Aksouh et al., 2001). Some authors reported that higher and lower temperatures, rather than intermediate temperatures, brought to an increase of GLS concentration; e.g., grown temperatures comprised between 7 and 13°C brought to an increase of glucoraphanin and lutein in broccoli; furthermore, act as a trigger for biosynthetic pathways (Schonhof et al., 2007). Moreover, broccoli sprouts grown at constant high (29-33°C) or low (11-16°C) temperatures had higher antioxidant content than sprouts grown at intermediate temperature (21.5°C) (Pereira et al., 2002). The same author confirms that the main antioxidants content is shown in sprouts that grow with a strong temperature range 30/15°C day/night.

Autumn/winter season plants, grown at lower temperature, lower light intensity, shorter days and higher water availability, tend to have the lowest total GLS and other phytochemicals concentration (Rosa et al., 1997; Vallejo et al., 2003a, Zhang et al., 2008; Cartea and Velasco, 2008; Björmark et al., 2011). An exception is represented by turnip that produces higher flavonoids and vitamin C content in autumn/winter season; this crop accumulates and produces the main phytochemicals with low/moderate temperature and considerable radiation, mainly in turnip tops (Aires et al., 2011; Francisco et al., 2012). More precisely, in *Brassica rapa* the correlation with temperature is bound also to plant portion; indeed, the number of days with a minimum temperature under 0°C was negatively correlated with total GLS content in turnip greens. In turnip top, GLS content was positively correlated with the number of days with a maximum temperature over 20°C. For what concern phenolics, no correlation was found between climatic factors and turnip greens, while in turnip tops total flavonoids and total phenolics content seem to be correlated with the number of days with a minimum temperature under 0 and 10°C, respectively (Francisco et al., 2011). In broccoli, the freezing temperature can positively influence the concentration of sulforaphane (Pék et al., 2013).

The biotic and abiotic factors that characterize the surrounding environment, can influence the quality of *Brassicaceae*. For what concern the biotic sphere, aphid infestation brought to an increased production of primary metabolites, including amino acids, as well as some secondary metabolites, as a plant defence mechanism against these pathogens (Cole, 1997). For what concern abiotic factors, water stress condition and metal exposure produce an initial increase of photosynthetic pigments, proteins, free amino acids and sugar content, followed by a subsequent decrease (Singh and Sinha, 2005). In details, a relation between copper stress and the production of amino acids was found because free amino acids production takes part to the detoxification from copper excess (Xiong et al., 2006). In *Brassica juncea,* accumulation of metals produces an increase of 35% of oil content (Singh and Sinha, 2005). A moderate salinity in water or soil affects the myrosinase-GLS system in broccoli, inducing the production of GLS; also, phenolic compounds increase in this stressful condition, but in case of strong salinity both GLS and phenolics decrease (Farnham et al., 2004; Rodriguez-Hernandez et al., 2014).

Ragusa and co-author (2016) investigate the effect of different germination temperatures (10, 20 and 30 °C) on the phytochemical content as well as on reducing and antioxidant capacity of broccoli and rocket sprouts. In both seeds and sprouts, the total GLS and ascorbic acid contents did not differ between vegetables, while broccoli exhibited exceptionally higher polyphenols and greater reducing and antioxidant capacity compared to rocket. In both species, an increase in germination temperature positively affected the glucosinolate content. Ascorbic acid increased during germination without a difference among the three tested temperatures. The phenol content increased in broccoli sprouts when they were grown at 30 °C, but the amount decreased at the highest temperatures in rocket. The reducing and antioxidant capacities increased with germination, and higher indexes were detected at 10 °C, particularly in rocket.

2.4. Agricultural factors

2.4.1. Cultivation system and soil composition

Cultivation system choice can influence the quality of vegetable product, in particular the concentration of primary and secondary metabolites in Brassica vegetables.

Some authors reported a higher antioxidant (phenolic compounds, in particular flavonoids) and GLS concentration in Brassicas growth in organic cultivation system than in conventional (Yang et al., 2000; Sousa et al., 2008; Naguib et al., 2012), as demonstrated in early harvested tronchuda cabbage (Vrchovska et al., 2006). This result could be linked to the fact that, under organic cultivation, crops are subjected to more biotic and abiotic stress; these stressing conditions cause an increase in the production of secondary metabolites as a mechanism of defence, and consequently allow to obtain vegetables with higher nutritional and antioxidant potential than in conventional system.

Several studies described an opposite situation and contrasting evidences about phytochemical enhancement in organic vegetables (Dangour et al., 2009; Hoefkens et al., 2010). Instead, Conversa et al. (2016) reported that the choice of cultivation systems does not modify the antioxidant properties of raw and processed products, but differences can be found in chlorophylls and carotenoids content of organic "cima di rapa" landraces. The lipophilic antioxidant content was improved in organic product while the hydrophilic component, that constituted the 99% of total antioxidant capacity, was not affected by the different crop management in "cima di rapa". However, organic system influenced the quality of products during storage: after 7 days of storage at 5°C, the organic "cima di rapa" maintained the best colour with high chlorophyll levels, probably due to a higher availability of nitrogen in organic management; but after 14 days of storage, the quality declined with a higher production of strong off-odour than conventional products.

Nitrate content was reported to be lower in organic than in conventional products (Koh et al., 2012).

Regarding the soil composition effect on Brassica quality, it was reported that the highest GLS and phenolic compounds content were detected in locations with the highest soil pH (around 5.5-5.6) and available potassium; the content can be also influenced by nitrogen and sulfur application in turnip (Francisco et al., 2011). On the contrary, in *B. rapa L. Subsp. Sylvestris,* flavonols (kaempferol and quercitin derivatives) were reduced by sulphur availability (De Pascale et al., 2007). Organic soil increases the total antioxidant content (Ju et al., 1980).

2.4.2. Irrigation

It was reported that a moderate water stress increases the concentration of bioactive compounds in Brassica, in partly due to an increased concentration per unit of dry weight; if the stress become intensive, the secondary metabolites production should decrease (Khan et al., 2011a). Phenolic compounds and GLS content increase in absence of irrigation, as a result of a reduction of vegetative growth, mainly in turnip, cabbage and broccoli (Zhang et al., 2008; Francisco et al., 2017; Bell and Wagstaff, 2017). The association between low availability of water in the soil during plant growth and postharvest cold storage brought to the best maintenance of antioxidant activity in Brassica (Cogo et al., 2011). Water stress condition affects also sugar contents, which increases in cabbage (Sasaki et al., 1998).

2.4.3. Plant density, intercropping and trap cropping

Plants density seems to affect the plant morphology and the phytochemical compound content: a higher density decreases the head size but increases the GLS content, because the competition for nutrients, in high density conditions, causes a stress on plants, stimulating the secondary metabolites production (Rosa et al., 1997).

Intercropping and trap cropping are strategies that allow weeds and pests control (Hooks and Johnson, 2003), but the presence of another crop can generate a stressing condition like plant competition for light, nutrients and water, decreasing their availability and affecting in different ways the production of phytochemicals by Brassicas.

The effect of intercropping depends by the choice of plants to be cultivated next to the main crops (Finch et al., 2003); e.g., intercropping white cabbage with red clover (*Trifolium pratense*) reduced the GLS content in white cabbage, but decreased the oviposition of *D. Floralis* (Björkam et al., 2008).

Trap crop must be naturally more attractive than the main crop, for attract and control insects; it can belong to the same or another species and variety or can differ for the growing stage. A particular type of trap crop is the so called “dead end”: it is highly attractive for insect’s oviposition, but the larvae could not survive on the plants. On this type of crops belongs the weed winter cress (yellow rocket, *B. vulgaris*), exerting its effect on *P. xylostella* (Badenes-Pérez et al., 2004).

The combination of intercropping and trap cropping is called “push-pull” and gives a synergic and powerful effect in pests and weeds control; it is also known as stimulo-deterrent-diversionary strategy that can use also the insect pathogenic fungi for reducing pest population (Cook et al., 2007).

2.4.4. Fertilization Practices

A correct fertilization plan is fundamental for obtaining high quality, healthy and safe vegetables. The nutritional and sensorial profile of Brassicas is conditioned by the availability of fertilizers and nutrients because they determine the biosynthesis of secondary metabolites.

Countless studies took care about the effect of sulphur fertilization on phytochemical concentration, mainly on GLS production considering their sulphur nature (Booth et al., 1991; Kim et al., 2002; Kopsell et al., 2003). There is a correlation between the increase of sulphur supply and the higher levels of total GLS (Kaur et al., 1990; Rosen et al., 2005; Li et al., 2007), in turnip (Kim et al., 2002), kale (Kopsell et al., 2003) and in broccoli, mainly if associated with reduction in water, at the expense of yield (Schreiner, 2002). Vallejo and co-authors (2003a) suggested that the effect of sulphur application on GLS varies with the development stage of broccoli plants and differs for each kind of GLS; in fact, they found an increase in total GLS content at the start of the inflorescence development, followed by a rapid decrease. Increasing sulphur fertilization brought to a positive impact in the synthesis of polyphenols, like flavonols and phenolic acids, increasing the total antioxidant activity in turnip top (*B. rapa ssp. sylvestis*) (De Pascale et al., 2007), and broccoli (Vallejo et al., 2003b). Sulphur fertilization in pre-harvest (from 2.6 mmol/L to 6.5 mmol/L) increases the lipophilic and hydrophilic antioxidant activity, but do not affect the nitrate and chlorophyll contents in ready-to-eat friariello product (Barbieri et al., 2009). Sulphur deficiency brought to an increased vulnerability of Brassica crops to diseases and fungal pathogens (Dubuis et al., 2005). Sulphur fertilization, besides improving the antioxidant activity, it is associated with a genotype-dependent significant reduction of leaf nitrate content, because enhances the incorporation of nitrogen into organic compounds and consequently it reduces the leaf nitrate concentration (De Pascale et al., 2007).

Nitrogen is the main constituent of chlorophyll structure, so its availability influences the content of carotenoids like lutein and β-carotene, indeed high NO_3-N:NH_4-N ratio led to a higher content of both (Kopsell et al., 2007). Consequently, also colour and pigmentation of leafy vegetables are improved (Conversa et al., 2016). Nitrogen fertilisation brought to a decrease of total GLS content (Rosen et al., 2005; Kim et al., 2002), but it acts differently according to the GLS type; in fact, abundant nitrogen applications increase progoitrin and decrease sinigrin concentration in *Brassica napus* (Zhao et al., 1994). A reduced nitrogen fertilisation brought to an increase of the bioactive compound content, mainly phenolics, because nitrogen stress triggers the gene expression of flavonoid pathway enzymes (Sousa et al., 2008). Combined fertilisation with NO_3^-:NH_4^+ is the optimal solution to maintain plant growth and increase the total GLS content (Robbins et al., 2005).

An optimal balance between nitrogen and sulphur fertilisation influences the biosynthesis of secondary metabolites (Zhu et al., 2009; Cartea et al., 2011). GLS, for example, can be enhanced by the presence of low nitrogen and high sulphur fertilizers: this balance influences the quantity and the quality of GLS produced, according to the corresponding amino acids synthetized (Rosa and Heaney, 1996). Some authors reported the effect of different nitrogen/sulphur combinations on GLS content in Brassicas, with increasing amount of nitrogen (80-320 kg/ha) applications. When enough sulphur was available (60 kg/ha), there weren't any effects on total GLS content, but their production moved to indolics; when the combination was with a low concentration of sulphur supply (10-20 kg/ha), the aromatic and aliphatic GLS decreased (Li et al., 2007). Increased nitrogen/sulphur ratio pushes the plants toward the vegetative growth, at the expense of GLS production (Kim et al., 2002). Fabek et al. (2012) showed that the type of fertilisation may influence the mineral composition in plants: nitrogen fertilisation was negatively associated with potassium (K) and calcium (Ca) content in broccoli, while sulphur fertilisation increased manganese (Mn) and zinc (Zn), and decreased copper (Cu). Applications of sodium selenate (Na_2SeO_4) produced an increase of GLS (Robbins et al., 2005).

Also salts availability can influence the phytochemical concentration in Brassicas, with salts stress increasing GLS content (Lopez-Berenguer et al., 2008). In details, selenium seems to increase the GLS content (in particular sulfuraphane), when applied up to a certain dose; above this level, it decreases the GLS production (Robbins et al., 2005).

In conclusion, there is a large possibility to improve the nutritional quality of Brassica vegetables through the implementation of the appropriate agronomic practices, but the effects of the treatments are strictly genotype-dependent, so a good choice of the genotype before starting the cultivation is needed (De Pascale et al., 2007).

2.4.5. Signalling molecules, biocontrol agents and biostimulants

Besides the classical fertilizers, in the last years new proposal products that bring several beneficial on crops, like improving safety, enhancing growth and production, improving the defence against weeds and pests and nutritional quality, were developing. Among these, signalling molecules, biocontrol agents, and biostimulants are increasing their spread and their application.

During growth, plants are exposed to various biotic (herbivory, fungal, bacterial and/or viral infection) and abiotic (metals, UV, temperature) stresses. Those factors lead to gene expression and biochemical changes, which finally result in an enhancement of the synthesis of primary and secondary metabolites. In this process, a number of signaling pathways are activated as *Brassica* defense responses, including SA (salicylic acid), JA (jasmonic acid), ethylene and ABA (abscisic acid) pathways (Jahangir et al., 2009a). Presence of signalling molecules clearly affects the GLS content in these species; indeed, GLS content analysis in leaves and cotyledons of *B. napus*, *B. rapa* and *B. juncea* revealed that content of glucobrassicin increased up to 20-fold after treatment with JA or MeJA (Methyl Jasmonate) (Bodnaryk, 1994). In contrast, treatment with ABA resulted in low levels of indole GLS in *B. napus* (Mollers et al., 1999).

Summing up, levels of hormones such as JA, SA and ABA seem to be related to the regulation of GLS and of other bioactive compounds content (Schreiner et al., 2011; Guo et al., 2013; Thiruvengadam et al., 2016). Consequently, hormonal elicitation can be a useful tool to induce the synthesis of bioactive compounds interesting for human health.

Concerning the application of biocontrol agents, Gallo et al. (2013) reported the effect of *Trichoderma* genus microbes on quality parameters of *Brassica rapa sylvestris var esculenta Hort.,* for increasing GLS content and promoting growth. They used strains of beneficial microorganisms (biocontrol agents) of *Trichoderma harzianum* TM10, T. *atroviride* P1, and their metabolites: harzianum acid and 6-pentyl-α-pyrone. The results affirmed that the use of *Trichoderma* and its metabolites brought to an increase of GLS in plants, probably due to their capability of inducing resistance mechanisms, stimulating the synthesis of salicylic and jasmonic acids and the cascade of events leading to the production of various metabolites; only the ascorbic acid was lower compared to control plants. *Trichoderma spp.* are free-living fungi available in soil and root ecosystems. They are opportunistic, avirulent plant symbionts, and parasites of other fungi; they are biocontrol agents (BCAs) used as biopesticides and antiobiotics.

In agriculture, besides biocontrol agents, also the utilisation of seaweeds extract, mycorrhizae, control pathogens and nematodes (Muelchen et al., 1990), humic acids like vermicompost foliar sprayed (Pant et al., 2009), and protein hydrolysates is growing under the term “biostimulants”, useful to increase the production of crops. Several studies associate their adjuvant properties also with the increase of bioactive compounds synthesis. Lola-Luz et al. (2013) showed an increasing effect of the application of a commercial extract of brown seaweed *Ascophyllum nodosum* on flavonoids and total phenolics concentration in cabbage, without relevant effect on crop yield.

Brassica species are able to contrast the main soil-borne agents thanks their secondary metabolites that act as biofumigants. A study reported the effectiveness of the flour of dry plants of *Brassica juncea, Eruca sativa, Raphanus sativus* and *Brassica macrocarpa*, in nematodes control (Meloidogyne spp.) on tomatoes. Tritated flour was distributed before planting (60 and 90 g m-2) and was successful for the sinigrin presence (Argento et al., 2013).

2.5. Post-harvest factors

Fresh vegetables are very perishable and sensible to post-harvest management that can strongly affect weight, firmness, colour, sensorial and organoleptic parameters and phytochemical compound contents. The packaged vegetables, like those in first evolved class, had a minor weight loss and an increasing shelf life than unpackaged fresh cut products.

The main studied Brassica vegetables during post-harvest are broccoli, considering the high perishability of their heads and florets; cold storage, modified atmosphere and post-harvest processes reduced the decrease of antioxidant potential (Lemoine et al., 2010).

2.5.1. Post-harvest treatments

In general, post-harvest treatments are useful for reduce and decrease the decay of antioxidant potential that normally occur after harvest. The most important post-harvest treatments are cold storage, modified and controlled atmosphere. Blanching is a pre-storage treatment, applied before freezing and other processes (Soengas et al., 2011).

2.5.1.1. COLD STORAGE

The main important action to do when fresh vegetables are harvested, and cut is the rapid reduction of their internal temperature; the most applied solution is the pre-cooling that consists in the application of ice over vegetables, or in putting them inside a fridge in the field, immediately after harvest. The short life of those vegetables can be increased by storing them at refrigerating temperatures. However, this strategy can assure quality standards only for a few days (Oliveira et al., 2015). Several studies confirmed that the best storage conditions for preserve vegetables and brassicas quality are:

- Relative humidity near the saturation level (98-100%);
- Cold temperature (4-5°C), e.g. in broccoli (Rodrigues and Rosa, 1999).

The storage at ambient temperature brought to rapid losses of phytochemical compounds like ascorbic acid, while some authors reported that storage at 4°C or 5°C for 7 days did not affect ascorbic acid (Favell, 1998) and flavonols contents (Leja et al., 2001). Storage of ready-to-eat cima di rapa at 5°C brought to an increase (by 1.5 times) of flavonols and total antioxidant content during 14 days of storage, probably given by the trigger of phenylalanine ammonia lyase (PAL), the enzyme necessary for phenols synthesis (Mittler, 2002). Higher antioxidant activity was observed in broccoli plants, kept at 4°C for 21 days, in respect control samples (Lemoine et al., 2007). Conversely, Conversa et al. (2016) reported a decrease in vitamin C (-56%) and in nitrate during storage period characterized by cold temperature (5°C), after packaging in heat-sealed bags of polypropylene (OPP) film, during the first 7 days of storage. A solution for decrease nitrate content in ready-to-eat friariello is to apply a storage period of 9 days at 4°C under light exposure; those conditions led to a decrease of nitrate content than in darkness storage. In fact, in darker storage, the weight loss is relevant, but the antioxidant capacity and nitrite content are not affected by light exposure (Barbieri et al., 2009). The content of individual and total GLS decreased in brassica vegetables (broccoli, Brussel sprouts, cauliflower and green cabbage) when stored in domestic refrigerator at 4-8°C for 7 days (Song and Thornalley, 2007).

Freezing is a common practice used in the food industry for extending the life cycle of fresh-cut vegetables; its main advantage is to preserve the nutritional quality and to minimize the loss of phytochemicals. Freezing is one of the best methods to preserve the nutritive constituents of raw Brassica vegetables (Gebczynski and Lisiewska, 2006). The vitamin C and polyphenols, non-essential and essential amino acids are greater retained in frozen than in canned product; vitamin C level remains stable for a storage period of 12 months at -20°C in broccoli and cauliflower (Korus 2012). Other works reported higher losses of vitamin C (up to 30%) at the same storage conditions (Puupponen-Pimia et al., 2003). Storage vegetables at -85°C could cause significant losses of GLS due to freeze-thaw fractures of plant cells, leading to enzymatic conversion of GLS to ITCs during thawing (Song and Thornalley, 2007).

For obtaining high-quality frozen vegetables, freezing process needs a pre-treatment, called blanching, as a steam or boiling heat treatment, for deactivating the main degradative enzymes like myrosinase, which destroys GLS during thawing (Rosa et al., 1997; Mithen et al., 2000). Freezing effect on GLS content is minor than blanching and boiling. Comparing freezing with fresh vegetables, the loss of phenolics, fiber and mineral is very small (Bouzari et al., 2015); the main losses are due to blanching pre-treatment rather than freezing process

(Mahn & Reyes, 2011). Blanching, followed by long term frozen storage, brought to a reduction of phenolics and phytochemicals content, and antioxidant potential, in cauliflower. In general freeze storage slightly affects the antioxidant potential over time (Volden et al., 2009 a, b).

2.5.1.2. BLANCHING

Blanching is a thermal pre-treatment applied before freezing and other post-harvest processes, used for deactivating degradation enzymes that brought, during storage, changes in texture, colour and odour of vegetables, affecting also the nutritional compounds; it was necessary for preserve quality during time. Blanching is a heat treatment that it can be done in different ways: steam, boiling water and microwave.

If, on one hand, the blanching pre-treatment is useful for deactivate enzymes like myrosinase, on the other it brought to losses of a high content of phytochemicals, mainly the heat sensible like vitamin C, and the hydrophilic (like polyphenols) in case of blanching in boiling water (Tanongkankit et al., 2010). Losses during blanching are plant-species dependent. E.g., steam blanching of broccoli florets decreased ascorbic acid concentration by 30% to 50% (Howard et al., 1999; Murcia et al., 2000). Viña et al. (2007) evaluated the effects of some pre-blanching treatments, like immersion in hot water (50°C) and microwave heating, in Brussels sprouts prior to blanching; they obtained an increase in radical scavenging activity.

For what concern the myrosinase deactivation, approximately the 90% of this enzyme is lost during blanching at 60°C for 10 min (Van Eylen et al., 2007). A solution for obtain the GLS hydrolysis during blanching is the addition of exogenous plant-based myrosinase, which guarantees the obtainment of ITCs during thawing (Matusheski et al., 2004; Dosz and Jeffrey, 2013). Another solution for obtain more ITCs is to deactivate the myrosinase cofactor (ESP), that become inactive at a minor temperature than myrosinase; blanching vegetables at 60°C for 5 minutes is useful for block ESP and obtain more ITCs. In normal conditions, ESP favoured nitrile production starting from GLS, removing them by ITCs useful production (Matusheski et al., 2004; Wang et al., 2012).

2.5.1.3. MODIFIED AND CONTROLLED ATMOSPHERE

Modified and controlled atmosphere are packaging strategies useful for preserve the nutritional and organoleptic quality during time and increase their shelf-life. Packaged vegetables show better phytochemical compounds retention than unpackaged ones. This result is due to the low oxygen content and the reduction of respiration rate in the package, and the reduction of cellular broken (cellular integrity is fundamental for preventing the mixing of GLS with myrosinase, so avoid their degradation) (Jones et al., 2006). In broccoli heads packaged with micro-perfored and non-perfored films, bioactive compounds concentrations remain similar to fresh vegetable, differently from the decrease in unpackaged controls (Serrano et al., 2006).

Several studies reported that a low oxygen atmosphere guarantees the maintenance of the original nutritional quality and phytochemicals concentration during the cold storage. Cefola and co-authors (2010a), studying ready-to-use vegetables, investigated the effects of four different atmosphere compositions (air, 3% O_2 in nitrogen, 3% O_2 + 10% CO_2 in air, and 10% CO_2 in air) on broccoli raab quality. They found that storage at low oxygen concentration and cold temperature (5°C) led to a better preservation and maintenance of nutritional parameters up to 17 days. In details, the initial phenolic, antioxidant and vitamin C contents were maintained during the entire cold storage in 3% O_2 in nitrogen. In a study of 2016, the same author reported the best combination of storage conditions; using PP/PA (microperforated polypropylene/polyamide) in A-MAP (active atmosphere achieved) with the use of CO2-absorbing sachets, reached an equilibrium condition very close to the optimal one for broccoli raab (5% O2 and ≤5% CO2) (Cefola et al., 2016b). A comparison between packaged and left-open packaged fresh cut “radicchio”, confirmed the beneficial effect of modified atmosphere in slowing the respiration rate of fresh cut vegetables, and in increasing the sensorial quality (Cefola et al., 2016a).

The objective of the use of modified atmosphere is to contrast the vegetables senescence, that consists in colour and firmness change, loss of chlorophyll, yellowing, flowering, visual quality deterioration, increasing in respiration and ammonia accumulation. Some studies find a relation between senescence and production of ethylene: brassica florets produce a higher ethylene quantity than leaves (Pogson and Morris, 1997). To favour the contrasting and inhibition of brassica senescence, it can be use 1-methylcyclopropene (1-MCP) for 24 h, particularly in fresh cut brassicas; applied in pre-processing, before to package the fresh material, it extends the shelf life and improves quality (Cefola et al., 2010b).

Ragusa and co-authors (2013) working with kale shoots found the best method to reduce the post-harvest senescence using 70% N2:30% CO2 modified atmosphere at 4°C: the product was kept in good condition for seven days of storage.

2.5.1.4. EDIBLE COATINGS

In recent years, studies on edible coatings and their application in pre and post-harvest, e.g. chitosan application, are increasing. Chitosan is a high molecular weight polymer, non-toxic, bioactive, useful for its fungicidal effect that helps the defence mechanism of plants accumulating antifungal hydrolates and phytoalexins. Chitosan had the same effects of MeJa on firmness, texture and antioxidants and phenolics contents of vegetables (Moreira et al., 2011). Chitosan films have a selective permeability to gasses (CO_2 and O_2) and good mechanical properties. However, the fact that chitosan films are highly permeable to water vapor limits their use; in fact, an effective control of moisture transfer is a desirable property for most foods, especially in moist environments.

Resuming, the positive effects of chitosan applications are:

- Counteract browning, restrain phenolic compounds and ascorbic acid increasing nutritional value;
- Help to incorporate functional ingredients like antimicrobials, minerals, antioxidants and vitamins. These combinations can either increase the effects of chitosan or reinforce the nutritional value of raw materials;
- Eco-packaging function: the film act as a barrier for oxygen and possesses a good resistance to breaking; the addition of essential oil increases the antimicrobial activity of chitosan.
- Antibacterial activity against foodborne pathogens (Alvarez et al., 2013).

Some authors found that not only chitosan, but also other edible coatings like carboxymethyl-cellulose, seemed to have a beneficial impact on quality retention of minimally processed broccoli, by retarding their weight loss, browning and yellowing processes, reducing stem hardening, microbial growth and improving total chlorophyll and ascorbic acid retention. Moreover, both edible coatings were able to inhibit the floret opening, which is an important quality characteristic for broccoli (Ansorena et al., 2011). Finally, both edible coatings and modified atmosphere packaging (MAP) treatment cause changes in atmosphere composition and respiration rate of lotus root slices, allowing the control of browning and improving the storage life of this fresh-cut vegetables (Elsabee et al., 2013).

2.5.2. Post-harvest processing

2.5.2.1. AIR-DRYING PROCESS

Drying is an ancient processing technique that evolved along years. It began with the sunlight drying, and through many intermediate steps, it finally evolved until the modern high-pressure drying process. This technique is cheaper than freeze-drying process, but possesses a lot of disadvantages, mainly because can strongly negatively affect the nutritional quality and the phytochemicals contents in vegetables. The efficiency of the process depends by the perfect combination between heat temperature (degree) and duration of process (minutes-hours). Many studies on the effect of air-drying process reported losses of 60% in phytochemical compounds content, mainly the thermo-labile compounds like vitamin C and phenolic content. When the blanching was made in boiling water, besides thermo-labile compounds, also all the hydrophilic compounds were lost (Biondi et al., 2018).

However, several authors reported an opposite trend on the effect of air-drying process, detecting a relevant increase of total antioxidant capacity. In broccoli, high temperature and short time of drying process maximised the antioxidant activity (Mrkic et al., 2006). Increase of antioxidant activity and high phenolic content was confirmed also by Lemoine and co-authors (2010) that combined UV-C germicidal lamps and hot air treatment at 48°C for 3 h, followed by storage condition of 0°C for 21 days. They found a diminished respiratory activity, indicating high tissue integrity, and after the storage period, treated samples showed higher levels of total sugars and total proteins than control.

The effect of increasing antioxidant capacity is a result of several situations in air-drying process:

- Increased extractability and release of compounds from the matrix after broken of tissue, or hydrolysis of phenolics (Mrkic et al., 2006);
- Production of new molecules with higher antioxidant potential than the primary and original compounds;
- Polymerization of phenolics, that led to the formation of macro-molecules with an higher oxidation state and consequently higher antioxidant potential than the original ones.
- Formation of novel compounds having antioxidant activity as products of Maillard's reaction, which takes place when vegetables and fruits are submitted to high heat temperature and prolonged storage (Nicoli et al., 1999).

2.5.2.2. FREEZE-DRYING PROCESS

Freeze-drying (FD), also known as lyophilisation, is a gentle dehydration technique representing the ideal process to produce high-value dried products. This technique is well-known for its ability to maintain the product quality (colour, shape, aroma and nutritional value) greater than any other drying method (Karam, 2016). Strength points of this technique are the low processing temperature (-20 to -80°C) and the absence of air oxygen (under vacuum) during processing, which minimize degradation reactions. The drying happens by sublimation (Oikonomopoulou and Krokida, 2013).

Freeze-dried materials are characterized by the lowest values of apparent density and the highest porosity (Ratti, 2001), minimal shrinkage, and negligible collapse (less than 10%); however, some authors have reported a decrease in β-carotene concentration by 26% and 43% in freeze-dried mango and watermelon respectively (Shofian et al., 2011). In addition, many authors have reported a maximal (up to 94% in certain cases and in all cases higher than 60%) ascorbic acid retention. Besides, the antioxidant activity was found to be increased by at least 13% (till 82%) in several freeze-dried products (Sablani et al., 2011; Shofian et al., 2011). Finally, it is important to highlight that unfortunately, this technique appears as a very lengthy and expensive preservation method, because of the low drying rates spawned by refrigeration and vacuum systems that increases energy costs (Liapis et al., 1996; Ratti, 2001).

2.5.2.3. COOKING

Cooking is the main post- harvest process applied by industries and by consumers and is strongly impacting on nutritional quality. The parameters most responsible of the nutritional profile modifications are: type of cooking, time and temperature of the process; indeed, it is possible to maintain unaltered sensorial attributes optimizing the cooking process (Bongoni et al., 2014). For what concern cooking of Brassica vegetables, there are a lot of different works in literature, with conflicting results. In general, the better method to cook Brassica vegetables minimizing losses of phytochemical compounds content is steaming, in respect to blanching, boiling, microwave cooking and stir-frying process (Vallejo et al., 2002, 2003; Moreno et al., 2006, 2007; Sikora et al., 2008; Volden et al., 2009b; Francisco et al., 2010). Borowski et al. (2005) proposed the best method to maintain desirable sensory attributes and greatest firmness, cooking broccoli in steam oven at 125°C, with 90% steam saturation for 8 min.

Vitamin C content strongly depends by cooking methods, because is a very thermo-labile compound and represent an indicator of the quality of the cooking process (Czarniecka-Skubina, 2002). In literature, a lot of opposite and contrast results of vitamin C content were found:

- High retention in microwave oven;
- Losses from 3.7% to 10.6%. with steam pressure cooker;

- Low retention with traditional cooking and microwave in broccoli (Zhang and Hamauzu, 2004);
- Canning is the worst preservation method for vitamin C retention in Brassica vegetables; indeed, Brussels sprouts blanched and canned show a reduction of 66% (Czarniecka 2002); Murcia and co-authors (2000) reported that canned broccoli retained only the 16% of original vitamin C.

