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Variation of Nutritional Quality Depending on Harvested Plant Portion of Broccoli and Black Cabbage

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Abstract: Brassicaceae plants are rich with antioxidant compounds that play a key role for human health. This study wants to characterize two Italian broccoli cultivars (Roya and Santee) and black cabbage, evaluating the variation of antioxidants in different portion and at different developmental stage of the plants: for broccoli, heads and stems were sampled, while for black cabbage, leaves and seeds were analyzed. Roja cultivar was also analyzed at the first and second harvest to evaluate the variation of phytochemical compounds over time. Nutritional and sensorial qualities were investigated. Black cabbage seeds showed higher value of total antioxidants, total phenols, and total anthocyanins than leaves. Similarly, phenolics and anthocyanins content in head was higher than in stem in broccoli. In Roja cultivar, the harvest date seemed to influence the antioxidant capacity and the phytochemical compounds content, with broccoli sampled in the second harvest showing better results for all the nutritional parameters. These local vegetables represent a significant source of antioxidants and may contribute to health benefits of the consumers.

Keywords: broccoli; black cabbage; sensorial quality; nutritional quality; antioxidant activity; total phenols; anthocyanins; plant portion; stage development

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1. Introduction

Italy is known in the world for its high-quality horticulture; among these, *Brassica* vegetables cover an important part of the market, with a total production of 420,630 t in 2020 (https://www.fao.org/faostat/en/#data/QCL, accessed on 12 March 2022). *Brassica* Italian crops production registered an increase of about 70,000 t since 2010.

A lot of studies showed a relation between the high consumption of *Brassica* vegetables and the reduction in the risk of age-related chronic illness, such as cardiovascular and other degenerative diseases [1]; they also reduce the risk of several types of cancer [2]. Broccoli and black cabbage are among the vegetable food with the highest antioxidant potential [3]. 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 [4]. These secondary metabolites have several functions in the plant, such as UV protection, pigmentation, and disease control, such as glucosinolates [5]. In *Brassica* genus, the main represented flavonols are quercetin, kaempferol, and isorhamnetin. Anthocyanins, in addition to conferring the blue and red pigmentation in broccoli sprouts and red cabbage, possess high antioxidant capacity [6]; among them, cyanidin-3-glucoside is the most represented in *Brassica* crops [7].

Several studies described the variation of the antioxidant activity and phenolics content in the plant portion: for example, in turnip plants, flower buds are the most active portion, followed by leaves and stems, while roots seem to have minor phenolic concentration [8]. Ferreres et al. [9] revealed that kale seeds have higher antioxidant potential than kale leaves, but leaves are the richest in phenolics. In *Brassica rapa*, Francisco et al. [10] reported that phenolic compounds content is higher in leaves harvested during the vegetative period than fructiferous stems (composed of flower, buds, and surrounding leaves), and flavonoids are the main represented compounds. These findings were confirmed by the same group [11], which found that total phenolic content is higher in green turnip than in turnip top. On the contrary, Schmidt et al. [12] reported that harvest period has only a minimal influence on flavonoid concentration in kale. Broccoli cultivars register an increase in phenolic compounds concentration during the inflorescence's development, with an interesting antioxidant capacity in flower, stem, and leaves [13,14].

The aim of this work was to analyze the content of phytochemical compounds in different edible plant portions of *Brassica* species at different developmental stages. In particular, in broccoli heads and stems were analyzed, while black cabbage leaves and seeds were evaluated.

To investigate the interaction between genotype and plant portion, two different cultivars of broccoli were compared. The effect of plant developmental stage on phytochemicals amount was also considered, comparing broccoli of the cultivar Roja from first and second harvest period.

