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Chapter 5

Agronomic Factors Influencing Brassica Productivity and Phytochemical Quality

Cristine Vanz Borges, Santino Seabra Junior, Franciely S. Ponce and Giuseppina Pace Pereira Lima

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Abstract

Agronomic practices and climatic factors affect the content and profile of phytochemicals. The effects of the environment, such as salinity, climate, and other abiotic factors, promote biochemical responses, inducing changes in the quantity and quality of polyphenol compounds, carotenoids, vitamins, glucosinolates, and polyamines, which are bioactive compounds. In plants, among the various functions, some phytochemicals can protect against biotic factors. Brassica vegetables are a source of several primary and secondary metabolism compounds, and they might be responsible for disease prevention. In addition, the increase of bioactive compounds in plant-based foods is important to the diet and consequently for the improvement of public health. In this chapter, we will point out the abiotic factors that affect the productive performance, quality, and chemical composition of different Brassica species and cultivars. We will also discuss its implications on plant protection and human health.

Keywords: environmental factors, cultivation conditions, polyphenol, carotenoids, glucosinolates

1. Introduction

The Brassica ceae family, previously known as Cruciferae, is composed of 338 genus and around 3700 species. The family includes many plants of economic importance, for the production of edible oil, such as the canola, forage rape (Brassica napus), and seasoning
plants, as the mustard and also various species for consumption, in which the leaf, stem, roots, and tubercles are edible parts. The main genus, *Brassica*, is formed by 37 species, which can be annual and biannual, including even weeds, wild plants, and domestic crops. *Brassica* vegetables originate from regions between the Mediterranean and the Sahara, where the climate consists of mild winters followed by hot and dry summers. Besides that, there are species inside the genus that are well adapted to colder regions, and many species are now considered naturalized in the entire world and are commonly observed in Western Europe, in the Mediterranean, and in temperate regions of Asia. In addition, many species also grow in as invasive weeds in the Americas (North and South) and Australasia [1].

The species of the *Brassica* genus were widely modified and domesticated by human beings and are vegetables cultivated worldwide [2], especially the varieties belonging to the species *Brassica oleracea*, which includes cabbage, tronchuda cabbage (*Brassica oleracea* L. var. costata DC), mustard, rocket, and Brussels sprout (*Brassica oleracea* L. var. gemmifera), among others *(Figure 1)*. Among these species, we also found broccoli, where the most consumed part is the inflorescence, the cauliflower, from which the floral peduncle is consumed and the tubercles, as the radish and the turnip.

*Brassica* vegetables have attracted great attention due to the presence of phytochemicals with recognized beneficial functions in the human organism, reducing the risk of diseases [3]. These vegetables are potential sources of anticarcinogenic and antioxidant compounds as the glucosinolates (GLS), vitamin C, phenolic acids, flavonols, anthocyanidins, carotenoids, and amino acids [4]. Most of the researches are focused on the content of secondary metabolites, mainly the glucosinolates. The benefits to human health from the ingestion of these vegetables, such as the reduced risk of degenerative diseases, are in great part attributed to the content of secondary metabolites substances of the plants [5]. Besides the human health aspects, these metabolites play a fundamental role in the plants’ defense against microorganisms, raising the interest in higher quantities of these secondary compounds as a strategy of increasing the protection of the cultures and reducing the use of agrochemicals.

Variations in the agronomic conditions (e.g., vegetal species, cultivars, development stage, plants organs, fertilization, and soil pH) and climatic factors (e.g., light intensity and water availability) are known for significantly affecting the phytochemical content and profile. The understanding of the effects of climatic and agronomic factors is necessary for increasing the predictability of the desired compounds, increasing the benefits related to the human health and to the plants’ protection (plague control) [6]. Although there is little information on the real influence of cultivation on the contents of glucosinolates and other important phytochemicals in *Brassicas*, it appears that the use of ecological practices can induce a rise in the content of these molecules. In this chapter, we provide a general view about the roles of the glucosinolates and other phytochemicals present in *Brassicaceae* and their implications in the plants’ protection, productivity, and human health, as well as emphasize the factors that affect the contents of these compounds in *Brassica* vegetables.
2. Agronomic factors and production

