Effect of plantation age on organoleptic traits of a typical Italian green asparagus crop

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Abstract Asparagus is a very common and important vegetable in Italy especially in the North-east regions where it is also a typical and historical product. For green asparagus, and in particular for typical cultivar grown in Northern Italy, the information on these issues are limited. For these reasons experiments have been carried out in order to evaluate nitrates, nitrites, polyphenols and pigments contents and antioxidants activity of marketable product of green asparagus cv Eros. The qualitative analysis on green shoots were performed on samples coming from 2 plantations with different age: 3 years old crop (YC) and 8 years old crop (OC). During harvest (from April till May), six samplings were performed. After each harvest, shoots were calibrated, washed and placed at 4 C° till next day. For qualitative analysis only extra class shoots (diameter >16 mm) were used. Shoots were weighted and then cut to 270 mm from the apex in order to separate not marketable portion from the commercial one. The latter fraction was cut in 3 parts 90 mm long (apical (A), intermediate (I) and basal (B)) to evaluate quality characteristics of each part. Results showed that OC asparagus had a higher content of dry matter and a greater weight. The nitrate content, which was always far below the recommended WHO limits, was not influenced by location, while considering the part of shoot, as expected, a decreasing trend from the base to the apex was observed. For nitrites there were no significant differences between locations and among shoot parts. The pigments content increased during the advancing of the harvest season in OC that showed the highest values. The content of these compounds strongly increased moving from the base to the top of the shoot and the chlorophyll a content was higher than the chlorophyll b and the pool xanthophylls + carotenoids. The antioxidant activity and polyphenol analysis showed that the upper part is the richest in these compounds mainly because directly exposed to stress situations.

Key words Asparagus officinalis L., quality, antioxidant, phenolics, vitamin C, nitrate

Asparagus is one of the most important perennial vegetables in the world. Europe and North America are the major production areas followed by Asia, South America, Australia and Africa. In Italy this crop is cultivated both for green and white asparagus production that are mainly marketed during spring (March-June). Asparagus is a good source of essential minerals, vitamins, amino acids and dietary fibers [15]. In addition, asparagus is rich in antioxidants, and the main components responsible for asparagus bioactivity are phenol (flavonoids), carotenoids, oligosaccharides and rutin, a drug which strengthens capillary walls [7;12]. However, spear nutritional quality is highly affected by environmental factors especially temperature and light, and fern growth [9;21]. Lill and Borst [8] demonstrated that shoots harvested in cool conditions were of higher postharvest quality than those harvested in warm conditions. Poll [11] found that the content of cellulose increased with the rise of temperature when it was not above 14 °C. However, little is known about the influence of plantation age and its interactions with agronomic practices on the nutritional quality of green asparagus during harvest time. Compositional profile of green asparagus spears was expected to be significantly affected by plantation age, and so it is important to know the extent of the seasonal fluctuations of nutritional composition. Consequently in the present study, fresh spears of two plantation with different age were harvested during production period to assess the seasonal changes in nutritional traits.
Materials and Methods

