

Review

Environmental Conditions and Agronomical Factors Influencing the Levels of Phytochemicals in *Brassica* Vegetables Responsible for Nutritional and Sensorial Properties

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Abstract: Recently, the consumption of healthy foods has been related to the prevention of cardiovascular, degenerative diseases and different forms of cancers, underlying the importance of the diet for the consumer's health. Fruits and vegetables contain phytochemicals that act as protective factors for the human body, through different mechanisms of action. Among vegetables, *Brassica* received a lot of attention in the last years for the phytochemical compounds content and antioxidant capacity that confer nutraceutical value to the product. The amount of healthy bioactive compounds present in the *Brassica* defines the nutritional quality. These molecules could belong to the class of antioxidant compounds (e.g., phenols, vitamin C, etc.), or to non-antioxidant compounds (e.g., minerals, glucosinolates, etc.). The amount of these compounds in *Brassica* vegetables could be influenced by several factors, depending on the genotypes, the environmental conditions and the cultivation techniques adopted. The aim of this study is to highlight the main phytochemical compounds present in brassicas used as a food vegetable that confer nutritional and sensorial quality to the final product, and to investigate the main factors that affect the phytochemical concentration and the overall quality of *Brassica* vegetables.

Keywords: phytochemical compounds; antioxidant capacity; *Brassica* spp.; vegetables; cultivation techniques; glucosinolates

1. Introduction

In recent years, the increasing incidence of cardiovascular, degenerative diseases and different forms of cancers has stimulated the interest of consumers in distinguishing healthy from unhealthy foods, as a consequence of the abandonment of Mediterranean diet which, in contrast to other eating regimes, was considered a model of healthy eating for years. The interest for consuming healthy food led to coining the new definition “functional food” for several foodstuffs. This term defines a food product that, in addition to carrying out the traditional alimentary function, also performs preventive and/or therapeutic effects against various human diseases, in particular chronic-degenerative diseases [1,2]. Fruits and vegetables contain phytochemicals that are responsible for these positive effects on human body.

Among vegetables, brassicas received a lot of attention in the last few years. They comprise a large and diverse group of widely consumed vegetables. *Brassica* is the Latin

name of a genus that is taxonomically placed within the *Brassicaceae* (*Cruciferae*). The main cultivated and most consumed as food vegetables *Brassica* species in the world are indicated in Table 1. Other closely related vegetables within the *Brassicaceae* family are also reported. The healthy potential of *Brassica* is bound to their phytochemical compounds. The main compounds responsible for healthy function are phenolic compounds, vitamins (C, B9, K), provitamin A (β -carotene), lutein and different types of glucosinolates [3]. The increasing interest for *Brassica* vegetables has been underlined by their economic importance (among the top 10 economic crops in the world [4]) and by the fact that there was an increase of about 11.5% of both the cultivated area and the production quantity from 2009 to 2019, with a slight increase in yield of about 1.5%. The Organization for Food and Agriculture of the United Nations (FAO) also reported that, in 2019, the global production of cauliflower, broccoli, cabbage and other Brassica crops was about 97 million tonnes, occupying a cultivated area of almost 4 million hectares. Asia accounts for more than 75% of the global *Brassica* vegetable production, with China producing almost a half of all of these vegetables (45 million tonnes). India, with more than 18 million tonnes, then Korea, Russia, and USA with more than 2 million tonnes, are also the biggest producers of cauliflower, broccoli, cabbage and other Brassica crops [5].

The present review summarizes the main chemical compounds responsible for the sensorial and nutritional quality of *Brassica* spp, with particular emphasis on the factors affecting their level.

Table 1. Main species, subspecies (ssp.) and varieties (var.) of *Brassicaceae* family crops consumed as food vegetable in the world [4,6–8].

Species	ssp./var.	Common Name/Italian Name
<i>Brassica oleracea</i> L.	<i>italica</i>	Broccoli
	<i>capitata</i>	Cabbage
	<i>capitata</i> f. <i>rubra</i>	Red cabbage
	<i>capitata</i> f. <i>alba</i>	White cabbage
	<i>botrytis</i>	Cauliflower
	<i>acephala sabellica</i>	Curly Kale, Red, Green and Russian curly kale
	<i>acephala laciniata</i>	
	<i>acephala</i>	Black Cabbage, Italian or Tuscan cabbage
	<i>gemmifera</i>	Brussels sprouts
	<i>gongylodes</i>	Kohlrabi
	<i>sabauda</i>	Savoy cabbage
	<i>alboglabra</i>	Chinese kale
	<i>costata</i>	Tronchuda cabbage
<i>Brassica rapa</i> L.	<i>rapa</i>	Turnip broccoli
	<i>sylvestris</i>	Turnip top, broccoli raab/cima di rapa, friarielli
	<i>pekinensis</i>	Chinese cabbage
	<i>chinensis</i>	Pak-choi, Chinese mustard
	<i>japonica</i>	Mizuna, curled mustard, Japanese greens
	<i>perviridis</i>	Tendergreen, Spinach mustard
	<i>rapifera</i>	Turnip
<i>Brassica napus</i> L.	<i>napus</i>	Rapeseed
	<i>napobrassica</i>	Swede/rutabaga
<i>Brassica kaber/Brassica arvensis/Sinapis arvensis</i>		Charlock/kaber
<i>Brassica alba/Sinapis alba, Brassica hirta</i>		White or yellow mustard
<i>Brassica nigra/Sinapis nigra</i>		Black mustard
<i>Brassica campestris</i>		Field mustard