The photosynthetic activity is the base for the production of reserves in the plant, which will constitute the biomass, a factor that can determine the vegetal development limits. The production depends on the interaction between the productive potential and the environmental
factors. The edaphoclimatic factors are directly related to productive responses that influence the flowering, hydric balance, respiration, and absorption of minerals. Latitude, altitude, rainfall, topography, and soil physics act indirectly on the production and other factors, such as solar radiation, temperature, water, and chemical elements of the soil act directly on the photosynthesis. The environmental factors, such as water, temperature, quality, and quantity of light hours, will determine the plants’ growth rate.

The temperature is a climatic factor that can limit the production of determined species in tropical and equatorial regions. Besides determining the growth and development, it establishes the end of the vegetative stage and the beginning of the productive stage in the biennial species, such as broccoli, cauliflower, Brussels sprout, among other species. The broccoli has a better productive development under average temperatures between 60 and 65°F, with a maximum of 75°F [7]. Prolonged periods of temperature above 77°C can retard the formation of inflorescence in plants that are in phase of vegetative growth, reducing the size and causing the development of leaves and bracts in the floral peduncles [8].

The temperature strongly influences the plants’ metabolic activity, and the stress caused by high and low temperatures can induce effects in the primary and secondary metabolism (Table 1). The heat or cold can affect the membrane fluidity, metabolism, and cytoskeleton rearrangement, consequently affecting the vegetative and reproductive tissues [9]. Abrupt increases of temperature can provoke excessively fast growth of the inflorescence and elongating the peduncle in certain cultivars [10]. Cultivations in conditions of high temperatures, where there are only few days with ideal temperatures for vernalization, the plants can continue to vegetate or to not produce commercial inflorescences, which means uneven bunches, the presence of bracts, and low compactness of the head and yellow coloration. Temperatures below the ideal level can prolong the cycle of provoke premature flowering in some species, as in the case of summer cauliflowers submitted to low temperatures [11]. Among the brassicas more tolerant to the cold, the minimum temperature for the germination and cultivation for species is 40°F and the maximum tolerated temperature can reach 105°F for turnip and kohlrabi, which are the most tolerant species to temperature in the germination phase. However, in the cultivation phase, the maximum temperature is situated around 75°F. In Brazil, some regions with mild temperatures and with mensal averages varying from 66 to 88°F, there are reports of commercial cultivation of broccoli, kale, rocket, and watercress [12]. However, cauliflower, cabbage, and radish are also cultivated in tropical regions. These cultivations are favored by the utilization of thermotolerant cultivars obtained by genetic improvement.

The plants can be modified to some degree, tolerating light stresses from either low or high temperatures when slowly submitted to the stress, leading to acclimatization. By contrast, plants that survive the exposition to conditions above the ideal temperature can produce chaperones, molecules that are related to the antioxidant activity, and solutes accumulation [9]. Low temperatures cause reduction in the enzymatic activity, rigidity of membranes, destabilization of protein complexes, compromise of photosynthesis, and rupture of the membranes. Cellular alterations associated to the tolerance to cold and/or freezing include the accumulation of sugar or compatible solutes, changes in the membrane composition, and synthesis of dehydrin-like proteins [9].
The stress by temperature can cause changes in the plants’ chemical constitution. Broccoli sprouts present increased glucosinolate contents when cultivated under high (84.2 or 89.6°F) or low temperatures (51.8 or 60.8°F), comparing the cultivated sprouts under ideal temperature (70.7°F) [13]. Similar to the sprouts, the broccoli leaves showed the highest glucosinolate level when cultivated under 53.6 or 89.6°F. This effect was also observed in younger cabbage plants. Under low temperatures, there is an increase of the glucosinolate levels in broccoli and watercress (Table 1). The combination of temperature and precipitation influences the