2. Materials and Methods

2.1. Plant Material

Vegetable materials used for these trials were provided by Valli di Marca Agricultural Company, located in the south of Marche Region (Italy), at 136 m.a.s.l. *Brassica* plants (broccoli and black cabbages) were cultivated in open field conditions, according to the typical cultivation system adopted in this area. Broccoli samples belonged to Roja and Santee cultivars and were harvested on 8 February, while Roja-II (second harvest) was sampled on the 26 February. After harvesting, samples were packaged in 300-g packs and immediately frozen at −20 °C. In a second step, broccoli samples were divided into heads and stems (approximately representing 25% and 75% of total fresh weight, respectively) for analyzing the antioxidant capacity, the phenols content, and the anthocyanins content in the two portions. Black cabbage seeds and leaves were also harvested (seeds were sampled during previous April). The list of plant samples collected and analyzed is reported in Table 1.

Table 1. Plant materials sampled.

Species	Samples (300 g)
Brassica oleracea* L. var. *italica	5 samples of Broccoli var. Roja* divided into heads and stems 5 samples of Broccoli var. Santee F1* divided into heads and stems 3 samples of Broccoli var. Roja*-II harvest divided into heads and stems
***Brassica oleracea* L. var. *Acephala* subvar. *Laciniata* L.**	2 samples of black cabbage leaves 2 samples of black cabbage seeds

2.2. 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 (Bosch, Munich, Germany) (except for the seeds) at 20.0 ± 0.5 °C. The measurement of vegetables soluble solids content (SSC) was performed using a hand-held refractometer (Atago, Tokio, Japan). For each sample, few drops of the previously obtained juice were put on the refractometer prism, and the SSC was recorded as °Brix. The refractometer prism was cleaned with distilled water after each sample. Vegetables Titratable Acidity (TA) was determined from 5 mL of the previously obtained juice diluted with 45 mL of distilled water. This solution was titrated by an automatic titrator (HI 84532 Fruit Juice—Titratable acidity—Hanna Instruments, Woonsocket, RI, USA), until the vegetable juice aqueous solution reached the neutral pH. The titratable acidity was expressed as % citric acid.

2.3. Vegetables Nutritional Quality

2.3.1. 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, dihydrogen potassium phosphate, dipotassium hydrogen phosphate, 2,4,6-tris(2-pyridyl)-striazine (TPTZ, 99%), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ferrous sulphate heptahydrate, potassium persulphate, 3,4,5-trihydroxybenzoic acid (gallic acid), and sodium hydroxide, were purchased from Sigma-Aldrich (Sigma-Aldrich s.r.l., Milan, Italy).

2.3.2. Vegetables Extraction

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 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 h, the suspensions were centrifuged at 2500 rpm for 15 min (Thermo Fisher Scientific Heraeus Megafuge 16R Centrifuge, Waltham, MA, USA) and the supernatants were collected and stored in six amber vials (for each sample), of 4 mL each, at −20 °C [15,16].

2.3.3. Total Antioxidant Capacity (TAC)

Vegetables TAC was evaluated using a FRAP (Ferric Reducing Antioxidant Power) assay, a method based on the rapid reduction in ferric-tripyridyltriazine (FeIII-TPTZ) in the blue-colored ferrous-tripyridyltriazine (FeII-TPTZ) by antioxidants present in the samples. The reduction in ferric–TPTZ was measured by the method of Benzie and Strain [17], modified by Deighton et al. [18] 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 (20 mM), and TPTZ (10 mM in 40 mM HCl). Briefly, the vegetable methanol extract was diluted 1:5 and vortexed. This solution was further diluted 1:10 adding the FRAP solution previously prepared, vortexed, and incubated in darkness for 4 min; after this process, 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). The calibration was calculated by linear regression from the dose–response curve of the Trolox standards.

2.3.4. Total Phenol Content (TPH)

The vegetable total phenol content was evaluated using the Folin–Ciocalteu reagent method [19], with gallic acid as the standard for the calibration curve. Briefly, glass test-tubes were filled with 3.5 mL water, and 150 µL of water-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).

2.3.5. Total Anthocyanin Content (ACY)

The vegetable total anthocyanin content was measured using the pH differential shift method [20]. 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 520 nm and 700 nm of wavelength by spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan).

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).