<i>Brassica carinata</i>	Ethiopian mustard, Abyssinian mustard, Texsel greens
<i>Brassica juncea</i>	Brown mustards
<i>Raphanus sativus</i> L.	Radish
<i>Raphanus raphanistrum</i> L.	Wild radish
<i>Nasturtium officinale</i> R. BR.	Watercress/Crescione d'acqua
<i>Eruca sativa</i> Mill.	Rocket/Rucola
<i>Eruca vesicaria</i> L.	Rocket Ruca, ruchetta
<i>Diplotaxis tenuifolia</i> L.	Wild rocket/Rughetta selvatica
<i>Diplotaxis muralis</i> L.	Wall rocket

2. Nutritional Quality

Nutritional quality could be defined as the value of the product for the consumer's physical, psychological, or emotional well-being. The first term of this extended definition concerns the effects of food determined by its phytochemicals, i.e., the sum of all beneficial and harmful compounds and their nutritional (or biological) aspects [9]. In the case of *Brassica* spp., these molecules could belong to the class of antioxidant compounds (e.g., phenols, vitamin C, etc.), exerting their health effects through the ability to 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. Antioxidant Compounds

Total antioxidant capacity (TAC) is the ability of food to preserve an oxidizable substrate, inactivate the radical species or reduce an oxidized antioxidant. 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 through different mechanisms and with different efficiency, depending on its chemical structure and the matrix it acts on. For this reason, TAC analysis is usually preferred to the 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 fruit and vegetables.

Brassica vegetables, i.e., broccoli and kale, showed higher antioxidant potential than other vegetable crops, such as spinach, carrots, potatoes, beans and onions. In general, among *Brassica* vegetables, Brussels sprouts, broccoli, and red cabbage belong to the group that has the highest antioxidant capacity. Common cabbage possesses the lowest antioxidant capacity [10,11]. Contrasting results were reported in cauliflower by Azuma et al. [12] and Wu et al. [13]. The analysis of TAC is influenced by the extraction method and the type of reactive species in the reaction mixture [12].

Many researchers studied and identified the main antioxidant molecules present in *Brassicaceae* [13–16]. These antioxidant compounds belong to two main groups: water-soluble antioxidants and lipo-soluble antioxidants [14,15]. Kurlich et al. [16] and Wu et al. [13] reported that hydrophilic antioxidants are responsible for 80–95% of TAC in *Brassicaceae*, while lipo-soluble antioxidants account for only 5–20%.

2.1.1. Water-Soluble Antioxidants

- Phenolic compounds

Phenolic compounds are the most widespread antioxidant family present in vegetables. This large group of compounds is particularly present in *Brassica* vegetables and constitutes the main source of antioxidants in these plants [14,17]. These plants produce them as secondary metabolites for protection from pest and insect attack.

Their importance in human health is related to antioxidant and anti-inflammatory properties that could have preventive and/or therapeutic effects against obesity, cancer,

and neurodegenerative and cardiovascular diseases [18]. Among *Brassica* species, kale and broccoli have the highest quantity of total polyphenols with about 13 mg gallic acid/g of dry weight [19,20].

Flavonoids represent common phenolic compounds in *Brassica*; they possess a lot of biological properties, e.g., antioxidant activity, a capillary protective effect, and an inhibitory effect elicited in various stages of tumours [14,21]. They are characterized by numerous subclasses, but the most important in *Brassica* 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 parts of leaves, seeds, shoots and sprouts leaves [22,23]. The most represented flavonols in *Brassica* vegetables are quercetin (up to 23 mg/100 g fresh product in kale), kaempferol (up to 47 mg/100 g fresh product in kale) and isorhamnetin (up to 24 mg/100 g fresh product in kale) [24]. Quercetin, found mainly in kale, is characterized by a strong antioxidant power (higher than vitamin C); it exerts its activity against free oxygen radicals and acts on the prevention of cardiovascular diseases and cancer, atherosclerosis and chronic inflammation, and the induction of enzymes that detoxify carcinogens [25,26]. 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 [27]. Kaempferol and quercetin, and in less amounts, myricetin, are the main represented flavonols in *B. rapa* subsp. *sylvestris*.
2. Flavones: Apigenin and luteolin are the only flavones detected in hydrolysed extracts of different *Brassica* vegetables (up to 45 and 12 µg/g of fresh weight in Chinese cabbage, respectively), excluding broccoli, where they were not detected [28].
3. Anthocyanins were detected in *Brassica* vegetables and described by several authors [13,29]. They are present only in bright coloured species and varieties with red, orange and purple pigmentation, such as 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 eight main types of anthocyanins (for a total of up to 190 mg Cyanidin-3-Glucoside equivalents/100 g of fresh weight) [30]; cyanidin-3-diglucoside is the most represented [31]. In broccoli, more than 17 anthocyanins were detected [29].

Among phenolic compounds, even if they are not hydro-soluble, it is worth mentioning 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 [16]. Some studies reported that lignans are mainly present in the kale family, broccoli and Brussel sprouts with lariciresinol (972, 599 and 493 µg/100 g fresh edible weight of Broccoli, Curly kale and Brussel sprouts, respectively) and pinioresinol (315, 1691 and 220 µg/100 g fresh edible weight of Broccoli, Curly kale and Brussel sprouts, respectively) being the most abundant [15,32].