<table>
<thead>
<tr>
<th>Stress factor</th>
<th>Productive and/or biochemical response</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
<td>[13]</td>
</tr>
<tr>
<td>Heat</td>
<td>Affects the glucosinolates content</td>
<td>[59]</td>
</tr>
<tr>
<td>Cold</td>
<td>Higher glucosinolates production</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>Higher glucosinolates production in broccoli under low temperatures</td>
<td>[60]</td>
</tr>
<tr>
<td>Thermal amplitude</td>
<td>Higher glucosinolates production in broccoli plants under temperatures between 53.6 and 89.6°F, compared to plants under temperature 71.6°F</td>
<td>[61]</td>
</tr>
<tr>
<td>Luminosity</td>
<td>Reduced levels of trypsin inhibitors in Brassica napus seedlings</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Reduced levels of glucosinolates in the leaves and roots in B. napus</td>
<td></td>
</tr>
<tr>
<td>Competition (population density and consortium)</td>
<td>Photoinhibition, thermal stress, and stomatal closing, leading to a reduction of net photosynthesis in brassicas, included B. napus.</td>
<td>[62]</td>
</tr>
<tr>
<td>Excess</td>
<td>Mustard plants (B. juncea) cultivated under shading screens of 50% showed lower quantities of ascorbic acid, larger foliar area, chlorophyll, carotenoids, N, NO₃, and a higher content of mineral nutrients in comparison to the complete solar luminosity</td>
<td>[63]</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>Affects the glucosinolates content</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>Spring broccoli growth in intermediate temperatures, high luminous intensity, longer days, and dry conditions have the highest total GLS content</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td>Glucosinolates levels in kale are not influenced by the photoperiod</td>
<td>[61]</td>
</tr>
<tr>
<td>Water</td>
<td>Growth reduction, lower yield of cabbage heads, and an increase of dry mass</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td>Kale: growth reduction, biomass reduction, increase of sorbitol, sucrose, verbascose and kestose levels, and a decrease of manitol</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>An increase in the sugar content in the phloem sap of broccoli submitted at hydric stress</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>Lower glucosinolate content in broccoli in hydric restriction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biomass reduction, an increase of nitrogen in the leaf, and a darker green leaf in Chinese cabbage</td>
<td>[67]</td>
</tr>
</tbody>
</table>
glucosinolate content in *brassicas* (white cabbage, red cabbage, savoy cabbage, Brussels sprouts, cauliflower, kale, kohlrabi, turnip, red radish, black radish, and white radish). High and low precipitations induce higher contents of glucosinolates, when compared to the same vegetables cultivated in a year with mild temperatures and higher precipitation [14].

The light is a factor that influences the *brassicas’* performance. The increase in the luminous intensity corresponds to a rise in the photosynthetic activity (within certain limits), while the decrease promotes a higher cellular elongation, resulting in etiolated plants. However, this response depends on the species’ susceptibility and on the plants’ density, producing a competition for light or on excessive shading obtained by the use of screens. The amount of energy intercepted is dependent on the characteristics of the cultivation system, row spacing, consortium, and even the architecture characteristics of each genotype, such as the leaf inclination. In the same plant it is possible to occur leaves exposed or not to the sun and with different quantic necessities, with different photosynthetic performance. In this context, there are species that show lower or higher stress when cultivated in a lower spacing or cultivated under consortium. In Ethiopian kale (*B. carinata*) and African nightshade (*Solanum scabrum*), cultivated in consortium and in ideal condition of irrigation and under hydric stress, there was an increase in the glucosinolates content in kale and the maintenance of the biomass