2.4. Statistical Analysis

Vegetable sensorial and nutritional parameters were analyzed in triplicate for each sample. The data for the titratable acidity, soluble solids content, 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 for $p \leq 0.05$ were considered significant.

3. Results and Discussion

3.1. Vegetables Sensorial Quality

Sensorial quality was analyzed on fresh material considering the whole samples of broccoli. The results of sensorial parameters are reported in Table 2. Broccoli var. Santee had the statistically highest value of SSC. Regarding the Titratable Acidity, the statistical analysis underlined how black cabbage samples had the highest values, while Roja-II samples showed the significantly highest pH value. Broccoli data confirm the results found by Nicoletto et al. [21]; 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), and lower for pH (5.67–5.83).

Table 2. Average values of total soluble solids content, titratable acidity, and pH of *Brassica* samples.

Vegetables	SSC [1] (°Brix)	TA [2] (% Citric Acid)	pH
Black Cabbage	9.55 ± 0.31^{b}	0.23 ± 0.02^{a}	5.88 ± 0.06^{d}
Roja-II [3]	8.97 ± 0.26^{b}	0.15 ± 0.01^{b}	6.77 ± 0.06^{a}
Roja [4]	9.56 ± 0.21^{b}	0.15 ± 0.01^{b}	6.53 ± 0.07^{b}
Santee [5]	10.62 ± 0.26^{a}	0.16 ± 0.01^{b}	6.35 ± 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] Roja: Broccoli var. Roja I harvest: [5] Santee: Broccoli var. Santee. Values with the same letter were not significantly different (Test SNK $p \leq 0.05$). n = 3.

Black cabbage belongs to the *Brassica oleracea* var. *acephala* group that includes kales. A lot of studies considered the sensorial aspects of kale family; Armesto et al. [22,23] and Martìnez et al. [24] reported similar amount of SSC, TA, and pH in kale.

According to these results, sensorial quality is affected by genotype. Regarding the developmental stage, the comparison between Roja and 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 Roja-II value being higher than Roja.

3.2. Vegetables Nutritional Quality

3.2.1. Black Cabbage and Whole Broccoli Nutritional Value

In Table 3, results about the average values of nutritional parameters for black cabbage seeds and leaves are reported. Furthermore, average values of nutritional parameters for each cultivar of broccoli are showed, comprising Roja harvested at two different developmental stages.

Black cabbage seeds showed a higher content of both the investigated phytochemical compounds (TPH and ACY) than leaves and, consequently, a statistically higher TAC value. These results were confirmed by Ferreres et al. [9], who performed characterization trials on kale, analysing the antioxidant activity of kale seeds and leaves. They found that seeds were rich in quercetin and isorhamnetin derivatives, not found in leaves, and in phenolic acids, conferring them a higher antioxidant capacity than leaves, which were rich in flavonols. The higher antioxidant capacity found in seeds is bound to their physiological functions as germination, permeability to water, protection by pathogen and insect attacks, storage, and protection of lipids from oxidation.

Table 3. Average values of total antioxidant capacity, phenolics and anthocyanins content of the different vegetables analyzed.

Vegetables	TEAC [1] (mM TE/kg fw)	TPH [2] (mg GA/kg fw)	ACY [3] (mg CYA-3-GLU/kg fw)
Black Cabbage Leaves	5.68 ± 0.21 [b]	1444.60 ± 23.82 [b]	3.25 ± 0.24 [b]
Black Cabbage Seeds	8.39 ± 0.53 [a]	1767.79 ± 12.67 [a]	16.87 ± 0.24 [a]
			n = 8
Santee [4]	4.65 ± 0.13 [B]	1038.74 ± 26.48 [B]	18.15 ± 2.33 [B]
Roja [5]	4.31 ± 0.15 [B]	1046.20 ± 28.73 [B]	23.42 ± 2.75 [B]
Roja-II [6]	8.86 ± 0.24 [A]	2021.17 ± 117.64 [A]	72.61 ± 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] Roja: Broccoli var. Roja I harvest; [6] Roja-II: Broccoli var. Roja-II harvest. Values with the same lowercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 3$). Lowercase letters refer to black cabbage analyses. Values with the same uppercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 3$). Uppercase letters refer to broccoli analyses.

