agriculture

MDPI

Article

Quality Evaluation of Wild and Cultivated Asparagus: A Comparison between Raw and Steamed Spears

Lucrezia Sergio [1], Francesca Boari [1,*], Donato Di Venere [1], Maria Gonnella [1,*], Vito Cantore [1] and Massimiliano Renna [1,2]

[1] CNR-Institute of Sciences of Food Production (ISPA), Via Amendola 122/O, 70126 Bari, Italy; lucrezia.sergio@ispa.cnr.it (L.S.); donato.divenere@ispa.cnr.it (D.D.V.); vito.cantore@ispa.cnr.it (V.C.); massimiliano.renna@uniba.it (M.R.)

[2] Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy

* Correspondence: francesca.boari@ispa.cnr.it (F.B.); maria.gonnella@ispa.cnr.it (M.G.)

Abstract: Asparagus is highly appreciated for its organoleptic and nutritional characteristics and wild genotypes are valuable components of traditional dishes. In this study, the physical and chemical traits of wild (green and violet) and cultivated asparagus ('Grande', 'Purple Passion', and 'Bianco di Bassano del Grappa'), both raw and steamed, were evaluated. Steaming did not affect the total phenols content with the exception of wild green (+49%) and 'Grande' (−31%). Only for wild violet asparagus steaming increased the total antioxidant activity (+46%). Chlorogenic acid and chicoric acid were found only in wild asparagus, while rutin was generally higher in colored cultivated asparagus than wild ones. The highest content of isorhamnetin-3-rutinoside was found in wild violet asparagus while only traces of this compound were detected in the cultivated ones. Steaming influenced the content of both chlorophylls and carotenoids in asparagus, also resulting in changes in the color parameters in cooked spears. Overall, the sugar content in wild asparagus was lower than in the cultivated ones and steaming had a low impact on this chemical trait. Principal component analysis highlighted the most evident separation between wild asparagus and cultivated ones. These results indicate that wild asparagus can be considered a nutritious and refined food, and provide specific information required for cooking process strategies in the agri-food industrial sector.

Keywords: *Asparagus acutifolius*; *Asparagus officinalis*; antioxidant activity; polyphenols; sugars; chlorophylls; carotenoid; color parameters; steam cooking; principal component analysis

Citation: Sergio, L.; Boari, F.; Di Venere, D.; Gonnella, M.; Cantore, V.; Renna, M. Quality Evaluation of Wild and Cultivated Asparagus: A Comparison between Raw and Steamed Spears. *Agriculture* **2021**, *11*, 1213. https://doi.org/10.3390/agriculture11121213

Academic Editor: Muraleedharan G. Nair

Received: 27 October 2021
Accepted: 29 November 2021
Published: 2 December 2021

1. Introduction

The asparagus spears from wild (*Asparagus acutifolius* L.) and cultivated (*Asparagus officinalis* L.) species are highly appreciated for their organoleptic and nutritional characteristics. This plant has a low caloric content (approximately 22–35 kcal 100 g^{-1}) [1], but it is very rich in bioactive compounds, with strong antioxidant properties [2,3], mainly represented by phenols, sterols, saponins, oligosaccharides, carotenoids, sulfurated acids, essential amino acids [4,5], fibers [6], minerals, and vitamins [1]. In particular, quercetin-3-rutinoside (rutin) has been reported as the most representative phenol in asparagus spears [7–9]. In a recent study, asparagus turned out to be the most important dietary source of quercetin (29%), following onions (41%) and ahead of green tea (8%) [10].

These compounds may play an important role in human health, reducing the risk of cancer [11,12], cardiovascular diseases [13], diabetes [14], and showing therapeutic activities such as being a diuretic and immunostimulant [15].

Wild asparagus is highly appreciated as a component of a wide range of traditional dishes [16]. Currently, the interest in wild asparagus is not only due to its taste and high nutritional value but also due to social and symbolic motives [17]. *A. acutifolius* is widespread in the Mediterranean region in brushland, plowed fields, and in uncultivated

areas close to paths, even in poor soils. Wild asparagus is usually found surrounded by other wild plants and near the base of olive, holm oak, or cork oak trees. This happens both because trees foster the spread of asparagus seeds by the animals that rest in the shelter of the foliage and because of the lack of plowing around the trees, which favors the rooting of the plants.

By carefully observing a bunch of wild asparagus, it is possible to notice a high variability in the chromatic characteristics and the degree of tenderness of the shoots. In practice, asparagus harvested in the sunniest areas are dark green to purplish and are more fibrous, whereas those collected in the undergrowth are a lighter green. This is predominantly determined by the micro environment in which each spear grew and, in particular, by the prevalence of direct or diffused solar radiation [18].

Wild asparagus could become a new crop with high income potential, especially for marginal areas where its cultivation fits perfectly within sustainable agriculture and biodiversity and environmental conservation approaches. Many studies are underway to make this species cultivable [19–22].

Asparagus cultivars show a high variability in agronomic and morpho-biochemical traits due to genotypic as well as agronomic and environmental factors. Fanasca et al. [23] reported the content of phenols, carotenoids, and vitamin C in four green asparagus cultivars. Slatnar et al. [9] compared the sugar, organic acid, anthocyanin, flavonol, flavone, and hydroxycinnamic acid content of six green and two purple asparagus cultivars. Sergio et al. [24] compared biometrical traits, ion composition, enzymes (peroxidase and polyphenoloxidase), sugars, phenols, and antioxidant activity in green and violet asparagus cultivars during cold storage.

Even cooked asparagus retains its nutrients well. In wild asparagus, Garcia-Herrera et al. [1] report that the loss of minerals (34–44%) is lower than that of other leafy vegetables (47–65%) and that, even after being boiled, *A. acutifolius* is an excellent source of some vitamins. An increase in antioxidant activity was also observed with some cooking methods [25,26]. The aim of our research was to compare the qualitative characteristics (dry matter, color parameters, chlorophylls, carotenoids, sugars, polyphenol composition, total phenols, and antioxidant activity) of two types of wild asparagus (green and violet) and three asparagus cultivars ('Grande', green; 'Purple Passion', violet; and 'Bianco di Bassano del Grappa', white), based on the hypothesis that wild products have better qualitative properties than cultivated ones and that cooking usually modify vegetable quality.

2. Materials and Methods

2.1. Chemical Reagents

High-performance liquid chromatography (HPLC)-grade water was prepared by a Milli-Q system (Millipore, Bedford, MA, USA). Methanol (HPLC grade), acetic acid, acetone, sodium chloride, sodium phosphate, potassium persulphate, and sodium carbonate were purchased from Carlo Erba Reagents (Milan, Italy). Glucose, fructose, sucrose, Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), chlorogenic acid, caffeic acid, and chicoric acid were purchased from Sigma-Aldrich (Milan, Italy). Quercetin 3-rutinoside (Q-3-rut, rutin) and isorhamnetin-3-rutinoside (I-3-rut) were purchased from Extrasynthèse (Genay, France). All HPLC standards had a chromatographic purity > 95%.

2.2. Plant Material

The wild asparagus spears were sampled in an oak (*Quercus* spp.) forest of Gravina in Puglia (40°51′ N–16°19′ E, Italy) in shady (green asparagus) and sunny (violet asparagus) areas (Figure 1A). Cultivated asparagus 'Grande' (green asparagus) and 'Purple Passion' (violet asparagus) were sampled in a field near Foggia (41°28′ N–15°34′ E, Italy), while 'Bianco di Bassano del Grappa' (white asparagus) was purchased from METRO (Italia Cash & Carry SpA, Milan, Italy) (Figure 1B). Immediately after harvesting, wild and cultivated ('Grande' and 'Purple Passion') asparagus spears were transferred to the laboratory under

refrigerated conditions (4.0 ± 1.0 °C). The asparagus 'Bianco di Bassano del Grappa' samples, purchased from METRO, were transferred to the laboratory under the same refrigeration conditions. The assessment of the color parameters and the extractions for biochemical analyses were performed within 20 h from arrival in the laboratory.