- Vitamin C and vitamin B9 (Folic Acid)

Vitamin C, or ascorbic acid, is a powerful antioxidant, widely present and studied in fruits; however, many recent works have been focused on the importance of vitamin C in vegetables, mostly in *Brassicaceae* family. In *Brassica* vegetables vitamin C concentration varies a lot among species and subspecies, and it is strictly genotype- and environment-dependent [33,34]. Vitamin C performs countless biological activities in the 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 [33,35].

Among *Brassica* genotypes, Brussel sprouts (76–192 mg/100 g edible portion) and kale (92–186 mg/100 g edible portion) seem to possess the highest content of vitamin C,

followed by broccoli (34–146 mg/100 g edible portion) and cauliflower (17–81 mg/100 g edible portion), while white cabbage (19–47 mg/100 g edible portion) possesses the lowest amount [14].

Vitamin B9 (Folic acid) is an important vitamin present in *Brassica*, mainly in raw broccoli (63 µg/100 g of edible portion), Brussel sprouts (61 µg/100 g of edible portion) and kale (141 µg/100 g of edible portion) [36], that act 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 in the prevention of megaloblastic anaemia, neuropsychiatric disorders and various forms of cancer in the foetus during pregnancy, also reducing the risk of neural tube defects [33,37]. These beneficial effects of folic acid, in particular on the pathogenesis of cancer, and neurological, haematological, and cardiovascular diseases may, in part, be due to its antioxidant activity, via its electron-accepting capacity [38,39].

2.1.2. Lipo-Soluble Antioxidants

Despite the low incidence of lipo-soluble antioxidants on the TAC of *Brassica*, several studies confirm the high content of lipo-soluble antioxidant in kale and broccoli, moderate in Brussels sprouts, and low amount in cauliflower and cabbage [33]. Among lipo-soluble antioxidants, carotenoids and vitamin E are the most important found in *Brassica* vegetables.

- Carotenoids

Carotenoids are responsible for the orange, yellow and red pigmentation of several fruits and vegetables, mainly carotenes and xanthophylls. The most represented carotenoids in *Brassica* vegetables are β -carotene, which the organism transforms to vitamin A, and lutein and zeaxanthin [14]. β -carotene prevents the insurgence of cancer and cardiovascular diseases, and decreases the risk of myocardial infarction, of immune dysfunction and age-related macular degeneration among smokers [33,40]. Muller [41] 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 the *Brassica oleracea* genus, kale possesses the highest content of carotenoids with over 10 mg/100 g of the edible portion [41].

The *Brassica* vegetable with the highest content of lutein and zeaxanthin is kale (3.04–39.55 mg/100 g); interesting contents were also found in broccoli and Brussels sprouts [14]. In *B. rapa* species, 16 carotenoids were identified by Wills and Rangga [42]; in *B. chinensis*, *parachinensis* and *pekinensis*, lutein and β -carotene are the most abundant carotenoids [15].

- Vitamin E

Vitamin E is formed by groups of compounds known as tocopherols and tocotrienols; in detail, α -tocopherol is the main compound found in *Brassica* vegetables, with the exception of cauliflower, that contains mainly γ -tocopherol [14,43]. Vitamin E performs a protective activity against coronary heart disease through the inhibition of LDL oxidation [44]. A high intake of vitamin E helps in the prevention of cancers, cardiovascular diseases, neurological disorders, and inflammatory diseases [33]. The content of vitamin E in *Brassica* species has been studied in the literature, as reported here 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) [43].

2.2. Micro- and Macro-Elements

Macro-elements, also called macronutrients, are those nutrients that the plants need in greater quantities for essential 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), and Selenium (Se), are required by plants in very small quantities and are known as microelements. 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 soil. The high capacity of *Brassicaceae* to accumulate the metals present in the soil led this family to be considered a good heavy metal hyperaccumulator, giving the significant number of genera (11) and species (90) of those kinds of plants belong to *Brassicaceae* family [45].

However, micro- and macro-elements also play important roles 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. Among *Brassicaceae*, kale is the richest in almost all the main macro and micro elements, with a particularly high amount of calcium (95–539 mg/100 g of edible portion), magnesium (20–67 mg/100 g of edible portion), phosphorus (13–92 mg/100 g of edible portion), potassium (20–491 mg/100 g of edible portion), zinc (0.3–0.9 mg/100 g of edible portion), iron (0.4–3.1 mg/100 g of edible portion), manganese (0.4–1.9 mg/100 g of edible portion), copper (0.02–1.03 mg/100 g of edible portion), and selenium (0–0.94 mg/100 g of edible portion). Mustard green is the richest in iron (1.64 mg/100 g of edible portion), Turnip in sodium (67 mg/100 g of edible portion) and Broccoli in selenium (2.5 mg/100 g of edible portion) [36,46].

2.3. Glucosinolates (GLS) and Isothiocyanates (ITCS)

Glucosinolates (GLS) are one of the most important secondary metabolites in *Brassicaceae* derived from amino acid biosynthesis [14,47]. GLS are glucosidic compounds containing sulphur, present in *Brassica* leaves, compartmentalized in the vacuole, at concentrations that are able to prevent the development of pathogens, diseases and pests [48]. Their concentrations vary among *Brassica* species [49], according to the developmental stage, tissue type, exposure to salt stress, environmental factors, or plant signalling molecules, including treatment with salicylic acid (SA), jasmonic acid (JA) and methyl-jasmonic acid (MeJA) [50–52]. However, their amount generally ranges from the 4.7–32.2 mg/100 g of Mustard spinach, 8.7–12.8 mg/100 g of Rocket, 9.7–33.7 mg/100 g of Chinese cabbage, up to the 65.4–151.1 mg/100 g of Kale, 149.4 mg/100 g of Chinese broccoli and 87.6–332.8 mg/100 g of Radish [53,54]. GLS can be divided into three chemical classes: arylaliphatic, indole and aliphatic, based on their amino acid precursor (aromatic amino acid, tryptophan and methionine, respectively) [55], as reported in Table 2. In *Brassica* vegetables, the most important GLS belong to the methionine-derived ones [56]. Some authors declared that the most popular food processing methods, such as boiling, blanching, and steaming, can significantly affect the final content of GLS. A mild-processing technique, such as blanching, is recommended in order to minimize the loss of GLS or their derivatives [57].