For what concerns GLS, the methods that greatly affect their contents in Brassica vegetables are boiling (Pereira et al., 2002), steaming, pressure cooking, microwaving (Vallejo et al 2002, but Verkerk and Dekker, 2004, affirmed the contrary) and stir-fry methods. All of them reduced the intake of GLS approximately by 30–60% (Rodrigues and Rosa, 1999; Verkerk and Dekker, 2004). GLS can be also leached in cooking water for enzymatic or thermal breakdown, around by 40 to 80% (Rosa and Heaney, 1993). The higher bioavailability of ITCs may be achieved by avoiding boiling of vegetables, because high temperature denatures myrosinase leading to a little production of ITCs. Jones et al. (2006) reported that the best way to maintain or reduce the loss of GLS is steaming for 2 min. however, also stir-frying can be useful, because the temperature of cooking oil was not so high and the frying process is rapid (3-5 min). This brought to a rapid inhibition of myrosinase without a degradation of GLS (Song and Thornalley, 2007). Indeed, the steam-blanching process results in an increase of GLS and antioxidant capacity (Carvajal et al., 2015).

In scientific literature, there are conflicting notions and results about the effect of cooking on the total antioxidant capacity. Some authors explain that these differences can be due by the analysed crop, indeed boiling broccoli and cauliflower led to an apparent higher increase in antioxidant capacity than cabbage. This is mainly explained with the production of redox-active secondary plant metabolites or breakdown products, the release of antioxidants from intracellular proteins, changes in plant cell wall structure, matrix modification, and more efficient release of antioxidants during homogenization (Wachtel-Galor et al., 2008). Several studies reported also an important effect of cooking method on the behaviour of antioxidant compounds. For example, cooking at moderate temperature (50-80°C) for a suitable period of time and subsequently cooking in boiling water led to a greater firmness than a direct cooking without precooking (Lin and Chang, 2005). Pellegrini et al. (2010) reported that in fresh broccoli, boiling and oven steaming generally led to an increase of antioxidant potential, while microwaving has a detrimental effect. Fresh *Brassica* retained phytochemicals and antioxidant activity better than frozen samples, more evidently in broccoli. Carotenoids are better retained with boiling in water than steaming and frying (Miglio et al., 2008; Ng et al., 2011). Roy et al. (2009) found that steaming increased the extractability of bioactive compounds and the antioxidant potential in broccoli.

In conclusion, boiling showed controversial and opposite results depending on crops studied. Steaming seems to be the best method for cooking Brassica vegetables without losing nutritional quality, followed by frying; the microwave process showed the worst effects. It is to cite that the fermenting process is frequently used for sauerkraut, the most famous fermented Brassica; this method brought to a decrease during storage of ITCs (Ciska and Pathak 2004). Also canning when used to store some Brassica vegetables, leads to a significant reduction of GLS contents (Dekker and Verkerk 2003).

3. RESEARCH SECTION

3.1. Aim of the study

The general aim of my PhD period was an investigation about factors determining brassica nutritional quality. The research has involved the study of many phytochemical compounds that constitute the nutritional quality of brassicacee vegetables, and of the factors that can influence and modify the quality of the final product. Once understood and known the factors that modified nutritional quality and phytochemical compounds content, it is possible to improve the nutritional quality of the first evolved class fresh products already commercialized by the company, but it is also possible to create and market new products with high nutritional value.

The specific objectives were divided for each research lines addressed:

Research line 1):

- Characterization of the nutritional quality and the antioxidant potential of Brassica vegetables and field grass for the first evolved class "Zeroscarti®" products;
- Investigation about the genotype-environment interaction on nutritional quality;
- Define a high-quality product, favouring the vegetables intake considering their simplicity of preparation and use.

Research line 2):

- Testing of the variation of phytochemicals content according to plant portion, developmental stage and harvest time of Brassicas.

Research line 3):

- Evaluation of the effect of post-harvest processes on nutritional quality of Brassica vegetables;
- Identification of the best process to maintain unaltered the nutritional quality of the very perishable fresh vegetables;
- Define new kind of Brassica products with high nutritional potential.

Research line 4):

- Testing the sensorial quality of Brassica vegetables subjected to different treatments;
- Investigation about the consumer's quality perception of Brassica products.

4. RESEARCH LINE I: CHARACTERIZATION AND COMPARISON OF RAW BRASSICA AND FIELD HERBS VEGETABLES NUTRITIONAL QUALITY

4.1. Introduction

Brassica vegetables and field herbs represent rustic and wild species characterized by a strong capacity of adaptation in hostile environments. They can be easily cultivated in the South of Marche Region where the pedoclimatic conditions are ideal and optimal for their growth, like mild climate and clay soils. In this area is very easy to find Brassica and grass fields as wild and spontaneous species.

The cultivation of these typical vegetables represents a good economic opportunity for the growers. In fact, in Italy the vegetables and fruits consumption is increased of the 3.1% from 2015 to 2016, as reported by Report Istat 2016 (https://www.istat.it/it/archivio/217356); this data confirm the healthy trend registered during the last years, that highlights the increasing attention of the consumers to healthy foods; so the farmers choice to cultivate Brassica and other vegetables is supported by an increasing demand for those products.

As previously affirmed, the first evolved class is an innovative production system that consists of the hand-harvesting of the edible younger portion of the vegetable product (constituted by new shoots and apical part of the plants) that is packaged, to provide to consumers a new product proposal of high antioxidant property that maintains and preserves the sensorial and nutritional quality during time. The hand harvest of plant portion allows also a reduction of soil compaction and an enrichment of soil organic matter during time, because the 80% of the plant remains on the ground acting as a cover and reducing the erosion risk. At the end of production time, the plants are milled and incorporated into the ground. Brassica crops play an important role as soil improvers; furthermore, they have a disinfesting action to soilborn pests and pathogens. This function is possible thanks to the biofumigation process, through the production of volatile sulphur compounds (Larkin and Griffin, 2007).

A lot of studies confirm the high quality of the younger portion. The antioxidant capacity and the concentration of phytochemical compounds strictly depend by the maturation stage of the plant; Kim et al. (2004) found that cabbage at juvenile phase has higher flavonols content than the mature phase. In detail, flower buds were found to be the most active part followed by leaves and steams (Fernandes et al., 2007; Soengas et al., 2011). For this reason, in this research were harvested only the younger jets of each Brassica species cultivated, for obtaining the highest antioxidant first evolved class products.

The importance of Brassica vegetables and field grass intake for humans is linked to their high concentration of phytochemical compounds and to their potential health protection. A strong attention for these vegetables was given by several biomedical studies, that shown the high capacity to prevent and reduce some chronic illness and cardiovascular and degenerative diseases, reporting anticancer activities (Herr and Buchler, 2010; Bjorkman et al., 2011; Soengas et al., 2011; Manchali et al., 2012).

The main aim of this research line was to investigate the sensorial and nutritional quality of several vegetables belonging to *Brassicacee* family and others, which constitute the raw material of the first evolved class fresh products, with the goal of provide to consumers high quality products and improve the vegetables intake because of their simplicity of preparation and use.

4.2. Materials and methods

4.2.1. Plant material

Vegetables characterization trial has been carried out in two years: 2016 and 2017. The fresh materials were provided by the agricultural company "Valli di Marca Agricultural Society", that realized the cultivation in different production areas. The first area is situated in the south of Marche Region (Italy), in a typical area specialized in Brassica cultivation called Valdaso (136 m.a.s.l.), where the vegetables were cultivated for both years; the second is Fucino upland (680 masl) area, located in Abruzzo region (Italy), in Mid–Adriatic climatic conditions where the vegetables were cultivated only for the second year (2017). The vegetables were cultivated in open field, following the agriculture cultivation system typical of this area. The fresh materials were sampled, harvesting only the edible portion (the younger shoots and leaves), according to "First evolved class and No-waste protocol" adopted by the company. The samples were selected, sliced and packaged. The table below (Tab. 4) shows the main species characterized in this study. In the first year all the species were analyzed, while in the second year the most commercially interesting Brassicas were repeated with the addition of two other brassicas (turnip top and black cabbage). 200 g of fresh material for each species were sampled and immediately frozen at -20°C, and subsequently analyzed. Table 5 reported the genotypes details of samples and the DAS (Day After Sowing) at harvest time; while in figure 3 are reported the climate graphs of the cultivation areas interested during the two years of trials.

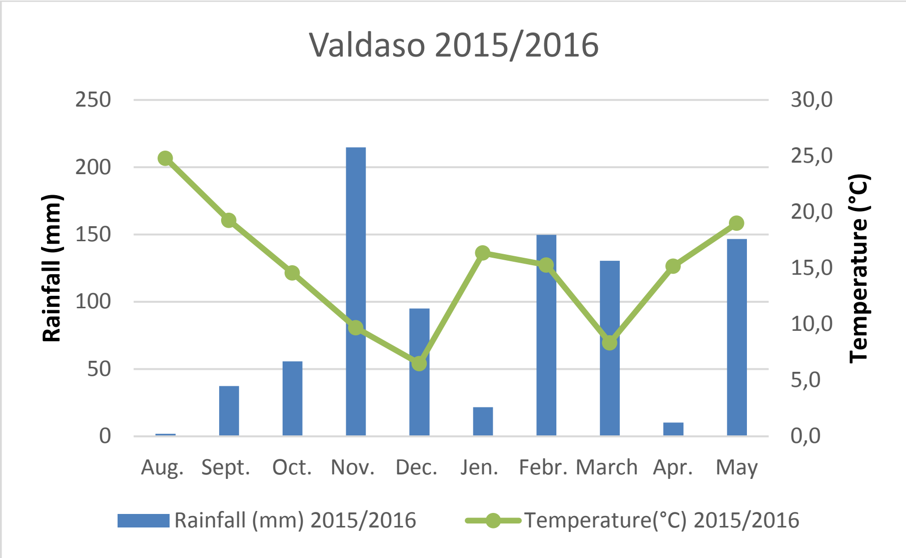
Table 4- Plant material analyzed during two years of trials belongs to *Brassicaceae* family and other field herbs (*I: 1st year;II: 2nd year).**

Species	**Vegetables**	**PROVENENCE**	**SSC-TA-pH**	**TAC-TPH-ACY**	**VITAMIN C**
Brassica rapa subsp. *sylvestris*	Turnip top	Valdaso	II	II	I-II
		Fucino	II	II	II
Brassica oleracea L. var. *acephala* subvar. *laciniata L.*	Black cabbage	Valdaso	II	II	I-II
		Fucino	II	II	II
Brassica oleracea L. var. *acephala* subvar. *sabellica L.*	Green Curly kale	Valdaso	I-II	I-II	I-II
		Fucino	II	II	-
	Red Curly kale	Valdaso	I-II	I-II	I
		Fucino	II	II	II
	Russian Curly kale	Valdaso	I-II	I-II	-
Brassica oleracea L. var. *italica*	Broccoli	Valdaso	I	I	I
Brassica oleracea L. convar. *botrytis var. cymosa*	“Spigariello” broccoli	Valdaso	I-II	I-II	I-II
		Fucino	II	II	-
	“Getti e foglie” broccoli	Valdaso	I-II	I-II	I
		Fucino	II	II	II
Brassica oleracea L. var. *sabauda*	Savoy Cabbage	Valdaso	I	I	-
Beta vulgaris var. *cicla*	Red Beet Green Beet	Valdaso	I	I	-
Cichorium intybus L.	Chicory	Valdaso	I	I	-
Raphanus raphanistrum L. subsp. *microcarpus (Lange)*	Radish-“Rapastrello”	Valdaso	I	I	-
Borago officinalis L.	Borage	Valdaso	I	I	-

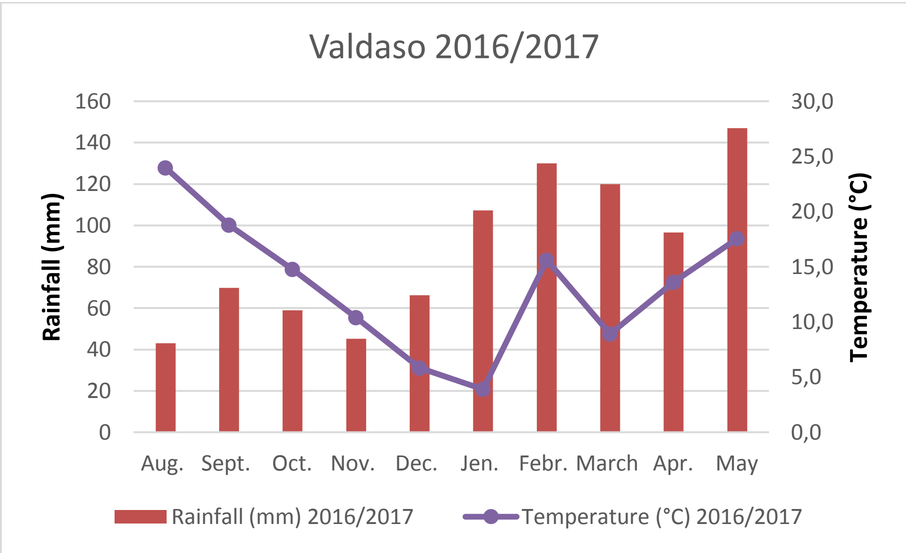
Table 5- Details of the samples harvested: Genotypes and DAS (Day After Sowing)

Vegetables analyzed	Genotypes,Varieties/ Seed company	Prov.	Sowing date	Harvest time	DAS
Turnip top	Var. Aprilatica /Larosa	Valdaso	1st 08/10/2015; 2nd 10/10/2016	1st 6/04/2016; 2nd 15/04/2017	1st 181; 2nd 187
		Fucino	2nd 16/05/2017	2nd 16/09/2017	2nd 123
Black cabbage	Var. Laciniato palmizio di Toscana/ Four	Valdaso	1st 13/09/2015; 2nd 01/10/2016	1st 02/12/2015; 2nd 03/01/2017	1st 80; 2nd 94
		Fucino	2nd 25/04/2017	2nd 14/06/2017	2nd 50
Green Curly kale	Kale dwarf green curled/ Maraldi	Valdaso	1st 28/08/2015; 2nd 05/09/2016	1st 26/11/2015; 2nd 04/12/2016	1st 90; 2nd 90
		Fucino	2nd 21/05/17	2nd 10/07/2017	2nd 50
Red Curly kale	Curly kale reddy/ Maraldi	Valdaso	1st 28/08/2015; 2nd 07/09/2016	1st 26/11/2015; 2nd 06/12/2016	1st 90; 2nd 90
		Fucino	2nd 24/06/2017	2nd 13/08/2017	2nd 50
Russian Curly kale	Kale red russian/ Maraldi	Valdaso	1st 28/08/2015; 2nd 07/09/2016	1st 26/11/2015; 2nd 06/12/2016	1st 90; 2nd 90
Broccoli	Broccoli sprouts var.Santee/ Bejo Italia	Valdaso	1st 31/08/15	1st 19/11/2015	1st 80
"Spigariello" broccoli	Cavolo broccolo a getti di Napoli/ Larosa	Valdaso	1st 28/08/2015; 2nd 10/09/2016	1st 26/11/2015; 2nd 09/12/2016	1st 90; 2nd 90
		Fucino	2nd 24/06/2017	2nd 23/08/2017	2nd 60
"Getti e foglie" broccoli	Broccoli getti e foglie / Larosa	Valdaso	1st 30/08/2015; 2nd 28/08/2016	1st 18/11/2015; 2nd 26/11/2016	1st 80; 2nd 90
		Fucino	2nd 24/06/2017	2nd 13/08/2017	2nd 50
Savoy Cabbage	Famosa F1/Bejo Italia	Valdaso	1st 01/09/2015	1st 10/12/2015	1st 100
Red Beet	Selec. Eolo/Four	Valdaso	1st 27/08/2015	1st 25/11/2015	1st 90
Green Beet		Valdaso	1st 27/08/2015	1st 25/11/2015	1st 90
Chicory	Wild species/ Ingegnoli	Valdaso	1st 15/09/2015	1st 14/12/2015	1st 90
Radish-"Rapastrello"	Wild species / Self production	Valdaso	1st 18/11/2015	1st 28/03/2016	1st 131
Borage	Borago officinalis/Four	Valdaso	1st 29/10/2015	1st 16/02/2016	1st 110

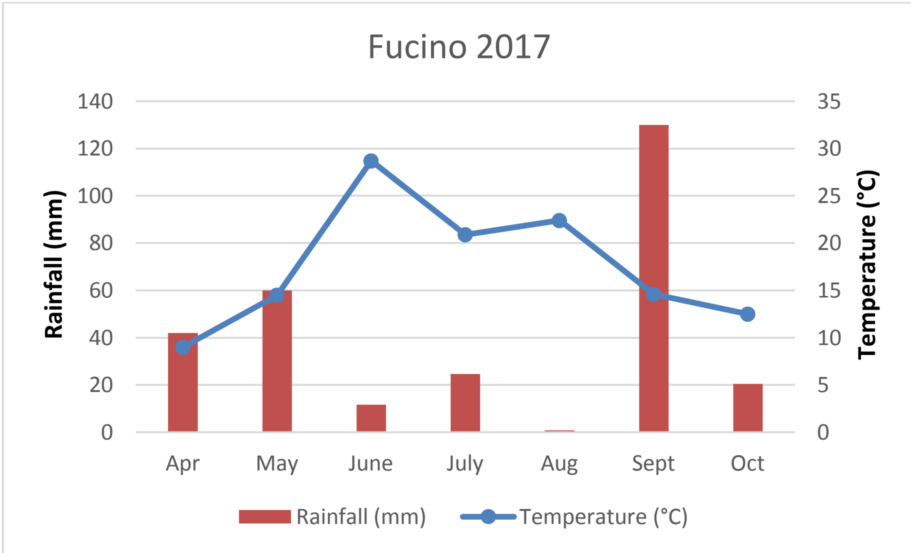
Figure 3- Climate graphs of the cultivation areas interested during the two years of trials. The graphs reported the total rainfall (mm) and the average of Temperature (°C) measured during the period of growth of the plants sampled. **a)** Valdaso climate graph of the 1st year of trial; **b)** Valdaso climate graph of the 2nd year of trial; **c)** Fucino climate graph referred to the 2nd year of trial.



a)



b)



c)

4.2.2. Chemicals

Methanol (99%, ACS-ISO) was purchased from Carlo Erba Reagents (Milan, Italy). Folin-Ciocalteu reagent, sodium carbonate (anhydrous), potassium chloride, sodium acetate, chloridric acid, glacial acetic acid, ferric chloride hexahydrate 2,4,6-tris(2-pyridyl)-striazine (TPTZ, 99%), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ferrous sulphate heptahydrate, 3,4,5-trihydroxybenzoic acid (gallic acid), vitamin C, were purchased from Sigma-Aldrich (Sigma-Aldrich s.r.l., Milan, Italy).

4.2.3. Vegetables sensorial quality

Vegetable materials were characterized for the soluble solid content, titratable acidity and the pH value; sensorial quality was performed at 20.0 ± 0.5 °C on fresh material.

Soluble solid content (SSC) - Vegetables soluble solid content was measured on fresh materials using a hand-held refractometer (Atago, Italy); results are expressed as °Brix.

Titratable acidity (TA) and pH - Vegetables titratable acidity and pH were measured with an automatic titrator (HI 84532 Fruit Juice Titratable Acidity- Hanna Instruments). To 5 ml of vegetables juice, extracted with a centrifuge and filtered, was added distilled water until reaching 50 ml of final volume, and TA was measured (1:10, v/v). Results are expressed in % citric acid. The instrument measured also the vegetables pH.

4.2.4. Vegetables nutritional quality

4.2.4.1. VEGETABLES EXTRACTION

4.2.4.1.1. Total Antioxidant Capacity, Total Phenol Content and Total Anthocyanin Content

The samples stored at -20°C were powdered by liquid nitrogen (1L for 100g) to obtain 10 g placed into test-tubes. The extraction goes on with the addition of 100 ml of extracting solution constituted by 20:80 water:methanol and 1% of acetic acid. Samples were homogenized using an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Germany). The homogenized suspensions were placed in a fridge at 4°C in the dark. After 48 hours, the suspensions were centrifuged at 2500 rpm for 15 min (Thermo Fisher Scientific Heraeus Megafuge 16R Centrifuge, Germany) and the recovered supernatants were collected and stored in six amber vials (for each sample), of 4 mL each, at -20°C waiting for analysis (Wang et al., 2000; Diamanti et al., 2012). The obtained samples were suitable for the analysis of Total Antioxidant Capacity, Total Phenol Content, and Total Anthocyanin Content.

4.2.4.1.2. Total Vitamin C Content

Total vitamin C (or ascorbic acid) was extracted and measured as described by Helsper et al. (2003) and Tulipani et al. (2008), with slight modifications. Briefly, total vitamin C of fresh samples was extracted by sonication of 1 g of raw product in 8 ml of extracting solution, composed of ice-cold water with 5% of metaphosphoric acid and 1 mM diethylenetriaminepentaacetic acid (DTPA). This step was followed by centrifugation at 4000 rpm for 15 min at 4°C, and then samples were filtered with 0.45 μm NY filters and immediately analyzed on an HPLC system.

4.2.4.2. VEGETABLES ANALYSES

4.2.4.2.1. Total Antioxidant Capacity (TAC)

The total antioxidant capacity of vegetables was evaluated using the FRAP (Ferric Reducing Antioxidant Power) assay. The reduction of ferric tripyridyltriazine (Fe+3 –TPTZ) was measured by the method of Benzie and Strain (1996) modified by Deighton et al. (2000) and optimized for Brassica vegetables. The FRAP reagent was freshly prepared by mixing 10:1:1 (v/v/v) of sodium acetate (300 mM acidified with acetic acid until pH 3.6), ferric chloride (20mM) and TPTZ (10mM in 40mM HCl). Briefly, the extract was diluted 1:5 and vortexed. This solution was further diluted 1:10 adding the FRAP solution previously prepared, vortexed and put in darkness for 4 min; the absorbance at 593 nm was measured by spectrophotometer (UV-1800 Shimadzu, UV Spectrophotometer, Kyoto, Japan). The results were expressed as mM Trolox Equivalent per kg of fresh weight.

4.2.4.2.2. Total Phenol Content (TPH)

Total phenols were evaluated using the Folin-Ciocalteu reagent method (Slinkard and Singleton, 1977), with gallic acid as the standard for the calibration curve. Briefly, each sample extract was diluted 1:3 in a glass test-tubes mixed with Folin-Ciocalteu reagent. After 3 min, the solution was added with sodium carbonate (20 %), vortexed and stored in the dark. The absorbance of the samples was measured at 760 nm after 60 min by spectrophotometer (UV-1800 Shimadzu, UV Spectrophotometer, Kyoto, Japan). The data were calculated and expressed as mg gallic acid per kg fresh sample, using a standard curve with the range of the standards ranging from 5 mM to 70 mM of gallic acid/L.

4.2.4.2.3. Total Anthocyanin Content (ACY)

Total anthocyanins were measured using the pH differential shift method (Giusti and Wrolstad, 2001). This assay is based on the characteristic change in intensity of the hue of the anthocyanins according to the pH shift. Briefly, the samples extracts were diluted 1:1 (except kale family diluted 1:3) with potassium chloride (pH 1) and, separately, with sodium acetate (pH 4.5) and the corresponding maximum absorbance for both solutions was measured (respectively at λ = 520 nm, and λ = 700 nm). The data were expressed as mg cyanidin 3-glucoside (the most representative anthocyanin in pigmented brassicas) per kg of fresh weight.

4.2.4.2.4. Total vitamin C content

The HPLC system (Jasco Inc, Easton, USA) was equipped with a C_{18} column, 15 cm x 0.46 cm, 5 μm particle size, and an Autosampler (Jasco Inc, Easton, USA). The mobile phase (aqueous solution of 50 mM phosphate buffered solution, pH 3.2) was slid into isocratic stream at a rate of 0.8 ml/min for 15 minutes. The HPLC system was coupled to a UV/VIS detector (Jasco Inc, Easton, USA) fixed at 244 nm wavelength, that is comprised in the range of best absorption of total vitamin C, and to which total vitamin C can be clearly identified as in our previous studies (Zhong et al., 2017). Total vitamin C quantification was made through a standard calibration curve, prepared by running increasing standard concentrations of vitamin C and measured in duplicate at the beginning and at the end of the analysis. Results were expressed as milligrams of total vitamin C per 100 grams of fresh weight. For each sample were made three replications.

4.2.5. Statistical analysis

The vegetable nutritional qualities were analyzed in triplicate for each sample. The data for TAC, TPH, ACY and total vitamin C were all analyzed with Statistica 7 (Stat Soft, Tulsa, OK – USA), using one-way analysis of variance (ANOVA), with each genotype as an independent variable. Significant differences within genotypes were calculated according to Student's Newman-Keuls tests, and differences at $p < 0.05$ were considered as significant.

4.3. Results and discussion

The results are referred to samples collected in fields located in the Valdaso area (Marche Region) in 2 consecutive years (2016 and 2017); data are reported as means of each year of study, and as means of the two years in the same area. Finally, the environment x genotype interaction is studied by analysing plant samples of the same genotypes grown in the second year (2017) in two different cultivation areas: Valdaso (Marche Region) and Fucino (Abruzzo Region). During the second year, the most commercially interesting brassicas were repeated with the addition of two other brassicas (turnip top and black cabbage).

4.3.1. Vegetables sensorial quality

The results showed a remarkable difference among the vegetables for each of the parameters analyzed, suggesting that both genus and species had a great influence in the sensorial parameters. Considering the SSC, samples belonging to *Brassicacee* family stand out for the highest contents in both years of study and in their average values (tables 6, 7 and 8), the curly kale group. In the first year of study, in addition to Brassica vegetables like broccoli, savoy cabbage, "getti e foglie", kales and "spigariello", the green beet showed an interesting value for SSC. "Getti e foglie" is the sample which presents the second highest value for SSC and the highest for TA in the first year of study (table 6), that confer to this vegetable a balanced taste.

Table 6- Mean values of sensorial parameters of vegetables cultivated during 1st year (2016) in Valdaso area

Vegetables 1st year-Valdaso	SSC[1] (°Brix)				TA[2] (% citric acid)				pH			
Red Beet	5.93	±	0.07	b	0.10	±	0.00	cd	6.60	±	0.06	b
Green Beet	8.07	±	0.59	a	0.12	±	0.01	bcd	6.50	±	0.15	bc
Borage	4.33	±	0.24	c	0.09	±	0.01	d	6.93	±	0.09	a
Broccoli	9.52	±	0.11	a	0.15	±	0.00	bc	6.41	±	0.03	bc
Savoy Cabbage	8.93	±	0.07	a	0.11	±	0.00	cd	6.37	±	0.03	bc
Chicory	5.93	±	0.07	b	0.10	±	0.00	cd	6.00	±	0.00	c
Getti e foglie	9.33	±	0.35	a	0.19	±	0.01	a	6.13	±	0.03	bc
Red Curly Kale	8.33	±	0.85	a	0.13	±	0.01	bcd	6.43	±	0.03	bc
Green Curly Kale	9.13	±	0.18	a	0.13	±	0.00	bcd	6.33	±	0.09	bc
Radish	6.80	±	0.12	b	0.11	±	0.01	cd	6.07	±	0.03	bc
Russian Kale	9.27	±	0.37	a	0.13	±	0.01	bcd	6.23	±	0.03	bc
Spigariello	8.27	±	0.44	a	0.16	±	0.03	ab	6.37	±	0.32	bc

In table are reported the average of [1]SSC: Soluble Solid Content; [2]TA: Titratable Acidity and pH ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n=3

Comparing results of brassicas samples analysed during the first and second year of study (tables 6 and 7), emerges that the variation of sensorial parameters wasn't strictly related to the genotype but is dependent also to other variables like year of cultivation. This means that environmental factors that can change from one year to another, like rainfall, temperature, global radiation and photoperiod length, could influence the sensorial parameters of genotypes cultivated at the same location for different years.

Indeed, the species with the higher values in the 1st year don't confirm the same trend in the 2nd year: e.g., "getti e foglie" sample, showed in table 7, had the lowest SSC, while in the first year it was among the highest. In table 7 are reported the results of the second year where curly and red kale possess the highest SSC and TA contents, this confer to this vegetable a balanced taste.

Table 7-Mean values of sensorial quality parameters of vegetables cultivated during 2nd year (2017) in Valdaso area

Vegetables 2nd year-Valdaso	SSC[1] (°Brix)		TA[2] (% citric acid)		pH	
Black Cabbage	10.80 ± 0.20	b	0.31 ± 0.07	ab	6.37 ± 0.12	a
Turnip Top	7.80 ± 0.12	d	0.24 ± 0.02	b	5.97 ± 0.03	b
Getti e foglie	8.87 ± 0.13	c	0.23 ± 0.02	b	6.13 ± 0.03	ab
Red Curly Kale	10.53 ± 0.18	b	0.26 ± 0.01	b	6.20 ± 0.12	ab
Green Curly Kale	12.00 ± 0.46	a	0.47 ± 0.10	a	5.70 ± 0.06	c
Russian Kale	11.60 ± 0.20	a	0.39 ± 0.05	ab	5.67 ± 0.03	c
Spigariello	9.60 ± 0.35	c	0.19 ± 0.01	b	6.23 ± 0.03	ab

In table are reported the average of [1]SSC: Soluble Solid Content; [2]TA: Titratable Acidity and pH ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n=3

Table 8- Mean values of sensorial parameters analyzed for two production cycles (2016/2017) in Valdaso area.

Valdaso Vegetables average 2016/2017	SSC[1] (°Brix)		TA[2] (% citric acid)		pH	
Getti e foglie	9.10 ± 0.20	b	0.21 ± 0.01	ab	6.13 ± 0.02	ab
Red Curly Kale	9.43 ± 0.63	b	0.20 ± 0.03	ab	6.32 ± 0.07	a
Green Curly Kale	10.57 ± 0.68	a	0.30 ± 0.09	a	6.02 ± 0.15	ab
Russian Kale	10.43 ± 0.55	a	0.26 ± 0.06	ab	5.95 ± 0.13	b
Spigariello	8.93 ± 0.39	b	0.18 ± 0.01	b	6.30 ± 0.15	a

In table are reported the average of [1]SSC: Soluble Solid Content; [2]TA: Titratable Acidity and pH ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n=6

The average of sensorial values of plant samples collected in Valdaso area for two years, reported in table 8, underline a balanced taste in all vegetables analysed, because there was a good ratio between SSC and TA. The greatest genotypes appreciated during two years of trial belong to kale family, with the most interesting SSC and TA contents detected.

When the cultivation area change, e.g. comparing plants grown in the two different environments (Fucino and Valdaso), the differences in the same genotype become more remarkable depending to the location of grow. Indeed, the content of SSC detected in green curly kale, turnip top and "spigariello" samples from Valdaso cultivation was statistically higher than those of the same plants but cultivated in Fucino area, while values of the other parameters measured were more constant and similar independently to the area of growth (table 9). Green, red curly kale and black cabbage belong to *Brassica oleracea acephala* species and showed the highest SSC, TA and pH during two years of trial. Higher pH level was detected in "spigariello" and "getti e foglie" too. The 2 years cultivation of the in Valdaso area provided samples having higher concentration of SSC than the values detected from samples grown in the Fucino area. All other parameters resulted the same independently to the area of cultivation.