Qualitative analysis of green shoots were carried out at the Department of Agronomy, Food, Natural Resources, Environment and Animals at University of Padova. Asparagus material came from experimental farm located in the province of Padova (45° 36’ 0” N, 11° 56’ 0” E). The experimental site was characterized by loamy soil and the asparagus plantation was realized using 12 months old root stock. In this trial asparagus shoots of different ages were evaluated: a young crop, at the first year of harvest (3 years after implantation) and a medium age crop after 5 years of harvest (8 years after implantation). The plantation adopted provides simple rows far between 2.30 m and a distance between plants on the row of 0.27 m, with a density of about 16000 plants ha\(^{-1}\). In both asparagus plantations 100 kg ha\(^{-1}\) of N, 90 kg ha\(^{-1}\) of P\(_2\)O\(_5\) and 160 kg ha\(^{-1}\) of K\(_2\)O were distributed. Nitrogen total amount was divided into two distributions: at the beginning of vegetative growth and at the end of shoots harvest time; whereas phosphorus was distributed in the autumn after the elimination of the dried plants. About qualitative analysis, 3 plots 25 m\(^2\) wide (5 x 5 m) for each asparagus plantation were identified within which during the harvest period shoots of the cultivar "Eros" were taken. The harvest period of shoots with commercial length began the 6\(^{th}\) of April and lasted until 15\(^{th}\) of May. In this period, six successive samplings denominated with S1, S2, S3, S4, S5, S6 corresponding to 10\(^{th}\) - 17\(^{th}\) - 24\(^{th}\) of April and 2\(^{nd}\) - 8\(^{th}\) - 15\(^{th}\) of May respectively were carried out. In each sampling only extra category (Ø> 16 mm) shoots were considered for qualitative analysis. Immediately after harvest shoots were placed in a refrigerator at 4 °C until the following day when samples were processed. This in order to simulate the usual storage practice adopted by growers before selling the product. Then the whole shoots were washed, weighed and cut to 270 mm from the apex so as to separate the waste portion from the marketable one. The marketable fraction was cut at the same time to obtain three equal parts (apical, middle and basal) in order to be able to assess any changes about qualitative properties passing from the apex to the basal part together with the waste. From each shoot 4 portions represented by the apical part, intermediate part, basal part and waste were obtained. Each sample was cut into 10 mm thick pieces and, subsequently, a portion was used to obtain the dry matter in a ventilated stove at 65 °C and the remainder was immediately frozen in a freezer at -80 °C to be used later for qualitative analysis. For each sample dry matter determinations, antioxidant activity (AOA), total phenols (TP), pigments (chlorophyll \(a\) and \(b\), carotenoids+xanthophylls), nitrate (NO\(_3\)) and nitrite (NO\(_2\)) were quantified.

Concerning the phenols extraction for analysis, asparagus tissues (5 g) were homogenized in methanol (20 mL) with an Ultra Turrax T25 at 13.500 rpm until uniform consistency. Samples were filtered (filter paper, Schleicher 589) and appropriate aliquots of extracts were assayed by a FC assay for total phenols (TPs) content. The TPs content was determined using the FC assay with gallic acid as calibration standard, by a Shimadzu UV-1800 spec-trophotometer (Columbia, MD, USA). The FC assay was the carried out by pipetting 200 µL of asparagus extract into a 10 mL PP tube. This was followed by additions of 1 mL of Folin-Ciocalteau’s reagent. The mixture was vortexed for 20-30 s and 800 µL of filtered 20% sodium carbonate solution was added after 1 min and before 8 min from the additions of the FC reagent. This was recorded as time zero, the mixture was then vortexed for 20-30 s after addition of sodium carbonate. After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm. The TPs content in the extracts was calculated from a standard calibration curves obtained with different concentrations of gallic acid, ranging from 0 to 600 µg mL\(^{-1}\) (correlation coefficient: \(R^2 = 0.9991\)). Results were expressed on the basis of mg of Gallic Acid Equivalent per kilogram (mg GAE kg\(^{-1}\)) of fresh asparagus [18].

About the determination of total antioxidant activity by Ferric Reducing Antioxidant Power, the assay was based on the methodology of Benzie and Strain [1]. The Ferric Reducing Antioxidant Power (FRAP) reagent was prepared fresh so that it contained 1 mM 2,4,6-triazine-2-tripyridyl (TPTZ) and 2 mM ferric chloride in 0.25 M sodium acetate at pH 3.6. A 100 µL aliquot of the methanol extract prepared as above was added to 1900 µL of FRAP reagent, mixed and allowed to react with sulfanilic acid, ranging from 0 to 600 µg mL\(^{-1}\) (correlation coefficient: \(R^2 = 0.9982\)) obtained by the additions of freshly prepared ammonium ferrous sulfate. FRAP values were calculated as µg mL\(^{-1}\) ferrous ion (ferric reducing power) from three determinations and are presented as mg kg\(^{-1}\) of Fe\(^{2+}\) E (ferrous ion equivalent).

Chlorophyll \(a\), \(b\) and xanthophylls + carotenoids were determined colorimetrically [22] and the following formulas where \(A\) is the absorbance: CHLa = (13.95 \(\times\) A665) - (6.88 \(\times\) A649); CHLB = (24.96 \(\times\) A649) - (7.32 \(\times\) A665); Xan + Car = [(1000 \(\times\) A470) - (2.05 \(\times\) CHLA) - (114.8 \(\times\) CHLB)] / 245. Results are expressed in µg g\(^{-1}\) fw.