Figure 1. (**A**) Wild violet (below) and wild green (above) asparagus. (**B**) 'Purple Passion' (left), 'Bianco di Bassano del Grappa' (center), and 'Grande' (right) cultivars of cultivated asparagus.

2.3. Sample Preparation and Steaming

The fibrous basal portion of the spears was trimmed with a sharp steel knife. Each sample was then washed with tap water and dried with paper towels. For each asparagus lot (wild as well as cultivated ones), two batches of about 1 kg each were selected, the first one for the analysis of the raw sample and the second one for the analysis of the cooked sample. Both batches (the second after steaming) were chopped into small pieces in order to obtain a randomized sample. The determination of the dry matter and of all biochemical parameters reported below was carried out in triplicate using this randomized vegetable material. Due to differences in the texture characteristics among spears, optimal steaming time and conditions were established according to preliminary trials. Asparagus spears were placed on a tray in a steam cooker (VC 101 630 Tefal, Italy), equipped with a tank containing 1 L of distilled water, covered with a lid, and cooked with water vapor (99.0 ± 1.0 °C) under atmospheric pressure. Steaming times were 5 min for wild asparagus, 7 min for 'Grande', 8 min for 'Purple Passion', and 9 min for 'Bianco di Bassano del Grappa'. After cooking, the samples were drained and rapidly cooled in an ice bath. We chose steaming because this cooking method allows to cook asparagus spears without causing mechanical damage to the sprouts and without or only minimal loss of nutrients by leaching into the cooking liquid.

2.4. Physico-Chemical Methods

To determine the color parameters, L (brightness), a^* (redness), and b^* (yellowness), a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in the reflectance mode and in the CIE L* a^* b^* color scale was used. Hue angle (h° = arctg b^*/a^*) and saturation (Chroma = C) were then calculated from the primary L, a^*, and b^* readings. The colorimeter was calibrated with a standard reference having values of L, a^*, and b^* corresponding to 97.55, 1.32, and 1.41, respectively. The color measurement was performed on three whole asparagus spears of each lot, at nine different points along each shoot. To calculate the weight change after cooking, samples were weighed individually before and after cooking. The result was expressed as a percentage change compared to the initial weight of the raw material.

To evaluate the dry matter (DM) percentage, three replicates of a 50 g sample from the randomized batch were weighed and maintained in a forced ventilation oven at 65 °C until a constant weight was achieved.

2.5. Biochemical Methods

2.5.1. Chlorophyll and Carotenoid Content

Three replicates of a 30 g sample from the randomized batch were finely chopped in a mortar with liquid nitrogen and then stored at −20 °C in the dark. To carry out the pigment extraction, the plant material was homogenized with 80% acetone (0.2 g mL^{-1}) and centrifuged for 10 min at 14,000 rpm. On the recovered supernatant, three spectrophotometric readings (i.e., 662, 646, and 470 nm) were performed. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (TC) were obtained by inserting the absorbance (Abs) values in the following equations [27]:

$$\text{Chl a } (\mu g\ mL^{-1}) = 12.21\ (\text{Abs } 662\ nm) - 2.81\ (\text{Abs } 645\ nm)$$

$$\text{Chl b } (\mu g\ mL^{-1}) = 20.13\ (\text{Abs } 645\ nm) - 5.03\ (\text{Abs } 662\ nm)$$

$$\text{TC } (\mu g\ mL^{-1}) = [1000\ (\text{Abs } 470\ nm) - 3.270\ \text{Chl a} - 104\ \text{Chl b}]/198$$

All extraction procedures were performed in refrigerated and low-light conditions.

2.5.2. Sugars

Three replicates of a 25 g sample from the randomized batch were homogenized and extracted for 1 h using 200 mL of deionized water at boiling temperature. The extract was filtered through a Whatman 1 paper filter, and then analyzed for glucose, fructose, and sucrose content using a method already used by Sergio et al. [24]. Briefly, a Dionex chromatographic system (ED40 electrochemical detector, GP50 gradient pump, PeakNet 5.11 software) and a CarboPacPA1 column (4 × 250 mm) (Dionex Corporation, Sunnyvale, CA, USA) were used. All chromatographic details were as already reported by Sergio et al. [24]. The glucose, fructose, and sucrose content was expressed as g 100 g^{-1} DM.

2.5.3. Phenols and Antioxidant Activity

Three replicates of a 25 g sample from the randomized batch were homogenized and extracted (twice for 1 h) with boiling methanol (1:5 *w/v*). The extract was filtered through a Whatman 1 paper filter, and then the methanol was removed under vacuum. The obtained residue was dissolved in methanol 50% in water (*v/v*) and used for total phenolic (TP) content evaluation, antioxidant activity (TAA) assay, and for the HPLC analysis of phenolic compounds. The TP concentration was determined spectrophotometrically using the Folin–Ciocalteu method as reported by Gatto et al. [28]. Briefly, an aliquot of each extract was mixed with 0.5 mL of Folin–Ciocalteu reagent; then, an aliquot of 1 mL of sodium carbonate solution (20% *w/v*) was added after 3 min. After incubation at 40 °C for 20 min, the absorbance at 750 nm was measured using a Cary 50 UV–vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). Caffeic acid was used as the reference standard. The concentration of TP was expressed as mg of caffeic acid equivalent (CAE) 100 g^{-1} DM. Individual phenolics were evaluated by HPLC using an Agilent 1100 Series liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with binary gradient pump (Agilent P/N G1312A) and spectrophotometric photodiode array detector (DAD) (Agilent P/N G1328A); data were processed using the Agilent ChemStation (Rev. A.06.03) software. All chromatographic details were as reported by Gatto et al. [28]. The TAA was assayed by the ABTS assay [29]. Briefly, the ABTS radical cation (ABTS•+) was produced by adding potassium persulphate (2.45 mM, final concentration) to a 7 mM ABTS solution. The mixture was allowed in the dark at room temperature for 12–16 h. The ABTS•+ solution was then diluted with absolute ethanol to an absorbance of 0.700 ± 0.020 at 734 nm (blank) at the temperature of 30 °C. An amount of extract producing a decrease from 20% to 80% of the blank absorbance was used. The absorbance of the samples was read exactly 2.5 min after initial mixing. The antiradical activity was expressed as mg Trolox 100 g^{-1} DM.

2.6. Statistical Analysis

The effect of genotype and steaming on the physico-chemical and biochemical parameters were tested by performing a two-way ANOVA analysis using the SAS GLM procedure (SAS software, Cary, NC, USA). Means were separated by the Student–Newman–Keuls (SNK) test. For a visual analysis of the data, a Principal Component Analysis (PCA) (XLStat, Addinsoft, Paris, France) was performed on mean centered and standardized (unit variance scaled) data prior to the analysis. The data matrix submitted to the PCA was made up of 10 observations (five genotypes per two matrix types, raw and cooked) and 14 variables. The results of the PCA are shown as biplots of scores (genotypes × matrices) and loadings (variables).

3. Results and Discussion

3.1. Dry Matter of Raw Asparagus and Weight Change after Steaming

The effect of genotypes on DM content is reported in Figure 2, whereas the interaction between genotypes and steaming treatment was not significant. Wild asparagus showed on average a DM content 70% higher than the cultivated ones. This result is expected in consideration of the smaller diameter of the wild shoot and its greater fibrousness due also to the more uncertain growth conditions and the absence of external inputs (i.e., fertilizers and irrigation). On average, steaming did not determine significant changes in DM content, as also reported by Gonnella et al. [30] and Sergio et al. [26].