Table 9-Means values of sensorial parameters collected during the 2nd year of trials in different environments (Fucino vs Valdaso area)

Vegetables	**Areas**	**SSC[1] (°Brix)**		**TA[2] (% citric acid)**		**pH**	
Black Cabbage	Fucino	9.73 ± 0.28	cd	0.30 ± 0.01	abc	6.07 ± 0.03	ab
	Valdaso	10.80 ± 0.20	abc	0.31 ± 0.07	abc	6.37 ± 0.12	a
Turnip Top	Fucino	4.23 ± 0.12	g	0.13 ± 0.02	c	6.03 ± 0.13	b
	Valdaso	7.80 ± 0.12	e	0.24 ± 0.02	bc	5.97 ± 0.03	bc
Getti e foglie	Fucino	7.73 ± 0.50	e	0.24 ± 0.01	bc	5.93 ± 0.03	bc
	Valdaso	8.87 ± 0.13	de	0.23 ± 0.02	bc	6.13 ± 0.03	ab
Red Curly Kale	Fucino	11.13 ± 0.72	ab	0.30 ± 0.01	abc	5.97 ± 0.03	bc
	Valdaso	10.53 ± 0.18	bc	0.26 ± 0.01	bc	6.20 ± 0.12	ab
Green Curly Kale	Fucino	9.53 ± 0.48	cd	0.38 ± 0.09	ab	6.07 ± 0.03	ab
	Valdaso	12.00 ± 0.46	a	0.47 ± 0.10	a	5.70 ± 0.06	c
Spigariello	Fucino	6.07 ± 0.07	f	0.23 ± 0.00	bc	6.20 ± 0.00	ab
	Valdaso	9.60 ± 0.35	cd	0.19 ± 0.01	bc	6.23 ± 0.03	ab
Fucino	-	8.07 ± 0.59	B	0.26 ± 0.02	N.S.	6.04 ± 0.03	N.S.
Valdaso	-	9.93 ± 0.34	A	0.28 ± 0.03	N.S.	6.10 ± 0.06	N.S.

In table are reported the average of [1]SSC: Soluble Solid Content; [2]TA: Titratable Acidity and pH ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). N.S: not significant n=3; N=18

4.3.2. Vegetables nutritional quality

4.3.2.1. TOTAL PHENOL CONTENT, TOTAL ANTHOCYANIN CONTENT, AND TOTAL ANTIOXIDANT CAPACITY

The nutritional data showed how environment and genotype influenced the phytochemical compounds content. In the tables reported below, it is possible to appreciate several differences among genotypes. According to table 10, in the first year of cultivation in Valdaso area, the highest values of antioxidant capacity was detected in borage, species that do not belong to *Brassicacee* family, followed by red curly kale and chicory. The same trend is reported for TPH where borage, chicory and radish had the highest antioxidant capacity. Interestingly, literature data for TPH of borage is 5210 mg GA/kg fw for wild species, while 2370 mg GA/kg fw for the cultivated ones (Abu-Qaoud et al., 2018), a very similar value to this study.

For what concern chicory and radish, a lot of studies verified their nutritional potential and phytochemical compounds presence, measuring their antioxidant capacity in correlation with total phenolic and flavonoid content. These studies introduced those species in the modern diet as a healthy alternative to the vegetables normally consumed (Kücükboyaci et al., 2012; Jancic et al., 2017).

Red curly kale, in the first year of trial in Valdaso area (table 10), expressed the highest content of anthocyanins, followed by broccoli and russian kale. All these genotypes are constituted by red and purple pigmentation. For the others genotypes the anthocyanins content isn't relevant due to their prevailing green colour.

As previously stated, during the second year the most commercially interesting Brassicas were repeated with the addition of two other brassicas (turnip top and black cabbage) (table 11). The results showed that green curly kale had the higher content of TAC and TPH followed by red curly kale for TAC and "spigariello" for TPH. Data from second year harvest showed higher values of TAC, TPH and ACY in kales than the first ones, except for red curly kale TPH. Red curly kale confirmed its ACY highest content also in the second year, followed by Russian kale that expressed some red and purple pigmentation.

Table 10 - Phenolics, anthocyanins contents and antioxidant capacity of different type of vegetables analysed during 1st year in Valdaso.

Vegetables 1°year-Valdaso	TAC[1] (µM Trolox/g fw)				TPH[2] (mg GA/kg fw)				ACY[3] (mg CYA-3-GLU/kg fw)			
Red Beet	2.92	±	0.14	e	1111.04	±	37.65	cd	0.00	±	0.00	e
Green Beet	2.03	±	0.04	e	715.48	±	24.83	e	0.24	±	0.11	e
Borage	11.94	±	0.19	a	2239.61	±	5.20	a	3.61	±	0.85	e
Broccolo	8.34	±	0.10	c	1744.37	±	29.71	b	51.05	±	0.71	b
Savoy Cabbage	2.58	±	0.16	e	869.70	±	60.70	de	2.00	±	0.14	e
Chicory	10.30	±	0.76	b	2493.13	±	151.72	a	1.78	±	0.33	e
Getti e foglie	4.09	±	0.04	d	1125.49	±	6.44	cd	0.54	±	0.24	e
Red Curly Kale	10.51	±	0.22	b	2210.15	±	11.94	a	207.32	±	1.16	a
Green Curly Kale	4.86	±	0.16	d	1427.28	±	50.51	c	0.50	±	0.12	e
Radish	8.43	±	0.08	c	2349.92	±	23.07	a	33.29	±	1.76	d
Russian Kale	5.43	±	0.20	d	1824.30	±	85.90	b	43.03	±	1.64	c
Spigariello	5.05	±	0.58	d	1255.81	±	95.06	c	1.52	±	0.31	e

In table are reported the average of [1]TAC: Total Antioxidant Capacity; [2]TPH: Total Phenols Content; [3]ACY: Total Anthocyanins Content ± their standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3

Table 11 - Phenolics, anthocyanins contents and antioxidant capacity of different type of vegetables analyzed during 2nd year in Valdaso.

Vegetables 2nd year-Valdaso	TAC[1] (µM Trolox/g fw)				TPH[2] (mg GA/kg fw)				ACY[3] (mg CYA-3-GLU/kg fw)			
Black Cabbage	8.43	±	0.24	d	1597.99	±	27.12	c	10.36	±	1.00	c
Turnip Top	4.53	±	0.09	e	860.21	±	26.16	d	0.73	±	0.29	c
Getti e foglie	4.21	±	0.08	e	701.39	±	10.07	e	2.97	±	0.57	c
Red Curly Kale	11.97	±	0.24	b	1582.49	±	27.73	c	347.21	±	5.89	a
Green Curly Kale	19.36	±	0.69	a	2788.77	±	27.51	a	3.09	±	1.18	c
Russian Kale	4.27	±	0.14	e	750.56	±	27.10	e	48.91	±	7.55	b
Spigariello	9.70	±	0.13	c	1706.96	±	35.20	b	5.23	±	0.32	c

In table are reported the average of [1]TAC: Total Antioxidant Capacity; [2]TPH: Total Phenols Content; [3]ACY: Total Anthocyanins Content ± their standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3

The average data of the two-years trial in Valdaso area (table 12) confirmed the results already found in each of the two years of study. In fact, kales family confirmed its highest content of TPH or ACY, according to the species considered. Consequently, both Red Curly and Green Curly Kales registered a high TAC value, as also reported in many studies

(Heimler et al., 2006; Olsen et al., 2012, Becerra Moreno et al., 2014). Therefore, the effect of the genotype resulted persistent independently of the year.

Table 12 - Phenolics, anthocyanins contents and antioxidant capacity of different type of vegetables analyzed during the two years of trials in Valdaso.

Valdaso Vegetables average 2016/2017	**TAC[1] (µM Trolox/g fw)**				**TPH[2] (mg GA/kg fw)**				**ACY[3] (mg CYA-3-GLU/kg fw)**			
Getti e foglie	4.18	±	0.06	c	807.41	±	48.02	e	2.36	±	0.51	c
Red Curly Kale	11.61	±	0.24	a	1739.41	±	73.17	b	312.24	±	16.24	a
Green Curly Kale	12.11	±	1.55	a	2108.02	±	144.71	a	1.79	±	0.64	c
Russian Kale	4.85	±	0.17	c	1287.43	±	120.30	d	45.97	±	3.83	b
Spigariello	7.38	±	0.57	b	1481.38	±	68.33	c	3.37	±	0.44	c

In table are reported the average of [1]TAC: Total Antioxidant Capacity; [2]TPH: Total Phenols Content; [3]ACY: Total Anthocyanins Content ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n=6

The 2 cultivation conditions (Valdaso and Fucino) highly influenced the phytochemical composition of the different genotypes tested in the second year of study. Data confirm the highest nutritional value of kales, given by the high values of TAC, TPH and ACY of green and red curly kale. For what concern TAC and TPH, Green curly kale cultivated in Valdaso showed the highest values. The TPH data obtained in this trial were similar to those previously obtained by Lafarga et al. (2018) (900-1600 mg GAE/kg fw), while were lower than those obtained by Olsen et al. (2012) (4240-5622 mg GAE/kg).

ACY results confirmed the high presence of anthocyanins group in Red curly kale variety, mainly in Fucino vegetables. Olsen et al. (2012) reported that in green curly kale the concentration of anthocyanins is negligible, while red curly kale possessed a higher ACY concentration than shown in this trial.

Summarizing, it is possible to affirm that the variation of phytochemical compounds content is explained by the interaction Environment x Genotype. In Brassica genus, the differences were evident among and within the species. Environmental factors like biotic and abiotic stress can also change the phytochemical contents, as demonstrated by the statistical differences of TAC, TPH and ACY mean values between Fucino and Valdaso vegetables (Table 13). The first two parameters were higher in Valdaso cultivation conditions, while the latter was higher in Fucino environment. Also the cultivation year could influence the phytochemical contents in the same growing area, as demonstrated in the Valdaso cultivation (table 10, 11). The importance of cultivation year and area has been confirmed by many studies (Cartea et al., 2011; Podsedek, 2007).

Table 13 – Effect of vegetable genotype x environment interaction on 2nd year Valdaso and Fucino samples on vegetables nutritional value.

Vegetables	Areas	TAC[1] (µM Trolox/g fw)		TPH[2] (mg GA/kg fw)		ACY[3] (mg CYA-3-GLU/kg fw)	
Black Cabbage	Fucino	4.92 ± 0.09	f	961.11 ± 35.19	e	3.92 ± 0.62	c
	Valdaso	8.43 ± 0.24	e	1597.99 ± 27.12	d	10.36 ± 1.00	c
Turnip Top	Fucino	3.03 ± 0.19	h	1038.06 ± 57.57	e	1.60 ± 0.12	c
	Valdaso	4.53 ± 0.09	f	860.21 ± 26.16	f	0.73 ± 0.29	c
Getti e foglie	Fucino	3.46 ± 0.08	gh	705.41 ± 13.85	g	3.54 ± 0.51	c
	Valdaso	4.21 ± 0.08	fg	701.39 ± 10.07	g	2.97 ± 0.57	c
Red Curly Kale	Fucino	13.68 ± 0.38	b	1966.52 ± 32.90	b	437.29 ± 22.64	a
	Valdaso	11.97 ± 0.24	c	1582.49 ± 27.73	d	347.21 ± 5.89	b
Green Curly Kale	Fucino	8.86 ± 0.33	e	1559.07 ± 53.34	d	0.00 ± 0.00	c
	Valdaso	19.36 ± 0.69	a	2788.77 ± 27.51	a	3.09 ± 1.18	c
Spigariello	Fucino	5.13 ± 0.06	f	1002.40 ± 26.71	e	3.92 ± 0.41	c
	Valdaso	9.70 ± 0.13	d	1706.96 ± 35.20	c	5.23 ± 0.32	c
Fucino	-	6.51 ± 0.45	B	1205.43 ± 52.78	B	75.05 ± 19.57	A
Valdaso	-	9.70 ± 0.62	A	1539.63 ± 81.15	A	61.60 ± 15.19	B

In table are reported the average of [1]TAC: Total Antioxidant Capacity; [2]TPH: Total Phenols Content; [3]ACY: Total Anthocyanins Content ± their standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n=3; N=18

4.3.2.2. TOTAL VITAMIN C CONTENT

Data obtained in this study confirmed that Brassica plants are a rich source of vitamin C, but the different species analyzed revealed a wide range of variation; indeed, vitamin C was strongly influenced by environment and genotype. The results illustrated below in fig. 4 and 5 are related to the content of vitamin C obtained in the first and second years of study, respectively.

In the first year of trial, are analyzed only the vegetables coming from Valdaso area, where the higher vitamin C content was detected in broccoli species, followed by kales group with green and red curly kale.

In the second year of trial a comparison between Valdaso and Fucino areas was made; the results indicated that Valdaso area was major brought to favour the vitamin C production in vegetables than Fucino; except for its turnip top that was the highest of second year.

Comparing the same genotypes during the two years in the same area emerged higher value during the second year expect for black cabbage, in Valdaso. Broccoli, turnip top and black cabbage possess the highest vitamin C content.

The amount of vitamin C registered for some vegetables is lower than reported in literature; e.g. Valdaso turnip top measured 7.30 and 10.50 mg/100g fw in the first and second year respectively, lower than 25-29 mg/100g fw reported by Conversa et al. (2016). Similarly, green and red curly kale registered in our study values of 4.26 and 17.57 mg/100g fw in Valdaso during the first year of cultivation, lower than 52 and 67 mg/100g fw reported by Olsen et al. (2012). Other kales analyzed in literature showed values comprised between 83 and 104 mg/100g of fw (Podsedek, 2007; Armesto et al., 2015).

However, several other vegetables analyzed in this study showed a higher concentration of vitamin C than reported in literature: e.g., Fucino turnip top, with 36.18 mg/100g fw, was better than those reported in Conversa et al. (2016). Similarly, Broccoli (80.35 mg/100g fw) showed a value slightly higher than found by Bahorun et al. (2004) (74.8 mg/100g fw). This variability in vitamin C concentration can be bound to genetic aspects and to climatic conditions of the cultivation areas. The vitamin C level was found to be widely variable in broccoli (4-fold) and in kale (2-fold) also in literature (Podsedek, 2007, Kaur et al*.,* 2006; Nicoletto et al*.,* 2016). Furthermore, several studies reported that the concentration of vitamin C varied also considering the plants portion: Lafarga et al., (2018) described a higher concentration in the inflorescences/leaves than stems in some varieties, while other studies found the opposite trend (Zhang and Hamauzu, 2004).

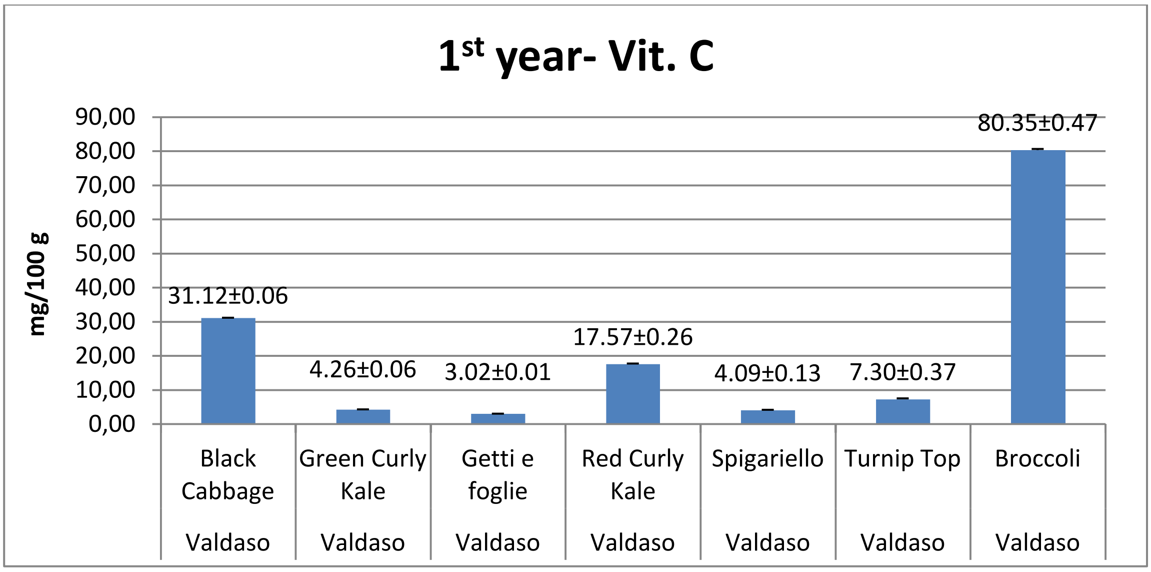


Figure 4- Average of Vitamin C (mg/100g fw) content in samples analyzed during the 1st year ± standard deviation. n=3

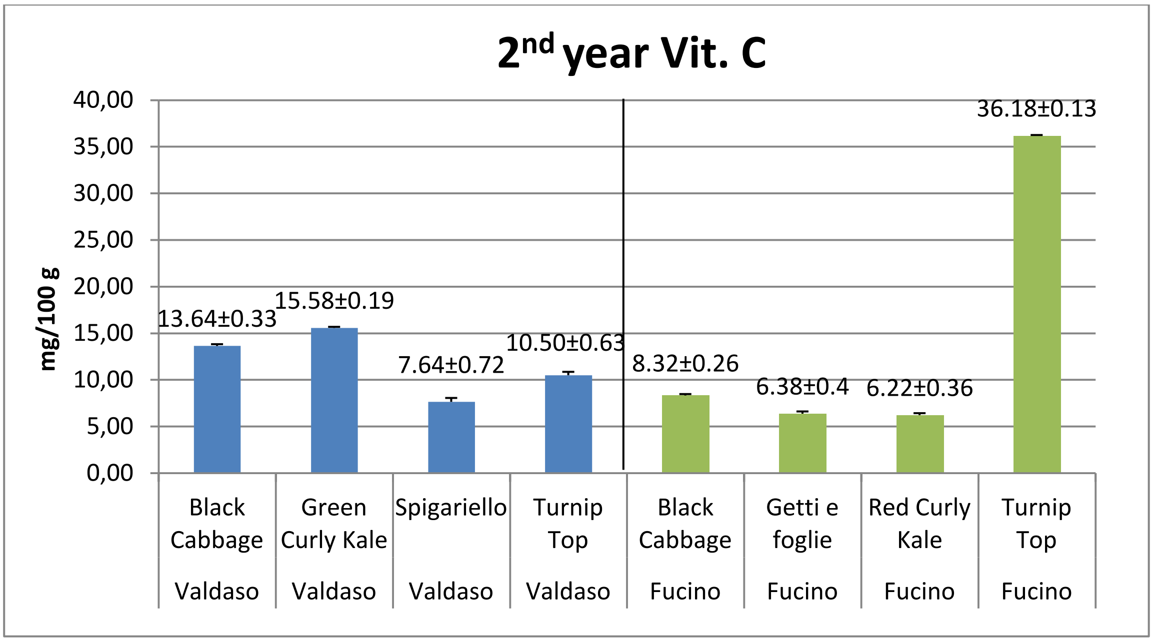


Figure 5- Average of Vitamin C content (mg/100g fw) in samples analyzed during the 2nd year ± standard deviation. n=3

4.4. Conclusions

In this study was confirmed the high sensorial, nutritional and antioxidant potential of *Brassicacee* plants and field herbs. It was reported the great variation among and inside the species influenced by the genotype and the environment (Podsedek, 2007). Several genotypes showed interesting and appreciated concentrations of phytochemical compounds like green and red curly kale, borage and chicory; instead, others presented lower contents like beets, “getti e foglie” and turnip top. For what concern the other species like chicory, borage, and radish, this study proved that the cultivated vegetables possess minor content of phytochemicals than the wild ones (Abu-Qaoud et al., 2018), and that they represented a wide source of antioxidants and phenols and can be used as alternative for the consumers.

For what concern the cultivation area, Valdaso promoted higher TAC values associated with higher TPH contents in vegetables than Fucino. About ACY, the red curly kale reached the highest value among all in Fucino. Considering the DAS (day after sowing) of samples harvested, emerged that those coming from Fucino were collected earlier than those of Valdaso area. This fact is explained with the increase of light during spring and summer season that accelerate the transition to the reproductive phase, reducing the life cycle length and anticipating the harvest time.

From the two years of research carried out, emerged the strongly predominance of curly kale group in terms of phytochemical compounds contents and nutritional property, in details the green for TAC and TPH and the red one for ACY; they possess interesting balance taste between SSC and TA analyzed too.

This study strengthens the commercial diffusion and proposal of the first evolved class vegetables; this product will satisfy the consumer's needs, maintaining high sensorial and nutritional quality, together with an ease of preparation and use.

5. RESEARCH LINE II: VARIATION OF NUTRITIONAL QUALITY DEPENDING ON HARVESTED PLANT PORTION OF BROCCOLI AND BLACK CABBAGE

5.1. Introduction

Italy is known in the word for its high-quality products and crops; among these, *Brassica* vegetables cover an important part of the market, with a total production of 828.258 t in 2017. However, *Brassica* Italian crops production suffered of a decreasing of 15% since 2010. The same percentage of decreasing is reported for the Marche Region, where the actual study was conducted, from 1.991 ha in 2010, to 731 ha in 2017, focusing on savoy cabbage, cauliflower and broccoli crops. By the way, an increase of other cabbage production was registered for this region (Istat- tav. C08-C09, 2010-2017).

A lot of studies showed a relation between the high consumption of *Brassica* vegetables and the reduction of the risk of age-related chronic illness such as cardiovascular and other degenerative diseases (Kris-Etherton et al., 2002); they reduced also the risk of several types of cancer (Bjorkman et al., 2011). Broccoli and black cabbage are among the vegetable food with the highest antioxidant potential (Zhou and Yu, 2006). The antioxidant and antiradical activity in *Brassica* vegetables is mainly represented by the large group of polyphenols, constituted by flavonoids (mainly flavonols and anthocyanins) and hydroxycinnamic acids. These secondary metabolites have several functions in the plant, like UV protection, pigmentation, and disease control, as glucosinolates (Pereira et al., 2002). In Brassica genus, the main represented flavonols are quercetin, kaempferol and isorhamnetin. Anthocyanins, besides to confer the blue and red pigmentation in broccoli sprouts and red cabbage, possess high antioxidant activity (Moreno et al., 2010); among them, cyanidin-3-glucoside is the mainly represented in Brassica crops (Lo Scalzo et al., 2008).

Several studies describe the variation of the antioxidant activity and phenolics content in the plant portion: e.g. in turnip plants, flower buds are the most active portion, followed by leaves and stems, while roots seem to have less interesting phenolic concentration (Fernandes et al., 2007). Ferreres et al. (2009) revealed that kale seeds had higher antioxidant potential than kale leaves, but leaves were the richest in phenolics. In *Brassica rapa*, Francisco et al. (2009) reported that phenolic compounds content is higher in leaves harvested during vegetative period than fructiferous stems (with flower, buds, and surrounding leaves), and flavonoids were the main represented compounds. These findings are confirmed by Francisco and collaborators (Francisco et al., 2011), who found that total phenolic content is higher in green turnip than in turnip top. On the contrary, Schmidt et al. (2010) found that harvest date has only a minimal influence on flavonoid concentration in kale. Broccoli cultivars have an increase of phenolic compounds during the inflorescences development, with an interesting antioxidant activity in flower, stem and leaf (Vallejo et al., 2003 b; Guo et al., 2001).

The aim of this work is to investigate the content of phytochemical compounds in different *Brassica* plant portions at different development stages for providing high antioxidant products to consumers. For this reason, broccoli plants were divided into heads and stems, while for black cabbage leaves and seeds were chosen. A comparison study between different varieties of broccoli was made too, to investigate the interaction between genotype and plant portion. Developmental stage was also kept in consideration, analyzing first and second harvest of product.

5.2. Materials and methods

5.2.1. Plant material

Vegetables materials used for these trials were provided by Valli di Marca Agricultural Farms that cultivated the raw materials in a typical agricultural area in the south of Marche Region (Italy) called Valdaso, situated in the middle Adriatic zone at 136 m. a. s. l.. *Brassica* plants were cultivated in open field conditions, according to the typical cultivation system adopted in this area. Broccoli samples belong to Roja and Santee cultivars were sowing on 20th November 2015 and were harvested on 8th February 2016, while Roja-II (second harvest) was collected on 26th February 2016. After harvesting, samples were packaged in 300 g of fresh weight and immediately frozen at -20°C. In a second step, before the analysis, broccoli samples were divided into heads and stems (approximately representing 25% and 75% of total fresh weight, respectively) for analyzing the different antioxidants, phenols and anthocyanins content in the two portions; black cabbage seeds and leaves were taken too (seeds were sampled during April 2016 while leaves on 10th March 2016). Table 14 shows the plant materials sampled and the day after sowing (DAS).

Table 14 - Plant materials sampled

Species	**Samples (300 g)**	**DAS**
Brassica oleracea L.var. italica *Bejo Italia	5 samples of Broccoli var. Roja* divided into heads and stems	80
	5 samples of Broccoli var. Santee F1* divided into heads and stems	80
	3 samples of Broccoli var. Roja* - II harvest- divided into heads and stems	98
Brassica oleracea L. var. acephala subvar. laciniata L.* ***Four sementi***	2 samples of black cabbage leaves	80
	2 samples of black cabbage seeds	-

5.2.2. Chemicals

Methanol (99%, ACS-ISO) was purchased from Carlo Erba Reagents (Milan, Italy). Folin-Ciocalteu reagent, sodium carbonate (anhydrous), potassium chloride, sodium acetate, chloridric acid, glacial acetic acid, ferric chloride hexahydrate, 2,4,6-tris(2-pyridyl)-striazine (TPTZ, 99%), ferrous sulphate heptahydrate, 3,4,5-trihydroxybenzoic acid (gallic acid), and sodium hydroxide, were purchased from Sigma-Aldrich (Sigma-Aldrich s.r.l., Milan, Italy).

5.2.3. Vegetables sensorial quality

The sensorial quality analysis was carried out on a vegetable juice extract obtained by the fresh material and prepared with a centrifuge for food (except for the seeds) and developed at 20.0 ± 0.5 °C. The measurement of vegetables soluble solids (SSC) was made using a hand-held refractometer (Atago, Italy). The refractive index was recorded as °Brix, with the refractometer prism cleaned with distilled water after each sample. Vegetables titratable acidity (TA) was determined from 5 ml juice diluted with distilled water (1:10, v/v) and automatically titrated by an automatic titrator (HI 84532 Fruit Juice- Titratable acidity- Hanna Instruments, Woonsocket, Rhode Island, Stati Uniti). The titratable acidity is expressed as % citric acid. This instrument allows also the pH measurement.

5.2.4. Vegetables nutritional quality

5.2.4.1. VEGETABLES EXTRACTION

5.2.4.1.1. Total Antioxidant Capacity, Total Phenol Content and Total Anthocyanin Content

Fresh materials stored at -20°C were sliced for obtaining 10 g of representative samples and placed into test tubes. 100 ml of methanol extracting solution, constituted by 20:80 water: methanol and 1% of acetic acid, were added to the samples. Samples were then homogenised using an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Germany). The homogenized suspensions were placed in a fridge at 4°C in the dark. After 48 hours, the suspensions were centrifuged at 2500 rpm for 15 min (Thermo Fisher Scientific Heraeus Megafuge 16R Centrifuge, Germany) and the recovered supernatants were collected and stored in six amber vials (for each sample), of 4 ml each, at -20°C (Wang et al., 2000; Diamanti et al., 2012).

5.2.4.2. VEGETABLES ANALYSES

5.2.4.2.1. Total Antioxidant Capacity (TAC)

Vegetables total antioxidant capacity (TAC) was evaluated using FRAP (*Ferric Reducing Antioxidant Power)* method; the reduction of ferric–TPTZ was measured by the method of Benzie and Strain (1996), modified by Deighton et al. (2000) and optimized for *Brassica* vegetables. The FRAP reagent was freshly prepared by mixing 10:1:1 (v/v/v) of sodium acetate (300 mM acidified with acetic acid until pH 3.6), ferric chloride (20mM) and TPTZ (10mM in 40mM HCl). Briefly, the vegetable methanolic extract was diluted 1:5 and vortexed. This solution was further diluted 1:10 adding the FRAP solution previously prepared, vortexed and put in darkness for 4 min; after this, the absorbance was measured at 593 nm by spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan).

The results were expressed as mM Trolox Equivalent per kg of fresh weight (mM TE/kg fw). Also in this case, the calibration was calculated by linear regression from the dose-response curve of the Trolox standards.

5.2.4.2.2. Total Phenol Content (TPH)

The vegetable total phenol content was evaluated using the Folin-Ciocalteu reagent method (Slinkard & Singleton, 1977), with gallic acid as the standard for the calibration curve. Briefly, glass test-tubes were filled with 3.5 ml water, and 150 μl diluted vegetable methanolic extract (1:3) was added. The absorbance of the samples was measured at 760 nm by spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan) after 60 min. The data were calculated and expressed as mg gallic acid per kg of fresh weight (mg GA/kg fw).

5.2.4.2.3. Total Anthocyanin Content (ACY)

The vegetable total anthocyanin content was measured using the pH differential shift method (Giusti & Wrolstad, 2001). This assay is based on the characteristic change in intensity of the hue of the anthocyanins according to the pH shift method. Briefly, the vegetable methanolic extracts were diluted (1:1) with potassium-chloride (pH 1.00) and with sodium acetate (pH 4.50); then the corresponding maximum absorbance of both solutions was measured at the wavelengths of 520 nm and 700 nm. The data are expressed as mg cyanidin 3-glucoside (the most represented anthocyanin in broccoli) per kg of fresh weight (mg Cya-Glu/kg fw).

5.2.5. Statistical analysis

Vegetable sensorial and nutritional qualities were analyzed in triplicate for each sample. The data for the fruit titratable acidity, soluble solids, FRAP, TPH and ACY were all analyzed through the STATISTICA 7 software (Stat Soft, Tulsa, OK - USA), using one-way analysis of variance (ANOVA), with each genotype or plant parts as an independent variable. Significant differences within genotypes or plant parts were calculated according to student's Newman-Keuls tests, and differences at $p < 0.05$ were considered significant.

5.3. Results and discussion

5.3.1. Vegetables sensorial quality

Sensorial quality was analyzed on fresh material considering the whole samples of broccoli. The results of sensorial analysis are reported in table 15. Broccoli var. Santee had the statistically highest soluble solids concentration. Regarding the Titratable acidity, the statistical analysis underlined how black cabbage samples had the highest titratable acidity, while Roja-II samples showed the statistically highest pH value. Broccoli data confirm the results found by Nicoletto and co-authors (Nicoletto et al., 2016); they reported similar value for SSC content (8.2-9.3°Brix), and higher value for what concern the TA (0.40-0.43 % citric acid); lower for pH (5.67-5.83).

Black cabbage belonged to the *Brassica oleracea var. acephala* group that includes kales. A lot of studies regarded the sensorial aspects of kale family; Armesto and co-authors, (2016, 2017) and Martinez et al. (2010) reported similar amount of SSC, TA and pH in kale.

According to those results, it is clear that the sensorial quality is affected by genotype. Regarding the development stage, the comparison of Roja with Roja-II did not show any significant difference for the SSC and TA parameters. The only significant difference was registered for the pH value, with the Roja-II being higher than Roja.