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<thead>
<tr>
<th>Stress factor</th>
<th>Productive and/or biochemical response</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>Growth reduction, Na or Cl accumulation, and lower productivity</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>A decrease of fresh matter in the aerial part of broccoli</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>An increase in GLS content and phenolic compounds</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>A drastic decrease in the vitamin C content in old broccoli leaves</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>A significant decrease in the vitamin C content in young broccoli leaves</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>Loss of flavonoids in old broccoli leaves;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss of turgor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accumulation of glucosinolates in <em>B. napus</em> L.;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduction in the nitrate content</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase or no effect in the nitrate content</td>
<td></td>
</tr>
<tr>
<td>Fertilization</td>
<td>Nitrogen (N)</td>
<td>Nitrogen fertilization influences the GLS metabolism in broccoli</td>
</tr>
<tr>
<td></td>
<td>Sulfur (S)</td>
<td>High level of sulfur provided an increase of polyphenol contents (flavonoids and phenolic acids) in <em>B. rapa</em> ssp. <em>sylvestris</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>An increase of the total glucosinolates with the increase of fertilization with sulfur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher quantities of sulfur and nitrogen combined did not provide higher contents of glucosinolates</td>
</tr>
</tbody>
</table>

Table 1. Effect of environmental factors in the production and biochemical compounds in *brassicas*.
production and nutritional characteristics. In addition, low irrigation induced the carotene levels in African nightshade, both under hydric stress and stress provided by the consortium. In opposition, hydric stress did not affect the glucosinolate content in *B. carinata* and the indole glucosinolates in *B. rapa* ssp. Rapiera [15]. These results suggest that the responses to drought and glucosinolates concentration can vary depending on the genotype.

3. *Brassicas'* phytochemical composition

*Brassica* vegetables are important sources of fibers, vitamins, and minerals. In addition, these vegetables are potential sources of anti-carcinogenic and antioxidant compounds, such as the glucosinolates, vitamin C, phenolic acids, flavonoids, anthocyanidins, carotenoids, and amino acids [4]. Most of the current researches are focused on the content of secondary metabolites, mainly of glucosinolates. Many epidemiologic studies indicate that a high ingestion of *brassicaceous* vegetables is associated to a reduced risk of cancer [5, 16], cardiovascular diseases [17], gut diseases (e.g., colite) [18], [19], and diabetes [20].

Many epidemiologic studies do not differ among the types of cruciferous vegetables, but the most common studies in the entire world include the broccoli, cauliflower, cabbages, bok choy, kale, watercress, turnip, and rocket [21]. Besides human health aspects, these metabolites play a fundamental role in the plants’ defense against microorganisms; thus, there is an increasing interest in raising the content of these secondary compounds as a strategy of increasing the protection to cultures and reducing the use of agrochemicals.

Even though there is little information on the real influence of the cultivation in the levels of glucosinolates and other important phytochemicals in *brassicas*, it seems that the use of ecological practices can induce a raise of these molecules. In addition, in most cases, it is fundamental to also study the impact of storage and cooking in these compounds, since *brassicas* are not consumed immediately after the harvest, in order to know the real benefits of these vegetables to the human health.

3.1. Glucosinolates

*Brassica* vegetables are the major source of glucosinolates that has been associated to its bioactivity, and these compounds may be responsible for their observed protecting effects. The glucosinolates are found in 16 families of dicotyledonous plants and in at least 120 different chemical structures that have been identified until now [21]. Depending on the chemical structure of the precursor amino acid, they are now classified into three groups: aliphatic, indole, and aromatic glucosinolates.

The glucosinolates profile and its modifications, together with specific products of hydrolyzation, are being discussed as a plant defense mechanism to deal with various abiotic and biotic stresses. Recent studies showed that glucosinolates, for example, breakdown products of 1-methoxy-indol-3-ylmethyl glucosinolate and 5-phenylpentyl isothiocyanate, exert mutagenic or genotoxic effects in mammalian and bacterial cell studies [22]. Studies
indicate that broccoli sprouts are sources of GLS (varying from 679.01 to 554.90 mg/100 g FW), and the predominant GLS is the glucosinolate glucoraphanin (GRA) (33% of the total GLS) [23].

Glucosinolates and isothiocyanates (products of glucosinolate hydrolysis) are produced by some plants in response to biotic stress. They are important as protective agents of the plants, due to their toxic or repelling effects against potential plagues (herbivores, bacteria, and fungi) [24]. Even though these compounds can be used as protection agents in plants, with a great importance in agriculture and horticulture, they are significantly important to human nutrition, due to the preventive effects on human health [24, 25]. These compounds are known for protecting against cancer in humans [26] and, in plants, these secondary metabolites and/or their breakdown products have different biological functions, like fungicidal, bactericidal, nematocidal, and allelopathic properties [27].