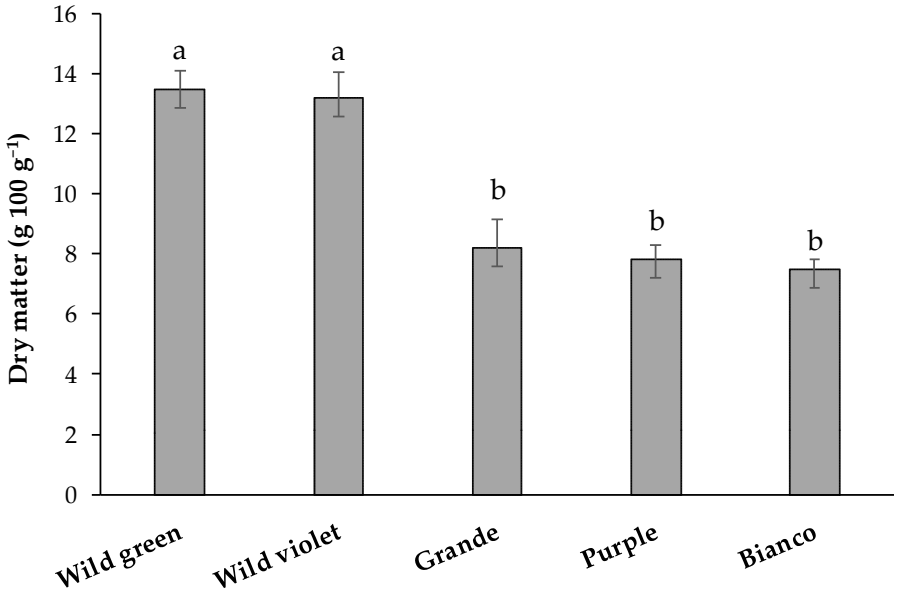

Figure 2. Average dry matter (DM) in different genotypes of raw asparagus ($p \leq 0.001$). The same letters indicate that mean values are not significantly different ($p = 0.05$). Vertical bars indicate standard deviation. Grande = 'Grande'; Purple = 'Purple Passion'; Bianco = 'Bianco di Bassano del Grappa'.

Steaming led to weight loss for all types of asparagus. The weight loss, which occurred following the steaming process, was more consistent in the cultivated asparagus than in the wild one. In particular, 'Bianco di Bassano del Grappa' had the greatest weight loss percent (6.93 ± 0.21) followed by 'Purple Passion' (3.29 ± 0.19), 'Grande' (2.67 ± 0.38), wild green (2.01 ± 0.41), and wild violet (1.12 ± 0.25).

3.2. Antioxidant Activity and Phenol Content

Steaming did not affect TAA in all genotypes, with the exception of wild violet asparagus, for which the TAA was raised by about 46% (Figure 3). This latter result might be explained by the remarkable increase of all recorded individual phenolics in wild violet asparagus after steaming (Table 1). Furthermore, no changes in TAA after steaming were also previously reported in wild green asparagus [25,26].

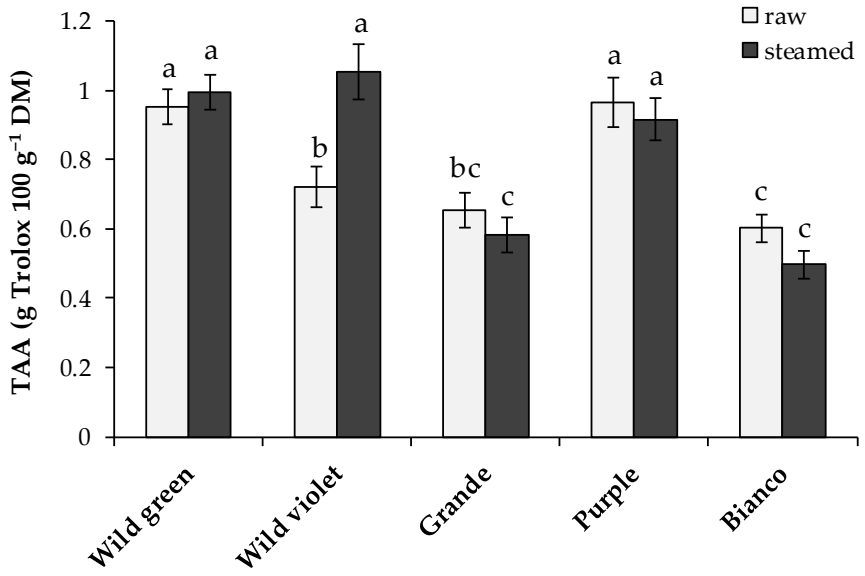

Figure 3. Total antioxidant activity (TAA) in raw and steamed asparagus ($p \leq 0.001$). The same letters indicate that the mean values are not significantly different ($p = 0.05$). Vertical bars indicate the standard deviation. Grande = 'Grande'; Purple = 'Purple Passion'; Bianco = 'Bianco di Bassano del Grappa'.

Table 1. Effects of steaming on phenolic composition in different genotypes of asparagus. SD = standard deviation.

Genotype	Type	Value	Chlorogenic Acid	Chicoric Acid	Rutin	I-3-Rutinoside
			(mg 100 g^{-1} DM)			
Wild green	Raw	Mean	66.76 b	82.08 a	80.41 e	95.69 d
		SD	6.01	7.88	7.94	7.02
	Steamed	Mean	77.11 b	79.20 a	116.32 d	137.15 c
		SD	6.95	6.9	9.88	10.03
Wild violet	Raw	Mean	70.16 b	30.94 b	203.03 c	154.16 b
		SD	7.04	3.25	20.11	11.38
	Steamed	Mean	84.63 a	78.46 a	220.19 bc	206.42 a
		SD	8.04	7.01	20.8	15.01
'Grande'	Raw	Mean	*n.d.*	*n.d.*	241.17 b	*tr.*
		SD	-	-	22	-
	Steamed	Mean	*n.d.*	*n.d.*	205.21 c	*tr.*
		SD	-	-	15.15	-
'Purple Passion'	Raw	Mean	*n.d.*	*n.d.*	310.40 a	*tr.*
		SD	-	-	24.2	-
	Steamed	Mean	*n.d.*	*n.d.*	318.04 a	*tr.*
		SD	-	-	24.75	-
'Bianco di Bassano del Grappa'	Raw	Mean	*n.d.*	*n.d.*	*n.d.*	*n.d.*
		SD	-	-	-	-
	Steamed	Mean	*n.d.*	*n.d.*	*n.d.*	*n.d.*
		SD	-	-	-	-
Significance			*	***	*	***

Significance: * and *** significant for $p \leq 0.05$ and $p \leq 0.001$, respectively. Different letters indicate statistically significant differences at $p = 0.05$; *n.d.* = not detected; *tr.* = traces, below the limit of quantification.

Steaming did not affect the TP content in the wild violet, 'Purple Passion', and 'Bianco di Bassano del Grappa' genotypes (Figure 4). Steaming increased the total phenols in wild green asparagus by about 49%, while it reduced them by about 31% in 'Grande'. (Figure 4). In general, changes in phenolic content in vegetables after cooking might

be caused by different processes: (i) oxidative degradation of phenolic acids (including enzymatic browning); (ii) release of free acids from conjugated forms; and (iii) formation of complex phenolic structures from related compounds, such as proteins, tannins, and anthocyanins [31]. In this specific case, steaming plays an important role in producing the final TP content of the cooked vegetable. In a previous study [26], no significant differences in TP content were found between the raw and steamed wild asparagus. On the contrary, in the present study, a tendency of an increase in phenolic content with steaming was observed in wild asparagus, probably due to a release of free phenolics into its fibrous structure. Indeed, in 'Grande', the observed decrease in TP content might be associated with the loss of rutin after steaming (Table 1).