Table 15 - Average of total soluble solids contents, titratable acidity and pH of *Brassica* samples analyzed

Vegetables	SSC[1] (°Brix)	TA[2] (% citric acid)	pH
Black Cabbage	$9.55 \pm 0.31_b$	$0.23 \pm 0.02_a$	$5.88 \pm 0.06_d$
Roja-II[3]	$8.97 \pm 0.26_b$	$0.15 \pm 0.01_b$	$6.77 \pm 0.06_a$
Roja[4]	$9.56 \pm 0.21_b$	$0.15 \pm 0.01_b$	$6.53 \pm 0.07_b$
Santee[5]	$10.62 \pm 0.26_a$	$0.16 \pm 0.01_b$	$6.35 \pm 0.02_c$

Average of [1]SSC: Soluble Solids Content; [2]TA: Titratable Acidity and pH ± standard deviation; [3]Roja-II: Broccoli var. Roja II°harvest; [4]Broccoli var. Roja I°harvest: [5]Broccoli var. Santee. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3

5.3.2. Vegetables nutritional quality

5.3.2.1. BLACK CABBAGE AND WHOLE BROCCOLI NUTRITIONAL VALUE

In table 16, results about the average values of nutritional parameters for black cabbage seeds and leaves are reported. Furthermore, average values of nutritional parameters for each variety of broccoli are showed, comprising Roja harvested at two different development stages.

Table 16 - Average of total antioxidant capacity, phenolics and anthocyanins content in the different vegetables analyzed.

Vegetables	**TAC[1] (mM Trolox/kg fw)**	**TPH[2] (mg GA/kg fw)**	**ACY[3] (mg CYA-3-GLU/kg fw)**
Black Cabbage Leaves	$5.68 \pm 0.21_b$	$1444.60 \pm 23.82_b$	$3.25 \pm 0.24_b$
Black Cabbage Seeds	$8.39 \pm 0.53_a$	$1767.79 \pm 12.67_a$	$16.87 \pm 0.24_a$
Santee[4]	$4.65 \pm 0.13_B$	$1038.74 \pm 26.48_B$	$18.15 \pm 2.33_B$
Roja[5]	$4.31 \pm 0.15_B$	$1046.20 \pm 28.73_B$	$23.42 \pm 2.75_B$
Roja-II[6]	$8.86 \pm 0.24_A$	$2021.17 \pm 117.64_A$	$72.61 \pm 9.05_A$

Average of [1]TAC: Total Antioxidant Capacity; [2] TPH: Total Phenol Content; [3] ACY: Total Anthocyanin Content ± standard deviation; [4] Santee: Broccoli var. Santee; [5] Broccoli var. Roja I° harvest; [6] Roja-II: Broccoli var. Roja II°harvest. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). Lowercase letters refer to black cabbage analyses; uppercase letters refer to broccoli analyses. n=3; N=3

Black cabbage seeds showed a higher content of both the investigated phytochemical compounds than leaves (TPH and ACY) and, consequently, a statistically higher TAC value. These results can be confirmed by Ferreres and collaborators (Ferreres et al., 2009), who performed characterization trials on kale, analysing the antioxidant activity of kale seeds and leaves. They found that seeds are rich in quercetin and isorhamnetin derivatives, not found in leaves, and in phenolic acids, conferring them a higher antioxidant capacity than leaves, rich in flavonols. This major antioxidant capacity found in seeds is bound to their physiological functions as germination, permeability to water, protection by pests and insect attacks, storage and protection of lipids from oxidation.

In our study, the effect of genotype on the phytochemical composition and the antioxidant capacity is not evident, giving that Roja (first harvest) and Santee cultivars did not show any statistically significant difference. This is due probably to the low amount of antioxidant compounds accumulated in the tissues of different cultivars analysed in this study. On the contrary, results on the effect of developmental stage on antioxidant capacity and phytochemical composition were very interesting. In fact, Broccoli Roja- II harvest showed a statistically higher content of TPH and ACY than Roja (first harvest), showing also a double value of antioxidant capacity than the latter. The harvest date can influence the antioxidant capacity of plants as reported by Soengas and co-authors (Soengas et al., 2011). Vallejo et al. (2003 b) reported that the content of phytochemical compounds, in particular phenolic compounds, increased with the exposure to sunlight. In fact, broccoli grow is very sensible to climate and light conditions (Björkman et al., 2011).

5.3.2.2. BROCCOLI'S HEAD AND STEM NUTRITIONAL QUALITY

5.3.2.2.1. Total Antioxidant Capacity (TAC)

In figure 6, FRAP showed contrasting results, because Roja and Santee possess the highest concentration of antioxidant in stem, in Roja II samples head value was the highest for the antioxidant capacity (9.91 ± 0.16 mM Trolox/kg fw). Similar FRAP results were obtained by Kaur et al. (2006) and Pellegrini (2010).

The total averages of different plant parts showed that stem possessed a higher FRAP value than head. In literature, Fernandes et al. (2007) found an opposite trend in turnip, reporting that flower buds possess the highest antioxidant capacity, followed by leaves and stems, where flavonols were mainly represented.

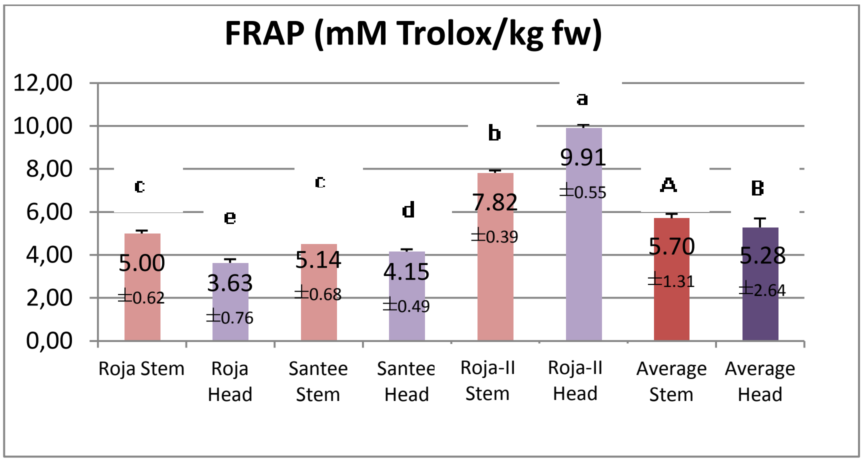


Figure 6- Average and standard deviation of total antioxidant capacity measured by Ferric Reducing Antioxidant Power (FRAP) of Broccoli analyzed: Roja, Santee and Roja II harvest divided into head and stem, and their average (represented with uppercase letters). Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3; N=9

5.3.2.2.2. Total Phenol Content (TPH)

In the analyzed broccoli, the range of phenol concentration varied from 891.26 to 2574.77 mg GA/kg of fw, as reported in figure 7. Total phenol content, that is responsible for the 80% of total antioxidant capacity, resulted to be highest in the broccoli heads, with an average value of 1499.35 mg GA/kg of fw. This trend was confirmed in all the analyzed genotypes, where TPH of heads are always statistically higher than the corresponding stems. Roja-II had a higher content of phenols in head and stem than Roja (first harvest), probably due to stage of development; this could mean that the late harvesting increases the quality of product as reported by Samec et al. (2011) and Soengas (2011). Our study confirms that total phenolic content depends by genotype and plant development stage; a lot of works reported similar findings (Bahorun et al., 2004; Kaur et al., 2006).

Usually TPH and FRAP results had the same trend, giving that phenolic compounds have a great incidence on the total antioxidant capacity of a food matrix. In this case, the higher TPH average content of head do not correspond to the average higher FRAP value of stem tissues; this result is probably due to the capacity of FRAP method to detect more antioxidant compounds over phenols, like ascorbic acid, carotenoids, vitamins and others, although the phenolics content represented the 80% of total antioxidant capacity as reported by Podsedek (2007). However, in Roja-II, TPH values of head was higher than TPH value of stem, and similarly FRAP value was higher in Roja-II head than stem.

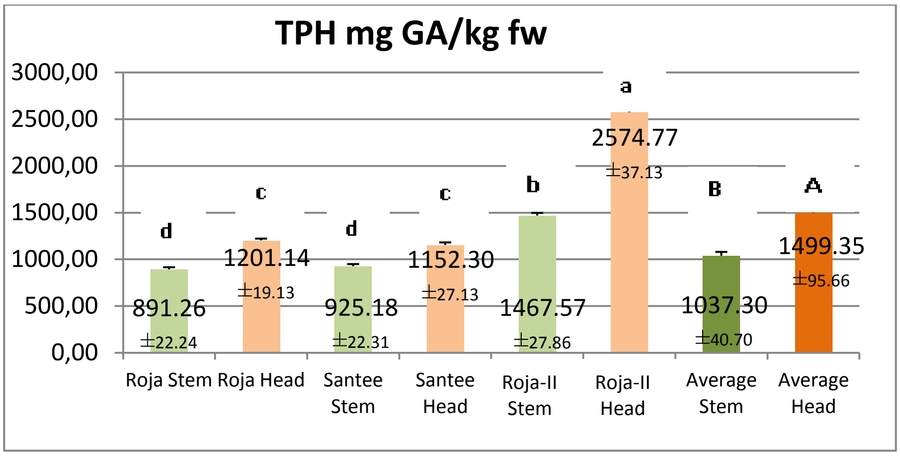


Figure 7- Average values of Total Phenol Content (TPH) with standard deviation of Broccoli analyzed: Roja, Santee and Roja-II harvest divided into head and stem, and their average (represented with uppercase letters). Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3; N=9.

5.3.2.2.3. Total anthocyanin content (ACY)

The highest value of anthocyanins content was detected in broccoli heads, as suggested by the slightly violaceous coloration of this part of broccoli (Figure 8). The highest concentration of anthocyanins has been registered for Roja-II broccoli head, probably due to an increasing of purple color intensity during plant development. However, also the less colored part of the plant (Stem) showed a statistically higher ACY content in Roja-II than in Roja (first harvest), indicating an increase of this compounds during development also in absence of purple coloration.

Stem showed an average amount of anthocyanins of 78% less than head content. Sotelo et al. (2014) found similar values in broccoli (2.2-6.3 mg CYA/kg fw); while Rodriguez-Hernandez et al. (2012), made a comparison between inflorescences and leaves, finding higher values than the present study.

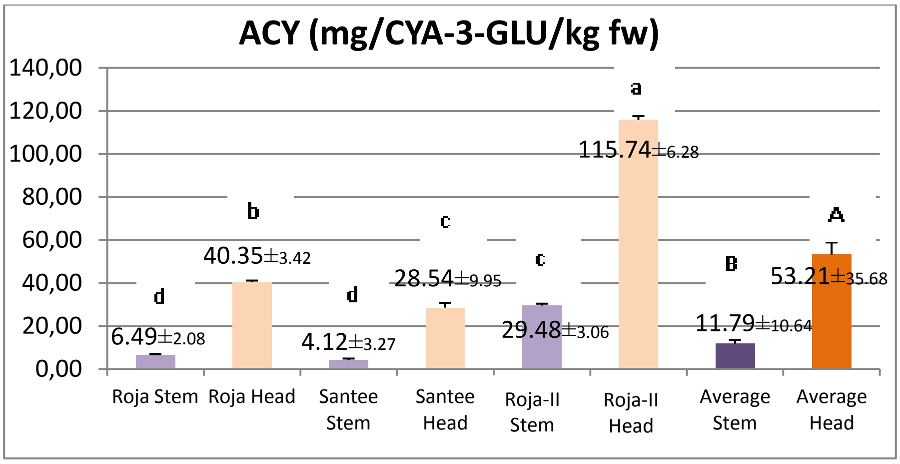


Figure 8- Average of Total Anthocyanin Content (ACY) with standard deviation of Broccoli analyzed: Roja, Santee and Roja-II harvest divided into head and stem, and their average (represented with uppercase letters). Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3; N=9.

5.4. Conclusions

The aim of the study was to analyse the nutritional properties of fresh *Brassica* plants to identify new high quality and phytochemical compounds-rich product to the consumers; indeed, the study on the variation of quality in different plant portion and development stage resulted interesting for the creation of some possible new products for the consumers.

Considering the plant portion, it is evident that in black cabbage, seeds possess a higher phytochemical compounds content than leaves. These results are not surprising if we consider that the function and role of seeds is to protect from oxidation their lipids, which are very important during germination when demand of oxygen is high (Sousa et al., 2007; Ferreres et al., 2009). For what concern broccoli, heads have a higher content of phytochemical than stems, but FRAP method revealed that the total antioxidant capacity followed the opposite trend.

Considering the different values between Roja and Santee, harvested at the same time, there are not statistically significant differences of phytochemical compounds content.

The big differences between Roja-II and Roja (first harvest) samples are probably due to development stage and harvesting time, because Roja-II were harvested approximately 20 days later; so the development stage and harvest time brought some phytochemical compounds content differences and suggest to keep on investigation and research about the behaviour of their concentration during time.

6. RESEARCH LINE III: COMPARISON STUDY ABOUT PROCESSING METHODS (POSTHARVEST TREATMENTS) AND THEIR EFFECTS OF THE NUTRITIONAL QUALITY OF DIFFERENT BRASSICA VEGETABLES

6.1. Introduction

Brassicaceae vegetables, also called crucifers, include different genera of cabbages, broccoli, cauliflowers, Brussel sprouts, kale, etc., which are consumed all over the world. These vegetables possess both antioxidant and anticarcinogenic properties for secondary substances content, like phenolics, glucosinolate, ascorbic acid. Several epidemiological studies indicate that a high intake of brassicaceous vegetables is associated with a reduced risk of several types of cancer, of age-related chronic illness such as cardiovascular diseases, diabetes and other degenerative disease (Podsedek, 2007; Mageney et al., 2017). Besides the Brassica species that is analyzed, the amount of the antioxidant compounds also depends by the plant organ harvested; flower buds were found to be the most active part, followed by leaves and stems and turnip roots, showing a significantly lower antioxidant capacity (Soengas et al., 2011). For example, young broccoli sprouts contain about 20-fold higher glucosinolate amount than the late vegetative stage (Fahey et al., 1997); the increase in phenolic compounds and ascorbic acid coincides with the inflorescence development (Vallejo et al., 2003 b). Brassica vegetables are characterized by the harvesting of the portion with the inflorescence and nearest leaves, or only leaves in acephalous plants, that are cut and prepared for obtain the marketable product. Therefore, the main problem of their production is to maintain unaltered the nutritional quality of fresh vegetables, very perishable after harvest for their high moisture content. Their short life can be increased by storing those vegetables at refrigerating temperatures. However, this strategy is able to assure quality standards only for a few days (Oliveira et al., 2015). The solutions for improving the shelf life period and the marketability of perishable foods are air-drying and freeze-drying processes.

This chapter refers to the following article already published:

Biondi F., Balducci F., Mazzoni L., Di Vittori L., Capocasa F., Mei E., Vagnoni M., Visciglio M., Mezzetti B. (2018). Comparison study about processing methods (postharvest treatments) and their effects of the nutritional quality of different Brassica vegetables. Acta Horticolturae 1202 ISHS 2018. DOI 10.17660/ActaHortic.2018.1202.19

Drying is an ancient process used to preserve food, it is favored due to low processing cost and speed of execution (Ratti, 2001). The basic objective in drying food products is the removal of water from the solids to a level at which microbial spoilage and deterioration resulting from chemical reactions is greatly minimized; this enables the product to be stored for longer periods. However, this process can lead to the degradation of naturally heat-sensitive substances, such as vitamins, antioxidants, minerals, pigments, and other bioactive compounds, due to high temperatures/times exposure (Krokida et al., 2003).

Freeze-drying, also known as lyophilization, is a better method of moisture removal, with final products of higher quality compared with air-drying. This method is based on dehydration of the frozen product by sublimation; the absence of liquid water, the absence of air oxygen during processing, and the ultra-freezing required for the process (Antal et al., 2017) stopped most microbiological reactions, giving a final product of excellent quality (color, shape, aroma and nutritional value) greater than any other drying method, with negligible losses of bioactive compounds (Ratti, 2001).

The aim of this work is to study the nutritional quality of several Brassica vegetables and evaluate the effects of air-drying and freeze-drying postharvest treatments on product quality.

6.2. Materials and methods

6.2.1. Plant material

In this study were evaluated different species of Brassica vegetables, cultivated during two production cycles (autumn 2016 and autumn 2017) in the peculiar open field condition of Fucino upland (680 masl) area, located in Abruzzo region (Italy), in Mid–Adriatic climatic conditions. In this area, Brassica crops are cultivated only in autumn and spring seasons because in winter the average temperature is very low for their cultivation. Cultural operations, fertilization, and weed control were made according to local practices. The Brassica vegetables analyzed were Black cabbage, Red curly kale, Green curly kale (*Brassica oleracea* L. spp. *acephala*); “Getti e foglie” and “Spigariello” broccoli (*Brassica oleracea* L. spp. *botrytis* var. *cymosa*) in the first year. During the second year was made a repetition of the previous species reported above, with the addition of Turnip top (*Brassica rapa* L. var. *sylvestris*) and Broccoli (*Brassica oleracea* L. spp. *italica*). These vegetables were analyzed fresh, air-dried and freeze-dried.

The fresh samples were provided by Valli di Marca Company that selected and cut the vegetables preparing 200 g of first evolved class products for each kind of vegetables; the samples were stored at -20°C after packaging in heat-sealed bags and then analyzed to detect their nutritional composition.

Three fresh samples for each type of vegetables analyzed (200 g) were prepared for the air-drying process performed by the company Farris srl (FG-Italy). The samples were washed with tap water and diced. Then, samples were cooked in boiling water at 100°C for few minutes, according to the vegetable species. Finally, samples were dried by oven at 100°C for some minutes and then the temperature was lowered until 58-60°C for 1.5 h. The process finished when the water activity reached 0.35-0.40, and the humidity of the product was 6-7%. Then samples were analyzed to determine the nutritional composition.

Three samples of 200 g fresh product, of each species analyzed, were frozen and stored at -20°C and lyophilized by Labconco Freezone 12L mod. 78670 (Kansas City, Missouri, USA) at -82°C of temperature and 0.1 hPa of pressure, for 5 days. Samples of dried brassica vegetables were milled into powder through the utilization of a mortar and were also analyzed to determine the nutritional composition.

Four replies were carried out for each sample analyzed.

6.2.2. Chemicals

Methanol (99%, ACS-ISO) was purchased from Carlo Erba Reagents (Milan, Italy). Folin-Ciocalteu reagent, sodium carbonate (anhydrous), potassium chloride, sodium acetate, chloridric acid, glacial acetic acid, ferric chloride hexahydrate 2,4,6-tris(2-pyridyl)-striazine (TPTZ, 99%), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ferrous sulphate heptahydrate, 3,4,5-trihydroxybenzoic acid (gallic acid), ascorbic acid, were purchased from Sigma-Aldrich (Sigma-Aldrich s.r.l., Milan, Italy).

6.2.3. Vegetables nutritional quality

6.2.3.1. VEGETABLES EXTRACTION

6.2.3.1.1. Total antioxidant capacity, Total phenol content, Total anthocyanin content

The fresh selected samples stored at -20°C were powdered by liquid nitrogen (1L for 100g); the extraction of phytochemical compounds was performed on 10 g of powder placed into test-tubes with 100 mL of extracting solution (20:80 water and methanol and 1% of acetic acid). Samples were then homogenized using an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Germany). The homogenized suspensions were placed in a fridge at 4°C in the dark. After 48 hours, the suspensions were centrifuged at 2500 rpm for 15 min (Rotofix32 centrifuge, Hettich Zentrifugen, Tuttlingen, Germany) and the recovered supernatants were collected and stored in six amber vials (for each sample), of 4 mL each. The supernatants were collected and stored at -20°C until the moment of analyses (Diamanti et al., 2012). The extraction of the air-dried and freeze-dried vegetables was performed on 2.50 g of samples instead of 10 g; the protocol applied was the same of the fresh material.

6.2.3.1.2. Ascorbic acid content

Ascorbic acid (or vitamin C content) was extracted and measured as described by Helsper et al. (2003) and Tulipani et al. (2008), with slight modifications. Briefly, ascorbic acid of fresh samples was extracted by sonication of 1 g of product in 8 mL of extracting solution, composed of ice-cold water with 5% of metaphosphoric acid and 1 mM DTPA. This step was followed by centrifugation at 4000 rpm for 15 min at 4°C, then samples were filtered with 0.45 μm nylon (NY) filters and immediately analyzed on an HPLC system.

6.2.3.2. VEGETABLES ANALYSES

6.2.3.2.1. Total Antioxidant Capacity (TAC)

Total Antioxidant Capacity (TAC) was measured by FRAP (Ferric Reducing Antioxidant Power) assay. The reduction of ferric tripyridyltriazine (Fe^{+3} –TPTZ) was measured by the method of Benzie and Strain (1996) modified by Deighton et al. (2000), in which the FRAP reagent was freshly prepared by mixing 10:1:1 (v/v/v) of sodium acetate (300 mM acidified with acetic acid until pH 3.6), ferric chloride (20mM) and TPTZ (10mM in 40mM HCl). Briefly, the sample extract was diluted 1:5 and vortexed. This solution was further diluted 1:10 adding the FRAP solution previously prepared, vortexed and put in darkness for 4 min; the absorbance at 593 nm was measured by spectrophotometer. The results were expressed as μM Trolox Equivalent per g of fresh weight.

6.2.3.2.2. Total Phenol Content (TPH)

The vegetable Total Phenol Content (TPH) was evaluated using the Folin-Ciocalteu reagent method (Slinkard and Singleton, 1977), that represent a good preliminary methodology for the evaluation of total polyphenols in *Brassica* vegetables (Radošević et al., 2017), with gallic acid as the standard for the calibration curve. Briefly, each sample extract was diluted 1:5 in a glass test-tubes mixed with Folin-Ciocalteu reagent. After 3 min, the solution was added with sodium carbonate (20 %), vortexed and stored in the dark. The absorbance of the samples was measured at 760 nm after 60 min. The data were calculated and expressed as mg gallic acid per kg fresh sample, using a standard curve built with the analysis of absorbance of increasing concentrations of gallic acid.

6.2.3.2.3. Total Anthocyanin Content (ACY)

The vegetable Total Anthocyanin Content (ACY) was measured using the pH differential shift method (Giusti and Wrolstad, 2001). This assay is based on the characteristic change in intensity of the hue of the anthocyanins according to the pH shift. Briefly, the samples extracts were diluted 1:1 (except kale family diluted 1:3) with potassium chloride (pH 1) and, separately, with sodium acetate (pH 4.5) and the corresponding maximum absorbance for both solutions was measured (respectively at $\lambda = 520$ nm, approximately and $\lambda = 700$ nm). The data were expressed as mg cyanidin 3-glucoside (the most representative anthocyanin in broccoli) per kg fresh weight (mg Cya3Glu/kg FW).

6.2.3.2.4. Ascorbic acid content

The HPLC system (Jasco Inc, Easton, USA) was equipped with a C_{18} column, 15 cm x 0.46, 5 µm and an Autosampler (Jasco Inc, Easton, USA). The mobile phase (aqueous solution of 50 mM phosphate buffered solution, pH 3.2) was slid into isocratic stream at a rate of 0.8 ml/min for 15 minutes. The HPLC system was coupled to a UV/VIS detector (Jasco Inc, Easton, USA) fixed at 244 nm wavelength, that is comprised in the range of best absorption of ascorbic acid, and to which ascorbic acid can be clearly identified as in our previous studies (Zhong et al., 2017). Ascorbic acid quantification was made through a standard calibration curve, prepared by running increasing standard concentrations of ascorbic acid and measured in duplicate at the beginning and end of the analysis. Results were expressed as milligrams of ascorbic acid per 100 grams of fresh weight (with Standard Deviation SD for three replications of each sample).

In the first year it was analyzed the ascorbic acid only on the fresh material while in the second year it was detected in all the samples prepared.

6.2.4. Statistical analysis

The vegetable nutritional qualities were analyzed in triplicate for each sample. The data for FRAP, TPH and ACY and ascorbic acid were all analyzed using one-way analysis of variance (ANOVA), with each genotype or each treatment as an independent variable. The two-ways analyses were performed keeping the genotypes and the treatments as independent variables. Significant differences within groups were calculated according to Student's Newman-Keuls tests, and differences at $p < 0.05$ were considered as significant.

6.3. Results and discussion of first year of trial

TPH provides a measure of all the phenol derivatives and phenolic compounds content in Brassica vegetables; they represent, with ascorbic acid, the 80% of the total antioxidant capacity of plants (Podsedek, 2007).

Table 17 - Phenolics, anthocyanins contents and antioxidant capacity in the different type of vegetables analyzed during first year of trial.

Vegetables	TPH[1] (mg GA/kg fw)	TAC[2] (µM Trolox/g fw)	ACY[3] (mg CYA-3-GLU/Kg fw)
Black Cabbage	1061.01 ± 351.15 $_b$	4.29 ± 0.72 $_b$	8.30 ± 7.57 $_a$
Getti e Foglie	771.10 ± 259.00 $_b$	3.08 ± 0.62 $_b$	2.59 ± 1.77 $_{ab}$
Red Curly Kale	1829.05 ± 1003.24 $_a$	8.40 ± 4.62 $_a$	5.04 ± 13.46 $_{ab}$
Green Curly Kale	1582.28 ± 875.18 $_{ab}$	6.24 ± 3.18 $_{ab}$	0.00 $_b$
Spigariello	935.31 ± 464.14 $_b$	3.39 ± 1.34 $_b$	2.76 ± 1.85 $_{ab}$

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content ± standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n= 9

Lako et al. (2007) confirmed that phenol compounds are the main phytochemical constituents responsible of antioxidant activity in plants. The highest concentration of phenols is found in red curly kale, while lower values are found in black cabbage, "getti e foglie" and "spigariello" (table 17). The fresh samples (F) have the main concentration of phenolic compounds, followed by lyophilized samples (L) and air-dryed samples (D) (table 18). The lyophilization treatment presents the 70% of phytochemical compounds retention, while air-drying process only 29%. Table 19 shows the effects of each treatments on the TPH content of different vegetables analyzed; the main losses of compounds is detected in air-drying process on "getti e foglie" (D), green curly kale (D) and "spigariello" (D). The main content of phenol compounds is detected in fresh red curly kale (F). Our results are supported by a previous study from Zhang and Hamauzu (2004), who reported the degradation and oxidation of polyphenols during thermal treatment as drying process.

Anthocyanins belong to the flavonoids group of phenolic compounds, and their presence in Brassica vegetables was previously demonstrated (Moreno et al., 2010). They are responsible of the pigmentation of red cabbage, purple cauliflower and purple broccoli. The main anthocyanins represented in Brassica vegetables are cyanidin derivatives. In this study, it was analyzed the total anthocyanin content in different Brassica crops: black cabbage shows the higher content of cyanidin-3-glucoside. It is followed by "getti e foglie", red curly kale and "spigariello" with values statistically similar each other; no anthocyanins are found in green curly kale, as reported in table 17. Clearly, green curly kale hasn't these compounds, because the leaves of this type of vegetable do not present any purple coloration. There are no significant differences between the anthocyanin content of material deriving from different processing methods (table 18). Data on fresh material are statistically like those deriving from the other treatments data, probably because the treatment of fresh vegetables did not strongly affect the average anthocyanins content. The main content of anthocyanins is registered for air-dried (D) black cabbage, (Table 19). The lowest anthocyanin content is detected in fresh (F) black cabbage, and fresh (F) and freeze-dried (L) green curly kale. According to those results, the effect of air-drying and lyophilisation on anthocyanin content is not completely understood; more studies and more accurate protocols of analysis are necessary for a better comprehension of this issue.

Table 18- Effects of different processing treatments on nutritional parameters in the first year.

Treatment	TPH[1] (mg GA/kg fw)	TAC[2] (µM Trolox/g fw)	ACY[3] (mg CYA-3-GLU/Kg fw)
F[4]	1858.61 ± 19.00 $_{a}$	7.21 ± 0.74 $_{a}$	3.53 ± 0.20 $_{n.s.}$
L[5]	1306.80 ± 36.01 $_{b}$	5.39 ± 0.11 $_{a}$	0.91 ± 0.51 $_{n.s.}$
D[6]	541.63 ± 31.07 $_{c}$	2.63 ± 0.12 $_{b}$	4.43 ± 1.36 $_{n.s.}$

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content; ± standard deviation. [4]F=fresh sample; [5]L=freeze drying. lyophilization; [6]D=air drying processing.
Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n.s: not significant. n= 15

A lot of studies showed high antioxidant capacity in Brussels sprouts, cabbage, kale, cauliflower, and broccoli; e.g. Cao and co-workers (1996) determine that kale is the richer vegetable source in terms of antioxidant compounds. In this study the results confirm the high content of antioxidants in the different vegetables analyzed, as reported in table 17. The red curly kale showed the highest total antioxidant capacity, while the lower were detected in black cabbage, "getti e foglie" and "spigariello". The freeze-dried material showed a very good recover of the initial TAC of analysed vegetables (table 18), with values statistically similar to fresh samples. Air-drying process showed the higher loss of antioxidant capacity with a reduction of 64% in respect to the fresh samples. Kaur and Kapoor (2001) explain this deterioration of natural antioxidant in food because of thermal processing. In fact, the antioxidants are heat sensitive molecules. These losses could be reduced using pre-treatments, like blanching the samples before the thermal process. Korus (2011) described a decrease of 60% of polyphenol content in air-dried kale but confirmed that blanching kale leaves for 2.5 min in water at 96–98°C before the convective drying led to the lowest loss of antioxidant compounds. In contrast with these results, Mrkìc et al. (2006), found that the antioxidant capacity is positively correlated with the drying temperature in broccoli air-dried. A good antioxidant activity is preserved using higher processing temperatures and shorter drying times. Some foods could maintain or enhance antioxidant properties by the development of new form of antioxidants (Nicoli et al., 1997). However, this enrichment is not observed in the present work, demonstrating that these applied processes do not lead to the formation of new antioxidant compounds. In this trial, the freeze-dried material reported a not significantly reduction of 25% in respect of the fresh sample; this limited reduction is explained by the low temperatures used during this processing method. The highest antioxidant capacity is detected in fresh red curly kale sample, while the lower retentions are found in "getti e foglie" for all treatments (F-L-D), air-dried red curly kale and green curly kale (D), freeze-dried and air-dried "spigariello" (L-D) (table 19).