Thus, factors influencing phytochemical content and profile in the production of brassicaceous plants are worth considering for both plant and human health. There are studies showing that the consumption of brassica vegetables has a direct relationship with cancer incidence reduction [28]. Besides GLS, these vegetables contain myrosinase, a thioglucoside glycosidase (EC 3.2.3.1) which is released from intracellular compartments when the vegetable tissue is damaged by cutting or chewing and induces GLS hydrolysis into isothiocyanates and nitriles, as the most important products. Sulforaphane is one of the investigated isothiocyanates and is particularly abundant in broccoli var. *italica* in the form of its corresponding glucosinolate glucoraphanin (GRA) (4-methylsulfinylbutyl glucosinolate) [29]. GRA can be converted into sulforaphane and glucobrassicin to indolyl-3-carbinol; both hydrolyzed derivatives are active against carcinogenesis as demonstrated by many in vitro experiments or in vivo studies [28, 30].

The quality and quantity of GLS differ among the plants species, among the different plant organs (tubercle or leaves), and in function of the ontogeny. The profile of these compounds is not only determined by the plant genetic constitution but also influenced by the environmental conditions [31]. Generally, high levels of GLS occur in response to temperature [32], exposition to different wavelengths [33], nutrients availability [34], and signaling molecules as the salicylic acid (SA) [25], jasmonic acid (JA), and methyl jasmonate (MeJA) [31, 35]. Exogenous applications of SA and its analogous acids, damage by herbivory or treatment with JA, induce increases of indole GLS in *B. napus* [36], *B. campestris* [37], and *B. juncea* [35]. Microorganism infection and/or mechanical damages can promote the biosynthesis of indole GLS and aromatic 2-phenylethyl GLS in *B. rapa* through synthesizing molecules (e.g., methyl jasmonate or jasmonic acid) [31]. In addition, many compounds such as phenolics, terpenoids, and compounds containing sulfur also regulate the biosynthesis [38].

Saline stress (150 mM NaCl) can reduce the total GLS levels, due to the decrease of both aliphatic GLP, as indole (GBS and MBGS), in broccoli sprouts [23]. This decrease is attributed to cell damages induced by Na accumulation [39]. However, studies with broccoli sprouts determined that the decrease in the GLS level in response to excessive contents of NaCl (44% in comparison to the control—0 mM NaCl) can be decreased by the application of MeJA, applied daily from the third day of growth of 10-day-old broccoli sprouts [23].
Brassica foods (e.g., cabbage, cauliflower, broccoli, Brussels sprouts, turnips, and kale) are consumed raw, frozen, or after domestic thermal processing (cooking). Generally, the conventional cooking methods, such as boiling, steaming, pressure cooking, and microwaving, reduce the content of glucosinolates to approximately 30–60%, depending on the method and analyzed compound [40]. Leaves (turning greens) and young-sprouting shoots (turning tops) of B. rapa cooked in steam showed maintenance of GLS content, compared to raw vegetable, by preventing leaching and solubilization of these metabolites. By contrast, conventional boiling and high-pressure cooking methods induced losses of GLS levels (64%), and the degradation of different GLS classes (e.g., aliphatic or indolic) was similar in both cooking methods [40]. However, some compounds with important pharmacologic activities can be formed after the thermal processing by hydrolysis (e.g., 2-aminothiophene and dimeric 1,4-dithiane-2,5-di-acetonitrile), increasing the bioactive potential in brassica vegetables [41].