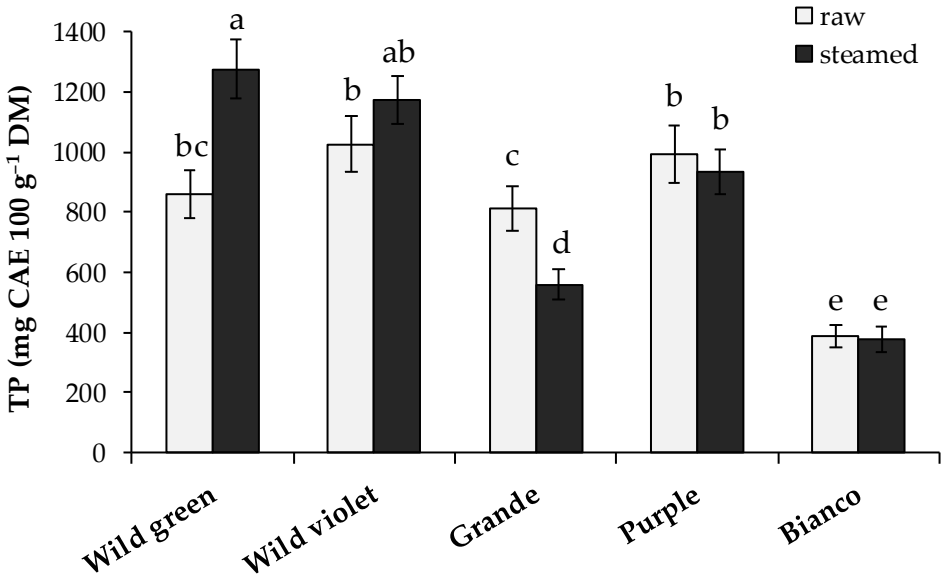

Figure 4. Total phenols (TP) content in raw and steamed asparagus ($p \leq 0.001$). The same letters indicate that the mean values are not significantly different ($p = 0.05$). Vertical bars indicate the standard deviation. Grande = 'Grande'; Purple = 'Purple Passion'; Bianco = 'Bianco di Bassano del Grappa'.

The quantification of different phenolic compounds is reported in Table 1. Chlorogenic acid and chicoric acid were found only in wild asparagus. Steaming did not affect the content of these phenolic compounds in wild green asparagus, while in the wild violet asparagus it increased the chlorogenic acid by about 21% and chicoric acid by about 154% (Table 1). The highest rutin content was found in 'Purple Passion', while it was not found in 'Bianco di Bassano del Grappa' (Table 1). Steaming did not affect the rutin content in wild violet asparagus, while in the wild green and 'Grande' it caused an increase of about 45% and a decrease of about 15%, respectively (Table 1). I-3-rutinoside was found in both wild asparagus genotypes, while only in very small amount (below the limit of quantification) in the 'Grande' and 'Purple' cultivated genotypes. In the wild genotypes its content was affected by steaming. Indeed, the content of this phenolic compound was 43 and 34% higher in steamed vs. raw wild green and violet asparagus, respectively (Table 1). It should be noted that all phenolic compounds found in the other genotypes were not found in 'Bianco di Bassano del Grappa'; in the latter, only a very small amount of an unidentified phenolic compound was detected, without significant differences in content caused by steaming.

Regarding the phenolic composition of wild asparagus, our findings are in agreement with results previously reported; actually, the presence of chlorogenic and chicoric acids, together with rutin and I-3-rutinoside as the main phenolic components, has been reported in wild asparagus [26,32]. Regarding cultivated asparagus, the presence of rutin as the main phenolic component has already been reported [7,23,24]. Changes in the content of individual phenolics as a consequence of the steaming were also found in good accordance

with data previously reported [26], confirming the goodness of the choice of steaming as the cooking method with the least impact on the phenolic compounds [26].

Generally speaking, structural differences between wild and cultivated genotypes could be responsible for a different balance between two opposite phenomena affecting the concentration of phenolics after steaming: degradation plus leaching, on the one hand, and matrix softening plus consequent higher extractability, resulting in a higher final concentration, on the other hand [31]. The first prevails in determining a higher susceptibility of the 'Grande' to lose rutin; the second causes a retention or even increase in all of the phenolic compounds in the wild asparagus.

3.3. Chlorophyll and Carotenoid Content

Steaming caused a significant reduction of chlorophylls and total carotenoids in all genotypes with the exception of 'Bianco di Bassano del Grappa', for which these pigments were not found (Figure 5).

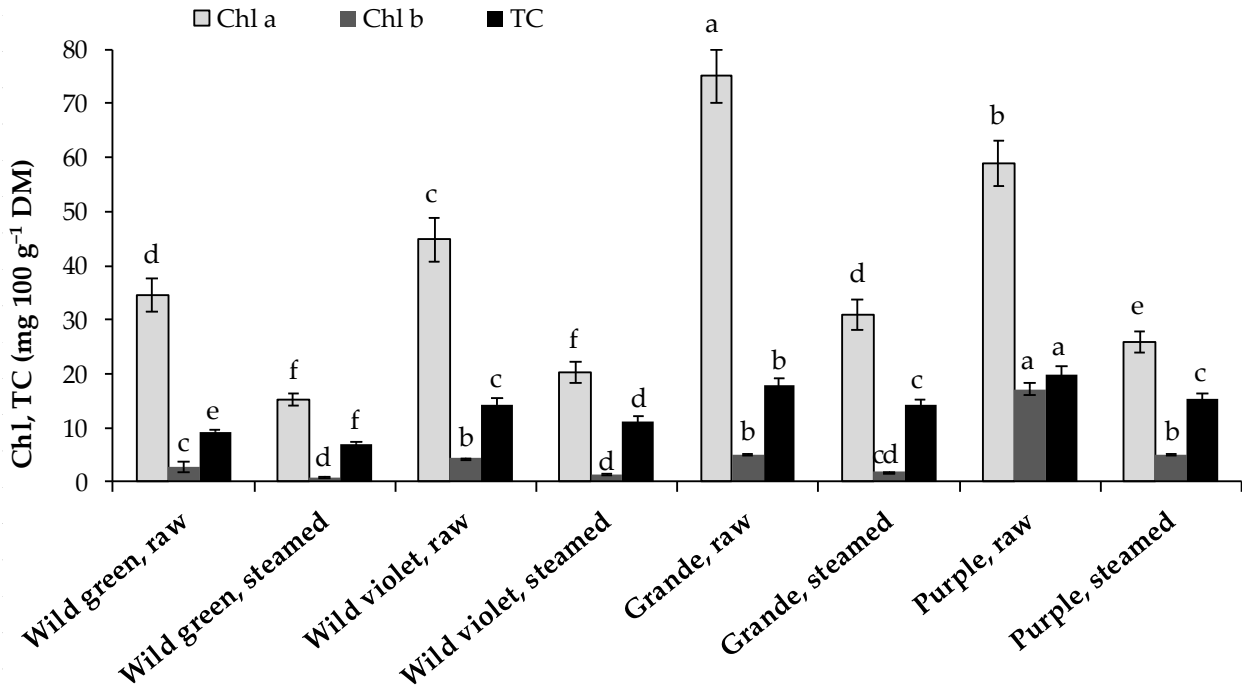

Figure 5. Content of chlorophyll (Chl) a and b, and total carotenoids (TC), in raw and steamed asparagus ($p \leq 0.001$). 'Bianco di Bassano del Grappa' is missing because the three pigments shown in the graph were not detected. For columns of the same parameter equal letters indicate that the mean values are not significantly different ($p = 0.05$). Vertical bars indicate the standard deviation. Grande = 'Grande'; Purple = 'Purple Passion'.