Table 2. Principal glucosinolates identified in leaves of *Brassica* vegetable crops.

Crop	Aliphatic Glucosinolates								Indole Glucosinolates					Arylaliphatic Glucosinolates	
	GIB	PRO	SIN	GAL	GRA	GNA	GBN	GIV	GER	GNL	GBS	NGBS	4HGBS	4MGBS	GST
<i>Brassica oleracea</i>															
White cabbage [58–61]	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Savoy cabbage [58–61]	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+
Red cabbage [58,60,61]	+	+	+	-	+	+	-	+	-	-	+	+	-	-	-
Kale [58,60–62]	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+
Collard [62]	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-
Tronchuda cabbage [60,63]	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Broccoli [62,64]	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Brussel sprouts [61,62,64]	+	+	+	-	+	+	-	+	-	-	+	+	-	-	-
Cauliflower [61,64]	+	+	+	-	+	-	-	+	-	-	+	+	-	-	-
Kohlrabi [61]	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-
<i>Brassica rapa</i>															
Turnip [59]	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+
Turnip greens [65]	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+
Turnip tops [66]	+	+	-	-	-	+	+	+	-	-	+	+	+	-	+
Chinese cabbage [59]	+	+	-	-	-	+	+	-	-	+	+	+	-	+	+
<i>Brassica napus</i>															
Swede [59]	-	+	-	-	+	-	+	-	-	+	+	+	+	+	+
Leaf rape [67]	-	+	-	+	-	+	+	+	-	+	+	+	-	+	+

GIB: glucoiberin (3-methylsulfinylpropyl GSL); PRO: progoitrin ((R)-2-hydroxybut-3-enyl GSL); SIN: sinigrin (prop-2-enyl GSL); GAL: glucoalysiin (5-methylsulphinylpentyl GSL); GRA: glucoraphanin (4-methylsulphinybutyl GSL); GNA: gluconapin (but-3-enyl GSL); GBN: glucobrassicinapin (pent-4-enyl GSL); GIV: glucoiberin (3-methylsulfinylpropyl GSL); GER: glucoerucin (4-methylsulfinylbutyl GSL); GNL: gluconapoleiferin (2-hydroxypent-4-enyl GSL); GBS: glucobrassicin (indol-3-ylmethyl GSL); NGBS: neoglucobrassicin (1-methoxyindol-3-ylmethyl GSL); 4HGBS: 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl GSL); 4MGBS: 4-methoxyglucobrassicin (4-methoxyindol-3-ylmethyl GSL); GST: gluconasturtiin (2-phenylethyl GSL).

GLS have no direct functions to human health: the health effects are exerted by their hydrolysis breakdown products, the isothiocyanates (ITCs). These 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 hydrolyses 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 the action of myrosinase produced by the human gut flora. Unfortunately, its activity and efficiency are lower than plant myrosinase [68,69]. It is possible to obtain many ITCs, and their production strictly depends on 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 [52,70].

ITCs are mainly responsible for the bitterness, and spicy and typical aroma and smell of *Brassica* vegetables [63]. They possess protective and preventing effects against several kinds of cancer e.g., prostate, intestinal, liver, lung, breast, and bladder, chronic inflammation and neurodegeneration, acting on the apoptotic phase of cell developmental cycle; they are also effective in the reduction in cholesterol [19,71,72].

The most studied ITCs in medical research is sulforaphane [73,74], mainly represented in broccoli and Brussel sprout. It is the most important ITCs considering its health benefits, it derives from the glucoraphanin [75]. Sulforaphane is an indirect antioxidant, because it acts as a catalyst in the stimulation of cellular antioxidant system. In particular, sulforaphane stimulates some enzymes active against tumoral cell proliferation [65,66].

3. Sensorial Quality

The quality of vegetables for the consumer not only concerns the nutritional aspects, but also includes the sensorial parameters that can be defined by several indicators.

The principal sensorial parameters are:

- *Firmness*, which indicates the resistance of vegetables to mechanical damages; it assumes a great importance during the post-harvest management.
- *Colour*, which indicates the freshness of the product and the quality of the storage conditions; it visually attracts the consumers [76].
- *Sweetness*, which is linked to the presence of glucose, fructose, and sucrose, and provides the sweet sensation to the consumer.
- *Acidity*, which indicates the acid sensation that the product stimulates in the consumer.

Considering *Brassica* vegetable quality, fundamental sensorial parameters are those related to aroma and taste. All of these parameters can be investigated through analytical measurements or the implementation of a panel test.

