TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2018) 42: 372-381
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doi:10.3906/tar-1711-27

# Variation in secondary metabolites contents of Spinoso Sardo artichoke (*Cynara cardunculus* L.) under different day lengths

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**Received:** 06.11.2017 • **Accepted/Published Online:** 11.07.2018 • **Final Version:** 11.10.2018

**Abstract:** Globe artichoke (*Cynara cardunculus* L. var. *scolymus* Fiori) is an economically important crop in the Mediterranean basin and particularly in Italy, which is a leading producer. In this research, we studied the effect of extended photoperiod (15 and 17 h) on polyphenolic, flavonoids, lignin concentrations, and antioxidant activity in edible and inedible organs of artichoke. The influence of day length on morphological traits and biomass accumulation was also assessed. The total phenolic content of whole plants grown under natural day length conditions exceeds that of photoperiod extended for 15 and 17 h by 31% and 32.4%, respectively. The decrease of total phenols was high in inner bracts at photoperiods of 15 and 17 h, whereas the differences among treatments in receptacle were negligible. Antioxidant activity and total flavonoids followed the same trend observed for total phenols. The amount of lignin was dependent on the tissue. In the control, the content of lignin ranged from 0.4 to 2.8 g/100 g of dry weight in receptacle and leaves of rosette, whereas in the 15-h treatment, it ranged from 0.3 to 5.1 g/100 g DW in floral steam and leaves of peduncle, respectively. Fifteen-hour day length significantly reduced the lignin content in receptacle and floral stem. Head morphology was little influenced by photoperiod, whereas significant differences were obtained in floral stem diameter, showing larger values in natural conditions compared to photoperiodic treatments. C and N were also affected by photoperiod.

**Key words:** Antioxidant activity, flavonoids, lignin, photoperiod, polyphenols

## 1. Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus,* Fiori) is a herbaceous perennial plant belonging to the *Asteraceae* family native to the Mediterranean basin. Globe artichoke is largely cultivated all over the world with a surface area of about 125,000 ha and a total yield of 1,634.219 t (FAO Statistical Database, 2015). Over the last 15 years, an increase of world production (~28%), with a substantial unvaried total surface used, has been registered. Although the production of globe artichoke heads is widely spread in the world, the Mediterranean basin still represents the principal cultivation area with about 93,875 ha (74.9% of the total world cultivation area). Italy is the leading producer with more than 58% of the Mediterranean region production (35,593 ha), followed by Egypt (~18,000 ha) and Spain (~16,000 ha). Globe artichoke is cultivated mostly for its large immature inflorescence (head), which constitutes about 35%–55% of the fresh weight (Lombardo et al., 2010, 2012). The enlarged receptacle and the tender inner bracts, commonly known as "heart", are a fundamental component of the Mediterranean diet and are used as fresh and prepared products in food industry. Lately, consumers have been paying more attention to food quality, with an increasing interest in crops with beneficial effects on human health. Globe artichoke has recently shown an increasing success, being considered as a functional food because of the high level of bioactive metabolites, such as polyphenols and flavonoids, present in leaves and inflorescences (Lattanzio et al., 2009; Ceccarelli et al., 2010; Lombardo et al., 2010, 2012; Dabbou et al., 2016). Globe artichoke heads are rich in caffeoylquinic acid derivatives (Pandino et al., 2010), particularly chlorogenic acid (Schütz et al., 2004), apigenin, lutein (Wang et al., 2003; Romani et al., 2006; Pandino et al., 2013a, 2013b), and different cyaniding caffeoylglucoside derivates (Wang et al., 2003; Schütz et al., 2004; Yoo et al., 2012). The importance of these compounds in human health seems to be related to their protective action against oxidative stress (Racchi et al., 2002). Today, the economic value of the globe artichoke is not only associated with head consumption, but also with the use of its by-products as a source of antioxidants