Among raw asparagus, the highest contents of Chl a and b were found, respectively, in 'Grande' and 'Purple Passion', while the lowest ones were found in wild green asparagus. 'Purple Passion' raw asparagus also showed the highest total carotenoid content, while raw wild green showed the lowest one (Figure 5). Similarly to what reported for raw asparagus, also in the steamed ones the highest contents of Chl a and b were found, respectively, in 'Grande' and 'Purple Passion', while the lowest ones were found in wild asparagus (both green and violet) (Figure 5). 'Grande' and 'Purple Passion' showed the highest content of total carotenoid among the steamed asparagus, while the lowest one was found in wild green asparagus (Figure 5).

The different content of these pigments in the studied cultivars and wild ecotypes could be attributed to both genetic and environmental factors. However, a study on the genetic distance between different populations of wild asparagus has not yet been carried out to evaluate the real differences. On the other hand, it could be probable that the wild

violet asparagus showed a significant increment in the content of Chl a (23%), Chl b (37%), and total carotenoids (35%) due to a higher exposition to the sun, since the violet type is more frequent in the areas with greater insolation than the green one. On the opposite, there is the absence of these pigments in the white asparagus cultivar due to its cultivation technique: the traditional growing method is to mound up soil over the plant row, before the spears start to grow and harvesting is done before the spear is exposed to light [33].

Genetic and environmental factors also influence the loss of these pigments during steaming, as the texture of the asparagus spears also affects the separation and breakdown of cells and thus the release of pigments. Indeed, as expected, steaming reduced the chlorophyll content more in cultivated asparagus (59%) than in the wild one (56%) (Figure 5), which generally have a more fibrous structure. After steaming, the total carotenoid content decreased by about 20–24%, in the following order: 'Grande' < wild violet < 'Purple Passion' < wild green. Our finding are consistent with those of Gonnella et al. [30], reporting for 'Grande' cooked by steaming a reduction in total chlorophyll of more than 57% and total carotenoids by 20%.

The content of chlorophyll a and b changed differently with the steaming; in particular, chlorophyll b showed a more severe reduction (63–71%) than chlorophyll a (55–59%). This results in an increase in the chlorophyll a/b ratio in accordance with Gonnella et al. [30], but in disagreement with Danowska-Oziewicz et al. [34], who for a steamed green asparagus cultivar found a reduction of Chl a (22%) but no change in Chl b.

The loss and/or degradation of chlorophylls during cooking was not only due to the separation and breakdown of the cells causing the pigment release but also due to the transformation of chlorophyll a into pheophitin a and chlorophyll b into pheophitin b [35]. Some authors in different green vegetables report greater lability, and therefore a greater susceptibility to pheophytinisation of Chl a [8,36] and others of Chl b [35].

For carotenoids, in addition to the release by cell breakdown that occurs with cooking, oxidation due to the double bonds presenting in their structure also has been hypothesized; this transformation would be highly dependent on oxygen, light access, and high temperature during processing [37].

3.4. Sugar Content

According to Slatnar et al. [9], fructose was the most abundant sugar in both the wild and cultivated genotypes, followed by glucose and lower amounts of sucrose; the latter was also reported by Di Maro et al. [31] (Figure 6). Overall, the sugar content in wild asparagus was lower than in the cultivated ones (Figure 6); this is probably due to the different structure of the shoot in the two genotypes, with a higher percentage of fibrous tissue in the wild than in the cultivated one. Steaming did not affect the glucose and fructose content in all genotypes with the exception of 'Purple Passion', for which steaming caused a reduction of about 47% for both glucose and fructose (Figure 6). Regarding sucrose, steaming did not affect the content in wild green asparagus and 'Grande', while for 'Purple Passion' and 'Bianco di Bassano del Grappa' it caused a reduction of 38% and 25%, respectively. On the other hand, the sucrose content in steamed wild violet asparagus was 40% higher than in the raw ones. On average, among the steamed asparagus, 'Bianco di Bassano del Grappa' showed a total sugar content higher than all the other genotypes (Figure 6).

The variation in sugar content due to steaming, as well as of other soluble components of a vegetable, could strongly depend on genetic differences in the tissue structure of the vegetable itself [31]. Therefore, the different behavior shown by each genotype towards these variations can be attributed to this factor. In particular, this might justify the poor ability to retain sugars during steaming, shown by 'Purple Passion', in which there was a significant decrease in all three soluble sugars. On the other hand, the same justification could be given, albeit with a reverse effect, for the slight but significant increase after steaming in the sucrose content recorded in wild violet.

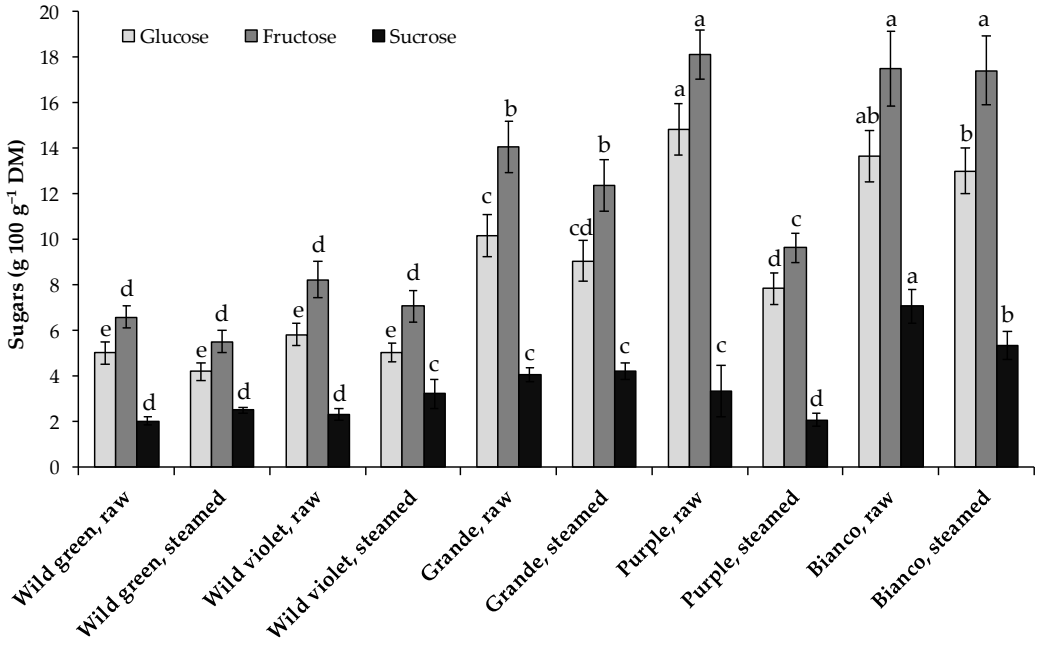

Figure 6. Content of glucose ($p \leq 0.001$), fructose ($p \leq 0.001$), and sucrose ($p \leq 0.01$) in raw and steamed asparagus. For columns of the same color the same letters indicate that mean values are not significantly different ($p = 0.05$). Vertical bars indicate the standard deviation. Grande = 'Grande'; Purple = 'Purple Passion'; Bianco = 'Bianco di Bassano del Grappa'.