Table 19 - Effect of genotype x treatment interaction on nutritional parameters in the first year of trial

Vegetables	Treatment	TPH[1] (mg GA/kg fw)	TAC[2] (µM Trolox/g fw)	ACY[3] (mg CYA/Kg fw)
Black Cabbage	F[4]	1442.01 ± 210.90 $_{d}$	4.92 ± 0.34 $_{d}$	0.00 $_{c}$
Black Cabbage	L[5]	1071.20 ± 15.02 $_{ef}$	4.56 ± 0.05 $_{de}$	2.48 ± 0.78 $_{abc}$
Black Cabbage	D[6]	669.15 ± 30.10 $_{gh}$	3.39 ± 0.1 $_{ef}$	18.25 ± 1.62 $_{a}$
Getti e Foglie	F[4]	1058.25 ± 81.23 $_{f}$	3.46 ± 0.31 $_{f}$	3.54 ± 1.07 $_{abc}$
Getti e Foglie	L[5]	784.13 ± 50.11 $_{g}$	3.48 ± 0.15 $_{f}$	3.33 ± 2.22 $_{abc}$
Getti e Foglie	D[6]	471.21 ± 22.61 $_{h}$	2.28 ± 0.11 $_{f}$	0.91 ± 0.33 $_{bc}$
Red Curly Kale	F[4]	2950.00 ± 20.01 $_{a}$	13.68 ± 1.52 $_{a}$	14.43 ± 22.95 $_{ab}$
Red Curly Kale	L[5]	1890.01 ± 28.90 $_{c}$	8.37 ± 0.15 $_{bc}$	0.54 ± 0.02 $_{bc}$
Red Curly Kale	D[6]	647.12 ± 11.20 $_{gh}$	3.15 ± 0.16 $_{f}$	0.15 ± 0.01 $_{bc}$
Green Curly Kale	F[4]	2339.31 ± 320.70 $_{b}$	8.86 ± 1.31 $_{b}$	0.00 $_{c}$
Green Curly Kale	L[5]	1949.21 ± 44.70 $_{c}$	7.73 ± 0.13 $_{c}$	0.00 $_{c}$
Green Curly Kale	D[6]	458.00 ± 54.00 $_{h}$	2.15 ± 0.21 $_{f}$	2.23 ± 1.29 $_{abc}$
Spigariello	F[4]	1504.01 ± 160.30 $_{d}$	5.13 ± 0.22 $_{d}$	3.92 ± 0.76 $_{bc}$
Spigariello	L[5]	840.02 ± 41.30 $_{eg}$	2.85 ± 0.09 $_{f}$	3.71 ± 1.65 $_{bc}$
Spigariello	D[6]	463.00 ± 37.80 $_{h}$	2.19 ± 0.03 $_{f}$	0.64 ± 0.56 $_{bc}$

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content ± standard deviation. [4]F=fresh sample; [5]L=freeze drying. lyophilization; [6]D=air drying processing. Values with the same letter were not significantly different (Test SNK p≥0.05). n= 3

The content of ascorbic acid varies significantly among Brassica vegetables and within their subspecies. Podsedek (2007) reported a comparison between several Brassica crops, e.g. in broccoli and cauliflower, ascorbic acid levels varied over a 4-fold levels of ascorbic acid, while Brussel sprouts and white cabbage varied 2.5-fold, and twice in kale; the lowest amount of ascorbic acid was detected in white cauliflower.

Table 20 - Content of Ascorbic acid (mg/100 g fw) in fresh samples in the first year of trial

Vegetables	Ascorbic acid (mg/100 g)
Black Cabbage	8.30 ± 0.27 b
Getti e Foglie	6.41 ± 0.41 d
Red Curly Kale	6.22 ± 0.37 d
Green Curly Kale	15.61 ± 0.19 a
Spigariello	7.63 ± 0.73 c

Average of ascorbic acid ± standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3

The cause of ascorbic acid content variation is related to differences in genotype (Vallejo et al., 2002). Our results (table 20) indicate that Green curly kale is the Brassica vegetable with the highest content of ascorbic acid, followed by black cabbage, “spigariello”, “getti e foglie” and red curly kale. Similar results were found by Singh et al., (2007), that reported values of ascorbic acid ranging from 5.61 mg/100 g, found in a cabbage cultivar, to 82.32 mg/100 g found in a broccoli cultivar.

6.4. Results and discussion of second year of trial

The second-year results reported the same trend obtained during the first year of trial; indeed, for what concern the TPH, the main content was detected in kales group, in particular in green and red curly kale. The vegetable with the lowest amount of TPH was “getti e foglie”, as reported in table 21. The comparison between treatments, shown in table 22, brought to a better retention of phytochemicals in fresh material (F) followed by lyophilized (L) and last air-dried (D) materials, confirming the first-year results. Green curly kale (F) retained the higher phenolic contents than others. The main losses of TPH happened in dried vegetables (table 23). In general, the values of second year of study are lower than ones measured during the first year of trial.

Table 21 - Phenolics. anthocyanins contents and antioxidant capacity in the different type of vegetables analyzed for second year of trial.

Vegetables	TPH[1] (mg GA/kg fw)				TAC[2] (µM Trolox/g fw)				ACY[3] (mg CYA/Kg fw)			
Turnip Top	828.04	±	40.16	b	4.77	±	0.12	bc	1.83	±	0.22	b
Broccoli	937.28	±	55.15	b	4.83	±	0.09	bc	3.86	±	0.27	b
Black Cabbage	929.19	±	63.21	b	6.52	±	0.28	b	9.20	±	0.97	b
Getti e Foglie	559.93	±	24.09	c	4.55	±	0.10	c	3.22	±	0.29	b
Red Curky Kale	1404.76	±	94.99	a	10.91	±	0.65	a	287.59	±	24.37	a
Green Curky Kale	1486.42	±	129.96	a	10.72	±	0.93	a	6.28	±	1.43	b
Spigariello	892.66	±	78.36	b	6.17	±	0.43	bc	3.77	±	0.30	b

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content ± standard deviation. Values with the same letter were not significantly different (Test SNK p≥0.05). n= 9

The measurement of ACY content showed that red curly kale strongly and markedly stands out (table 21), revealing the higher ACY value in all three treatments, in descending order: red curly kale (F), followed by (L) and finally (D) (table 23). The other genotype combinations did not report any significantly differences (table 21), neither for all the treatments applied (table 23). ACY are best retained in fresh material (F) than other treatments, which present the same effects on ACY content (table 22). From the comparison with the first-year, it emerges that, although the red curly kale possessed a good level of ACY, the best result was obtained in black cabbage genotype. The only explanation is that the genotype-environment interaction, e.g. climatic conditions, rainfall, temperatures during growth, availability of nutrients, deeply affected the phytochemicals contents in different genotypes, as reported by several authors (De Pascale et al., 2007; Francisco et al., 2009; Soengas et al., 2011).

Table 22 - Effects of different processing treatments on quality parameters for second year.

Treatments	TPH[1] (mg GA/kg fw)				TAC[2] (µM Trolox/g fw)				ACY[3] (mg CYA/Kg fw)			
F[4]	1407.12	±	47.58	a	9.05	±	0.40	a	70.88	±	12.77	a
L[5]	887.13	±	32.40	b	6.13	±	0.22	b	32.67	±	8.06	b
D[6]	387.63	±	8.41	c	4.07	±	0.08	c	15.58	±	2.31	b

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content ± standard deviation. [4]F=fresh sample; [5]L=freeze drying. lyophilization; [6]D=air drying processing. Values with the same letter were not significantly different (Test SNK p≥0.05). n= 21

For what concern the TAC in the second year of trial, are confirmed the results obtained in the first one, with the higher content detected in red and green curly kale vegetables, while the lower content was measured in “getti e foglie”, as reported in table 21. F was confirmed as the best way to appreciate the higher retention of antioxidants, followed by L and finally D, as shown in table 22. The higher TAC was detected in green and red curly kale (F); the lower values were registered in “spigariello” and green curly kale (D) (table 23). In the first year, the main antioxidant retention was also reported in red curly kale (F). In general, TAC values of second year repetition were slightly higher than the first-year ones.

Table 23 - Effect of vegetable genotype x treatment interaction on nutritional quality in the second year of trial

Vegetables	T.	TPH[1] (mg GA/kg fw)				TAC[2] (µM Trolox/g fw)				ACY[3] (mg CYA/Kg fw)			
Turnip Top	F[4]	1024.11	±	28.36	d	4.98	±	0.19	cd	2.10	±	0.18	d
Turnip Top	L[5]	860.21	±	26.16	de	5.24	±	0.03	cd	0.54	±	0.02	d
Turnip Top	D[6]	403.73	±	8.16	g	3.87	±	0.03	cd	2.58	±	0.70	d
Broccoli	F[4]	1284.21	±	18.19	c	4.22	±	0.04	cd	4.00	±	0.41	d
Broccoli	L[5]	1022.97	±	5.64	d	4.83	±	0.01	cd	5.36	±	0.17	d
Broccoli	D[6]	504.66	±	6.98	fg	5.44	±	0.06	cd	2.23	±	0.18	d
Black Cabbage	F[4]	1279.55	±	69.86	c	7.88	±	0.39	b	7.64	±	0.88	d
Black Cabbage	L[5]	711.45	±	2.52	ef	5.75	±	0.01	cd	2.76	±	0.28	d
Black Cabbage	D[6]	446.20	±	5.27	g	4.59	±	0.03	cd	18.75	±	0.41	d
Getti e Foglie	F[4]	705.41	±	8.38	ef	5.04	±	0.10	cd	4.04	±	0.38	d
Getti e Foglie	L[5]	515.07	±	8.10	fg	4.64	±	0.04	cd	3.29	±	0.59	d
Getti e Foglie	D[6]	313.85	±	4.45	g	3.48	±	0.03	cd	1.51	±	0.23	d
Red Curky Kale	F[4]	1966.52	±	45.23	b	14.88	±	0.28	a	437.79	±	14.80	a
Red Curky Kale	L[5]	1254.90	±	4.91	c	9.53	±	0.06	b	212.44	±	2.18	b
Red Curky Kale	D[6]	431.09	±	3.94	g	4.35	±	0.04	cd	62.32	±	1.41	c
Green Curky Kale	F[4]	2173.92	±	131.52	a	15.31	±	1.16	a	2.04	±	0.66	d
Green Curky Kale	L[5]	1292.59	±	7.23	c	8.89	±	0.04	b	0.50	±	0.00	d
Green Curky Kale	D[6]	305.22	±	9.01	g	3.35	±	0.05	d	20.53	±	2.92	d
Spigariello	F[4]	1354.68	±	76.57	c	8.62	±	0.48	b	5.07	±	0.29	d
Spigariello	L[5]	552.68	±	7.31	fg	4.03	±	0.02	cd	3.79	±	0.44	d
Spigariello	D[6]	308.62	±	6.45	g	3.40	±	0.01	d	1.16	±	0.18	d

Average of [1]TPH: Total Phenol Content; [2]TAC: Total Antioxidant Capacity; [3]ACY: Total Anthocyanin Content ± standard deviation. [4]F=fresh sample; [5]L=freeze drying. lyophilization; [6]D=air drying processing. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n= 3

Vegetable with the higher ascorbic acid content in the second year of trial was broccoli, while the lower was "getti e foglie", as reported in table 24. Values detected during the first year were higher than these, while broccoli and turnip top samples were analyzed only in the second year of trial.

Table 24 - Content of Ascorbic acid (mg/100 g fw) in fresh samples in the second year of trial

Vegetables	Ascorbic acid (mg/100 g)			
Broccoli	63.72	±	4.57	a
Black Cabbage	13.75	±	2.23	b
Turnip Top	14.21	±	2.57	b
Getti e foglie	3.40	±	0.45	c
Red Curky Kale	9.66	±	1.25	bc
Green Curky Kale	8.12	±	1.39	bc
Spigariello	5.22	±	0.56	bc

Average of ascorbic acid ± standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=9

Table 25 - Effects of different processing treatments on ascorbic acid in the second year of trial

Treatments	Ascorbic acid (mg/100 g)			
F[1]	17.16	±	3.10	n.s.
L[2]	17.04	±	3.93	n.s.
D[3]	13.12	±	3.07	n.s.

Average of ascorbic acid ± standard deviation. [1]F=fresh sample; [2]L=freeze drying. lyophilization; [3]D=air drying processing. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n.s: not significant. n=21

The comparison between treatments did not show any significant difference for the vitamin C content (table 25). In table 26, Broccoli (F), followed by broccoli (L) and finally broccoli (D), were the samples with the higher retention of ascorbic acid; for the other samples, genotype-treatment interaction did not show any significant difference. The freeze-drying process guarantees a good retention of ascorbic acid, approximately of the 80%, while air-drying process maintain only the 60% of vitamin C, as reported also by Ratti (2001). Similar values for fresh broccoli were detected by Hrncirik et al. (2001).

Table 26 - Effect of vegetable genotype x treatment interaction on ascorbic acid in the second year of trial

Vegetables	Treatment	Ascorbic acid (mg/100 g)			
Broccoli	F[1]	85.55	±	0.28	a
Broccoli	L[2]	70.13	±	0.62	b
Broccoli	D[3]	49.60	±	0.16	c
Black Cabbage	F[1]	17.69	±	3.44	d
Black Cabbage	L[2]	14.50	±	5.25	d
Black Cabbage	D[3]	7.08	±	0.22	d
Turnip Top	F[1]	17.99	±	4.57	d
Turnip Top	L[2]	16.90	±	1.62	d
Turnip Top	D[3]	7.20	±	1.28	d
Getti e Foglie	F[1]	4.70	±	0.76	d
Getti e Foglie	L[2]	3.85	±	0.45	d
Getti e Foglie	D[3]	1.88	±	0.12	d
Red Curly Kale	F[1]	11.90	±	2.54	d
Red Curly Kale	L[2]	9.75	±	1.03	d
Red Curly Kale	D[3]	5.00	±	1.14	d
Green Curly Kale	F[1]	9.92	±	2.53	d
Green Curly Kale	L[2]	8.13	±	0.27	d
Green Curly Kale	D[3]	4.50	±	0.88	d
Spigariello	F[1]	5.87	±	0.82	d
Spigariello	L[2]	5.81	±	1.03	d
Spigariello	D[3]	3.35	±	0.24	d

Average of [1]F=fresh sample; [2]L=freeze drying. lyophilization; [3]D=air drying processing ±standard deviation. Values with the same letter were not significantly different (Test SNK $p \geq 0.05$). n=3

6.5. Conclusions

The main differences on the phytochemical contents in Brassica tissues regard the genotypes and treatments tested, with the red curly kale having the statistically highest TPH and TAC values, in the first year of trial. In the same season, black cabbage showed the highest ACY contents, while green curly kale the highest content of ascorbic acid. Regarding treatments, lyophilization showed statistically higher values than air-drying process for TPH and TAC.

In the second year of trial the antioxidant potential of red and green curly kale was confirmed, giving that they possessed the higher value in TAC and TPH, respectively. The red curly kale also showed the highest content in ACY. Broccoli and turnip top, added to the analysis in the second year of trial, showed an interesting and relatively high TPH and TAC values; moreover, broccoli possessed the highest ascorbic acid content.

The nature of antioxidant compounds is thermo sensitive; they have a low stability during heat treatments and their presence is an indicator of food processing quality. The freeze-drying process is considered the best method to maintain unaltered the quality of nutritional compounds; the retention of antioxidant capacity and phenolics compounds is approximately between 70-80%, even if for some nutritional thermo sensitive compounds, the low temperature process can promote their deterioration. The air-drying process lead to the depletion of most of the phytochemical compounds, with 70% and 60% of losses of phenolic compounds and antioxidant capacity, respectively. For ascorbic acid the depletion can vary between 40 and 60%. Those reductions depend by the temperature applied and the length of drying process. In this trial, it was also found an increase of total anthocyanins content in the air-dried material for some vegetables analyzed.

Post-harvest treatments are necessary for maintaining the quality of vegetables during shelf life. Their use can lead to a reduction of waste and a good management of the surplus vegetables, allowing easy transportation and storage of the final products obtained. Processing treatment could decrease the nutritional quality of fresh vegetables, with air-drying being more negative than the freeze-drying process. However, the main advantage of processing treatments, besides the ease of vegetables storage, is that the amount of dried vegetables consumed in a single serving corresponded to a 10-fold amount of fresh vegetables, allowing a higher intake of phytochemicals to the consumer also with small portion assumed. The higher intake of phytochemicals will allow the consumers to better exploit the positive healthy effects of those compounds, although a better characterization of the analyzed vegetables is recommended for the future. These results provide concrete information on the content of bioactive compounds of the different vegetables and on the processing methods that guarantee their higher stability. These data can be used to differentiate in the label the characteristics of each product depending to the content of bioactive compounds that can have a positive effect on consumer health. The higher stability of bioactive compounds in dried vegetables, open the possibility to create new products for approaching mostly the young consumers to this kind of vegetables, to be used also in combination of meat, pasta and eggs, for an new easy to access, healthy diet.

7. RESEARCH LINE IV: SENSORIAL ANALYSIS OF *BRASSICACEAE* VEGETABLES

7.1. Introduction

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 2004).

The sensorial analysis possesses many applications, e.g., the investigation about sensorial quality of products sold by the companies, to test the level of appreciation of new type of products provided to consumers, to evaluate the effect of different treatments applied in post-harvest and the quality perception of consumers.

The main problems of this approach to measure sensorial quality are due to the subjectivity of tasters, that brought to high variable data generated by mood and motivation of people, innate physiological sensitivity to sensory stimulation, history and familiarity with similar products; therefore, the main problem is the reproducibility (Stone and Sidel, 1993; Lawless et al., 1998).

In the sensorial analysis, flavor is an overall impression of odor, taste and texture, and in Brassica vegetables it represents a mark of recognizability of the whole family. Typical taste and odor are determined by several compounds, e.g. the bitterness is provided by glucosinolates (GLS), more precisely by their hydrolysis products, the isothiocianates (ITCs) with sulphurous aminoacids. In particular, sinigrin, glucoiberin and glucoraphanin are also responsible of the aftertaste persistence; they confer sulphurous and pungent taste of brassica products too. Several studies confirm that the bitterness derives from a synergistic activity of various phytochemicals such as the hydrolysis of aliphatic and indolic GLS, the phenolic compounds and the flavonoids. Portion of the plant and degree of ripeness can influence the GLS content and consequently brassica flavor (Hansen et al., 1997; Baik et al., 2003; Jones and Sanders, 2002).

Typical odor of brassica vegetables is characterized by volatile compounds like pentanol, pentanal, hexanal, heptanal and nonanal; these aldehydes and alcohols account for 90-95% of the total volatiles content, formed by oxidative breakdowns of free fatty acids by the action of lipoxygenases. These products can be volatile or not and get free during chewing and cooking. Alcohols and aldehydes are responsible of the green or grassy aroma (Buttery et al., 1976; Chin and Lindsay 1994; Good, 1998).

The sweetness is conferred by glucose, fructose, and sucrose present in the vegetables; moreover, a correlation between the sugar and the GLS contents has been reported. If the GLS concentration is high, the sweetness sensation decreases in broccoli and cauliflower, and *vice versa*. Salty taste is correlated with quercetin, while acidity with GLS and total phenolics contents (Schonhof et al., 2004).

The aims of this work is to test the variation of the sensorial quality of Brassica vegetables subjected to different process, and investigate about the consumer's quality perception of Brassica products.

7.2. Materials and methods

7.2.1. Plant material

Vegetables material was provided by Valli di Marca agricultural company and was cultivated in Fucino upland (680 m. a. s. l.) following the first evolved class "Zeroscarti®" cultivation protocol and management. The plant material was constituted by the most produced and sold vegetables of the company, and mainly appreciated by consumers: Broccoli, Turnip top, Black Cabbage, Green Curly Kale, Red Curly Kale, "Getti e Foglie" and "Spigariello" broccoli. The fresh material was sampled in packages of 200g, washed, cleaned and frozen until the day of preparation and cooking for the sensorial analysis. A similar amount of fresh material was frozen and then destined to the freeze-drying process, obtaining the freeze-dried samples. The air-dried material was provided by the same company, but the air-drying process was applied by Farris srl (Foggia, Italy), a company specialized in this sector that conferred the final packaged dried product for the trial. All the samples (fresh, air-dried and freeze-dried) were cooked and rehydrated following accurate protocols (described below) before the sensorial analysis.

7.2.2. Brassicaceae descriptors definition

For evaluating the sensorial quality of *Brassicaceae* vegetables, some main sensorial descriptors were identified. Several authors (Jones and Sanders, 2002; Pasini et al., 2011; Helland et al., 2016) reported many information about sensorial descriptors for Brassicas, but in this trial only the main representative for Brassica vegetables, reported in table 27, were chosen.

Table 27 - Descriptors chosen for the evaluation of sensorial quality of brassica vegetables

Aroma:	Aroma intensity	**Taste:**	Bitter
	Pungent		Pungent
	Sulphurous		Sulphurous
	Green/grassy		Acid
	Off-flavor		Salty
	Abnormal aroma		Sweet
			After-taste
			Untypical taste
			Fibrosity

7.2.3. Panel group choice and training period

The panel group was composed of 16 young people between the ages of 21 and 38. For the panel representativeness and validity, the minimum number of participants (11 participants) was always guaranteed for all the duration of the test.

The training period was done following the guide lines and the information reported in ISO 3972 (2013); ISO 8586 (2014); ISO 8589 (2014). The recognition of main odor and taste were made administrating to panelists the references substances reported in table 28 during the training period. The reference substances concentrations were retrieved in scientific literature consulting Jones and Sanders (2002), adapting to this panel test.

Table 28- Reference substances used for training panelists

Description	**Reference**
Vinegar-like, pungent	Acetic acid
Pungent	Pepper
Green-grassy	Cis-3-hexenal
Sweet	Sucrose
Salty	Sodium chloride
Sour, acid	Citric acid
Bitter	Caffeine

7.2.4. Panel test evaluation

7.2.4.1. SAMPLES PREPARATION

Samples for the sensorial analysis were prepared cooking them in boiling water (fig. 9) and adding a defined salt concentration; for each tasted vegetable material, time and details of cooking procedure are reported in table 29. Each vegetable possessed a different code, as reported in table 30. The panel group didn't know the nature and treatments applied of each tested vegetable.

Figure 9 - The boiling cooked of samples.

Table 29 - Cooking protocols and codes adopted for the preparation of samples before panel test

Vegetables	Code	Cooking Water (ml)	Salt (g)	Samples weight (g)	Cooking time (min)
Air-dried material	1-7	500	3	20	6
Fresh material	8-14	1000	6	200	10
Freeze-dried material	15-21	500	3	20	5

Table 30 - Samples treatment and test codes

Vegetables Code	Air-dried	Fresh-frozen	Freeze-dried
Broccoli	**1**	**8**	**15**
Turnip Top	**2**	**9**	**16**
Black Cabbage	**3**	**10**	**17**
Green Curly Kale	**4**	**11**	**18**
Red Curly Kale	**5**	**12**	**19**
Getti e Foglie	**6**	**13**	**20**
Spigariello	**7**	**14**	**21**

Below is reported, in figure 10, 11, 12, the preparation scheme of vegetables for the sensory analysis, with the images of the starting materials and the corresponding samples ready for the panel test.

Figure 10- Preparation of air-dried samples; above) the starting air-dried packaged material; below) the corresponding air-dried samples cooked and ready for the administration with the specific code.

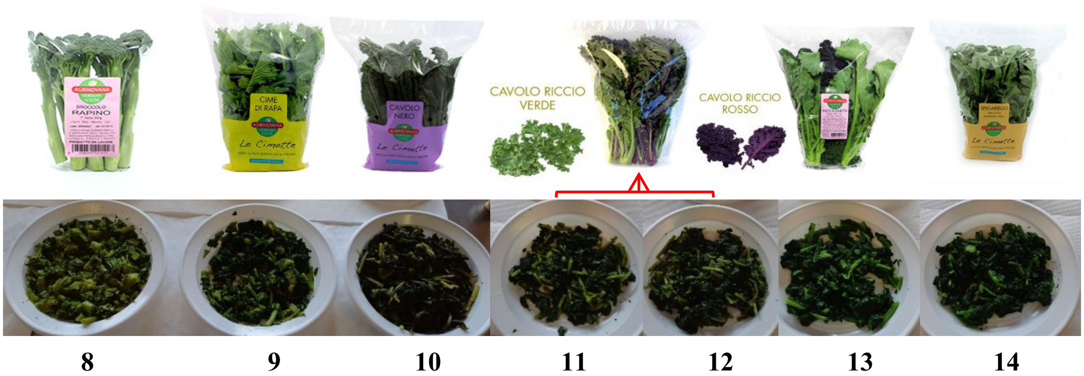


Figure 11- Preparation of fresh samples; above) the starting fresh packaged material; below) the corresponding fresh samples cooked and ready for the administration with the specific code.

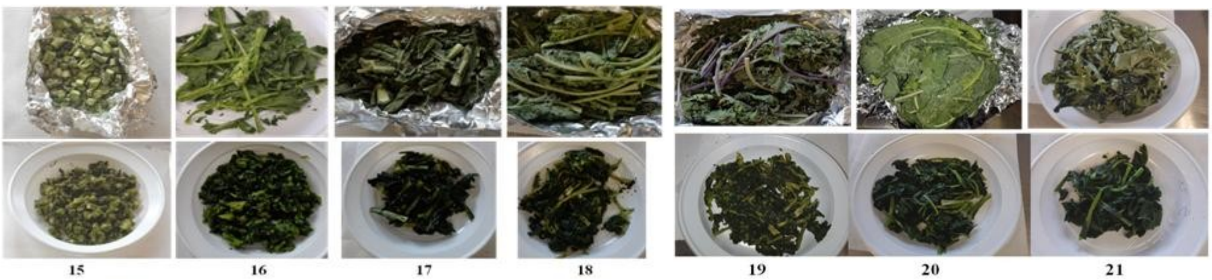


Figure 12- Preparation of freeze-dried samples; above) the starting freeze-dried material; below) the corresponding freeze-dried samples cooked and ready for the administration with the specific code.

7.2.4.2. ADMINISTRATION AND ASSESSMENT: SCALE OF VALUES DEFINITION

To assess the sensorial quality of Brassicas, an evaluation form to be used by panelists, was realized. For the analytical test, aroma and taste are identified with the following traits:

- AROMA: aroma intensity, pungent, sulphurous, green/grassy, off-flavor, abnormal aroma.
- TASTE: bitter, pungent, sulphurous, acid, salty, sweet, aftertaste, untypical taste, fibrosity.

For the hedonic test part, the tasters were asked to express the acceptance of the product; moreover, a space for collecting comments/opinion of panelists was added, following the panel rules find in ISO 8586 (2014).

The descriptors previously described are reported in a numerical (integer) scale of 9 points (Lawless and Malone, 1986). The scale form is divided in aroma descriptors, with a minimum value of 1 (none) and maximum value of 9 points (high), and taste descriptors starting with 1 (none) and finishing with 9 (high). The panel test was performed in a sensorial room (figure 13) specific for food and beverage tastings, according to ISO 8589 (2014).

Figure 13- Room specific for the panel test with a group of panelists during the evaluation

7.3. Results and discussion

For each sample administrated, each member of panelist group was asked to fill the evaluation form previously described. At the end of the trial, the results were collected and analyzed calculating the average values of aroma and taste descriptors and of acceptance for each sample. Data are represented with radar graphs and grouped for vegetables: broccoli (fig. 14), turnip top (fig.15), black cabbage (fig. 16), green curly kale (fig.17), red curly kale (fig.18), “getti e foglie” (fig.19) and “spigariello” (fig.20). In details, in each radar graph are reported the comparison of the same vegetable differently treated.

For what concern broccoli samples shown in figure 14, emerged that air-dried broccoli reached the best value of acceptance of the whole sensorial test, with an average of 7 points; fresh and freeze-dried broccoli reached the same value, lower than air-dried broccoli. The fresh material preserved better aroma and flavor than treated broccoli. Pungent and sulphurous taste and aroma were not so intense. In general, this vegetable was quite appreciated by panel group, because strong off flavor, untypical and abnormal aroma did not emerge. The sensorial profile can differ among the varieties of the same species, as indicated by Baik and co-authors (2003). They found that sensory and GLS profiles significantly differed among broccoli varieties. They also found a little evidence that the GLS were responsible for the distinctive flavor notes of cooked broccoli, but only in the fresh ones.

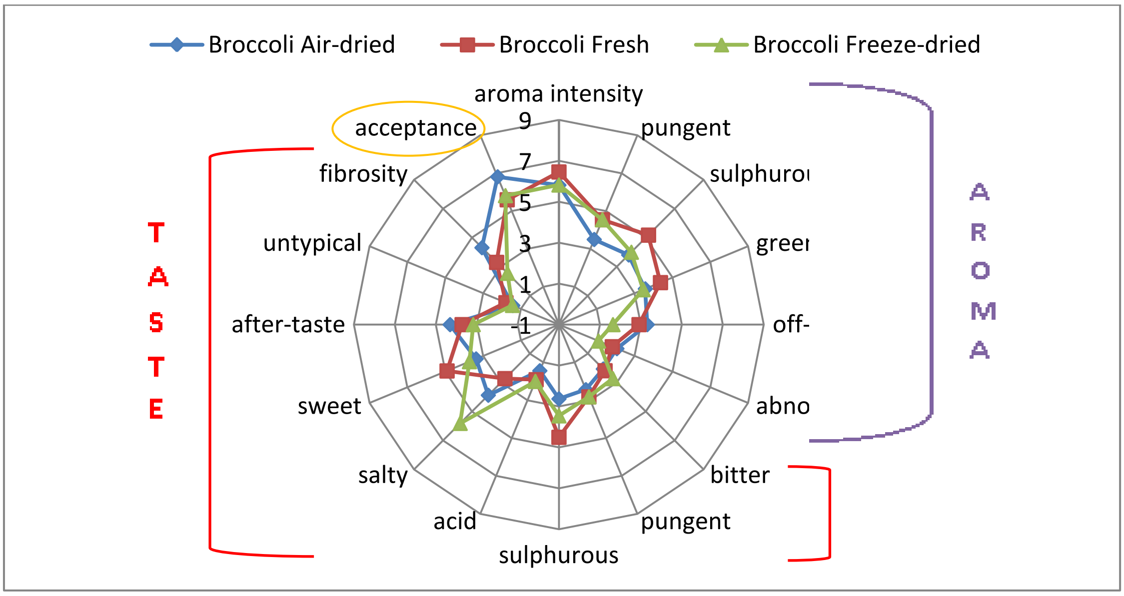


Figure 14- Radar chart about the average values of Broccoli panel test results.

In figure 15 is represented the average values of turnip top descriptors, with the best acceptance reached by the fresh material (6 points). In this case, the aroma seemed to be mainly expressed by the fresh material-derived samples. Sulphurous and bitter tastes were quite evident, in particular in freeze-dried-derived material. Turnip top possessed a persistent after-taste, probably due to the high sulphurous and bitter taste. The intensity of sulfurous and pungent aroma and of the bitter taste are important factors, because they can influence the consumers' preference about turnip top intake. In fact, if these attributes are to strong and intense, the appreciation of consumers decreases, mainly for young people, as studied by Jones and Sanders (2002). They developed a preparation method that masked the bitterness in turnip leaves and found that bitterness and aftertaste changed with variety and maturity stage of plants. Several studies researched and reported the sensorial quality of turnip top and leaves, defining their attributes and the molecules that characterized them (Padilla et al., 2007; Francisco et al., 2009; Arias-Carmona et al., 2012).