3.1. *Brassica* Aroma

The typical aroma is one of the main reasons for the consumers' rejection against *Brassica* vegetables [77]. Raw vegetables are rich in aroma compounds, which are usually produced because of enzymatic reactions. The typical sulphurous and pungent odour of *Brassicaceae* crops are often attributed to GSL/ITC content. These traits predominantly stem from sulphur-compound degradation products, such as from S-methyl-L-cysteine sulfoxide (SMCSO) [78], and formation can be facilitated by factors, such as bacterial metabolism, plant senescence, cooking, and enzymatic breakdown because of tissue damage (e.g., cutting) [79–81]. Sulphides are generally undesirable odour attributes [82], and compounds such as methanethiol, dimethyl sulphide (DMS), dimethyl trisulphide (DMTS), and dimethyl disulphide (DMDS) are regularly linked with sulphurous aromas and overcooked off-flavours. The main responsible of the fresh cabbage odour is the allyl isothiocyanate, a hydrolysis product of sinigrin thanks to the action of myrosinase [83,84]. Additionally, green note is a particularly important characteristic to recognize in *Brassica*

and is conferred by alcohols and aldehydes formed by the enzymatic degradation of free fatty acids [83].

Cooking is the main adopted form to eat *Brassica*, because makes those vegetables more easily digestible and causes a flavour change in them, increasing the consumers' acceptance [85]. Reductions in alcohols, aldehydes and nitriles concentration were reported in cooked *Brassica*, as well as of the sulphides amount (except in broccoli) [85]. The concentration of isothiocyanate was found to increase after cooking [85]. Additionally, the storage of vegetables in frozen form could impact their volatile profile, in particular, influencing the alcohol, aldehydes, and isothiocyanates content [85].

3.2. *Brassica* Taste

As previously stated, *Brassica* vegetables contain health-related compounds that possess undesirable sensory characteristics. Bitterness is particularly accentuated in *Brassica*; this sensation is caused by ITCs that derive from sinigrin, gluconapin, progoitrin, glucobrassicin, neoglucobrassicin at different intensities [86,87]. Many studies identified the relation between the bitter taste and sinigrin and goitrin in cooked Brussels sprout, and between bitterness and sinigrin and neoglucobrassicin in cooked cauliflower [88]. Several studies affirm that the GLS and their breakdown products are not the only ones responsible for the bitter taste and *Brassica* aroma, but these resulted from a synergistic activity of various phytochemicals (indole hydrolysis products, flavonoids, etc.) [65,89].

The overall taste of *Brassica* vegetables is not only linked to bitter compounds but derives from the interaction between the bitter and the sweet tastes [88]. Some evidence demonstrated how the taste is the main driver of liking a food product [90,91], and that there is an innate preference for sweet taste in respect to bitter and sour taste [92,93]. This explains why a bitter taste in vegetables could deter most consumers from buying them. Some studies demonstrated that consumers prefer *Brassica* with low amounts of bitter GLS and higher concentrations of sucrose and, more generally, that the sweet taste is a favourable characteristic for the consumer's appreciation of *Brassica* [87,91].

4. Factors Influencing the Phytochemical Compounds of *Brassica* Vegetables

The quality of the final product can be influenced by several factors such as genetic, environmental, and agricultural (Figure 1).

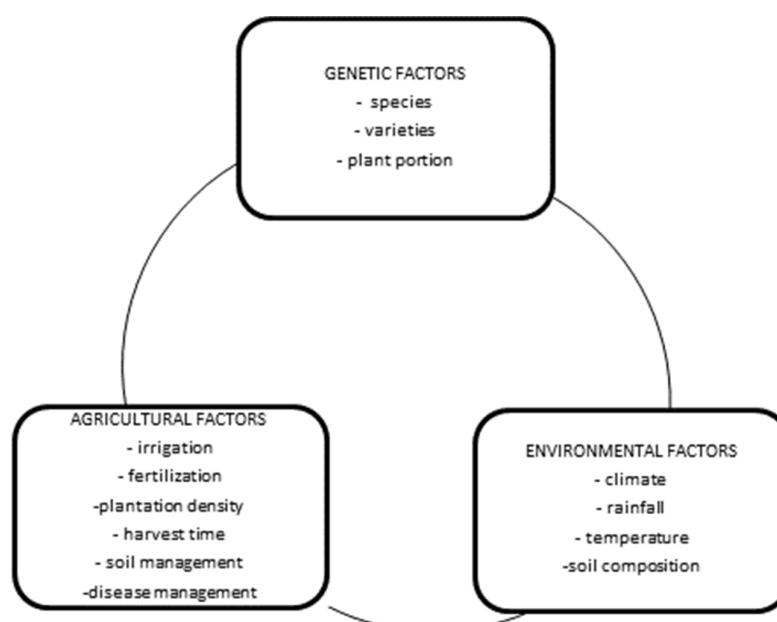


Figure 1. Factors that influence the quality of the final product.

4.1. Genetic Factors

As for all other crops, *Brassica* quality is influenced by several factors, but the principal is represented by the genotype characteristics. Many breeding programs are working towards the creation and selection of new better productive and qualitative genotypes. These programs are particularly implemented in the Mediterranean Area, where many spontaneous and wild *Brassica* provide genetic diversity and variability, allowing for the development of new pre-breeding and advanced breeding materials. Particularly in Italy and Spain, it is possible to find countless ecotypes and populations of *Brassica oleracea* and *Brassica rapa* species, handed down by generations of farmers [49].

These two species have been widely studied and showed a wide diversity in terms of nutritional quality.

4.1.1. *Brassica Oleracea* Species

Kale could be considered the ancestor of several *B. oleracea* vegetable crops because it has been found to be very similar to the *B. oleracea* wild type and to several wild *Brassica* species ($n = 9$) [94].