In our study, the effect of genotype on the phytochemical composition and the antioxidant capacity was not evident, given that Roja (first harvest) and Santee cultivars did not show any significant difference. This was due, probably, to the low amount of antioxidant compounds accumulated in the tissues of these cultivars. 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), associated with doubled value of antioxidant capacity. The influence of the harvest date on the antioxidant capacity of these type of plants was reported by Soengas et al. [25]. Vallejo et al. [13] reported that the content of phytochemical compounds, in particular phenolic compounds, increased with the exposure to sunlight. In fact, the growth of broccoli is very sensitive to climate and light conditions [2].

3.2.2. Broccoli's Head and Stem Nutritional Quality

In Figure 1, FRAP analysis showed contrasting results: in fact, Roja and Santee possessed the highest concentration of antioxidant in stem, while in Roja-II head was the plant part with the highest antioxidant capacity (9.91 ± 0.16 mM Trolox/kg fw), thus highlighting that with maturation there is a greater accumulation of nutritional substances in this part of plants. Similar FRAP results were obtained by Kaur et al. [26] (on heads) and Pellegrini et al. [27] (on whole material).

The total average of different plant parts showed that stem possessed a higher FRAP value than head. In the literature, Fernandes et al. [8] found an opposite trend in turnip, reporting that flower buds possess the highest antioxidant capacity, followed by leaves and stems, where flavonols were the most represented compound.

In the analyzed broccoli, the range of phenol concentration varied from 891.26 to 2574.77 mg GA/kg of fw (Figure 2). 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 were 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 confirms that the late harvesting has a positive effect on the quality of the product, as reported by Šamec et al. [28] and Soengas [25]. Our study confirms that total phenolic content is influenced by genotype and plant developmental stage, in accordance with previous studies [26,29].