3.5. Color Traits

The color traits of different genotypes of asparagus (raw and steamed) are reported in Table 2. Steaming caused a decrease in the L value in all genotypes with the exception of purple-like asparagus (both 'Purple Passion' and wild violet). Among raw asparagus, the highest and lowest L value were found, respectively, in 'Bianco di Bassano del Grappa' and wild green, while the highest and lowest L value in steamed asparagus were found, respectively, in the cultivar 'Bianco di Bassano del Grappa' and the purple-like types (both 'Purple Passion' and wild violet). With the exception of green-like asparagus (both 'Grande' and wild green), steaming caused a more negative value of the *a** parameter. At the same time, steaming caused an increase in both the *b** and C values in purple-like asparagus (both 'Purple Passion' and wild violet). Steaming did not affect the h° value in 'Grande' and wild green asparagus but it caused an increase in all the other samples. The L parameter (lightness) indicates a value of luminosity (between 0 and 100), which is the property according to which each color can be considered as equivalent to a member of the grey scale, between black (0) and white (100) [38]. The parameter *a** takes positive values for reddish colors and negative values for the greenish ones, whereas *b** takes positive values for yellowish colors and negative values for the bluish ones. The C parameter, considered the quantitative attribute of colorfulness, is used to determine the degree of difference of a hue in comparison to a grey color with the same lightness. The higher the chroma values, the higher is the color intensity of samples perceived by humans [39]. Hue angle (h°), considered the qualitative attribute of color, is the attribute according to which colors have been traditionally defined as reddish, greenish, etc., and it is used to define the difference of a certain color with reference to grey color with the same lightness [39].

Color is an important quality attribute for vegetable products, and it influences consumer's choice and preferences. It is important to highlight that the color of vegetables is derived from natural pigments, many of which change depending on genotype as well as being a consequence of the applied food processing. Actually, color may be considered also an indicator of heat treatment and can be used to predict the corresponding quality deterioration resulting from heat exposure [40–42]. The primary pigments that are responsible for the color quality in vegetables are chlorophylls (green), carotenoids (yellow, orange, and

red), water-soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red) [43]. In agreement, results of our study highlight that steaming influenced the content of both chlorophylls and carotenoids in asparagus spears, translating also in changes in the color parameters in steamed spears. Actually, we found a negative correlation between h° and Chl b (R = −0.827; $p = 0.05$) as well as between L and total carotenoids (R = −0.768; $p = 0.05$).

Table 2. Effects of steaming on color traits in different genotypes of asparagus. SD = standard deviation.

Genotype	Type	Value	L	*a**	*b**	C	h°
Wild green	Raw	Mean	46.94 d	−16.47 f	32.42 a	36.37 a	116.92 b
		SD	0.94	0.76	0.13	0.46	0.98
	Steamed	Mean	40.15 e	−14.03 e	28.82 b	32.06 b	115.93 b
		SD	1.43	1.41	1.53	1.96	1.23
Wild violet	Raw	Mean	26.77 g	8.17 a	9.16 f	12.28 d	48.27 e
		SD	1.57	0.11	0.34	0.31	0.83
	Steamed	Mean	32.05 f	−10.80 d	19.11 d	21.95 c	119.47 b
		SD	0.92	0.12	0.34	0.33	0.37
'Grande'	Raw	Mean	52.15 c	−16.12 f	32.97 a	36.69 a	116.05 b
		SD	1.14	0.41	0.2	0.36	0.44
	Steamed	Mean	41.71 e	−13.81 e	27.34 c	30.63 b	116.78 b
		SD	0.55	1.03	1.06	1.41	0.83
'Purple Passion'	Raw	Mean	25.86g	9.02 a	1.81 h	9.21 e	11.46 f
		SD	0.43	0.86	0.34	1.38	2.71
	Steamed	Mean	31.22 f	−3.43 c	11.98 e	12.47 d	105.79 c
		SD	1.25	1.04	1.16	1.38	3.29
'Bianco di Bassano del Grappa'	Raw	Mean	78.85 a	−1.22 b	11.23 e	11.30 d	96.21 d
		SD	1.06	0.13	0.23	0.22	0.79
	Steamed	Mean	63.43 b	−4.34 c	6.00 g	7.40 f	125.86 a
		SD	1.1	0.19	0.15	0.23	0.53
Significance			***	***	***	***	***

Significance: *** significant for $p \leq 0.001$. Different letters indicate statistically significant differences at $p = 0.05$.

3.6. Principal Component Analysis

The PCA gives a graphical representation of the separation of the effects that can be analyzed through ANOVA. Indeed, the results given by the ANOVA can be also found in the biplot shown in Figure 7. The biplot shows scores (observations) and loadings (variables) at the same time, allowing an easier view of their distribution. The most evident separation in this biplot is that between the wild asparagus samples (raw and steamed) on the left (the negative side of PC1) and all the cultivated asparagus on the right (the positive side of PC1). The main variables affecting PC1 are sugars, positively correlated to this PC, and most of the antioxidant compounds (chlorogenic, chicoric acids, and I-3-rut) and DM on the opposite side. The first ones are highly correlated with the white asparagus, both raw and steamed, effectively rich in reducing sugars and sucrose. The variables negatively influencing PC1 are highly correlated to the wild asparagus, green and violet, where the listed compounds were found (Table 1), and characterized by the highest DM content (Figure 2). Regarding PC2, the most influencing variables are chlorophyll a and b, TC, and rutin, which among the t hose positively correlated to this PC, whereas L and h° are among the negatively correlated variables. Indeed, the white asparagus and L are in the same quarter of the biplot, since it showed the highest value of L (Table 2). On the other hand, purple asparagus, especially raw, was highly correlated to the compounds (chlorophyll a and b, TC, and rutin) placed on the positive side of PC2.

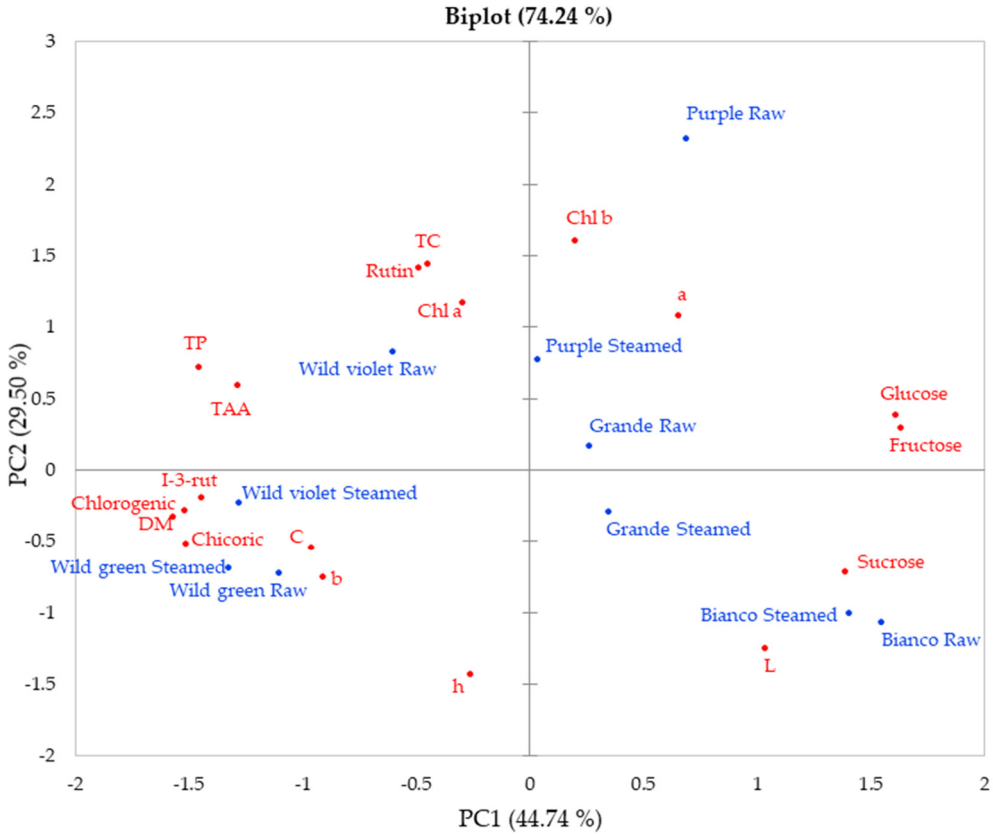

Figure 7. PCA biplot (PC1 vs. PC2) describing the distribution of the chemical-physical parameters in relation to the asparagus genotypes and their status (raw or steamed). DM, dry matter; L, brightness; *a**, redness; *b**, yellowness; h°, hue angle; C, saturation; TAA, total antioxidant activity; TP, total phenolics; Chlorogenic, chlorogenic acid; Chicoric, chicoric acid; I-3-rut, I-3-rutinoside; Chl a, chlorophyll a; Chl b, chlorophyll b; TC, total carotenoids; Grande, 'Grande'; Purple, 'Purple Passion'; Bianco, 'Bianco di Bassano del Grappa'.