Nitrate content was determined colorimetrically [2]. Approximately 0.2 g asparagus dry matter was boiled for 30 minutes and then filtered through filter paper Schleicher 589. The extract was used after cooling. The standard curve was developed with KNO\(_3\). Nitrite content was evaluated using the same extract as that for NO\(_3\) [19]. In the method, nitrite is allowed to react with sulfanilamide in an acid solution (10 g L\(^{-1}\) HCl 3N). The resulting compound reacts with N-(1-naphyl)-ethylen diamine dihydrochloride (0.2 g L\(^{-1}\) in H\(_2\)O) to form a pink solution, whose absorbance
is measured at 540 nm (Shimadzu UV-1800). The standard curve was developed with KNO₃ 0.002M.

Statistical analysis was performed according to a factorial design with the factorial combination of 2 plantation age (1 year and 5 year of harvest) x 6 harvest dates (S1, S2, S3, S4, S5, S6) x 4 shoot parts (apical, middle, basal and waste) to obtain 48 treatments with 3 field replications (blocks). Data were analyzed using ANOVA. In the case of a significant F-value, the means were compared by Tukey’s HSD test at the significance level of P ≤ 0.01.

Data on air temperature (at 2 m), RH, rainfall for the trial period as well as the global radiation were detected in the agro-meteorological station ARPAV (Regional Agency for Environmental Prevention and Protection of Veneto) located near the experimental area. Soil temperature at -0.15 m was recorded via a geo-probe installed near the shoots sampling point. As regards the maximum air temperatures (Fig. 1), recorded values during the test were between 15 °C and 20 °C for the first three samplings rising up to 25 °C for subsequent. In respect of minimum air temperatures, values were between 4 °C and 8 °C until mid-April to reach 12 °C in mid-May. Rainfall data (Fig. 3/4) showed that the trial period was characterized by a good rainfall amount (124.2 mm) and larger rainfall events occurred in the second week of April and around 5th and 20th of May. About the RH, always the same figure shows that maximum values were always just below 100% whereas the minimum ones, were more heterogeneous following the distribution and the rainfall amount. Concerning thermal levels recorded in the field using geo-probe (Fig. 2) showed maximum temperatures measured at 19:30 h about 15 °C until mid-April then increased to 25 °C at the end of the following month. The minimum values (7:30 h) below 15 °C were recorded up to 28th of April and have never exceeded 20 °C.

Fig. 1. Five-day averages of maximum and minimum temperature, relative humidity and five-day cumulative rainfall recorded during the experiment.

Fig. 2. Daily maximum and minimum soil temperatures (-0.1 m) recorded during the experiment.

Results

Yield values showed that the shoot average weight (Tab. 1), after reaching the highest value (38.6 g) in S2, decreased with advancing of the season. In S6 data weights decreased to 30.1 g with a decrease of 22.1%. Shoots weight was also influenced by the plantation age, indeed the product harvested in the younger crop (YC) showed 38.9 g per shoot and 29.0 g for the older crop (OC) (-25.3%). Shoots weight harvested on OC (Fig. 3A), was always lower than those of YC. The weighting values obtained for OC during the production period, proved rather constant whereas in YC, after an increase found from S1 to S2, there has been a steady contration of the shoots average weight to S6.

The dry matter concentration (Tab. 1) ranged between 8% and 10%. S1 and S2 showed the highest percentages (9.4%) decreasing significantly in the later harvest reaching 8.4%. About crop age the dry matter percentage was higher in shoots produced in OC that presented a concentration of 9.0% against 8.4% of those coming from YC. Concerning shoot subdivisions, it is evident that the apical one was characterized by the higher dry matter concentration (10.5%), followed by waste and the basal and intermediate parts with the lowest values. Concerning the evolution of dry matter content in the different shoot parts (Fig. 4A), it responded significantly during the harvest period. Indeed, after the first three samplings, intermediate part slightly increased and the waste one decreased whereas apical and basal sections did not differ.