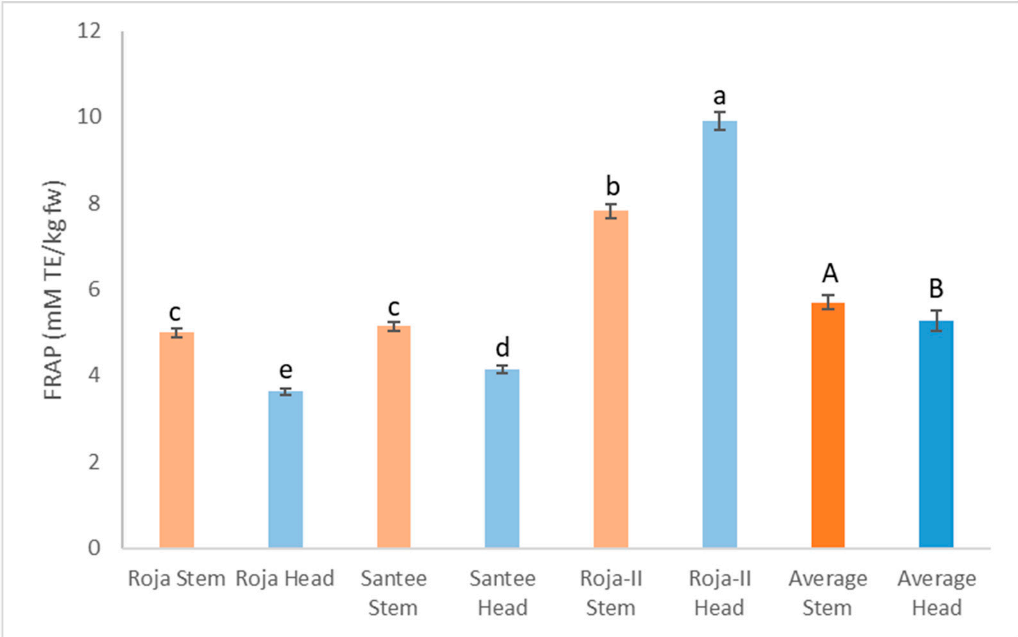

Figure 1. Average values and standard deviation of total antioxidant capacity measured by Ferric Reducing Antioxidant Power (FRAP) of Broccoli analyzed: Roja, Santee and Roja-II harvest divided into head and stem, and their average. Values with the same lowercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 3$). Lowercase letters refer to Roja, Santee and Roja-II harvest divided into head and stem. Values with the same uppercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 9$). Uppercase letters refer to head and stem average values.

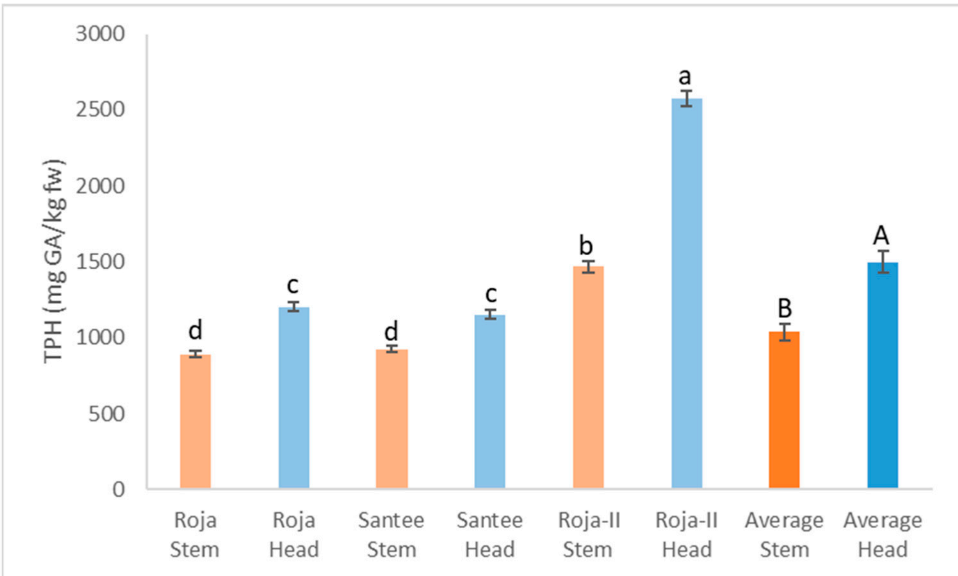

Figure 2. Average values of Total Phenol Content (TPH) with standard deviation of different cultivars and type of tissues of Broccoli: Roja, Santee and Roja-II harvest divided into head and stem, and their average. Values with the same lowercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 3$). Lowercase letters refer to Roja, Santee and Roja II harvest divided into head and stem. Values with the same uppercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 9$). Uppercase letters refer to head and stem average values.

Phenolic compounds have a great incidence on the total antioxidant capacity of a food matrix: for this reason, TPH and FRAP results usually have the same trend. In our case, the higher TPH average content of head did not correspond to a higher average FRAP value of the same tissue; this result was probably due to the capacity of FRAP method to detect other antioxidant compounds over phenols, such as ascorbic acid, carotenoids, vitamins, and others, although the phenolics content represented 80% of the total antioxidant capacity as reported by Podsedek [30]. However, in Roja-II, TPH values of head was higher than stem, as well as FRAP value was higher in Roja-II head than stem.

The highest value of anthocyanins content was detected in broccoli heads (Figure 3), as suggested by their slightly violaceous coloration. The highest concentration of anthocyanins has been registered for Roja-II broccoli head, probably due to an increase in purple color intensity during plant development. However, the less colored part of the plant (Stem) also showed a statistically higher ACY content in Roja-II than in Roja (first harvest), suggesting an increased accumulation of these compounds during development, likewise in absence of purple coloration.