4. Conclusions

In this study, for the first time, some quality traits of wild and cultivated asparagus spears were compared before and after steaming. The results indicate that wild asparagus has a qualitatively richer phenolic compounds composition than the cultivated ones; this in some cases could have determined an increase in antioxidant activity following steaming. On the other hand, wild asparagus generally showed lower values of chlorophylls, carotenoids, and sugars (glucose, fructose, and sucrose) than cultivated asparagus. Steaming influenced the content of both chlorophylls and carotenoids in asparagus spears, also causing changes in the color parameters in steamed spears. Overall, results of the present study indicate that wild asparagus can be considered a nutritious and refined food. Our results also provide specific information usually useful for cooking process strategies in the agri-food industrial sector. Our findings are of particular interest taking into consideration the increasing market demand for wild asparagus due to their high nutritional value. Nevertheless, more information about the effect of storage on the preservation of quality in steamed wild asparagus will be required before any hypothetical industrial implementation.

Author Contributions: Conceptualization, L.S., F.B., D.D.V., V.C. and M.R.; methodology, L.S., F.B., D.D.V. and M.R.; validation, L.S., V.C. and M.R.; formal analysis, M.G.; investigation, L.S., F.B., D.D.V., M.G. and M.R.; resources, F.B., M.G. and V.C.; data curation, L.S., D.D.V., M.G. and M.R.; writing—original draft preparation, L.S., F.B., D.D.V., M.G. and M.R.; writing—review and editing, L.S., F.B., M.G. and M.R.; visualization, L.S., D.D.V., M.G. and M.R.; supervision, L.S., V.C. and

M.R.; project administration, V.C.; funding acquisition, V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Project "Recupero ambientale del Bosco di Gravina in Puglia e valorizzazione della sua biodiversità attraverso la creazione di microfiliere gastronomiche" (BoBioGa), P.S.R. Puglia 2014/2020-Misura 16–Cooperazione-Sottomisura 16.2 "Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie"-D.A.G. n. 194 del 12/09/2018, pubblicata nel B.U.R.P. n. 121 del 20/09/2018", funding number sfc:B89J20000150009.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors thank Domenico Genchi for IT support, Simonetta Martena for providing administrative technical collaboration, and Pasquale Del Vecchio for bibliometric and data management support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. García-Herrera, P.; Morales, P.; Cámara, M.; Fernández-Ruiz, V.; Tardío, J.; Sánchez-Mata, M.C. Nutritional and phytochemical composition of mediterranean wild vegetables after culinary treatment. *Foods* **2020**, *9*, 1761. [CrossRef]
2. Solana, M.; Boschiero, I.; Dall'Acqua, S.; Bertucco, A. A comparison between supercritical fluid and pressurized liquid extraction methods for obtaining phenolic compounds from *Asparagus officinalis* L. *J. Supercrit. Fluids* **2015**, *100*, 201–208. [CrossRef]
3. Lee, J.W.; Lee, J.H.; Yu, I.H.; Gorinstein, S.; Bae, J.H.; Ku, Y.G. Bioactive compounds, antioxidant and binding activities and spear yield of *Asparagus officinalis* L. *Plant Foods Hum. Nutr.* **2014**, *69*, 175–181. [CrossRef] [PubMed]
4. Ferrara, L.; Dosi, R.; Di Maro, A.; Guida, V.; Cefarelli, G.; Pacifico, S.; Mastellone, C.; Fiorentino, A.; Rosati, A.; Parente, A. Nutritional values, metabolic profile and radical scavenging capacities of wild asparagus (*A. acutifolius* L.). *J. Food Compos. Anal.* **2011**, *24*, 326–333. [CrossRef]
5. Słupski, J.; Korus, A.; Lisiewska, Z.; Kmiecik, W. Content of amino acids and the quality of protein in as-eaten green asparagus (*Asparagus officinalis* L.) products. *Int. J. Food Sci. Technol.* **2010**, *45*, 733–739. [CrossRef]
6. Fuentes-Alventosa, J.M.; Rodríguez-Gutiérrez, G.; Jaramillo-Carmona, S.; Espejo-Calvo, J.A.; Rodríguez-Arcos, R.; Fernández-Bolaños, J.; Guillén-Bejarano, R.; Jiménez-Araujo, A. Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chem.* **2009**, *113*, 665–671. [CrossRef]
7. Sergio, L.; Cantore, V.; Spremulli, L.; Pinto, L.; Baruzzi, F.; Di Venere, D.; Boari, F. Effect of cooking and packaging conditions on quality of semi-dried green asparagus during cold storage. *LWT-Food Sci. Technol.* **2018**, *89*, 712–718. [CrossRef]
8. Mazzeo, T.; Paciulli, M.; Chiavaro, E.; Visconti, A.; Fogliano, V.; Ganino, T.; Pellegrini, N. Impact of the industrial freezing process on selected vegetables -Part II. Colour and bioactive compounds. *Food Res. Int.* **2015**, *75*, 89–97. [CrossRef] [PubMed]
9. Slatnar, A.; Mikulic-Petkovsek, M.; Stampar, F.; Veberic, R.; Horvat, J.; Jakse, M.; Sircelj, H. Game of tones: Sugars, organic acids, and phenolics in green and purple asparagus (*Asparagus officinalis* L.) cultivars. *Turk. J. Agric. For.* **2018**, *42*, 55–66. [CrossRef]
10. Mitchell, S.C.; Waring, R.H. Asparagusic acid. *Phytochemistry* **2014**, *97*, 5–10. [CrossRef]
11. Wang, J.; Liu, Y.; Zhao, J.; Zhang, W.; Pang, X. Saponins extracted from by-product of Asparagus officinalis L. suppress tumour cell migration and invasion through targeting Rho GTPase signalling pathway. *J. Sci. Food Agric.* **2013**, *93*, 1492–1498. [CrossRef]
12. Jaramillo, S.; Muriana, F.J.G.; Guillen, R.; Jimenez-Araujo, A.; Rodriguez-Arcos, R.; Lopez, S. Saponins from edible spears of wild asparagus inhibit AKT, p70S6K, and ERK signalling, and induce apoptosis through G0/G1 cell cycle arrest in human colon cancer HCT-116 cells. *J. Funct. Foods* **2016**, *26*, 1–10. [CrossRef]
13. Palfi, M.; Tomic-obrdalj, H.; Horvat, D. Healthy vegetables for healthy heart: Asparagus. *Cardiol. Croat.* **2014**, *9*, 142–149.
14. Hafizur, R.M.; Kabir, N.; Chishti, S. *Asparagus officinalis* extract controls blood glucose by improving insulin secretion and β-cell function in streptozotocin-induced type 2 diabetic rats. *Br. J. Nutr.* **2012**, *108*, 1586–1595. [CrossRef] [PubMed]
15. Guarrera, P.M.; Savo, V. Perceived health properties of wild and cultivated food plants in local and popular traditions of Italy: A review. *J. Ethnopharmacol.* **2013**, *146*, 659–680. [CrossRef] [PubMed]
16. Ghirardini, M.P.; Carli, M.; del Vecchio, N.; Rovati, A.; Cova, O.; Valigi, F.; Agnetti, G.; Macconi, M.; Adamo, D.; Traina, M.; et al. The importance of a taste. A comparative study on wild food plant consumption in twenty-one local communities in Italy. *J. Ethnobiol. Ethnomed.* **2007**, *3*, 22. [CrossRef]
17. Acosta-Naranjo, R.; Guzmán-Troncoso, A.J.; Gómez-Melara, J. The persistence of wild edible plants in agroforestry systems: The case of wild asparagus in Southern Extremadura (Spain). *Agrofor. Syst.* **2020**, *94*, 2391–2400. [CrossRef]
18. Maeda, T.; Honda, K.; Sonoda, T.; Motoki, S.; Inoue, K.; Suzuki, T.; Oosawa, K.; Suzuki, M. Light condition influences rutin and polyphenol contents in asparagus spears in the mother-fern culture system during the summer-autumn harvest. *J. Jpn. Soc. Hortic. Sci.* **2010**, *79*, 161–167. [CrossRef]