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or valuable biomass for bioenergy purposes (Ledda et al., 2013). Crop residues from artichoke production: leaves, bracts, and floral stems could be used for animal feedstuff, and as a source of fiber and natural antioxidants employed in pharmaceutical industries or to functionalize food (Lattanzio et al., 2009; Ceccarelli et al., 2010). Secondary metabolites in artichoke heads are influenced by genotype, harvest time, and climatic conditions during plant growth and flower development (Lattanzio, 2003; Ceccarelli et al., 2010; Lombardo et al., 2010). Temperature and harvest time affected the qualitative and quantitative content of polyphenols in Violetto di Sicilia cultivar growing in field conditions (Pandino et al., 2013a). The effect of day length on secondary metabolites in globe artichoke has not been studied in detail, and fragmented information is available on total polyphenols, flavonoids, and antioxidant activity in plant tissues. Besides the beneficial effect on human health, consumers are also interested in the palatability of the edible fraction of the globe artichoke head. Lignin is the second most abundant secondary metabolite in plants (Boerjan et al., 2003), and it represents an important component of plant cell wall. Lignin is a secondary metabolite belonging to the phenolic compounds, representing the second most abundant polymer after cellulose (Boerjan, 2003). The functional significance of lignin is associated mainly with mechanical support (allowing plants to stand), with water transport in the xylem vases, and with defense mechanism against pests (Boudet, 2000). Lignin affects the texture of globe artichoke tissues. Globe artichoke heads with a high lignin content are not appreciated by consumers, since it makes tissues less tender and ligneous (Lattanzio, 2003). However, there is an increasing body of literature focusing on the use of lignin as material for binder industries and for the production of resins and polymers (Park et al., 2008; Stewart, 2008). Besides the genetic background, several abiotic and biotic stresses could alter lignin production in plants. In particular, environmental conditions like low temperatures, water deficiency, and UV radiation strongly influence lignin content in several plant species (Moura et al., 2010). As far as we know, globe artichoke lignin content has been only partially explored (Fernández et al., 2006) and no experiments have been conducted to evaluate its concentration in different tissues.

The aim of the present study was to analyze the concentrations of polyphenols, flavonoids, lignin, and the antioxidant activity in globe artichoke plants exposed to extended photoperiods (15 and 17 h). The influence of day length on the distribution patterns of C and N, morphological traits, and biomass accumulation were also assessed. The importance of globe artichoke in the Mediterranean diet and the increasing interest in globe artichoke by-products, rich sources of bioactive compounds, prompted us to study the effects of photoperiod regimes on the contents of secondary metabolites of edible (floral stem, receptacle, inner bracts) and inedible (leaves of rosette, leaves of peduncle, outer bracts) tissues.

## 2. Materials and methods

### 2.1. Field experiment: site, soil, experimental design, and environmental conditions

The experiment was carried out at Uta, Sardinia (Italy) (39°N; 9°E; 6 m a.s.l.). The soil was an approximately 0.5 m layer of clay-loam. The climate is typically Mediterranean, with a mean seasonal rainfall of 427 mm. The hottest months are July and August (30.0 °C/19.0 °C maximum and minimum temperatures) and the coldest is January (14.0 °C/6.0 °C maximum and minimum temperatures). The 'Spinoso sardo', an early cultivar with spines on both leaves and bracts, harvestable from November to spring in the Mediterranean basin, was cultivated in 2014/2015 under natural or extended (15 h and 17 h) photoperiod conditions in a split plot design. The day length in the control treatment varied from 14.2 h at the emergence of the crop (beginning of August) to 11.1 h at harvest (February). A spacing of 9.0 m was maintained between photoperiodic treatments to remove possible interactions of light effects. Photoperiod extension was achieved by suspending a 400-W high-pressure sodium lamp between the two central rows of the photoperiod plots. The level of light reaching the uppermost leaves was adequate to saturate the photoperiod response of most crop plants (Craufurd et al., 1999). Photoperiod extension was maintained until flowering. The length of the photoperiod was defined as the number of hours between the sunrise and sunset when the inclination of the sun was no more than 6 degrees below the horizon (Weir et al., 1984). For each treatment, average, minimum, and maximum daily temperatures in the 10 days before harvest were recorded at a meteorological station located at the experimental site.

### 2.2. Preparation of samples, morphological traits, and data collection

Semidormant offshoots ("ovuli"), uniform in terms of age, shape, and size, were planted on 25 July 2014, 0.8 m apart within rows, and 1.2 m apart among rows. Sixteen offshoots were planted for each day length treatment.

Crop management followed standard commercial practices for globe artichoke. A drip irrigation system was used to irrigate the soil when 60% of the soil water content was lost by evapotranspiration (ETc). ETc was calculated according to the Penman–Monteith method and corrected for the crop coefficient (Kc) dependent on crop growth stage. The nutrient management was performed as basal dressing (N 50 kg $ha^{-1}$, $P_2O_5$ 140 kg $ha^{-1}$, and $K_2O$ 140 kg $ha^{-1}$) and twice (October and January) during the growing season (N 180 kg $ha^{-1}$).