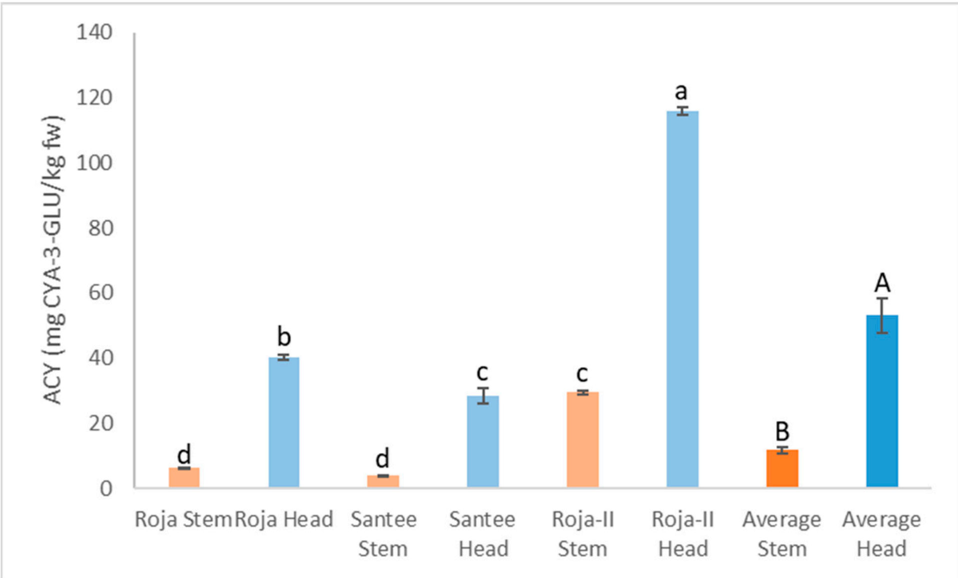

Figure 3. Average of total anthocyanin content (ACY) with standard deviation of different cultivars and type of tissues of Broccoli: Roja, Santee and Roja-II harvest divided into head and stem, and their average. Values with the same lowercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 3$). Lowercase letters refer to Roja, Santee and Roja-II harvest divided into head and stem. Values with the same uppercase letter were not significantly different (Test SNK $p \leq 0.05$, $n = 9$). Uppercase letters refer to head and stem average values.

Stem tissue had 78% lower accumulation of anthocyanins than head tissue. Sotelo et al. [31] found similar values in broccoli (2.2–6.3 mg CYA/kg fw), while Rodriguez-Hernandez et al. [32] made a comparison between inflorescences and leaves in purple sprouting broccoli, finding higher values than the present study.

4. Conclusions

The aim of the study was to analyze the nutritional properties of fresh *Brassica* plants to identify new high-quality products for the consumer, with richest content of phytochemical compounds with health benefits for the consumer. Indeed, the study on the variation of quality in different plant portion and developmental stage resulted interesting for the creation of some possible new products for the consumers. Considering the plant portion, it was evident that in black cabbage, seeds possess higher content of phytochemical compounds 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 [9,33]. Related to the concerns of broccoli, heads had a higher phytochemicals content than stems, but the FRAP method revealed that the total antioxidant capacity followed the opposite trend. More deep analyses could reveal the factors that determine the different values of antioxidant capacity among the two developmental stages. Regarding TPH and ACY values, the Roja and Santee tissues harvested at the same time showed the same content of these antioxidant compound, not resulting in a significant difference. The big differences highlighted between Roja-II and Roja (first harvest) samples were probably due to developmental stage and harvesting time, because Roja-II was harvested approximately 20 days later than Roja (first harvest). The developmental stage and the harvest time brought some phytochemical compounds content differences: this evidence deserves to be further investigated, to better understand the behavior of phytochemicals concentration during time.

Therefore, as a conclusion, these results clearly indicate that to identify a new high sensorial and nutritional quality fresh or processed *Brassica* product it is important to identify the most appropriate cultivar, the type of tissue to be used and also the harvesting time.

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