19. Lo Porto, C.; Sergio, L.; Boari, F.; Logrieco, A.F.; Cantore, V. Cold plasma pretreatment improves the germination of wild asparagus (*Asparagus acutifolius* L.) seeds. *Sci. Hortic.* **2019**, *256*, 108554. [CrossRef]
20. Benincasa, P.; Tei, F.; Rosati, A. Plant density and genotype effects on wild asparagus (*Asparagus acutifolius* L.) spear yield and quality. *HortScience* **2007**, *42*, 1163–1166. [CrossRef]
21. Conversa, G.; Elia, A. Effect of seed age, stratification, and soaking on germination of wild asparagus (*Asparagus acutifolius* L.). *Sci. Hortic.* **2009**, *119*, 241–245. [CrossRef]
22. Conversa, G.; Lazzizera, C.; Elia, A. Effects of after-ripening, stratification and GA3 on dormancy release and on germination of wild asparagus (*Asparagus acutifolius* L.) seeds. *Sci. Hortic.* **2010**, *125*, 196–202. [CrossRef]
23. Fanasca, S.; Rouphael, Y.; Venneria, E.; Azzini, E.; Durazzo, A.; Maiani, G. Antioxidant properties of raw and cooked spears of green asparagus cultivars. *Int. J. Food Sci. Technol.* **2009**, *44*, 1017–1023. [CrossRef]
24. Sergio, L.; Gonnella, M.; Renna, M.; Linsalata, V.; Gatto, M.A.; Boari, F.; Di Venere, D. Biochemical traits of asparagus cultivars and quality changes in two differently coloured genotypes during cold storage. *LWT* **2019**, *101*, 427–434. [CrossRef]
25. Boari, F.; Cefola, M.; Di Gioia, F.; Pace, B.; Serio, F.; Cantore, V. Effect of cooking methods on antioxidant activity and nitrate content of selected wild Mediterranean plants. *Int. J. Food Sci. Nutr.* **2013**, *64*, 870–876. [CrossRef] [PubMed]
26. Sergio, L.; Boari, F.; Pieralice, M.; Linsalata, V.; Cantore, V.; Di Venere, D. Bioactive phenolics and antioxidant capacity of some wild edible greens as affected by different cooking treatments. *Foods* **2020**, *9*, 1320. [CrossRef]
27. Wellburn, A.R. The Spectral Determination of Chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [CrossRef]
28. Gatto, M.A.; Sergio, L.; Ippolito, A.; Di Venere, D. Phenolic extracts from wild edible plants to control postharvest diseases of sweet cherry fruit. *Postharvest Biol. Technol.* **2016**, *120*, 180–187. [CrossRef]
29. Pellegrini, N.; Re, R.; Yang, M.; Rice-Evans, C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-azinobis(3-ethylenebenzothiazoline-6- sulfonic acid radical cation decolorization assay. *Methods Enzymol.* **1998**, *299*, 379–389. [CrossRef]
30. Gonnella, M.; Durante, M.; Caretto, S.; D'Imperio, M.; Renna, M. Quality assessment of ready-to-eat asparagus spears as affected by conventional and sous-vide cooking methods. *LWT-Food Sci. Technol.* **2018**, *92*, 161–168. [CrossRef]
31. Palermo, M.; Pellegrini, N.; Fogliano, V. The effect of cooking on the phytochemical content of vegetables. *J. Sci. Food Agric.* **2014**, *94*, 1057–1070. [CrossRef] [PubMed]
32. Di Maro, A.; Pacifico, S.; Fiorentino, A.; Galasso, S.; Gallicchio, M.; Guida, V.; Severino, V.; Monaco, P.; Parente, A. Raviscanina wild asparagus (*Asparagus acutifolius* L.): A nutritionally valuable crop with antioxidant and antiproliferative properties. *Food Res. Int.* **2013**, *53*, 180–188. [CrossRef]
33. Papoulias, E.; Siomos, A.S.; Koukounaras, A.; Gerasopoulos, D.; Kazakis, E. Effects of genetic, pre- and post-harvest factors on phenolic content and antioxidant capacity of White Asparagus spears. *Int. J. Mol. Sci.* **2009**, *10*, 5370–5380. [CrossRef] [PubMed]
34. Danowska-Oziewicz, M.; Narwojsz, A.; Draszanowska, A.; Marat, N. The effects of cooking method on selected quality traits of broccoli and green asparagus. *Int. J. Food Sci. Technol.* **2020**, *55*, 127–135. [CrossRef]
35. Turkmen, N.; Poyrazoglu, E.S.; Sari, F.; Velioglu, Y.S. Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. *Int. J. Food Sci. Technol.* **2006**, *41*, 281–288. [CrossRef]
36. Tenorio, M.; Villanueva, M.; Sagardoy, M. Changes in carotenoids and chlorophylls in fresh green asparagus (*Asparagus officinalis* L.) stored under modified atmosphere packaging. *J. Sci. Food Agric.* **2004**, *84*, 357–365. [CrossRef]
37. Pénicaud, C.; Achir, N.; Dhuique-Mayer, C.; Dornier, M.; Bohuon, P. Degradation of β-carotene during fruit and vegetable processing or storage: Reaction mechanisms and kinetic aspects: A review. *Fruits* **2011**, *66*, 417–440. [CrossRef]
38. Granato, D.; Masson, M.L. Instrumental color and sensory acceptance of soy-based emulsions: A response surface approach. *Ciênc. Tecnol. Aliment.* **2010**, *30*, 1090–1096. [CrossRef]
39. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.J. Colour measurement and analysis in fresh and processed foods: A review. *Food Bioprocess Technol.* **2013**, *6*, 36–60. [CrossRef]
40. Renna, M.; Pace, B.; Cefola, M.; Santamaria, P.; Serio, F.; Gonnella, M. Comparison of two jam making methods to preserve the quality of colored carrots. *LWT-Food Sci. Technol.* **2013**, *53*, 547–554. [CrossRef]
41. Shin, S.; Bhowmik, S.R. Thermal kinetics of color changes in pea puree. *J. Food Eng.* **1995**, *24*, 77–86. [CrossRef]
42. Lozano, J.E.; Ibarz, A. Colour changes in concentrated fruit pulp during heating at high temperatures. *J. Food Eng.* **1997**, *31*, 365–373. [CrossRef]
43. Barrett, D.M.; Beaulieu, J.C.; Shewfelt, R. Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 369–389. [CrossRef] [PubMed]