The antioxidant concentration showed values which, although not very different during harvest time, expressed significant differences. The higher AOA was registered in S4 (2882 mg Fe²⁺ E kg⁻¹ fw) and the lowest one (2375 mg Fe²⁺ E kg⁻¹ fw) in S2 (Tab. 1).
Crop age significantly affected the AOA and OC shoots presented the highest value, 13.0% more if compared to YC shoots. About shoot parts significant differences were observed and the higher AOA was recorded in the apical portion, followed by the intermediate one, basal and the waste. Within the marketable part a big difference of nearly 70% was observed between apical and basal part. During the harvest time the OC product presented the lower AOA content in S1 and S6, whereas YC showed the higher amount in the same samplings (Fig. 3B).

Total phenols (TP) content showed significant differences among harvest dates despite changes among values are very small and included in a narrow range. Plantation age did not significantly affect the TP content even if asparagus shoots from OC apparently showed higher values than YC. Shoot portions presented significant differences with increasing values shifting from the waste to the apical part with a difference of 74.6% (Tab. 1).

Concerning pigments (Tab. 2), chlorophyll a (CA), chlorophyll b (CB), and xanthophylls + carotenoids (XC) were determined. During harvest time CB did not show significant differences staying around 4 µg g⁻¹ fw, whereas CA and XC were statistically different among samplings. On average the CA content was always greater than CB and XC during the experiment increasing from S1 to S6. The basal portion instead gradually increased in S3 and S4 to decrease again in the end. Regarding the waste portion two peaks were found: the first in S1 (3.80 g g⁻¹ fw) and the second one in S4 (3.69 g g⁻¹ fw) that was followed by a contraction to S6. The plantation age affected CB content during harvesting season (Fig. 3C). Indeed, in the first three samplings an increase of 44.9% was registered for OC, whereas in YC there was a values reduction in the same period to reach OC values in the final harvests. Concerning the CX concentration, as already observed for CA and CB, the more substantial quantities were found in the apical zone of the shoot in both environments of cultivation. The interaction "harvest time x shoot part" (Fig. 4B), showed that CA increased in all parts of the shoot during the season, but with diversified trends. The CB concentration in the shoot during harvest season (Fig. 4C) was always higher in the apical part. In the middle part, after an initial and rapid increase from S1 to S2, CB content progressively decreased to 2.77 g g⁻¹ fw in S6. The basal portion decreased to 2.77 g g⁻¹ fw in S6. The basal portion instead gradually increased in S3 and S4 to decrease again in the end. Regarding the waste portion two peaks were found: the first in S1 (3.80 g g⁻¹ fw) and the second one in S4 (3.69 g g⁻¹ fw) that was followed by a contraction to S6. The plantation age affected CB content during harvesting season (Fig. 3C). Indeed, in the first three samplings an increase of 44.9% was registered for OC, whereas in YC there was a values reduction in the same period to reach OC values in the final harvests. Concerning the CX concentration, as already observed for CA and CB, the more substantial quantities were found in the apical zone of the shoot in both environments of cultivation. The interaction "harvest time x shoot part" (Fig. 4D) is justified by the different intensity and concentration trends in different parts of the shoot.

Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot weight (g)</th>
<th>Dry matter (%)</th>
<th>AOA (mg Fe²⁺E kg⁻¹ fw)</th>
<th>TP (mg GAE kg⁻¹ fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plantation age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 years old (YC)</td>
<td>38.9 a</td>
<td>8.4 b</td>
<td>2393 b</td>
<td>265 a</td>
</tr>
<tr>
<td>8 years old (OC)</td>
<td>29.0 b</td>
<td>9.0 a</td>
<td>2752 a</td>
<td>273 a</td>
</tr>
<tr>
<td><strong>Harvest time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 (10/4)</td>
<td>31.5 c</td>
<td>9.3 a</td>
<td>2573 bc</td>
<td>271 ab</td>
</tr>
<tr>
<td>S2 (17/4)</td>
<td>38.6 a</td>
<td>9.4 a</td>
<td>2375 c</td>
<td>290 a</td>
</tr>
<tr>
<td>S3 (24/4)</td>
<td>36.8 ab</td>
<td>8.4 b</td>
<td>2445 bc</td>
<td>255 b</td>
</tr>
<tr>
<td>S4 (2/5)</td>
<td>33.8 bc</td>
<td>8.5 b</td>
<td>2882 a</td>
<td>271 ab</td>
</tr>
<tr>
<td>S5 (8/5)</td>
<td>33.0 bc</td>
<td>8.3 b</td>
<td>2554 bc</td>
<td>272 ab</td>
</tr>
<tr>
<td>S6 (15/5)</td>
<td>30.1 c</td>
<td>8.3 b</td>
<td>2606 b</td>
<td>254 b</td>
</tr>
<tr>
<td><strong>Shoot parts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>waste</td>
<td>9.6 b</td>
<td></td>
<td>1156 d</td>
<td>136 d</td>
</tr>
<tr>
<td>basal</td>
<td>7.4 c</td>
<td></td>
<td>1578 c</td>
<td>160 c</td>
</tr>
<tr>
<td>intermediate</td>
<td>7.3 c</td>
<td></td>
<td>2362 b</td>
<td>243 b</td>
</tr>
<tr>
<td>apical</td>
<td>10.5 a</td>
<td></td>
<td>5193 a</td>
<td>535 a</td>
</tr>
</tbody>
</table>

Within columns, values with no letter in common significantly differ at P ≤ 0.01 (Tukey HSD test).
The nitrate (NO$_3$) content in the whole shoot reached the maximum level in S2 (409 mg kg$^{-1}$ fw), then it decreased till the end of harvest period 179 mg kg$^{-1}$ fw (Tab. 2). No significant differences were observed about age crop effect. Concerning shoot portions there was a gradual decrease of NO$_3$ content (-46.9%) from waste to the apical part. The latter one (Fig. 4E), maintained a nitrate content always lower than other parts with the largest difference in S1. The basal part showed the higher peak concentration in S2 (506 mg kg$^{-1}$ fw) and from S3 to S6, except for the intermediate part, the NO$_3$ content in the other portions decreased.

The nitrite (NO$_2$) content (Tab. 2) showed no significant differences between different harvest dates with values ranging from 0.90 mg kg$^{-1}$ fw (S3) and 0.68 mg kg$^{-1}$ fw (S6). Also between two plantation age no significant changes were observed even if shoots harvested on OC apparently showed higher NO$_2$ concentration (+17.1%) compared to YC. The different parts of the shoot, did not show differences statistically different, even if the shoot marketable portions seemed to present higher values than waste. In relation to the different effect of harvest time on NO$_2$ content in shoot parts (Fig. 4F), the results were quite heterogeneous. The waste and basal portion showed an opposite trend compared to intermediate and apical parts. In the first case an increase up to S3 was registered, then values decreased in S4; the intermediate and apical portions, instead, showed an initial decrease in S2 to rise up again in S4. During the final stages of the harvest period the NO$_2$ amount was more homogeneous between 0.4 and 0.8 mg kg$^{-1}$ fw.

**Discussion and conclusions**

The yield data recorded during the experiment allowed to observe that, starting from S2, the value mean weight of the shoots was significantly reduced. This result is justified by the fact that, during the shoots production, the storage substances present in the root system were depleted giving less nutrients to growing sprout. Moreover plantation age deeply affected yield as reported also by other authors [10;23;4]. Indeed, for YC, we worked on a crop system at the first year of harvest, probably more rich of reserve substances in the early stages of the harvest cycle as underused for production. Such behavior has been confirmed by the interaction "harvest time x plantation age", which showed the OC production more stable over time and less sensitive to continuous cuts of the shoots; this contrary to what was recorded for YC where young plants did not well react to frequent harvests showing a quick decline in production after S2.