Several differences among varieties within this species 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 [95]. Although the methodologies of analysis used in many studies were different, all of them agree on the lower content of phytochemicals in white cabbage, in respect to broccoli, Brussel sprouts, curly kale and red cabbage. There are controversial results regarding cauliflower as it showed high activity in liposomal phospholipid suspension system, but low activity in oxygen radical absorption capacity (ORAC method) [14,15].

As mentioned above, the variability is also expressed among genotypes of the same species in broccoli [96], cauliflower [97], cabbage [26] depending on their characteristics; in general, the higher content of antioxidants is detected in the varieties with red or purple pigmentation. Broccoli is important for its cancer-protective compounds; in particular, for its content of glucoraphanin, and its active form sulforaphane. Sicilian landraces of violet cauliflower could be considered an environmentally friendly crop, being characterized by high plant rusticity and adaptability to the Mediterranean climatic condition that allows one to limit the use of pesticides and fertilizers for its cultivation [98].

4.1.2. *Brassica rapa* Species and Other Cruciferous Crops

Brassica rapa species include turnip tops and leaves (“cima di rapa” and “friariello”), turnip, pak choi, Chinese cabbage, choy-sum and mizuna, evidencing a wide variability among close species [99] and varieties of the same species [100]. Phenolic compounds are mainly affected by the interaction between environment and genotype; this means that their variability strictly depends on the environmental conditions, hence they possess low heritability.

Choy sum, a *Brassica rapa* variety, showed the highest antioxidant potential compared to broccoli, cabbage, and cauliflower [101]. Some studies found that watercress showed a higher antioxidant potential in comparison to salad rocket; however, these two varieties, together with wild rocket and mizuna, are good sources of antioxidants [102,103]. In *B. rapa*, the aliphatic glucosinolates (GLS) is the predominant form, with gluconapin as the most abundant, followed by glucobrassicinapin [104]. *B. rapa* varieties have shown a high concentration of isorhamnetin, irrespective of the plant organs considered [105].

4.1.3. Plant Portion and Plant Developmental Stage

The variation in nutritional and phytochemical content does not differ only among species and varieties of the same species, but also can change during the growth period [6] and based on plant portion [50], as reported below.

For most of these crops, the more interesting parts of the plant for 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 capacity than leaves [22]. In turnip, flower buds registered the highest antioxidant content, in respect to leaves, stems and roots [106].

GLS content also differs based on plant portion. Seeds possess the highest concentration of GLS, followed by inflorescences, siliques, leaves, roots, stems and petioles [107]. Indeed, the concentration of aliphatic GLS in kale (*B. oleracea acephala*) leaves increases over 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 [50].

A comparison study on turnip tops and turnip greens also reported several and appreciated differences in phytochemicals compounds. Turnip tops gave a higher GLS value (26.02 $\mu\text{mol/g dw}$) than turnip greens (17.78 $\mu\text{mol/g dw}$). The opposite trend was reported for total phenolic, whereby turnip greens showed a higher content (43.81 $\mu\text{mol/g dw}$) than turnip tops (37.53 $\mu\text{mol/g dw}$) [104].

Several studies confirmed the possibility to detect differences within the same portion of plant. In tronchuda cabbage, the mainly consumed portion are the internal leaves, utilised for salad or cooking; nonetheless, these have an antioxidant capacity lower than the external ones, which are usually discarded [22]. The same results were observed in Chinese cabbage, whereby the variation in bioactive compounds was also evident 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 [108].

The stage of growth can influence the content and concentration of phytochemical compounds in *Brassica*, and the knowledge of this aspect is fundamental in choosing the proper harvesting moment for obtaining products with the highest quality. Indeed, the juvenile cabbage possesses more flavonols than the mature one [26].

Total GLS content also varies in the function of the stage of growth and increases from vegetative to reproductive stages and maturity. Consequently, the highest content is found either in flower buds, or in leaves harvested at the optimum consumption stage, 180 days after the sowing of kale [50]. In broccoli heads, the highest glucoraphanin content was also observed 180 days after sowing, with a following decline during flowering [109].

Vallejo et al. [110] found an increase in ascorbic acid and phenol compounds during the development of the inflorescence in three broccoli cultivars.

Carotenoids are also affected by the plant developmental stage. In kale, the highest content of lutein was registered in 1- to 2-week-old leaves, and the highest content of β -carotene was found in 2- to 3-week-old leaves [111].

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 the lower antioxidant capacity of the sprouts [112].

4.2. Environmental and Agricultural Factors

Seasonal variation, light exposure, temperature, water availability [113], phytosanitary measures, sowing date and harvesting period [114] are all factors linked to environmental conditions that can influence the quality, in particular nutritional content and profile, of *Brassica* vegetables [6]. Different responses to seasonal variations were reported in several *Brassica* crops, such as broccoli, kale, and turnip [115]; this effect is determined mainly by temperatures and day length during the period before harvest.

Countless studies agree that spring season crops, growing at intermediate temperatures, high light intensity, during longer days and in dry conditions (or low average of rainfall) during their vegetative period, contain an increased total GLS and phytochemicals concentration [50,104,114]. For example, in canola (*Brassica napus*), it was found that GLS concentration increased when a temperature of 40 °C was maintained for 4 h on five successive days, giving a total of 15-degree days of stress (15 DD/40 °C) [116]. Some authors reported that higher and lower temperatures, rather than intermediate temperatures, brought about an increase in GLS concentration, e.g., growing temperatures between 7 °C and 13 °C brought about an increase in glucoraphanin and lutein in broccoli; furthermore, they acted as a trigger for biosynthetic pathways [117]. 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) [118]. The same authors confirm that the main antioxidant content is observed in sprouts that grow with a strong temperature range of 30/15 °C day/night.