The thermal treatment causes denaturation of enzymes that catalyze the degradation of nutrients and some metabolites. When brassica vegetables are chopped, ground, or chewed, there is a rupture of the tissues, and the GLSs enter in contact with the myrosinase, inducing the conversion to isothiocyanates, nitriles, thiocyanates, epithionitriles, oxazolidine-2-thiones, and epithioalkanes [42]. The hydrolysis products, mainly during the storage and processing, as well as the myrosinase activity of the gut microbiota, can affect the total content and bioavailability of these compounds [26]. In addition, the glucosinolates are water-soluble compounds and are generally lost by leaching, in methods that use water for cooking.

3.2. Polyphenols

The phenolic compounds are a group of secondary metabolites present in the vegetal kingdom. The most disseminated and diversified groups of polyphenols are the flavonoids, which have C6-C3-C6 flavone skeleton. The flavonoids are important phenolic phytochemicals containing a basic structure of two aromatic benzene rings separated by a heterocyclic-oxygenated ring [43]. The flavonoids and the hydroxycinnamic acids are widely distributed in plants and are important bioactive compounds in the human diet. The dietetic flavonoids have antiviral, anti-inflammatory, antihistaminic, and antioxidant properties. Flavonoids and phenolic acids are the most characterized groups of phenolic compounds in brassicas and can protect the plants against UV radiation, microorganisms, and predator insects [44]. In cabbage, many (poly)phenolic compounds were identified, including myricetin, quercetin, kaempferol, luteolin, delphinidin, cyanidin, and pelargonidin [45].

Generally, the phenolic compounds are produced through the phenylpropanoid pathway. Biotic or abiotic stresses, such as elicitors, were reported for inducing alterations in the phenolic compounds contents, as described in broccoli sprouts [23, 46]. In addition, the quality and quantity of the phenols differ among the plant species and among the plant organs. For example, broccoli sprouts have higher phenolic levels (1133.85 mg/100 g FW), when compared with mature broccoli inflorescences (63.4 mg/100 g FW) [45]. Most of the phenolic compounds present in broccoli sprouts are the hydroxycinnamic acids (sinapic acid derivatives), approximately 98% of the total phenolics found [23].
In saline-stress conditions, there is a possibility for a decrease to occur up to 30% in the phenolic compounds’ content in *brassicas* (e.g., broccoli sprout). It is important to highlight that the increase or decrease of these compounds in this situation depends on the plant sensibility to salt and on the development stage when the plant was submitted to the stress [23]. The exogenous application of elicitors can also induce the phenolic compounds’ biosynthesis, affecting the contents of antioxidant and nutritional compounds in *brassicas*. This technique can be a viable tool to obtain vegetables with higher levels of these bioactive compounds. Studies with broccoli sprouts showed that the prolonged application of low concentrations of SA and MeJa during the sprouting significantly increased the content of phenolic compounds. Exogenous SA (50 μM) applied for 5 days or 100 μM SA for 7 days achieved flavonoids-enriched broccoli sprouts by 24 and 33%, respectively. A 10 μM MeJa was a highly efficient treatment, promoting increases of 31 and 23% in the concentration of flavonoids and total phenolics, respectively [47].

The culinary process is a source of several alterations, both physical and biochemical, modifying the phytochemical constituents present in the vegetables, resulting in changes in the nutritional values [48] of *brassicas*. During the cooking process, the phenolic compounds are highly reactive and can be significantly modified, including the release of conjugated compounds (bound forms), oxidation, degradation, and polymerization [49]. Generally, the effect of boiling in *brassica* vegetables can lead to significant polyphenol losses. During the boiling, because the phenolic compounds are water-soluble, there might be losses by leaching, besides the breaking of these compounds during the thermal processing. The analysis of the water used in experiments with boiled *brassica* vegetables (e.g., in watercress) shows the presence of total phenols in the water (9.35 ± 0.12 mg GAE/g DW), confirming the loss by leaching (raw watercress – 14.86 ± 2.02 mg GAE/g DW). The quantity of phenols found in the water and in cooked material (residual phenols) is not different from the quantity present in raw watercress [50]. In opposition, in these studies, a minimum deleterious effect was demonstrated when microwaving and steaming were used in the content of phenolic compounds. This minimum effect occurs according to the quantity of water used and to the inactivation of oxidative enzymes, which prevent the rupture and the degradation of the phenolic biosynthesis [50].