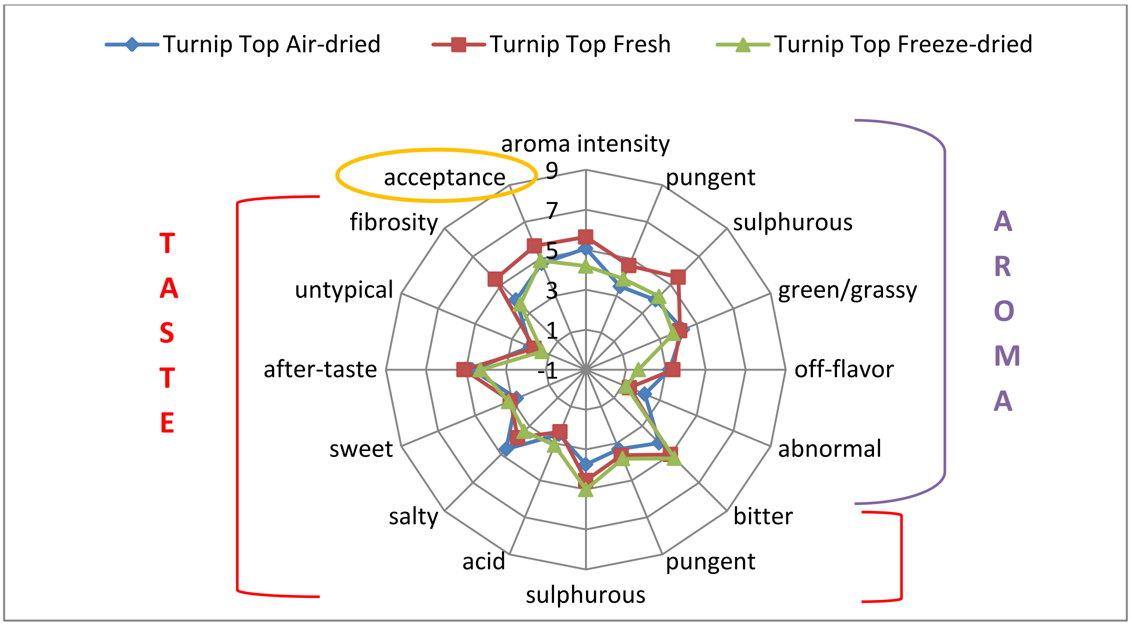


Figure 15- Radar chart about the average values of turnip top panel test results.

For *Brassica oleracea acephala* group, the most evident characteristic is the high fibrosity, probably due to fact that they are composed only by fleshy leaves, more fibrous than the "heads" typical of other species.

Black cabbage showed its higher acceptance when is presented as air-dried derived material (figure 16). Strong aroma intensity was detected in the fresh samples; the freeze-dried ones accentuated the sweet and sulphurous taste. Off-flavor, abnormal aroma and untypical taste were not detected. Despite the cooking, the fresh material remained very fibrous.

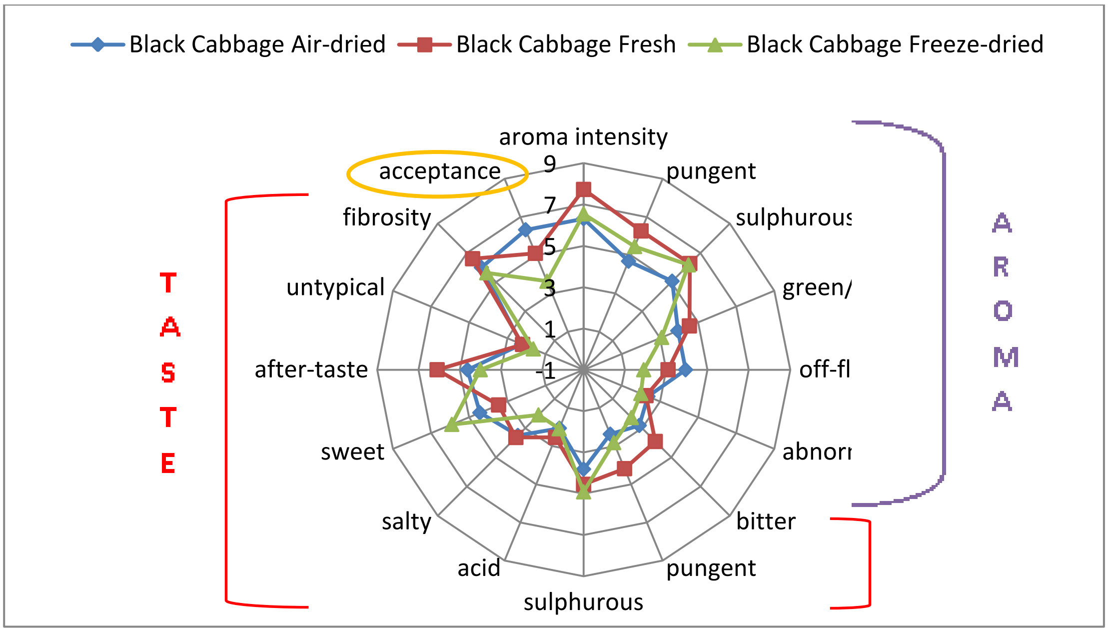


Figure 16- Radar chart about the average values of black cabbage panel test results.

Green curly kale is shown in figure 17. It presented 6 points of acceptance for the air-dried sample, which represented the most appreciated form for this vegetable. It was also detected a relevant fibrosity in freeze-dried derived samples.

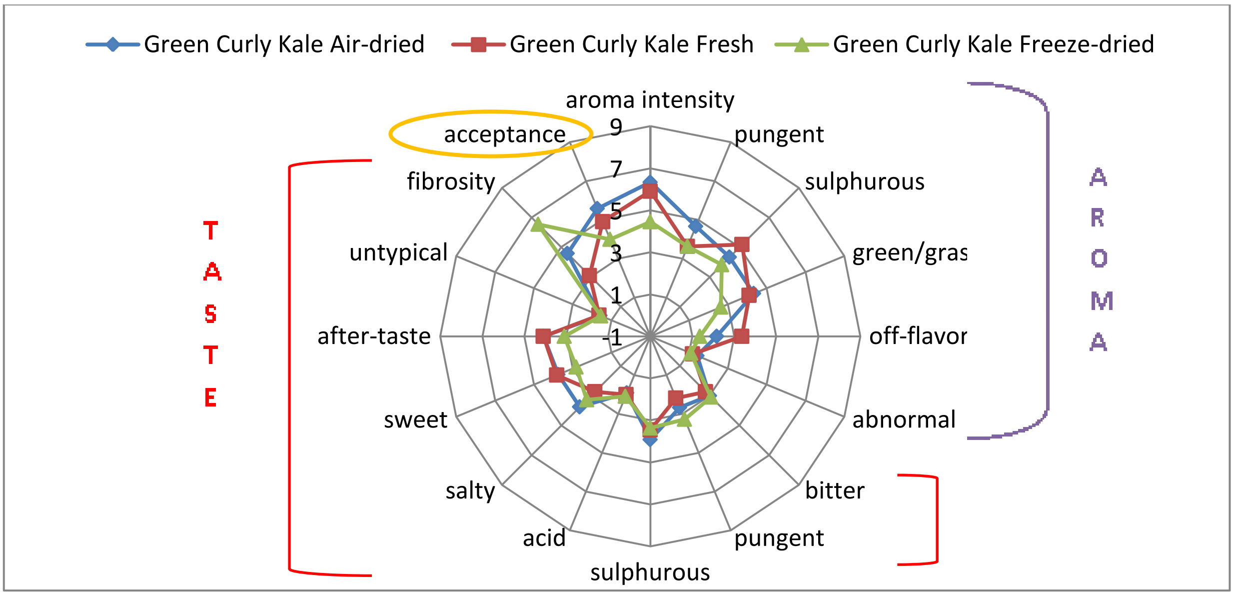


Figure 17- Radar chart about the average values of Green Curly Kale panel test results.

Red curly kale (figure 18) showed low level of acceptance, between 4 and 5 points, with fresh and air-dried materials being the most appreciated. Is it to underline that, in freeze-dried derived samples, a high level of fibrositis was detected (7 points).

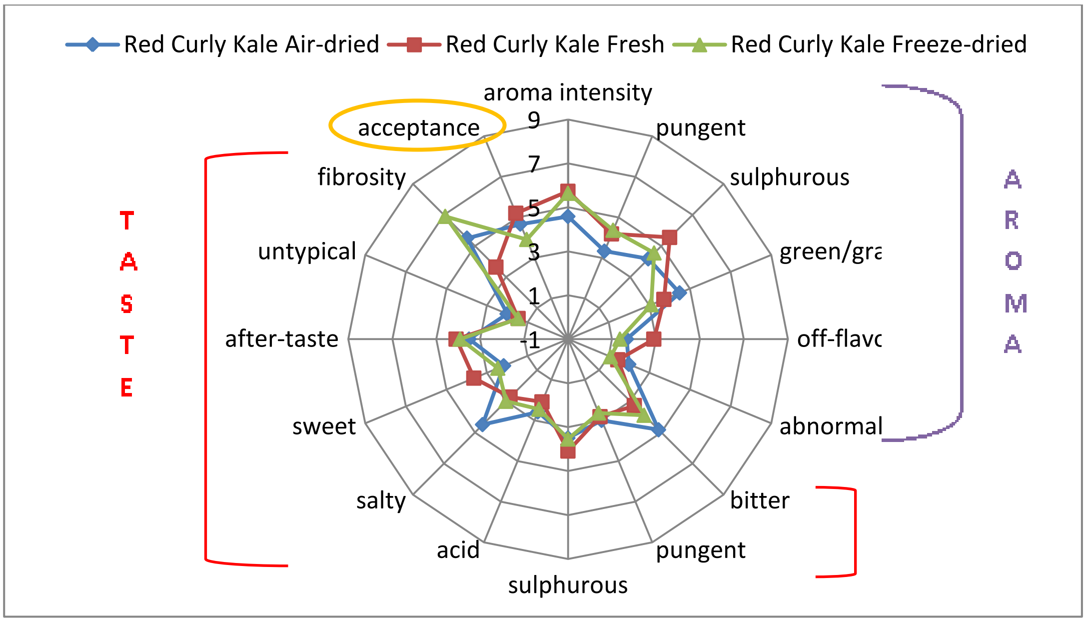


Figure 18- Radar chart about the average values of Red Curly Kale panel test results.

“Getti e foglie” and “spigariello” broccoli belong to the same species and possess similar value for aroma intensity, sulphurous taste and after-taste persistence. Among all the samples analyzed in this panel test, they showed the lowest levels of acceptance, with 3 and 2 points respectively, both for the freeze-dried derived vegetables (figures 19 and 20). The peaks registered in freeze-dried material for the aroma intensity, sulphurous taste and odor were probably due to the capacity of freeze-drying process to concentrate and exalt the phytochemical contents, in particular molecules responsible of flavor and taste.

“Getti e foglie”, represented in figure 19, showed its best panel acceptance on the fresh material, with 6 points of average. Aroma intensity, sulphurous taste and aroma were mainly preserved in freeze-dried samples.

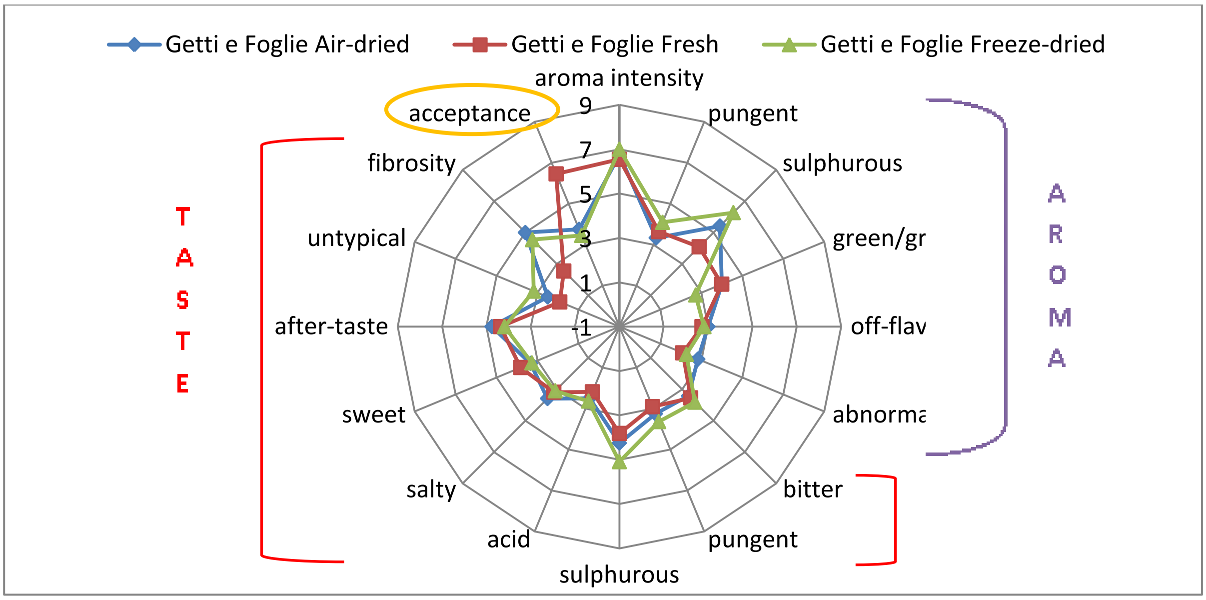


Figure 19- Radar chart about the average values of “Getti e Foglie” panel test results.

“Spigariello” broccoli was the worst vegetable of this panel test in terms of acceptance, with only 4, 4, and 2 average values for air-dried, fresh and freeze-dried materials, respectively. In some comments collected, members of panel reported soil and burnt odor and taste, probably due to overcooking.

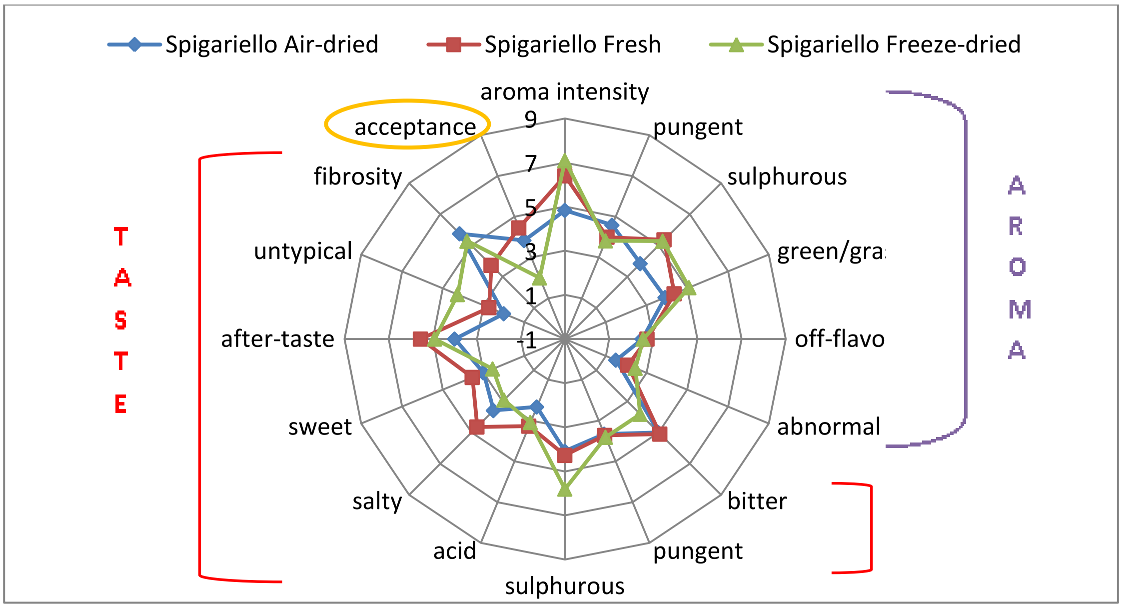


Figure 20- Radar chart about the average values of “Spigariello” panel test results.

In figure 21 it was reported a comparison between samples differently treated, indicating the average values based on the treatments received by the vegetables before cooking. Radar graph reported a similar trend for all descriptors except for the acceptance, with 5, 5, and 4 points for air-dried, fresh and freeze-dried material, respectively.

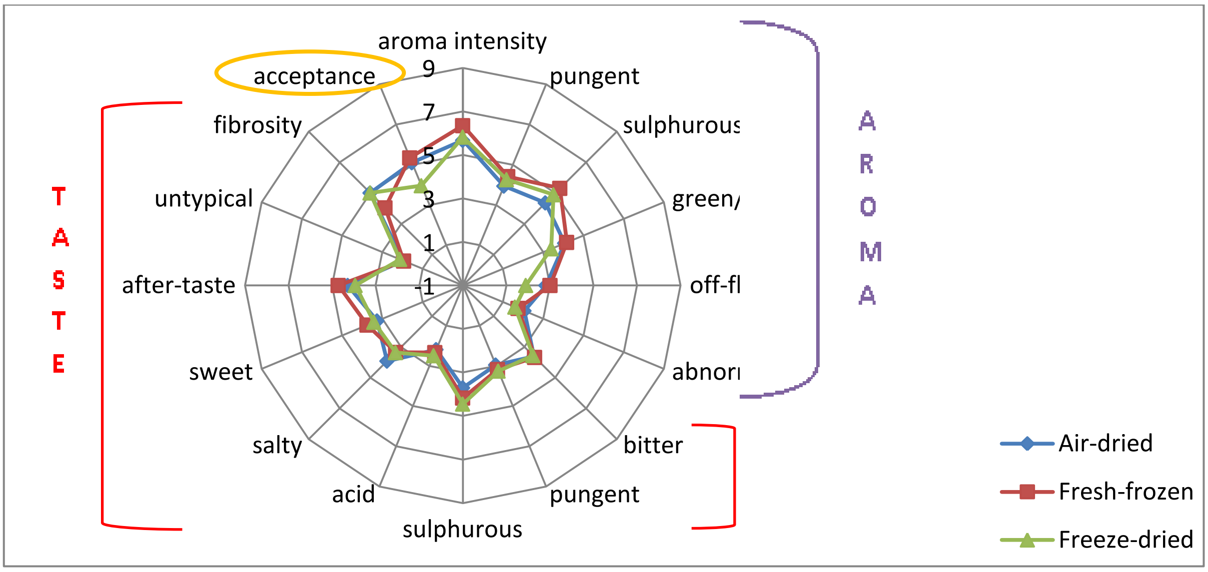


Figure 21 - Average values of descriptors evaluated by the panelists based on vegetables treatment before cooking.

7.4. Conclusions

These results confirm an interesting sensorial quality of brassicas analyzed. The treatments applied for the preparation and storage of vegetables can influence the sensorial quality of the final products. The panelist group mainly appreciated the air-dried and fresh samples, but the best was air-dried broccoli. The worst was freeze-dried "spigariello".

These data confirm the importance to investigate the consumer perception of quality before the introduction and commercialization of new products, because not always the high nutritional quality analyzed in laboratory corresponds to a positive sensorial quality perceived by consumers. This because the high concentration of some high nutritional interest compounds can also bring changes in quality perception of the product (eg. acidity for higher content of vitamins).

Indeed, many studies confirmed that freeze-drying process is the best post-harvest process applicable to maintain unaltered the nutritional quality of vegetables; this process subtracts humidity concentrating the phytochemicals compounds and consequently enhance flavor, aroma and taste. For this reason, the sensorial characteristics of freeze-dried vegetables can be too strong and pronounced for the panel tasters, indeed in this study they resulted the less appreciated. In conclusion, the freeze-dried is the best process for maintain high nutritional quality, but the risk is to obtain a lower consumer appreciation because with a too strong sensorial perception in comparison with the fresh products. Even from this study it can be confirmed that the sensorial analysis, based on programmed panel tests, remain an important tool to understand the consumer behavior and preference for any kind of new products.

8. CONCLUSION OF THE STUDY

An increasing number of medical studies are focusing the attention on the correlation between the higher intake of brassica vegetables and the decreased risk of several human chronic pathologies. This health-promoting activity of *Brassicaceae* is mainly due to the presence of bioactive compounds characteristic of this genus. This study presented an investigation on the presence of those bioactive compounds, analyzing the nutritional quality and the factors that can modify their concentration in Brassica vegetables, like genotype, environmental conditions and pre-storage treatments. From the correct management and application of those factors it is possible to obtain high quality and healthy products, and to propose new ones.

First evolved class “Zeroscarti®” product is a good solution for maintain and preserve high nutritional quality and high phytochemical compounds content in the fresh vegetables, increasing their shelf life. Moreover, this study highlights the possibility to find interesting nutritional quality also in brassicas production waste, surplus, and plant portion normally not used for the intake, that can be destined to new commercial proposals, uses or as functional foods.

One of the strengths of brassica vegetables is that they belong to a big family, constituted by a wide genetic variability with marked differences among genus, and species. This variability brought to a great intraspecific and interspecific differentiation of phytochemical compounds contents. Evaluating the genotype-environment interaction, it was reported the variation of phytochemicals concentration is strongly influenced by climatic condition, temperature, rainfall, nutrients availability etc., thus to differentiate the market characteristics of the variety depending to the cultivation environment and system.

In this study, the genotypes that showed the highest content of phytochemical compounds with antioxidant capacity belong to *Brassica oleracea acephala* species, in particular the green curly kale for TPH and TAC, and red curly kale for the ACY contents, followed by black cabbage. These vegetables showed also a good balanced taste for what concern SSC, TA and pH. Among the analyzed field herbs, borage and chicory possessed the highest antioxidant capacity and total phenol contents. Vitamin C, one of the main responsible of the samples TAC, was mainly detected in broccoli, followed by black cabbage and turnip top.

Considering portion and development stage of plant, it was demonstrated that seeds possess highest antioxidant capacity than leaves. For what concern broccoli, heads have a higher content of phytochemical than stems, but FRAP method revealed that the total antioxidant capacity followed the opposite trend. The comparison between two varieties of broccoli (Roja and Santee) did not bring statistically significant differences for the antioxidant capacity, while the harvested time, in the same variety, affects it (e.g. Roja II-harvest than Roja).

The comparison between two cultivation areas defined Valdaso and Fucino showed a better TPH and TAC average values in the Brassicas deriving from the first environment, while for ACY, Fucino vegetables possessed the highest contents. Several differences were appreciated between areas and years of cultivation.

The results observed on the fresh vegetables can be used to characterize only products that reach the market as fresh product in first evolved class. For a long-term market, they need to store and in this case some treatments are needed, in particular to remove the water from the vegetable matrix and prolong the shelf-life of the product. In this study, the effects of two treatments, the air-drying and the freeze-drying, on the quality of vegetables were investigated, in comparison with fresh products. The best method for preserve the nutritional quality and at the same time extend the shelf life resulted the freeze-drying process, even if its main disadvantage is the high cost of the process. Freeze-drying process allow a retention of antioxidant capacity and phenolics compounds approximately between 70-80%, even if for some nutritional thermo sensitive compounds, the low temperature process can promote their deterioration. The higher TPH and TAC content was detected in freeze-dried *Brassica oleracea achepala* group constituted by black cabbage, green and red curly kales. The air-drying process lead to the depletion of most of the phytochemical compounds, with 70% and 60% of losses of phenolic compounds and antioxidant capacity, respectively (broccoli, black cabbage and red curly kale maintained the better nutritional properties). For ascorbic acid the depletion can vary between 40 and 60%. Those reductions depend by the temperature applied and the length of drying process. In this trial, it was also found an increase of total anthocyanins content in the air-dried material for some vegetables analyzed (e.g. black cabbage).

Furthermore, if a company wants to produce a new typology of product to introduce into the market, a deep evaluation of the sensorial quality and possible consumer appreciation should be performed before its commercialization. This because not always to the high nutritional quality corresponds a high sensorial appreciation from the consumers. Panel test is the main tool to be used for having this type of information. For this reason, a panel test was performed in this study, which revealed a high appreciation of dried broccoli, despite the air-drying process is the worst to preserve the nutritional quality. In general, fresh material resulted to better exalt aroma and aftertaste persistence of brassicas, while the freeze-dried process has been perceived as the worst treatment for the vegetables acceptance by consumers.

Putting together the information deriving from the different research lines of this study, the company can carry on different ways:

- Strengthen the commercial diffusion and proposal of the first evolved class fresh vegetables combining the advantages found in this study. The choice of the better genotypes (e.g. green and red curly kales for brassicas and borage for field herbs); the harvest of the young portion at the correct time and finally, the right management during storage, with packaging use and cold temperature (4-5°C), that help to maintain unaltered the nutritional and sensorial quality and prolonged the shelf life.

  This product will satisfy the consumer's needs, maintaining high sensorial and nutritional quality, together with an ease of preparation and use.

- Start to produce new dried products to use brassicas as an additive in pasta, meat or eggs, for added nutritional value in the normal diet. The most challenging future proposal is to realize new high-quality freeze-dried products, facing the problem of the low consumer acceptance for the aroma intensity typical of brassicas, in particular the freeze-dried ones.

  If the consumer will be informed and made conscious of the healthy potential of the phytochemical compounds present in the brassicas, may be willing to accept these products despite the aroma.

9. REFERENCES

- **Abu-Qaoud H., Shawarb N., Hussen F., Jaradat N., Shtaya M. (2018)**. Comparison of qualitative, quantitative analysis and antioxidant potential between wild and cultivated *Borago officinalis* leaves from Palestine. Pakistan Journal of Pharmaceutical Sciences. 31(3), 953-959.
- **Ackland M.L., Van De Waarsenburg S., Jones R. (2005).** Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. *In vivo.* 19, 69-76.
- **Aires A., Fernandes C., Carvalho R., Bennet R.N., Saavedra M.J., Rosa E.A.S. (2011).** Seasonal effects on bioactive compounds and antioxidant capacity of six economically important Brassica vegetables. Molecules. 16, 6816-6832.
- **Aksouh N.M., Jacobs B.C., Stoddard F.L., Mailer R.J. (2001)**. Response of canola to different heat stresses. Australian Journal of Agricultural ResearchVolume 52, Issue 8, 2001, Pages 817-824. DOI: 10.1071/AR00120
- **Alegria C., Gonçalves E.M., Moldão-Martins M., Cisneros-Zevallos L., Abreu M. (2016)** Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. Postharvest Biology and Technology. 120, 232–239**.** http://dx.doi.org/10.1016/j.postharvbio.2016.07.004.
- **Alvarez M.V., Ponce A.G., Moreira M.R. (2013).** Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. Food Science and Technology. 50, 78-87. http://dx.doi.org/10.1016/j.lwt.2012.06.021
- **Angelino D., Dosz E.B., Sun J., Hoeflinger J.L., Van Tassell M.L., Chen P., Harnly J.M.,Miller M.J., Jeffery E.H. (2015)**. Myrosinase-dependent and -independent formation and control of isothiocyanate products of glucosinolate hydrolysis. Frontiers in Plant Science. 6, 831.
- **Angelino D., Jeffery E. (2014)**. Glucosinolate hydrolysis and bioavailability of resulting isothiocyanates: focus on glucoraphanin. Journal of Functional Foods. 7, 67–76.
- **Anilakumar K.R., Khanum F., Bawa A.S. (2006)**. Dietary role of glucosinolate derivatives: a review. J Food Sci Technol- Mysore. 43, 8–17.
- **Ansorena M.R., Marcovich N.E., Roura S.I. (2011).** Impact of edible coatings and mild heat shocks on quality of minimally processed broccoli (*Brassica oleracea* L.) during refrigerated storage. Postharvest biology and technology. *ISSN* : 0925-5214.
- **Antal T., Tarek M., Tarek-Tilistyák J., Kerekes B. (2017).** Comparative effects of three different drying methods on drying kinetics and quality of Jerusalem artichoke (*Helianthus tuberosus* L.). J. of Food Processing and Preservation. Vol. 41 http://dx.doi.org/ 10.1111/jfpp.12971.
- **Argento S., Raccuia S.A., Melilli M.G., Toscano V., Branca F. (2013).** Brassicas and their glucosinolate content for the biological control of root-knot nematodes in protected cultivation. Acta Hortic. 1005, 539-544. DOI: 10.17660/ActaHortic.2013.1005.66).
- **Arias-Carmona M.D., Romero-Rodriguez M.A., Munoz-Ferreiro N., Vazquez-Oderiz M.L. (2012).** Sensory analysis of protected geographical indication products: an example with turnip greens and tops. J of Sensory Studies. 27, 482-489. doi:10.1111/joss,12013

- **Armesto J., Carballo J., Martínez S. (2015).** Physicochemical and phytochemical properties of two phenotypes of Galega kale (*Brassica oleracea*. var. *acephala* cv. Galega). Journal of Food Biochemistry. 39 (4), 1, 439-448. DOI: 10.1111/jfbc.12151
- **Armesto J., Gómez-Limia L., Carballo J., Martínez S. (2016).** Effects of different cooking methods on some chemical and sensory properties of Galega kale. International Journal of Food Science and Technology. 51,(9) 2071-2080. DOI: 10.1111/ijfs.13181
- **Armesto J., Gómez-Limia L., Carballo J., Martínez S. (2017).** Impact of vacuum cooking and boiling and refrigerated storage on the quality of Galega kale (Brassica oleracea var. acephala cv. Galega). LWT - Food Science and Technology. 79, (1) 267-277. DOI: 10.1016/j.lwt.2017.01.050
- **Azuma K., Ippoushi K., Ito H., Higashio H., Terao J. (1999).** Evaluation of antioxidative activity of vegetable extracts in linoleic acid emulsion and phospholipid bilayers. Journal of the Science of Food and Agriculture, 79, 2010–2016.
- **Badenes-Pérez F.R., Shelton A.M., Nault B.A. (2004)**. Evaluating trap crops for diamondback moth (L.) *Plutella xylostella* (Lepidoptera: *Plutellidae*). J. Econ. Entomol. 97, 1365–1372.
- **Baenas N., Moreno D.A., García-Viguera C. (2012)** Selecting Sprouts of Brassicaceae for Optimum Phytochemical Composition. J. Agric. Food Chem. 60, 11409−11420. dx.doi.org/10.1021/jf302863c
- **Bahorun T., Luximon-Ramma A., Crozier A., Aruoma, O.I. (2004).** Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. Journal of the Science of Food and Agriculture, 84, 1553–1561.
- **Baik H.Y., Juvik J.A., Jeffery E.H., Wallig M.A., Kushad M., Klein B.P. (2003).** Relating glucosinolate content and flavor of broccoli cultivars. J Food Sci. 68, 1043-1050.
- **Bailey L.B., Rampersaud G.C., Kauwell G.P.A. (2003).** Folic acid supplements and fortification affect the risk for neural tube defects, vascular disease and cancer: Evolving science. J of Nutrition. 133, 1961S-1968S.
- **Baldi L., Casati D. (2009).** Un distretto della IV gamma? Il comparto che vende tempo libero. Agriregionieuropa, 5(16), 50-52.
- **Barbieri G., Bottino A., Orsini F., De Pascale S. (2009)** Sulfur fertilization and light exposure during storage are critical determinants of the nutritional value of ready-to-eat friariello campano (*Brassica rapa* L. subsp. *sylvestris*). J Sci Food Agric 2009; 89: 2261–2266. DOI 10.1002/jsfa.3718
- **Becerra-Moreno A., Alanís-Garza P.A., Mora-Nieves J.L., Mora-Mora J.P., Jacobo-Velázquez D.A. (2014).** Kale: An excellent source of vitamin C, pro-vitamin A, lutein and glucosinolates, CyTA - Journal of Food. 12:3, 298-303, DOI: 10.1080/19476337.2013.850743
- **Bell L., Wagstaff C. (2017).** Enhancement of glucosinolate and isothiocyanate profiles in *Brassicaceae* Crops: Addressing Challenges in Breeding for Cultivation, Storage, and Consumer-Related Traits. J. Agric. Food Chem., 65, 9379-9403. DOI: 10.1021/acs.jafc.7b03628.
- **Bellostas N., Kachlicki P., Sørensen J.C., Sørensen H. (2007**). Glucosinolate profiling of seeds and sprouts of B. oleracea varieties used for food. Sci. Hort. Amsterdam. 114, 234–242.
- **Benzie I.F.F, Strain J.J. (1996).** The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay. Anal. Biochem.. 239. 70–76 https://doi.org/10.1006/abio.1996.0292.