Autumn/winter season crops, grown at lower temperature, lower light intensity, shorter days, and higher water availability, tend to have the lowest total GLS and other phytochemicals concentration [119,120]. An exception is represented by a turnip that produces higher flavonoids and vitamin C content in the autumn/winter season; this crop accumulates and produces the main phytochemicals with low/moderate temperature and considerable radiation, mainly in turnip tops [121]. More precisely, in *Brassica rapa*, the correlation with temperature is also bound to the plant portion; indeed, the number of days with a minimum temperature below 0 °C was negatively correlated with total GLS content in turnip greens. In turnip tops, GLS content was positively correlated with the number of days with a maximum temperature above 20 °C. In the case of phenolics, no correlation was found between climatic factors and turnip greens, while in turnip tops, total flavonoids and total phenolics content seemed to be correlated with the number of days with a minimum temperature below 0 and 10 °C, respectively [104]. In broccoli, freezing temperature can positively influence the concentration of sulforaphane [122].

The biotic and abiotic factors that characterize the surrounding environment can influence the quality of *Brassicaceae*. With respect to biotic sphere, aphid infestation brought about an increased production of primary metabolites, including amino acids, as well as some secondary metabolites, as a plant defence mechanism against these pathogens. Concerning abiotic factors, the water stress condition and metal exposure produce an initial increase in photosynthetic pigments, proteins, free amino acids and sugar content, followed by a subsequent decrease [123]. In detail, a relation between copper stress and the production of amino acids was found as free amino acid production takes part in the detoxification from excess copper [124]. In *Brassica juncea*, the accumulation of metals produces a 35% increase in oil content [123]. 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 the case of strong salinity both GLS and phenolics decrease [125]. Seedlings of *Brassica oleracea* L. var. *italica* subjected to water shortage (applied by increasing the time between two irrigation events) showed a decrease in inflorescence chlorophylls, carotenoids, ascorbic acid, total phenols and total soluble carbohydrates [126].

Ragusa and co-author [127] investigated 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 a greater reduction in 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 grown at 30 °C, while the reverse was true in rocket. The antioxidant

capacities increased with germination, and higher indexes were detected at 10 °C, particularly in rocket.

4.2.1. Cultivation System and Soil Composition

The cultivation system influences 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 *Brassica* growth in organic cultivation system than in conventional systems [128], as demonstrated in early harvested tronchuda cabbage [129]. This result could be linked to the fact that, under organic cultivation, crops are subjected to more biotic and abiotic stress; these stressing conditions lead to an increase in the production of secondary metabolites as a defence mechanism, and consequently obtaining vegetables with higher nutritional and antioxidant potential than in a conventional system.

Several studies described an opposite situation and contrasting evidence about phytochemical enhancement in organic vegetables [130,131]. In fact, Conversa et al. [132] reported that the choice of cultivation systems does not modify the antioxidant properties of raw and processed products, but differences can be found in the chlorophyll and carotenoid contents of organic “cima di rapa” landraces. The lipophilic antioxidant content was improved in organic product while the hydrophilic component, which constituted 99% of the total antioxidant capacity, was not affected by the different crop management in “cima di rapa”. However, the 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; on the contrary, the quality declined with a higher production of strong off-odour after 14 days of storage, in comparison to the conventional products.

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 and available potassium; the content can be also influenced by nitrogen and sulphur applications in turnip [104]. On the contrary, in *B. rapa* L. Subsp. *Sylvestris*, flavonols (kaempferol and quercetin derivatives) were reduced by sulphur availability [113].

4.2.2. Water Stress

It was reported that a moderate water stress increases the concentration of bioactive compounds in *Brassica*, partly due to an increased concentration per unit of dry weight; if the stress becomes intensive, the secondary metabolite production should decrease [133]. Phenolic compounds and GLS content increase in the absence of irrigation, because of a reduction in vegetative growth, mainly in turnip, cabbage and broccoli [6]. The association between low availability of water in the soil during plant growth and postharvest cold storage brought about the best maintenance of antioxidant activity in *Brassica*. Water stress conditions also affect sugar content, as it is increased in cabbage [134].

4.2.3. Plant Density, Intercropping and Trap Cropping

Plant density seems to affect the plant morphology and 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 stress on plants which, in turn, stimulates the production of secondary metabolites [120].

Intercropping and trap cropping are strategies utilised for weed and pest control [135]; however, the presence of another crop can generate stressful conditions, such as plant competition for light, nutrients, and water, decreasing their availability and, hence, affecting the accumulation of phytochemicals in *Brassica* plant tissue.

4.2.4. Fertilization Practices

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

Countless studies have been conducted on the effect of sulphur fertilization on phytochemical concentration, mainly on GLS production, considering their sulphurous nature [136,137]. There is a correlation between the increase in sulphur supply and higher levels of total GLS [138], in turnip [136], kale [137] and broccoli, mainly when associated with a reduction in water, at the expense of yield [139]. Vallejo and co-authors [119] 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 thereafter. Increasing sulphur fertilization brought about a positive impact in the synthesis of polyphenols, such as flavonols and phenolic acids, increasing the total antioxidant capacity in turnip top (*B. rapa* ssp. *Sylvestris*) [113], and broccoli [110]. Sulphur fertilization in pre-harvest (from 2.6 mmol/L to 6.5 mmol/L) increases the lipophilic and hydrophilic antioxidant capacity but does not affect the nitrate and chlorophyll contents in ready-to-eat “friariello” product [140]. Sulphur deficiency induced an increased vulnerability of *Brassica* crops to diseases and fungal pathogens [141]. Sulphur fertilization, besides improving the antioxidant activity, it is also associated with a genotype-dependent significant reduction in leaf nitrate content, since it enhances the incorporation of nitrogen into organic compounds and consequently reducing the leaf nitrate concentration [113].