Shoots dry matter content was higher in OC and this result shows that the product is constituted by more fibrous tissue, especially in the basal and waste parts. The high proportion of dry matter found in the apical portion is mainly due to the considerable presence of bracteal leaves almost absent in the basal and intermediate fractions and characterized by little hydrated tissues. High content has also occurred in the waste part as characterized by tissue with a structural
function, partially lignified and to support the shoot during its growth. With regard to the chemical analysis the NO$_3$ content was markedly higher than what reported in literature [9]. Furthermore, the continuous decrease from S2 till the end of the experiment is in contrast with what stated by other authors [17] who registered a marked NO$_3$ increase. This is probably due to the low light intensity (cloudy weather) during the first 2 harvest that limited the enzyme nitrate reductase action. From S3 the reduction of nitrate to nitrite in a consistent manner as instead occurred in the later samplings when conditions have improved in terms of light intensity and day-length. This result is also due to the reduction of the nitrogen amount available to the plant as the fertilizers supply is normally carried out at the end of the harvest period, before fern expansion during the summertime. About shoot parts, the basal and waste one, were those with higher nitrates content as they are the most vascularized tissues [14]. Indeed they are more interested by the nutrients flow to the apical part and less involved by light. Intermediate and apical part did not exhibit high NO$_3$ content because, in addition to being less vascularized, are much more well-lighted and nitrate is reduced more intensively [6]. Moreover a high percentage of nitrates are promptly used by the higher shoot part to produce new tissues.

The NO$_2$ content was not affected by harvest time, plantation age and shoot parts. This may be attributed to the fact that the nitrite reductase degraded sufficiently nitrates maintaining a relatively low concentration, markedly smaller than other references [17].

The pigment content showed increasing trend for CA during harvest time unlike CB and XC that were more stable and significantly lower. The increasing CA content is mainly due to the growing temperature and light intensity during samplings. In addition OC showed the highest quantities of all pigments with increasing values from the waste to the apical part of shoot.

The total antioxidant activity showed significant differences among harvests especially in S4 where the content increased. This is probably due to the stress suffered by the plant [16], both in terms of water since there have been intense rainfall events, and in terms of average temperatures that in the first part of growing cycle was around 15 °C, quite low compared to the optimum level of 18-28 °C [5]. Concerning plantation age significant differences were observed in OC that manifested the highest concentration for AOA. As regards the AOA it can be stated that asparagus presented higher concentrations if compared to broccoli [20]. The apical part of shoot showed the highest AOA that the other parts in accordance with other authors [9] and this is attributable to the greater exposure of the apical portion to low temperature which increase the anthocyanins content [5]. Sakaguchi et al. [13] reported the strong AOA of asparagus due to the presence of rutin and vitamin C. The same authors stated that asparagus AOA is one of the highest compared to other most consumed vegetables. In asparagus juice these substances have a greater bioavailability than when they are raw or cooked, this is because the shoots are subject to a very rapid process
of lignification during post-harvest. Using asparagus in the diet can increase the positive effects of a diet low in calories, improving blood pressure and blood values [3].

The TP concentrations showed significant differences among samplings and they gradually decreased from S2 to S6. About crop age no significant differences were observed and, as antioxidants, the apical part presented the highest content of polyphenols. In any case, the data obtained were lower than those reported for other varieties [17].

In conclusion we can say that: asparagus harvested on OC had a higher content of dry matter, but a higher and more homogeneous shoot weight than YC. The apical part expressed the highest dry matter values followed by the waste and the other two portions. Concerning antioxidants, OC shoots showed significantly higher levels and the apical part AOA is drastically different, up to three times higher than the others. Chlorophyll a and xanthophylls + carotenoids were more concentrated in OC asparagus whereas about chlorophyll b no differences were observed in relation to crop age. The chlorophyll a was the pigment mostly present in the shoot and, the apical part, was more pigmented than the other which showed depigmentation proceeding from the apex towards the base. The nitrates and nitrites content found in the shoots harvested in OC and YC showed no significant differences and the amount of these compounds is lower than the maximum acceptable daily intake level allowed by the European Union (ADI). The shoot, in respect of the nitrate content, showed a decreasing
gradient going from base to apex. This condition is a good thing in terms of nutritional and health whereas high intake of nitrates and nitrites can cause serious diseases of the circulatory, digestive and respiratory systems. Consequently it is possible to say that asparagus has good and useful quality properties for the consumer in general and these can be affected by many aspects of both agronomic and morphological level.

References