- **Biondi F., Balducci F., Mazzoni L., Di Vittori L., Capocasa F., Mei E., Vagnoni M., Visciglio M., Mezzetti B. (2018).** Comparison study about processing methods (postharvest treatments) and their effects of the nutritional quality of different Brassica vegetables. Acta Horticolturae. 1202 ISHS 2018. DOI 10.17660/ActaHortic.2018.1202.19
- **Bjorkman M., Klingen I., Birch A., Bones A., Bruce T., Johansen T.J., Meadow R., Molmann J., Seljasen R., Smart L.E., Stewart D. (2011).** Phyto-chemicals of *Brassicaceae* in plant protection and human health. Influences of climate, environment and agronomic practice. Phytochemistry72, 538-556. doi:10.1016/j.phytochem.2011.01.014.
- **Björkman M., Hopkins R., Rämert B., (2008**). Combined effect of intercropping and turniproot fly (Delia floralis) larval feeding on the glucosinolate concentrations in cabbage roots and foliage. J. Chem. Ecol. 34, 1368-1376.
- **Bodnaryk R.P. (1994).** Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35, 301–305.
- **Bonasia A., Conversa G., Lazzizera C., Elia A. (2013).** Pre-harvest nitrogen and Azoxystrobin application enhances postharvest shelf-life in butterhead lettuce. Postharvest Biol. Technol. 85, 67–76, http://dx.doi.org/10.1016/j.postharvbio.2013.04.012.
- **Bongoni R., Verkerk R., Steenbekkers B., Dekker M., and Stieger M. (2014).** Evaluation of different cooking conditions on broccoli (*Brassica oleracea* var. *italica*) to improve the nutritional value and consumer acceptance. Plant Foods for Human Nutrition. 69(3), 228-234. doi:10.1007/s11130-014-0420-2
- **Booth E.J., Walker K.C., Griffiths D.W. (1991)**. A time course study of the effect of sulfur on glucosinolates in oilseed rape (*Brassica napus*) from the vegetative stage to maturity. J Sci Food Agric. 56, 479–493.
- **Borowski J., Narwojsz A., Borowska E.J., Majewska K. (2005)**. The effect of thermal processing on sensory properties, texture attributes and pectic changes in broccoli. Czech Journal of Food Sciences. 33, 254–260.
- **Bouzari A., Holstege D., Barrett D.M. (2015).** Mineral, fiber, and total phenolic retention in eight fruits and vegetables: a comparison of refrigerated and frozen storage. Journal of Agricultural and Food Chemistry. 63, 951–956.
- **Branca F., Ragusa L., Tribulato A. (2013).** Diversity of Kale Growing in Europe as a Basis for Crop Improvement. Acta Hort. 1005, ISHS 2013
- **Branca F., Ragusa L., Tribulato A., Velasco P. Cartea M.E. (2013a).** Glucosinolate profile in different mediterranean brassica species (N=9). Acta Hortic. 1005, 279-284. DOI: 10.17660/ActaHortic.2013.1005.31).
- **Branca F., Chiarenza G.L., Pinio M., Alonso Alos M., Argento S. (2013b).** Characteristics and seed production of sicilian landraces of violet cauliflower. Acta Hortic. 1005, 519-524. DOI: 10.17660/ActaHortic.2013.1005.63
- **Branca F., Ragusa L., Lo Scalzo R., Picchi V. Argento S. (2013c).** Phytochemical profile of kohlrabi (*Brassica oleracea l. var. gongylodes*) cultivated in italy. Acta Hortic. 1005, 285-292. DOI: 10.17660/ActaHortic.2013.1005.32

- **Brown P.D., Tokuhisa J.G., Reichelt M., Gershenzon J. (2003)**. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry. 62, 471–481.
- **Burow M., Zhang Z.Y.,Ober J.A., Lambrix V.M., Wittstock U., Gershenzon J., Kliebenstein D.J. (2008).** ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in Arabidopsis. Phytochemistry. 69, 663–671.
- **Buttery R.G., Guadagni D.G., Ling L.C., Seifert R.M., Lipton W. (1976).** Additional volatile components of cabbage, broccoli and cauliflower. J Agric Food Chem. 24, 829-32.
- **Calderon-Montano J.M., Burgos-Moron E., Pérez-Guerrero C., Lòpez-Lazaro M. (2011).** A review on the dietary flavonoid kaempferol. Mini-review in Medicinal Chemistry. 11, 298-344.
- **Cao G., Sofic E., Prior R. (1996).** Antioxidant activity of Tea and common Vegetables. J. Agric. Food Chem. 44. 3426-3431 https://doi.org/10.1021/jf9602535.
- **Carlson D.G, Daxenbichler M.E., Tookey H.L. (1987).** Glucosinolates in turnip tops and roots: cultivars grown for greens and/or roots. J Am Soc Hort Sci 112:179–183.
- **Cartea M.E, Velasco P. (2008).** Glucosinolates in Brassica foods: bioavailability in food and significance for human health. Phytochem Rev 7:213-229
- **Cartea M.E., Francisco M., Soengas P., and Velasco P. (2011).** Phenolic Compounds in Brassica Vegetables. Molecules 2011, 16, 251-280; doi:10.3390/molecules16010251
- **Cartea M.E., Rodrìguez V.M., Velasco P., de Haro A., Ordàs A. (2007 a).** Variation of glucosinolates and nutritional value in nabicol (Brassica napus pabularia group). Euphytica.
- **Cartea M.E., Velasco P., Obregòn S., del Rìo M., Padilla G., de Haro A. (2007 b).** Seasonal variation in glucosinolate content in Brassica oleracea crops grown in northwestern Spain. Phytochemistry.
- **Carvajal M., Martínez-Ballesta M.C., Moreno D.A., Bernabeu J., García-Viguera C. (2015).** Seed coating increase broccoli nutrient content and availability after cooking. Journal of Agricultural Science. 7, 182–191.
- **Cefola M., Amodio M.L., Colelli G. (2016 b).** Design of the correct modified atmosphere packaging for fresh-cut broccoli raab. Acta Hortic. 1141. ISHS 2016. DOI 10.17660/ActaHortic.2016.1141.12
- **Cefola M., Amodio M.L., Cornacchia R., Rinaldi R., Vanadia S., Colelli G. (2010 a).** Effect of atmosphere composition on the quality of ready-to-use broccoli raab (*Brassica rapa L.)*. J Sci Food Agric. 2010 Apr 15; 90(5):789-97. doi: 10.1002/jsfa.3885.
- **Cefola M., Amodio M.L., Rinaldi R, Vanadia S., Colelli G. (2010 b).** Exposure to 1-methylcyclopropene (1-MCP) delays the effects of ethylene on fresh-cut broccoli raab (Brassica rapa L.). Postharvest Biology and Technology 58 (2010) 29–35. doi:10.1016/j.postharvbio.2010.05.001
- **Cefola M., Carbone V., Minasi P., Pace B. (2016 a).** Phenolic profiles and postharvest quality changes of fresh-cut radicchio (Cichorium intybus L.): nutrient value in fresh vs. stored leaves. Journal of Food Composition and Analysis 51 (2016) 76–84. http://dx.doi.org/10.1016/j.jfca.2016.06.004
- **Chin H.W., Lindsay R.C. (1994).** Modulation of volatile sulfur compounds in cruciferous vegetables. In: Mussinan C.J., Keelan M.E., editors. Sulfur compounds in food Washington, DC. American Chemical Society. p 90-104.

- **Ciancaleoni S., Chiarenza G.L., Raggi L., Branca F., Negri V.(2014).** Diversity characterisation of broccoli (Brassica oleracea L.var. italica Plenck) landraces for their on-farm (in situ) safeguard and use in breeding programs. Genet Resour Crop Evol 61:451–464. DOI 10.1007/s10722-013-0049-2
- **Ciska E., Martyniak-Przybyszewska B., Kozlowska H. (2000).** Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J Agric Food Chem 48:2862–2867.
- **Ciska E., Pathak D.R. (2004)**. Glucosinolate derivatives in stored fermented cabbage. J Agric Food Chem. 52, 7938–7943.
- **Clarke J.D., Hsu A., Riedl K., Bella D., Schwartz S.J., Stevens J.F., Ho E. (2011).** Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. Pharmacological Research. 64, 456–463.
- **Cogo S.L.P., Chaves F.C., Schirmer M.A., Zambiazi R.C., Nora L., Silva J.A., Rombaldi C.V. (2011).** Low soil water content during growth contributes to preservation of green colour and bioactive compounds of cold-stored broccoli (*Brassica oleracea* L.) florets. Postharvest Biology and Technology. 60, 158-163.
- **Cohen J., Kristal R., Stanford J. (2000).** Fruit and vegetable intakes and prostate cancer. Journal of the National Cancer Institute, 9, 61–68.
- **Cole R.A. (1997).** The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. Entomol. Exp. Appl. 85, 121-133.
- **Conversa G., Bonasia A., Lazzizera C., Elia A. (2016).** Bio-physical, physiologiacal, and nutritional aspects of ready-to-use cima di rapa (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) as affected by conventional and organic growing systems and storage time. Scientia Horticulturae 213, 76-86. http://dx.doi.org/10.1016/j.scienta.2016.10.021
- **Cook S.M., Khan Z.R., Pickett J.A. (2007).** The use of push–pull strategies in integrated pest management. Ann. Rev. Entomol. 52, 375–400.
- **Czarniecka-Skubina, E. (2002).** Effect of the material form, storage and cooking methods on the quality of Brussels sprouts. Polish Journal of Food and Nutrition Sciences. 11/52(3), 75–82.
- **Czeczot H. (2000).** Biological activities of flavonoids—A review. Polish Journal of Food and Nutrition Sciences, 950(4), 3–13.
- **Dangour A.D., Dodhia S.K., Hayter A.,Allen E., Lock K., Uauy R. (2009).** Nutritional quality of organic foods: a systematic review. Am. J. Clin. Nutr. 90, 680-685. http://dx.doi.org/10.3945/ajcn.2009.28041.
- **Dauchet L., Amouyel P., Hercberg S., Dallongeville J. (2006).** Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. J. Nutr. 136 (10), 2588-93.
- **Davey M.W., Van Montagu M., Inze D., Sanmartin M., Kanellis A., Smirnoff N. (2000).** Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture, 80, 825–860.

- **De Pascale S., Maggio A., Pernice R., Fogliano V., Barbieri G. (2007).** Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. Europ. J. Agronomy 26, 418-424. Doi:10.1016/j.eja.2006.12.009
- **Deighton N., Brennan R., Finn C., Davies H.V. (2000).** Antioxidant properties of domesticated and wild Rubus species. Sci. Food Agri. 80, 1307–1313 https://doi.org/10.1002/1097-0010(200007)80:9<1307::AID-JSFA638>3.0.CO;2-P
- **Dekker M. and Verkerk R. (2003).** Dealing with variability in food production chains: a tool to enhance the sensitivity of epidemiological studies on phytochemicals. Eur J Nutr. 42, 67–72.
- **Di Vittori L., Mazzoni L., Battino M., Mezzetti B. (2018).** Pre-harvest factors influencing the quality of berries. Scientia Horticulturae-Volume 233, Pages 310-322. https://doi.org/10.1016/j.scienta.2018.01.058.
- **Diamanti J., Capocasa F., Balducci F., Battino M., Hancock J., Mezzetti B. (2012).** Increasing Strawberry Fruit Sensorial and Nutritional Quality Using Wild and Cultivated Germplasm. PLOS ONE, 10.1371/journal.pone.0046470. https://doi.org/10.1371/journal.pone.0046470
- **Diamanti J., Mazzoni L., Balducci F., Cappelletti R., Capocasa F., Battino M., Dobson G., Stewart D., Mezzetti B. (2014).** Use of wild genotypes in breeding program increases strawberry fruit sensorial and nutritional quality. J Agric Food Chem 62(18):3944–3953. doi: 10.1021/jf500708x.
- **Dinkova-Kostova A.T., Kostov R.V. (2012)**. Glucosinolates and isothiocyanates in health and disease. Trends in Molecular Medicine. 18, 337–347.
- **Dosz E.B., Jeffery E.H. (2013).** Modifying the processing and handling of frozen broccoli for increased sulforaphane formation. Journal of Food Science. 78, 1459–1463.
- **Drewnowski A., Henderson S.A., Barratt-Fornell A. (2001)**. Genetic taste markers and food preferences. Drug Metabol Dispos. 29, 535–538.
- **Dubuis P.H., Marazzi C., Städler E., Mauch F. (2005).** Sulphur deficiency causes a reduction in antimicrobial potential and leads to increased disease susceptibility of oilseed rape. J. Phytopathol. 153, 27–36.
- **Dyrby M., Westergaard N., Stapelfeldt H. (2001).** Light and heat sensitivity of red cabbage extract in soft drink model system. Food Chem. 72, 431-437.
- **Elsabee M.Z., Abdou E.S. (2013).** Chitosan based edible films and coatings: A review. Materials Science and Engineering: C. Volume 33, Issue 4, 1 May 2013, Pages 1819-1841. http://dx.doi.org/10.1016/j.msec.2013.01.010
- **Engelen-Eigles G., Holden G., Cohen J.D., Gardner G. (2006)**. The effect of temperature, photoperiod, and light quality on glucnasturtiin concentration in watercress (*Nasturtium officinale*). J Agric Food Chem. 54, 328–334.
- **Fabek S., Toth N., Redovnikovic I.R., Custic M.H., Benko B., Žutic I. (2012)** The effect of nitrogen fertilization on nitrate accumulation, and the content of minerals and glucosinolates in broccoli cultivars. Food Technology and Biotechnology, 50, 9.
- **Fahey J.W., Haristoy X., Dolan P.M., Kensler T.W., Scholtus I., Stephenson K.K., Talalay P., Lozniewski A., (2002).** Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains

of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc. Natl. Acad. Sci. USA 99, 7610–7615.

- **Fahey J.W., Zhang Y., Talalay P. (1997).** Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. U.S.A. 94. 10367-10372 https://doi.org/10.1073/pnas.94.19.10367
- **FAOSTAT. (2011).** Food and Agricultur Organization of the United Nations. FAOSTAT database. Top exports of Cabbages and Other Brassicas. URL http://faostat.fao.org/site/342/default.aspx.
- **FAOSTAT. (2018).** Food and Agricultur Organization of the United Nations. FAOSTAT database. Top exports of Cabbages and Other Brassicas. URL http://faostat.fao.org/site/342/default.aspx
- **Farnham M.W., Wilson P.E., Stephenson K.K., Fahey J.W. (2004).** Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. Plant Breeding. 123, 60-65.
- **Favell D. J. (1998).** A comparison of the vitamin C content of fresh and frozen vegetables. Food Chemistry. 62, 59–64.
- **Fernandes F., Valentão P., Sousa C., Pereira J.A., Seabra R.M., Andrade P.B. (2007)** Chemical and antioxidative assessment of dietary turnip (Brassica rapa var. rapa L.). Food Chemistry 105, 1003–1010. doi:10.1016/j.foodchem.2007.04.063.
- **Ferreres F., Fernandes F., Sousa C., Valentão P., Pereira J.A., Andrade P.B. (2009).** Metabolic and Bioactivity Insights into Brassica oleracea var. Acephala. J. Agric. Food Chem. 57, 8884–8892. DOI:10.1021/jf902661g
- **Fieldsend J., Milford G.F.J. (1994).** Changes in glucosinolates during crop development in single-and double low genotypes of winter oilseed rape (*Brassica napus*): production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. Ann Appl Biol. 124, 531–542.
- **Finch S., Billiald H., Collier R.H. (2003)**. Companion planting – do aromatic plants disrupt host-plant finding by the cabbage root fly and the onion fly more effectively than nonaromatic plants? Entomol. Exp. Appl. 109, 183–195.
- **Florindo A.A., Brownson R.C., Mielke G.I., Gomes G.A., Parra D.C., Siqueira F.V., Hallal P.C. (2015).** Association of knowledge, preventive counseling and personal health behaviors on physical activity and consumption of fruits or vegetables in community health workers. BMC public health, 15(1), 1.
- **Francisco M., Cartea M.E., Butrón A.M., Sotelo T., Velasco P. (2012).** Environmental and genetic effects on yield and secondary metabolite production in *Brassica rapa* crops. J of Agric and Food Chem. 60, 5507-5514.
- **Francisco M., Cartea M.E., Soengas P., Velasco P. (2011).** Effect of Genotype and Environmental Conditions on Health-Promoting Compounds in Brassica rapa. J. Agric. Food Chem. 2011, 59, 2421–2431 DOI: 10.1021/jf103492r
- **Francisco M., Moreno D.A., Cartea M.E., Ferreres F., Garcia-Viguera C., Velasco P. (2009).** Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable Brassica rapa. J. Chromatogr. A 1216, 6611-6619.

- **Francisco M., Tortosa M., Martinez-Ballesta M., Velasco P., Garcia-Viguera C., Moreno D.A (2017).** Nutritional and phytochemical value of Brassica crops from the agri-food perspective. Ann Appl Biol 170, 273-285. Doi: 10.1111/aab.12318
- **Francisco M., Velasco P., Moreno D.A., Garcia-Viguera C., Cartea M.E. (2010)**. Cooking methods of *Brassica rapa* affect the preservation of glucosinolates, phenolics and vitamin C. Food Research International. 43, 1455–1463.
- **Gallo M., Esposito G., Ferracane R., Vinale F., Naviglio D. (2013).** Beneficial effects of *Trichoderma* genus microbes on qualitative parameters of *Brassica rapa* L. subsp. *sylvestris* L. Janch. Var. *esculenta* Hort, Eur Food Res Technol. 236, 1063-1071. Doi 10.1007/s00217-013-1971-4.
- **Gebczynski P., Lisiewska Z. (2006).** Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. Innov. Food Sci. Emerg. Technol. 7, 239–245.
- **Giamoustaris A., Mithen R. (1995).** The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. Ann. Appl. Biol. 126, 347–363.
- **Giusti M., Wrolstad R.E. (2001).** Characterization and Measurement of anthocyanins by UV-visible Spectroscopy. In Current Protocals in Food Analytical Chemistry. 1st ed.; Wrolstad. R.E.. Ed.; John Wiley & Sons. Inc.: New York. NY. USA; pp. F1.2.1–F1.2.13 https://doi.org/10.1002/0471142913.faf0102s00
- **Good S.K. (1998).** Flavor characterization of two broccoli cultivars after different refrigerated storage conditions (MSc Thesis). Urbana-Champaign, Ill.: Univ. of Illinois. 134 p. Available from Univ. of Illinois Libraries, Urbana, IL 61801.
- **Griffiths D.W., Birch A.N.E., Hillman J.R. (1998).** Antinutritional compounds in the Brassicaceae. Analysis, biosynthesis, chemistry and dietary effects. J Hort Sci Biotech. 73, 1–18.
- **Grubb C.D., Abel S. (2006).** Glucosinolate metabolism and its control. Trends Plant Sci. 11, 89–100.
- **Guo J.T., Lee H.L., Chiang S.H., Lin F.I., Chang C.Y. (2001).** Antioxidant properties of the extracts from different parts of broccoli in Taiwan. J of Food and Drug Analysis. 9, 96-101.
- **Guo R., Shen W., Qian H., Zhang M., Liu L., Wang Q. (2013)**. Jasmonic acid and glucose synergistically modulate the accumulation of glucosinolates in Arabidopsis thaliana. Journal of Experimental Botany. 64, 5707–5719.
- **Hansen M., Laustsen A.M., Olsen C.E., Poll L., Sorensen H. (1997).** Chemical and sensory quality of broccoli (*Brassica oleracea* L. var *italica*). J Food Qual. 20,441-459.
- **He F.J., Nowson C.A., MacGregor G.A. (2006).** Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. Lancet. 367(9507), 320-6.
- **Heimler D., Vignolini P., Dini M.G., Vincieri F.F., Romani A. (2006).** Antiradical activity and polyphenol composition of local *Brassicaceae* edible varieties. *Food Chem. 99*, 464-469.
- **Helland H.S., Leufvén A., Bengtsson G.B,. Pettersen M.K., Lea P., Wold A.B. (2016).** Storage of fresh-cut swede and turnip: Effect of temperature, including sub-zero temperature, and packaging material on sensory attributes, sugars and glucosinolates. Postharvest Biology and Technology. 111, 370–379. http://dx.doi.org/10.1016/j.postharvbio.2015.09.011.

- **Helsper J.P.F.G., De Vos C.H.R., Maas F.M., Jonker H.H., Van den Broeck H.C., Jordi W., Pot C.S., Keizer L.C.P., Schapendonk A.H.C.M. (2003).** Response of selected antioxidants and pigments in tissues of Rosa hybrida and Fuchsia hybrida to supplemental UV-A exposure. Physiol. Planta. 117, 171– 187 https://doi.org/10.1034/j.1399-3054.2003.00037.x
- **Herr I., Buchler M.W. (2010).** Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. Cancer Treatment Reviews. 36, 377–383.
- **Hoefkens C., Sioen I., Baert K., De Meulenaer B., De Henauw S.,Vandekinderen I., et al., (2010).** Consuming organic versus conventional vegetables: the effect on nutrient and contaminant intakes. Food Chem Toxicol. 48, 3058-3066. http://dx.doi.org/10.1016/j.fct.2010.07.044.
- **Hooks C.R.R., Johnson M.W. (2003)**. Impact of agricultural diversification on the insect community of cruciferous crops. Crop Prot. 22, 223–238.
- **Howard L.A., Wong A.D., Perry A.K., Klein B.P. (1999).** B-carotene and ascorbic acid retention in fresh and processed vegetables. Journal of Food Science. 64, 929–936.
- **Hrncirik K., Valusek J., Velisek J. (2001).** Investigation of ascorbigen as a breakdown product of glucobrassicin autolysis in Brassica vegetables. European Food Research and Technology. 212, 576-581.
- **Huang D., Ou B., Hampsch-Woodill M., Flanagan J.A., Prior R.L. (2002).** High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J Agric Food Chem. 50(16), 4437-44.
- **Huang D., Ou B., Prior R.L. (2005).** The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 53(6), 1841–1856. http://dx. doi.org/10.1021/jf030723c.
- **ISO 3972: 2013-12.** Sensorische Analyse - Methodologie - Bestimmung der Geschmacksempfindlichkeit
- **ISO 8586:2014-05.** Sensorische Analyse - Allgemeiner Leitfaden für die Auswahl. Schulung und Überprüfung ausgewählter Prüfer und Sensoriker.
- **ISO 8589: 2014-10** Sensorische Analyse –Allgemeiner Leitfaden für die Gestaltung von Prüfräumen
- **Istat**- tav. C08-C09, 2010-2017
- **Jagdish S., Upadhyay A.K., Singh S., Rai M. (2009).** Total phenolics content and free radical scavenging activity of Brassica vegetables. J of Food Science and Tech-Mysore. 46, 595-597.
- **Jahangir M., Abdel-Farida I.B., Kima H.K., Choia Y.H., Verpoortea R. (2009 a).** Healthy and unhealthy plants: The effect of stress on the metabolism of Brassicaceae. Environmental and Experimental Botany 67, 23–33.
- **Jahangir M., Kim H.K., Choi J.H., Verpoorte R. (2009 b)** Health-Affecting Compounds in Brassicaceae. Comprehensive Reviews In Food Science And Food Safety-*Vol. 8.*
- **Jancic D., Todorovic V., Sircelj H., Dodevska M., Beljkas B., Znidarcic D., Sobajic S. (2017).** Biologically active compounds and antioxidant capacity of *Cichorium intybus* L. leaves from Montenegro. Italian Journal of Food Science.Vol. 29, Issue 4, 24, Pages 627-643.
- **Jeffery, E.H., Brown, A.F., Kurilich, A.C., Keek, A.S., Matusheski, N., Klein, B.P., et al. (2003).** Variation in content of bioactive components in broccoli. Study review. Journal of Food Composition and Analysis, 16, 323–330.

- **Jenks M.A., Rashotte A.M., Tuttle H.A., Feldmann K.A. (1996).** Mutants in *Arabidopsis thaliana* altered in epicuticular wax and leaf morphology. Plant Physiol. 110, 377-385.
- **Jenks M.A., Tuttle H.A., Eigenbrode S.D., Feldmann K.A. (1995).** Leaf epicuticular waxes of the Eceriferum mutants in *Arabidopsis.* Plant Physiol. 108, 369-377.
- **Jones G., Sanders O.G. (2002).** A sensory profile of turnip greens as affected by variety and maturity. J Food Sci. 67, 3126-3129.
- **Jones R.B., Faragher J.D., Winkler S. (2006).** A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. Postharvest Biology and Technology. 41, 1–8.
- **Ju H.Y., Chong C., Bible B.B., Mullin W.J. (1980)**. Seasonal variation in glucosinolate composition of rutabaga and turnip. Can J Plant Sci. 60, 1295–1302.
- **Karam M.C., Petit J., Zimmer D., Djantou E.B., Scher J. (2016).** Effects of drying and grinding in production of fruit and vegetable powders: A review. J of Food Engineering 188, 32-49.
- **Kaur C., Kapoor H.C. (2001).** Antioxidants in fruits and vegetables- the millennium's health. Int. J. Food Sci. Tech. 36, 703-725. https://doi.org/10.1046/j.1365-2621.2001.00513.x
- **Kaur C., Kumar K., Anil D., Kapoor H.C. (2006).** Variations in antioxidant activity in broccoli (Brassica oleracea L.) cultivars.
- **Kaur S., Gupta S.K., Sukhija P.S., Munshi S.K. (1990).** Accumulation of glucosinolates in developingmustard (*Brassica juncea* L.) seeds in response to sulfur application. Plant Sci. 66, 181–184.
- **Keum Y.S., Jeong W.S., Kong A.N.T. (2004)**. Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. Mutation Research, 555, 191–202.
- **Khan M.A.M., Ulrichs C., Mewis I. (2011a).** Drought stress- Impact on glucosinolate profile and performance of phloem feeding cruciferous insects. Acta Horticulturae, 917,111–117.
- **Kim D.O., Padilla-Zakour O.I., Griffiths P.D. (2004).** Flavonoids and antioxidant capacity of various cabbage genotypes at juvenile stage. Journal of Food Science 69, C685-C689.
- **Kim S.J., Kawaguchi S., Watanabe Y. (2003).** Glucosinolates in vegetative tissues and seeds of twelve cultivars of vegetable turnip rape (Brassica rapa L.) Soil Sci Plant Nutr. 49:337–346.
- **Kim S.J., Matsuo T.,Watanabe M.,Watanabe Y. (2002).** Effect of nitrogen and sulphur application on the glucosinolate content in vegetable turnip rape (*Brassica rapa* L.). Soil Sci. Plant Nutr. 48, 43–49.
- **Koh E., Charoenprasert S., Mitchell A.E. (2012).** Effect of organic and conventional cropping systems on ascorbic acid, vitamin C, flavonoids, nitrate, and oxalatein 27 varieties of spinach (*Spinacia oleracea* L.). J. Agric. Food Chem. 60, 3144–3150. http://dx.doi.org/10.1021/jf300051f.
- **Kopsell D.A., Kopsell D.E., Curran-Celentano J. (2007).** Carotenoid pigments in kale are influenced by nitrogen concentration and form. J. Sci. Food Agric. 87, 900– 907.
- **Kopsell D.E., Kopsell D.A., RandleW.A., Coolong T.W., Sams C.E., Curran-Celentano J. (2003)**. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. J. Agric. Food Chem. 51, 5319–5325.
- **Korus A. (2012).** Effect of technological processing and preservation method on amino acid content and protein quality in kale (*Brassica oleracea* var. *acephala*) leaves. Journal of the Science of Food and Agriculture. 92, 618–625.