Nitrogen is the main constituent of chlorophyll structure: for this reason, its availability influences the content of carotenoids such as lutein and β -carotene, indeed high $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio led to a higher content of both [142]. Consequently, the colour and pigmentation of leafy vegetables are also improved [132]. Nitrogen fertilisation led to a decrease in the total GLS content [136]; nonetheless, it acts differently according to the type of GLS; in fact, abundant nitrogen applications increase progoitrin and decrease sinigrin concentration in *Brassica napus* [143]. A reduced nitrogen fertilisation generated an increase in the bioactive compound content, mainly phenolics, as nitrogen stress triggers the gene expression of flavonoid pathway enzymes [128]. Combined fertilisation with $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ is the optimal solution to maintain plant growth and increase the total GLS content [144].

An optimal balance between nitrogen and sulphur fertilisation influences the biosynthesis of secondary metabolites [21]. 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 synthesized. Some authors reported the effect of different nitrogen/sulphur combinations on GLS content in *Brassica*, with an increasing amount of nitrogen (80–320 kg/ha) applications. When enough sulphur was available (60 kg/ha), there were no 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 arylaliphatic and aliphatic GLS decreased [138]. Increased nitrogen/sulphur ratio pushes the plants towards the vegetative growth, at the expense of GLS production [136]. Fabek et al. [145] showed that the type of fertilisation may influence 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 in GLS [144].

Similarly, microelements availability can influence the phytochemical concentration in *Brassica*, with salts stress increasing GLS content. In detail, selenium seems to increase the GLS content (in particular sulphuraphane), when applied up to a certain dose; above this level, it decreases the GLS production [51].

Furthermore, some *Brassica* species are used as metal hyperaccumulator, and the type and amount of metal in the soil affects the concentration of glucosinolates in plant tissues. In particular, it was reported that glucosinolate concentrations in roots and shoots of *Thlaspi caerulescens* responded in different way to enhanced Zn accumulation: decreased glucosinolate levels were observed in leaves of plants accumulating high Zn concentrations, while increased levels were detected in roots, with Zn accumulation [146]. Similarly, the content of total glucosinolates, mostly due to indolic glucosinolates as glucobrassicin, was increased only in the roots of Chinese cabbage, when subjected to high soil copper stress [147]. In two *B. juncea* cultivars subjected to high arsenic levels, the increased levels of thiol related proteins, sulphur content and phytochemicals (phenolics and ascorbic acid) in leaves allow us to better tolerate the oxidative stress induced in the plant; different response pattern of total and individual GSLs content was observed in both cultivars under arsenic stress [148].

Besides the classical fertilizers, in the last years, new proposed products that are beneficial on crops, such as improving safety, enhancing growth and production, improving the defence against weeds and pests and nutritional quality, were developed. Among these, signalling molecules, biocontrol agents, and biostimulants are now gaining high interest for improving plant resilience and quality. Leaves and cotyledons of *B. napus*, *B. rapa* and *B. juncea* showed an up to 20-fold increase in glucobrassicin content after treatment with JA (Jasmonic Acid), or MeJA (Methyl Jasmonate) [149]. In contrast, treatment with ABA (Absciscic Acid) reduced the accumulation of indole GLS in *B. napus* [150].

In summary, the levels of hormones, such as JA, SA (Salicylic Acid) and ABA, seem to be related to the regulation of GLS and of other bioactive compound content [151]. 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. [152] affirmed that the use of *Trichoderma* and its metabolites led to an increase in GLS in plants. This could probably be 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 ascorbic acid was lower compared to control plants.

Additionally, in *Brassica* spp. cultivation is increasing the use of biocontrol agents, there is also the utilisation of seaweeds extract, mycorrhizae, nematodes [153], humic acids such as vermicompost foliar sprayed [154], and protein hydrolysates, all compounds now classified as “biostimulants”, useful to increase plant yield and the accumulation of bioactive compounds [155].

Brassica species can contrast the main soil-borne agents thanks to 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. Minced flour was distributed before planting (60 and 90 g m⁻²) and was successful for the sinigrin presence [156].

5. Conclusions

Brassica vegetables are a good source of many phytochemical compounds that exert positive effects on the final consumer's health. This study presented an investigation on the presence of these bioactive compounds, analysing how they affect the sensorial and nutritional quality, and on the factors that can modify their concentration in *Brassica* food vegetables, such as genetic, environmental and agronomic factors.

There is a large possibility to improve the nutritional and sensorial quality of *Brassica* vegetables through the implementation of appropriate agronomic practices; nevertheless, the effects of the treatments are strictly genotype-dependent, and a good selection of the genotype before the start of cultivation is required. Furthermore, the environmental

factors could influence to different extents the quality of *Brassica* genotypes, and they should be considered in the evaluation of the phytochemical compounds amount.

All this information is useful for developing new fresh and processed products with increased nutritional and sensorial quality, according to the final users' needs and the final purpose of consumption. If the consumer will be informed and made conscious of the healthy potential of the phytochemical compounds present in *Brassica*, they may be willing to accept these products despite the bitter taste and the intense aroma, which are often responsible for a low consumer acceptance.

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