### 3.3. Carotenoids

Carotenoids are a class of phytornutrients that are responsible for the colors red, orange, and light yellow in many vegetables and fruits. Most of the *brassica* vegetables contain carotenoids, such as β-carotene, lutein, zeaxanthin, neoxanthin, violaxanthin, and folate, which have important antioxidant, anticarcinogenic properties, and provitamin A [51]. Carotenoids have shown to have functions during the photosynthesis and show an important role in defense mechanisms apart from the essential nutrients. These compounds are involved in biotic and abiotic stresses response and development, acting as signaling molecules, and in addition, they are related to processes such as photomorphogenesis, nonphotochemical quenching and lipid peroxidation, and attracting pollinators [52].

*Brassica* vegetables are rich in carotenoids and, among the varieties of *B. oleracea*, kale has the highest levels of lutein and beta-carotene [51]. In Chinese cabbage (*B. rapa* ssp. pekinensis),
lutein and β-carotene are mainly distributed in the older leaves and in the flowers, while the zeaxanthin and violaxanthin levels are relatively low [53].

In order for the carotenoid absorption to occur by gut enterocytes, the mechanical and/or enzymatic disruption of the food matrix is necessary. In addition, due to the hydrophobic character of these chemical molecules, the formation of micelles before its absorption is also necessary [54]. Since the carotenoids in fruits and vegetables are present in the chromoplasts, their substructure and the cell wall are the main barriers to the bioavailability of these compounds [55]. Thus, thermal processing as the boiling or the steaming can have positive effects in bioavailability, collaborating to the food matrix disruption, even though negative effects caused by the carotenoids degradation were also reported [56].

The processing methods used in *brassica* vegetables generally increase the carotenoid content [50]. The thermal processing can cause quantitative and qualitative changes by isomerization processes. An increase in the carotenoid content in *brassica* vegetables, such as broccoli, Brussels sprouts, cabbage, cauliflower, and watercress, after boiling and steaming, were reported in many studies [33, 50]. The increase in the total carotenoid content after thermal treatments can also be explained by changes in the cell wall, due to the cellulose degradation, improving the extraction of these compounds, as a result of the denaturation of carotenoids/protein complexes caused after the thermal processing [57]. However, high temperatures can lead to isomerization processes, decreasing the food nutritional values. β-carotene and lutein degradation for the formation of cis-isomer (4–40%) during the thermal processing was described in some studies with *brassica* vegetables (e.g., broccoli and kale) [58]. Thus, a higher retention of cis-isomers was registered in *brassica* vegetables thermally processed in comparison to trans-isomers, leading to losses in the vitamin A content in these foods.

4. Conclusion

*Brassica* vegetables have attracted increasing attention due to the presence of phytochemicals with beneficial recognized functions to the human organism, reducing the risk of diseases. Variations in the agronomic conditions (e.g., vegetal species, cultivars, development stages, plant organs, fertilization, soil, and pH) and climatic factors (e.g., luminous intensity and water availability) are known for affecting the content and the profile of compounds from the secondary metabolism.

Many studies show that stress can lead to the accumulation of bioactive compounds in plants, generating the production of foods with more benefits to the human health. In contrast, the growth and development are affected, because there is a reallocation of primary metabolites for the formation of secondary metabolites. This reflects in the biomass production and, certainly, in the species production. However, these metabolites, such as GLS, phenolic compounds, and carotenoids, play a fundamental role in the plants’ defense against microorganisms, possibly leading to a better adaptation of the plants to the environment and, consequently, to the reduction in the use of agrochemicals. The current knowledge of the climatic factors that affect the content and profile of these phytochemicals in *Brassicaceae* is of
scientific and economical interest and can be the base to elaborate strategies for producing plants more resistant to plagues and diseases, reducing the use of agrochemicals and increasing the productivity with a higher nutraceutical potential.

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Conflict of interest

The authors affirm that there is no conflict of interest.

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