- **Korus. A. (2011).** Effect of preliminary processing. method of drying and storage temperature on the level of antioxidants in kale (*Brassica oleracea* L. var. *acephala)* leaves. LWT—Food Sci. Tech. 44(8), 1711–1716. https://doi.org/10.1016/j.lwt.2011.03.014
- **Kris-Etherton P.M., Etherton T.D., Carlson J., Gardner C. (2002).** Recent discoveries in inclusive food-based approaches and dietary patterns for reduction in risk for cardiovascular disease. Current Opinion in Lipidology.13, 397–407
- **Krokida M.K., Karathanos V.T., Maroulis Z.B., Marinos-Kouris D. (2003).** Drying kinetics of some vegetables. J. Food Eng. 59, 391-403. https://doi.org/10.1016/S0260-8774(02)00498-3
- **Kücükboyaci N., Güvenc A., Turan N.N., Aydin A. (2012).** Antioxidant activity and total phenolic content of aqueous extract from *Raphanus raphanistrum* L. Turkish Journal of Pharmaceutical Sciences. Vol. 9, Issue 1, Pages 93-100
- **Kurilich A.C., Tsau G.J., Brown A., Howard L., Klein B.P., Jeffery E.H., Kushad M., Walling M.A., Juvik J.A. (1999)**. Carotene, tocopherol, and ascorbate contents in subspecies of Brassica oleracea. Journal of Agricultural and Food Chemistry 47,1576-1581
- **Kurilich, A.C., Jeffery, E.H., Juvik, J.A., Wallig, M.A., Klein, B.P. (2002).** Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. Journal of Agriculture and Food Chemistry. 50, 5053–5057.
- **Kushad M.M., Brown A.F., Kurilich A.C., Juvik J.A., Klein B., Wallig M.A., Jeffery E.H. (1999).** Variation of glucosinolates in vegetable subspecies of Brassica oleracea. J Food Agric Chem. 47, 1541–1548.
- **Lafarga T., Viñas I., Bobo G., Simó J., Aguiló-Aguayo I. (2018).** Effect of steaming and sous vide processing on the total phenolic content, vitamin C and antioxidant potential of the genus Brassica. Innovative Food Science and Emerging Technologies 47, 412–420. https://doi.org/10.1016/j.ifset.2018.04.008
- **Lako J., Trenerry V.C., Wahlqvist M., Wattanapenpaiboon N., Sotheeswaran. S., Premier R. (2007).** Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem. 101, 1727-1741. https://doi.org/10.1016/j.foodchem.2006.01.031
- **Larkin R.P., Griffin T.S. (2007).** Control of soilborne potato diseases using Brassica green manures. Crop Protection. 26, 1067–1077. doi:10.1016/j.cropro.2006.10.004.
- **Lawless H.T., Heymann H. (1998).** Sensory Evaluation of Food. Principles and Practices. International Thomson Publishing. New York.
- **Lawless H.T., Malone J.G. (1986).** A comparison of scaling methods: sensivity, replicates and relative measurement. Journal of Sensory Studies. 1, 85-86
- **Lefsrud M., Kopsell D., Wenzel A., Sheehan J. (2007).** Changes in kale (*Brassica oleracea* L. var. *acephala*) carotenoid content and chlorophyll pigment concentrations during leaf ontogeny. Sci. Hort. Amsterdam. 112, 136–141.
- **Leja M., Mareczek A., Starzynska A., Rozek S. (2001).** Antioxidant ability of broccoli flower buds during short-term storage. Food Chemistry, 72, 219–222

- **Lemoine M.L., Chaves A.R. Martìnez G.A., (2010).** Influence of a combined hot air and UV-C treatment on the antioxidant system of minimally processed broccoli (brassica oleracea L. var. italica). LWT- Food Sci and Tech. 43, 1313-1319.
- **Lemoine M.L., Civello P.M., Martinez G.A., Chaves A.R. (2007)**. Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. *italica*). J. Sci. Food Agric. 87. 1132–1139.
- **Li S., Schonhof I., Krumbein A., Li L., Stutzel H., Schreiner M. (2007)**. Glucosinolate concentration in turnip (Brassica rapa ssp. rapifera L.) roots as affected by nitrogen and sulfur supply. J. Agric. Food Chem. 55, 8452–8457.
- **Liapis A.I., Pim M.L., Bruttini R. (1996).** Research and development needs and opportunities in freeze drying. Dry. Technol. 14 (6), 1265e1300.
- **Lim Y.Y., Lim T.T., Tee J.J. (2007).** Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry. 103(3), 1003–1008. http://dx.doi. org/10.1016/j.foodchem.2006.08.038.
- **Lin C.H., Chang C.Y. (2005).** Textural change and antioxidant properties of broccoli under different cooking treatments. Food Chem. 90, 9-15.
- **Lo Piero A.R., Lo Cicero L., Ragusa L., Branca F. (2013).** Change in the expression of anthocyanin pathway genes in developing inflorescences of sicilian landraces of pigmented broccoli and cauliflower. Acta Hortic. 1005, 253-260. DOI: 10.17660/ActaHortic.2013.1005.28
- **Lo Scalzo R., Genna A., Branca F., Chedin M., Chassaigne H. (2008).** Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*B. oleracea* L. var. *capitata*) and its stability in relation to thermal treatments. Food Chem. 2008, 107, 136-144.
- **Lo Scalzo R., Picchi V., Migliori C.A., Campanelli G., Leteo F., Ferrari V., Di Cesare L.F. (2013).** Variations in the phytochemical contents and antioxidant capacity of organically and conventionally grown Italian cauliflower (*Brassica oleracea* L. var. *botrytis*): results from a three-year field study. J of Agric and Food Chem. 61, 10335-10344.
- **Lola-Luz T., Hennequart F., Gaffney M. (2013)**. Enhancement of phenolic and flavonoid compounds in cabbage (*Brassica oleraceae*) following application of commercial seaweed extracts of the brown seaweed, (*Ascophyllum nodosum*). Agricultural and Food Science. 22, 288–295.
- **López-Berenguer C., Martínez-BallestaM.C., García-Viguera C., Carvajal M. (2008)** Leaf water balance mediated by aquaporins under salt stress and associated glucosinolate synthesis in broccoli. Plant Science. 174, 321–328.
- **Mageney V., Neugart S., Albach D.C. (2017).** A Guide to the Variability of Flavonoids in Brassica oleracea. Molecules. 22, 252. http://dx.doi.org/ 10.3390/molecules22020252
- **Mahn A., Reyes A. (2011).** An overview of health-promoting compounds of broccoli (*Brassica oleracea* var. *italica*) and the effect of processing. Food Science and Technology. 18, 503–514.
- **Manchali S., Murthy K.C., Patil B.S. (2012).** Crucial facts about health benefits of popular cruciferous vegetables. Journal of Functional Foods, 4, 94–106.
- **Martınez S., Olmos I., Carballo J., Franco I. (2010).** Quality parameters of Brassica spp. grown in northwest Spain. International Journal of Food Science and Technology 2010. 45. 776–783. doi:10.1111/j.1365-2621.2010.02198.x

- **Martìnez-Sanchez A., Gil-Izquierdo A., Gil M.I., Ferreres F. (2008).** A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. J of Agric and Food Chem. 56, 2330-2340.
- **Martinez-Villaluenga C., Peñas E., Ciska E., Piskula M.K., Kozlowska H., Vidal-Valverde C., Frias J. (2010)** Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds. Food Chemistry 120. 710–716. doi:10.1016/j.foodchem.2009.10.067
- **Matusheski N.V. and Jeffery E.H. (2001)**. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. Journal of Agricultural and Food Chemistry. 49, 5743–5749.
- **Matusheski N.V., Juvik J.A., Jeffery E.H. (2004).** Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. Phytochemistry. 65, 1273–1281.
- **Miglio C., Chiavaro E., Visconti A., Fogliano V., Pellegrini N. (2008).** Effect of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. J of Agric and Food Chem. 56, 139-147.
- **Mikkelsen M.D., Petersen B.L., Glawischnig E., Jensen A.B., Andreasson E., Halkier B.A. (2003).** Modulation of CYP79 genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. Plant Physiol. 131, 298–308.
- **Milder I.E.J., Arts I.C.W., Van De Putte B., Venema D.P., Hollman P.C.H. (2005).** Lignin contents of Dutch plant foods: A database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. British J of Nutrition 93, 393-402.
- **Miller N.J., Rice-Evans C., Davis M.J. (1993).** A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science 83, 407–412.
- **Ministero di agricoltura, industria e commercio (fino al 1923);** Istituto di economia e statistica agraria (anni 1924-1926); Istat, Stima delle superfici e produzioni delle coltivazioni agrarie, floricole e delle piante intere da vaso (dal 1927) (https://www.istat.it/).
- **Mithen R. (2001).** Glucosinolates—biochemistry, genetics and biological activity. Plant Growth Regul. 34, 91–103.
- **Mithen R., Faulkner K., Magrath R., Rose P., Williamson G., Marquez J. (2003)**. Development of isothiocyanate-enriched broccoli and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Gen. 106, 727–734.
- **Mithen R.F., Dekker M., Verkerk R., Rabot S., Johnson I.T. (2000)**. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J Sci Food Agric. 80, 967–984.
- **Mittler R. (2002).** Oxidative stress, antioxidants and stress tolerance. Trends PlantSci. 7, 405–410. http://dx.doi.org/10.1016/S1360-1385(02)02312-9.
- **Mollers C., Nehlin L., Glimelius K., Iqbal M.C.M. (1999).** Influence of in vitro culture conditions on glucosinolate composition of microspore-derived embryos of Brassica napus. Physiol. Plant. 107, 441–446.

- **Moreira M.R., Roura S.I., Ponce A. (2011).** Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. Food Science and Technology. 44, 2335-2341. doi:10.1016/j.lwt.2011.04.009
- **Moreno D.A., Carvajal M., Lopez-Berenguer C., García-Viguera C. (2006).** Chemical and biological characterisation of nutraceutical compounds of broccoli. J of Pharmaceutical and Biomedical Analysis. 41, 1508-1522.
- **Moreno D.A., López-Berenguer C., Carvajal M., García- Viguera C. (2007)**. Health benefits of broccoli. Influence of pre- and postharvest factors on bioactive compounds. Food. 1, 297–312.
- **Moreno D.A., Pérez-Balibrea S., Ferreres F., Gil-Izquierdo A., García-Viguera C., (2010).** Acylated anthocyanins in broccoli sprouts. Food Chem.. 123. 358-363 https://doi.org/10.1016/j.foodchem.2010.04.044
- **Mrkìc V., Cocci E., Dalla Rosa M., Sacchetti G. (2006).** Effect of drying conditions on bioactive compounds and antioxidant activity of broccoli (*Brassica oleracea* L.). J. Sci. Food Agric. 86, 1559–1566 http://dx.doi.org/ 10.1002/jsfa.2554
- **Muelchen A.M., Rand R.E., Parke J.L. (1990).** Evaluation of crucifer manures for controlling aphanomyces root rot of peas. Plant Dis. 74, 651–654.
- **Muller H. (1997)**. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. Food research and technology 204,88-94
- **Murcia M.A., Lopez-Ayerra B., Martinez-Tome M., Vera A.M., Garcia-Carmona F. (2000).** Evolution of ascorbic acid and peroxidise during industrial processing of broccoli. Journal of the Science of Food and Agriculture. 80, 1882–1886.
- **Naguib A.E.M.M., El-Baz F.K., Salama Z.A., Hanaa H.A.E.B., Ali H.F., Gaafar A.A. (2012).** Enhancement of phenolics, flavonoids and glucosinolates of broccoli (*Brassica oleracea* var. *italica*) as antioxidants in response to organic and bio-organic fertilizers. J of the Saudi Society of Agric Science. 11, 135-142.
- **Ng Z.X., Chai J.W., Kuppusamy U.R. (2011).** Customized cooking method improves total antioxidant activity in selected vegetables. Intern. J. of Food Sci and Nutrition. 62, 158-163.
- **Nicoletto C., Santagata S., Pino S., Sambo P. (2016).** Antioxidant characterization of different italian broccoli landraces. Hortic. Bras. 34, 074-079. doi-http://dx.doi.org/10.1590/S0102-053620160000100011.
- **Nicoli M.C., Anese M., Parpinel M. (1999).** Influence of processing on the antioxidant properties of fruit and vegetables. Trends in Food Science & Technology. 10, 94-100. P I I : S0924-2244(99)00023-0
- **Nicoli M.C., Anese M., Parpinel M.T., Franceschi S., Lerici C.R. (1997).** Loss and/or formation of antioxidants during food processing and storage. Cancer Lett. 114 (1e2), 71-74. https://doi.org/10.1016/S0304-3835(97)04628-4
- **Nilsson J., Olsson K., Engqvist G., Ekvall J., Olsson M., Nyman M., Åkesson B. (2006).** Variation in the content of glucosinolates, hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular-weight carbohydrates in Brassica vegetables. J. Sci. Food Agric. 86, 528–538.
- **Oikonomopoulou V.P., Krokida M.K. (2013**). Novel aspects of formation of food structure during drying. Dry. Technol. 31, 990-1007.

- **Oliveira S.M., Ramos I.N., Brandão T.R.S., Silva C.L.M. (2015).** Effect of air-drying temperature on the quality and bioactive characteristics of dried galega kale (*Brassica oleracea* L. var. *acephala*). J. Food Process. Preserv. 39, 2485–2496 https://doi.org/10.1111/jfpp.12498
- **Olsen H., Grimmer S., Aaby K., Saha S., Borge A. (2012).** Antiproliferative Effects of Fresh and Thermal Processed Green and Red Cultivars of Curly Kale (*Brassica oleracea* L. convar. *acephala* var.*sabellica*). J Agric. Food Chem., 60, 7375−7383. dx.doi.org/10.1021/jf300875f.
- **Ou B.X., Huang D.J., Hampsch-Woodill M., Flanagan J.A., Deemer E.K. (2002).** Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J of Agric and Food Chem. 50, 3122-3128.
- **Padilla G, Cartea ME, Velasco P, de Haro A, Ordàs A. (2007)** Variation of glucosinolates in vegetable crops of Brassica rapa. Phytochemistry 68:536–545.
- **Pant A.P., Radovich T.J.K., Hue N.V., Talcott S.T., Krenek K.A. (2009).** Vermicompost extracts influence growth, mineral nutrients, phytonutrients and antioxidant activity in pak choi (*Brassica rapa* cv. *bonsai*, *Chinensis* group) grown under vermicompost and chemical fertiliser. J of the Sc of Food and Agric. 89, 2383-2392.
- **Pasini F., Verardo V., Cerretani L., Cabonia M.F., D'Antuono L.F. (2011).** Rocket salad (*Diplotaxis* and *Eruca* spp.) sensory analysis and relation with glucosinolate and phenolic content. J Sci Food Agric. 91, 2858–2864. DOI 10.1002/jsfa.4535
- **Pék Z., Daood H., Nagyné M.G., Neményi A., Helyes L. (2013).** Effect of environmental conditions and water status on the bioactive compounds of broccoli. Central European J of Biology. 8, 777-787.
- **Pellegrini N., Chiavaro E., Gardana C., Mazzeo T., Continuo D., Gallo M.,Riso P., Fogliano V., Porrini M. (2010).** Effect of different cooking methods on color, phytochemical concentration, and antioxidant capacity of raw and frozen Brassica vegetables. Journal of Agricultural and Food Chemistry. 58, 4310-4321. DOI:10.1021/jf904306r
- **Pereira F.M.V., Rosa E., Fahey J.W., Stephenson K.K., Carvalho R., Aires A. (2002).** Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. *italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. J of Agric and Food Chem. 50, 6239-6244.
- **Perez-Balibrea S., Moreno D.A., Garcia-Viguera C. (2011).** Genotypic effects on the phytochemical quality of seeds and sprouts from commercial broccoli cultivar. Food Chem. 125, 348-354.
- **Piironen, V., Syvaoja, E.-L., Varo, P., Salminen, K., & Koivistoinen, P. (1986).** Tocopherols and tocotrienols in Finnish foods: Vegetables, fruits, and berries. Journal of the Agriculture and Food Chemistry, 34, 742–746.
- **Podsedek A. (2007).** Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT- Food Sci. and Tech.. 40. 1-11 https://doi.org/10.1016/j.lwt.2005.07.023.
- **Pogson B.J., Morris S.C. (1997).** Consequences of cool storage of broccoli on physiological and biochemical changes and subsequent senescence at 20°C. J. Amer. Soc. Hort. Sci. 122(4), 553-558.

- **Puupponen-Pimia R., Hakkinen S.T., Aarni M., Suortti T., Lampi A.M., Eurola M., et al. (2003).** Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. J of the Science of Food and Agric. 83, 1389–1402.
- **Radošević K., Srček V.G., Bubalo M.C., Brnci S.R., Takács K., Redovnikovi I. (2017).** Assessment of glucosinolates. antioxidative and antiproliferative activity of broccoli and collard extracts. Journal of Food Composition and Analysis. 61, 59–66. https://doi.org/10.1016/j.jfca.2017.02.001.
- **Ragusa L., Branca F., Licciardello F., Restuccia C., Melilli M.G., Argento S., Raccuia S.A. (2013).** Development of Modified Atmosphere Packages on the Quality of Sicilian Kale (Brassica oleracea var. acephala) Shoots. Acta Hort. 1005, ISHS 2013
- **Ragusa L., Picchi V., Tribulato A., Cavallaro C., Lo Scalzo R., Branca F. (2016).** The effect of the germination temperature on the phytochemical content of broccoli and rocket sprouts. Int J Food Sci Nutr. 2017; 68(4):411-420. doi: 10.1080/09637486.2016.1248907
- **Ratti C. (2001).** Hot air and freeze-drying of high-value foods: a review. J. Food Eng. 49 (4). 311-319 https://doi.org/10.1016/S0260-8774(00)00228-4
- **Renaud E.N.C., Lammerts Van Bueren E.T., Myers J.R., Paulo M.J., Van Eeuwijk F.A., Zhu N., Juvik J.A. (2014).** Variation in broccoli cultivar phytochemical content under organic and conventional management systems: implications in breeding for nutrition. PloS One 9, e95683.
- **Rice-Evans C., Sampson J., Bramley P.M., Holloway, D.E. (1997).** Why do we expect carotenoids to be antioxidants in vivo. Free Radical Research, 26, 381–398.
- **Robbins R.J., Keck A.S., Banuelos G., Finley J.W. (2005).** Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. J. Med. Food 8, 204–214.
- **Rodrigues A.S. and Rosa E.A.S. (1999)**. Effect of postharvest treatments on the level of glucosinolates in broccoli. J Sci Food Agric. 79, 1028–1032.
- **Rodriguez-Hernandez M., Moreno D.A., Carvajal M., Garcìa-Viguera C., Martìnez-Ballesta M. (2012).** Natural Antioxidants in Purple Sprouting Broccoli under Mediterranean Climate. Journal of Food Science Vol. 77, Nr. 10,. doi: 10.1111/j.1750-3841.2012.02886.x
- **Rodriguez-Hernandez M.C., Moreno D.A., Carvajal M., Martìnez-Ballesta M.C. (2014).** Genotype influences sulfur metabolism in broccoli (*Brassica oleracea* L.) under elevated CO2 and NaCl stress. Plant and Cell Physiology. 55, 2047-2059.
- **Rodríguez-Mateos A., Vauzour D., Krueger C.G., Shanmuganayagam D., Reed J., Calani L., Mena P., Del Rio D., Crozier A. (2014).** Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Archives of Toxicology. 88, 1803–1853.
- **Rosa E.A.S. (1997).** Glucosinolates from flower buds of Portuguese Brassica crops. Phytochemistry 44: 1415–1419.
- **Rosa E.A.S., Heaney R.K. (1993).** The effect of cooking and processing on the glucosinolate content: studies on four varieties of Portuguese cabbage and hybrid white cabbage. J Sci Food Agric. 62, 259–265.
- **Rosa E.A.S., Heaney R.K. (1996).** Seasonal variation in protein, mineral and glucosinolate composition of Portuguese cabbage and kale. Anim Feed Sci Technol 57:111–127.

- **Rosa E.A.S., Heaney R.K., Fenwick G.R., Portas C.A.M. (1997).** Glucosinolates in crop plants. Horticultural Rev 19: 99–215.
- **Rosa E.A.S., Rodrigues A.S. (2001).** Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late season. Hortscience 36, 56-59.
- **Rose P., Huang Q., Ong C.N., Whiteman M. (2005)**. Broccoli and watercress suppress matrix metalloproteinase-9 activity and invasiveness of human MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol. 209, 105–113.
- **Rosen C.J., Fritz V.A., Gardner G.M., Hecht S.S., Carmella S.G., Kenney P.M. (2005).** Cabbage yield and glucosinolate concentrations as affected by nitrogen and sulfur fertility. HortScience, 40, 1493–1498.
- **Roy M.K., Juneja L.R., Isobe S., Tsushida T. (2009).** Steam processed broccoli (*Brassica oleracea*) has higher antioxidant activity in chemical and cellular assay systems. Food chem. 114, 263-269.
- **Rybarczyk A., Wold B.A., Hansen M.K., Hagen S.F. (2014).** Vitamin C in broccoli (Brassica oleracea L. var. italica) flower buds as affected by postharvest light, UV-B irradiation and temperature. Postharvest Biology and Technology 98:82–89. DOI: 10.1016/j.postharvbio.2014.06.017
- **Sablani S.S., Andrews P.K., Davies N.M., Walters T., Saez H., Bastarrachea L. (2011).** Effects of air and freeze drying on phytochemical content of conventional and organic berries. Dry. Technol. 29 (2), 205e216.
- **Šamec D., Piljac-Žegarac J., Bogović M., Habjanič K., Grùz J. (2011)** Antioxidant potency of white (Brassica oleracea L. var. capitata) and Chinese (Brassica rapa L. var. pekinensis (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. Scientia Horticulturae 128 78–83. doi:10.1016/j.scienta.2011.01.009.
- **Sasaki H., Ichimura K., Okada K., Oda M. (1998).** Freezing tolerance and soluble sugar contents affected by water stress during cold-acclimation and de-acclimation in cabbage seedlings. Sci. Hortic. 76, 161–169.
- **Schmidt S., Zietz M., Schreiner M., Rohn S., Kroh L.W., Krumbein A. (2010).** Genotypic and climatic influences on the concentration and composition of flavonoids in kale (Brassica oleracea var. sabellica). Food Chem. 119, 1293-1299
- **Schonhof I., Klaring H.P., Krumbein A., Claußen W., Schreiner M. (2007).** Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agriculture, Ecosystems Environment. 119, 103-111.
- **Schonhof I., Krumbein A., Brückner B. (2004).** Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. Nahrung/Food 48, 25 – 33. DOI: 10.1002/food.200300329
- **Schreiner M., Huyskens-Keil S., Peters P., Schonhof I., Krumbein A., Widell S. (2002).** Seasonal climate effects on root colour and compounds of red radish. J. Sci. Food Agric. 82, 1325–1333.
- **Schreiner M., Krumbein A., Knorr D., Smetanska I. (2011).** Enhanced glucosinolates in root exudates of *Brassica rapa* ssp. *rapa* mediated by salicylic acid and methyl jasmonate. Journal of Agricultural and Food Chemistry. 59, 1400–1405.

- **Seong G.U., Hwang I.W., Chung S.K. (2016).** Antioxidant capacities and polyphenolics of Chinese cabbage (Brassica rapa L. Ssp. Pekinensis) leaves. Food Chem. 199/ 612-618. DOI: 10.1016/j.foodchem.2015.12.066
- **Serrano M., Martìnez-Romero D., Guillen F., Castillo S., Valero D. (2006).** Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Postharvest Biology and Technology. 39, 61-68.
- **Shofian N.M., Hamid A.A., Osman A., Saari N., Anwar F., Dek M.S.P., Hairuddin M.R. (2011).** Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. Int. J. Mol. Sci. 12 (7), 4678-4692.
- **Sikora E., Cieslik E., Leszczynska T., Filipiak-Florkiewicz A., Pisulewski P.M. (2008).** The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. Food Chem. 107, 55-59.
- **Singh J., Upadhyay A.K., Prasad K., Bahadur A., Rai M. (2007).** Variability of carotenes. vitamin C. E and phenolics in Brassica vegetables. J. Food Compost. Anal.. 20 (2). 106–112 https://doi.org/10.1016/j.jfca.2006.08.002
- **Singh S., Sinha S., (2005).** Accumulation of metals and its effects in *Brassica juncea* L. Czern. (cv. Rohini) grown on various amendments of tannery waste. Ecotox. Environ. Safe. 62, 118-127.
- **Sisti M., Amagliani G., Brandi G. (2003).** Antifungal activity of *Brassica oleracea* var. *botrytis* fresh aqueous juice. Fitoterapia. 74, 453–458.
- **Slinkard K., Singleton V.L. (1977).** Total phenol analysis: automation and comparison with manual methods. Amer J Enol and Vitic. 28, 49–55.
- **Snowdon R., Lühs W., Friedt W. (2007**). Oilseed rape. Oilseeds 2, 55–114.
- **Soengas P., Sotelo T., Velasco P., Cartea M.E. (2011).** Antioxidant Properties of *Brassica* Vegetables. *Functional Plant Science and Biotechnology* 43-55
- **Sones K., Heaney R.K., Fenwick G.R. (1984).** The glucosinolate content of UK vegetables: cabbage (Brasica oleracea), swede (B. napus) and turnip (B. campestris). Food Additive and Contaminants 3:289–296
- **Song L.J., Thornalley P.J. (2007)**. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. Food Chem. Toxicol. 45, 216–224.
- **Sotelo T., Cartea M.E., Velasco P., Soengas P. (2014).** Identification of Antioxidant Capacity - Related QTLs in Brassica oleracea. PLoS ONE 9(9): e107290. https://doi.org/10.1371/journal.pone.0107290
- **Sousa C., Lopes G., Pereira D.M., Taveira M., Valentao P., Seabra R.M., Pereira J. A. (2007).** Baptista, P.; Ferreres, F.; Andrade,P. B. Screening of antioxidant compounds during sprouting of Brassica oleracea L. var. costata DC. Comb. Chem. High Throughput Screen. 10, 377–386
- **Sousa C., Pereira D.M., Pereira J. A., Bento A., Rodrigues M.A., Dopico-Garcìa S., Valentao P., Lopes G., Ferreres F., Seabra R.M., Andrade P.B. (2008).** Multivariate analysis of tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) phenolics: influence of fertilizers. J of Agric and Food Chem. 56, 2231-2239.

- **Stampfer M.J., Rimm E.B. (1995).** Epidemiologic evidence for vitamin E in prevention of cardiovascular disease. American Journal of Clinical Nutrition, 62, 1365–1369
- **Stone H., Sidel J.L. (1993).** Sensory Evaluation Practices. Academic. 2. Edition. San Deigo.
- **Stone H., Sidel J.L. (2004).** Introduction to Sensory Evaluation, Editor(s): Herbert Stone, Joel L. Sidel, In Food Science and Technology, Sensory Evaluation Practices (Third Edition), Academic Press. Pages 1-19, ISBN 9780126726909, https://doi.org/10.1016/B978-012672690-9/50005-6.
- **Tanongkankit Y., Chiewchan N., Devahastin S. (2010).** Effect of processing on antioxidants and their activity in dietary fiber powder from cabbage outer leaves. Drying Technology. 28, 1063-1071.
- **Terzo M.N., Pezzino F., Amodeo L., Catalano D., Viola M., Tribulato A., Travali S., Branca F. (2018).** Evaluation of a sicilian black broccoli extract on in vitro cell models. Acta Hortic. 1202. ISHS 2018. Doi 10.17660/ActaHortic.2018.1202.20
- **Thiruvengadam M., Baskar V., Kim S.H., Chung I.M. (2016).** Effects of abscisic acid, jasmonic acid and salicylic acid on the content of phytochemicals and their gene expression profiles and biological activity in turnip (*Brassica rapa* ssp. *rapa*). Plant Growth Regulation. 1–14. doi:10.1007/s10725-016-0178-7
- **Torres-Contreras A.M., Nair V., Cisneros-Zevallos L., and Jacobo-Velázquez D.A. (2017).** Stability of Bioactive Compounds in Broccoli as Affected by Cutting Styles and Storage Time. Molecules 2017, 22, 636; doi:10.3390/molecules22040636.
- **Tulipani S., Mezzetti B., Capocasa, F., Bompadre S., Beekwilder J., Ric De Vos C.H., Capanoglu E., Bovy A., Battino M. (2008).** Antioxidants phenolic compounds and nutritional quality of different strawberry genotypes. J. Agric. Food Chem. 56, 696–704 https://doi.org/10.1021/jf0719959
- **Vale A.P., Cidade H., Pinto M., Oliveira M.B.P.P (2014).** Effect of sprouting and light cycle on antioxidant activity of Brassica oleracea varieties. Food Chemistry 165 (2014) 379–387. http://dx.doi.org/10.1016/j.foodchem.2014.05.122
- **Vallejo F., Tomas-Barberan F., Garcia-Viguera C. (2003a).** Healthpromoting compounds in broccoli as influenced by refrigerated transport and retail sale period. Journal of Agricultural and Food Chemistry, 51, 3029–3034.
- **Vallejo F., Tomas-Barberan F., Garcia-Viguera C. (2003b).** Changes in broccoli (Brassica oleracea L. var. italica) health-promoting compounds with inflorescence development. J. Agric. Food Chem. 51. 3776-3782 https://doi.org/10.1021/jf0212338.
- **Vallejo F., Tomas-Barberan F.A., Garcia-Viguera C. (2002).** Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. J. Sci. Food Agric. 82. 1293-1297 https://doi.org/10.1002/jsfa.1183
- **Van Dam N., Tytgat T., Kirkegaard J., (2009)**. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. Phytochem. Rev. 8, 171-186.
- **Van Eylen D., Oey I., Hendrickx M., Van Loey A. (2007)**. Kinetics of the stability of broccoli (*Brassica oleracea* cv *italica*) myrosinase and isothiocyanates in broccoli juice during pressure/temperature treatments. Journal of Agricultural and Food Chemistry. 55, 2163–2170.
- **VanEtten CH, Daxenbichler ME, Tookey HL, Kwolek WF, Williams PH, Yoder OC (1980)** Glucosinolates: otential toxicants in cabbage cultivars. J Am Soc Hort Sci 105:710–714

- **Vavilov N.I. (1926).** Studies on the origin of cultivated plants. Bull. Appl. Bot. Genet. Pl. Breed., 16, 139-248.
- **Velasco P., Cartea M.E., Gonzalez C., Vilar M., Ordàs A. (2007).** Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). J of Agricultural and Food Chem. 55,955-962.
- **Verkerk R., Dekker M. (2004)**. Glucosinolates and myrosinase activity in red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* DC.) after various microwave treatments. J Agric Food Chem. 52, 7318–7323.
- **Viña S.Z., Olivera D.F., Marani C.M., Ferreyra R.M., Mugridge A., Chaves A.R., Mascheroni R.H. (2007).** Quality of Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) as affected by blanching method, J of Food Engineering. 80, 218-225.
- **Volden J., Bengtsson G.B., Wicklund T. (2009a).** Glucosinolates, L-ascorbic acid, total phenols, anthocyanins, antioxidant capacities and colour in cauliflower (*Brassica oleracea* L.ssp. *botrytis*); effects of long –term freezer storage. Food Chem 112, 967-976.
- **Volden J., Borge G.I.A., Hansen M., Wicklund T. Bengtsson G.B. (2009b).** Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (*Brassica oleracea* L.ssp. *botrytis*). LWT-Food Sci and Tech 42, 63-73.
- **Vrchoska V., Sousa C., Valentao P., Ferreres F., Pereira J.A., Seabra R.M., Andrade P.B. (2006).** Antioxidative properties of tronchuda cabbage (*Brassica oleracea* L. Var. *costata* DC) external leaves against DPPH, superoxide radical, hydroxyl radical and hypochlorous acid. Food Chem. 98, 416-425.
- **Wachtel-Galor S., Wong K.W., Benzie I.F.F. (2008).** The effect of cooking on Brassica vegetables. Food Chem. 110, 706-710.
- **Wang G.C., Farnham M., Jeffery E.H. (2012).** Impact of thermal processing on sulforaphane yield from broccoli (*Brassica oleracea* L. ssp. *italica*). Journal of Agricultural and Food Chemistry. 60, 6743–6748.
- **Wang S.Y., Lin H.S., (2000).** Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J Agric Food Chem. 48, 140–146.
- **Wills R.B.H., Rangga A. (1996).** Determination of carotenoids in Chinese vegetables. Food Chem. 56, 451-455.
- **Wu X., Beecher G.R., Holden J.M., Haytowitz D.B., Gebhardt, S.E., Prior, R.L. (2004).** Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agriculture and Food Chemistry, 52, 4026–4037.
- **Wurr D.C.E., Fellows J.R., Phelps K. (1996).** Investigating trends in vegetable crop response to increasing temperature associated with climate change. Sci. Hort. Amsterdam 66, 255–263.
- **Xiong Z.T, Liu C., Geng B. (2006).** Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotox. Environ. Safe. 64, 273-280.
- **Yang J.H., Mau J.L., Ko P.T., Huang L.C. (2000).** Antioxidant properties of fermented soybean broth. Food and Chem. 71, 249-254.
- **Zhang D.L. and Hamauzu Y. (2004).** Phenolics. ascorbic acid. carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem.. 88. 503-509 https://doi.org/10.1016/j.foodchem.2004.01.065

- **Zhang H., Schonhof I., Krumbein A., Gutezeit B., Li L., Stutzel H., Schreiner M. (2008).** Water supply and growing season influence glucosinolate concentration and composition in turnip root (*Brassica rapa* ssp. r*apifera* L.). J of Plant Nutrition and Soil vScience. 171, 255-265.
- **Zhao F., Evans E.J., Bilsborrow P.E., Syers J.K. (1994).** Influence of nitrogen and sulphur on the glucosinolate profile of rapeseed (*Brassica napus*). J. Sci. Food Agric. 64, 295–304.
- **Zhong C.F., Mazzoni L., Balducci F., Di Vittori L., Capocasa F., Giampieri F., Mezzetti B. (2017).** Evaluation of vitamin C content in fruit and leaves of different strawberry genotypes. Acta Hortic. 1156 https://doi.org/10.17660/ActaHortic.2017.1156.56
- **Zhou K., Yu L. (2006).** Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT-Food Science and Tech. 39, 1155-1162.
- **Zhu W.F., Lin X.Y., Jin C.W., Zhang Y.S., Fang P. (2009).** Effects of nitrogen application rates on antioxidant contents and antioxidative activities in Chinese cabbage (*Brassica chinensis* L.). Journal of Zhejiang University, 35, 299–306.

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