In regards to weed and pest control, the usual crop management of globe artichoke culture for head production was used. Gibberellic acid was not supplied. For each

treatment, 15 plants, including the globe artichoke heads, floral stems (0.15 m), the tenth leaves of the stem, and the first leaves of rosette were collected at market maturity. All heads were weighed and head length/diameter (HL/D) ratio was calculated. Plants (3 replications of 5 plants each) were manually separated into outer and inner bracts (OB, IB), receptacle (R), rosette and peduncle leaves (LR, LP), and floral stem (FS), then air-dried and stored at –80 °C until analysis. Data were presented and discussed considering each plant organ separately, and following food industry requirements, dividing plants into edible (IB, R, and FS) and inedible (OB, LR, and LP) organs. Data considering FS as inedible part were also provided.

### 2.3. Evaluation of carbon (C), nitrogen (N), C/N ratio, and lignin

The elemental composition (C, N) was determined using an elemental analyzer (Leco CHN 628) in 0.1 g of lyophilized plant matter. Data were expressed as percentage of C (%) and N (%). Three replicates for each treatment as well as for each organ were used.

Lignin determination was performed following Hammerschmidt (1985) protocol with Mulas et al. (1996) modifications. The absorbance was read at 280 nm with an Agilent 8453 UV-Vis spectroscopy system. The results were expressed as g of lignin/100 g of dry weight (DW) on the basis of the lignin extinction coefficient $\varepsilon = 8.70 l/g \times cm$ (Brinkmann et al., 2002). Data presented are the mean and standard deviation of three independent experiments.

### 2.4. Preparation of extracts

One hundred milligrams of Freeze-dried globe artichoke were extracted with 5 mL of methanol/water solution (80% MeOH). The samples were shaken for 30 min, then centrifuged (6,000 rpm for 10 min), and filtered with n. 4 Whatman filter paper. The methanolic extracts were used for the assessment of total phenols, flavonoid concentration, and antioxidant activity.

### 2.5. Determination of total polyphenols, flavonoids, and antioxidant activity

Total polyphenols (TP), total flavonoids content (TF), and antioxidant activity (AA) were measured to study the influence of day length on artichoke tissues.

Total phenolic concentration was determined with the Folin–Ciocalteu method according to Fadda et al. (2014). Diluted extracts were mixed with Folin–Ciocalteu reagent (1:1) and after 3 min with 4 mL of a sodium carbonate ($Na_2CO_3$) solution (75 g/L). The mixture was shaken and adjusted to a final volume of 10 mL with water. Samples were stored in the dark at room temperature for 2 h, and then the absorbance was read at 750 nm with an Agilent 8453 UV-Vis spectroscopy system. Results were expressed as gallic acid (GA) equivalents (mg GA/g of dry weight, DW) using gallic acid as external standard (1–10 mg/L, $R^2 = 0.99$). Total flavonoids were determined as in Fadda et al. (2014). The absorbance was read at 510 nm with an Agilent 8453 UV-Vis spectroscopy system. The results were expressed as mg of catechin equivalents (CE)/g of DW (catechin 2–20 μg/mL, $R^2 = 0.99$). Radical scavenging activity was spectrophotometrically determined with the DPPH method according to Fadda et al. (2014) with some modifications. The extracts (1.9 mL) were mixed with 100 μL of DPPH solution (1 mM in methanol). The mixture was stored in the dark at room temperature for 1 h and UV-Vis-VIS readings were carried out with a spectrophotometer Agilent 8453 at 517 nm. The antiradical activity was expressed as TEAC units (μmol trolox/g of dry weight) using a Trolox calibration curve (5–20 μM, $R^2 = 0.99$).

### 2.6. Statistical analysis

One-way analysis of variance (ANOVA) was performed on morphological traits, secondary metabolites, lignin content, C and N content, and C/N ratio parameters. Means were separated by Fisher's least significance test at $P \leq 0.05$. Two-way analysis of variance using GLM was also run on morphological and chemical data. All statistical analyses were carried out using Graph Pad Prism 7 for Windows software (GraphPad software, Inc. La Jolla, CA, USA).

## 3. Results and discussion

### 3.1. Plant growth and head morphology

The morphological parameters of globe artichoke head were little influenced by photoperiodic conditions. The head size (head lenght, HL; head diameter, HD) and shape (HL/D) of plants grown under extended day lengths were similar to those of the control group (Table 1). HL/D ratio is a relatively constant cultivar trait. HL/D ratio of control and treated heads was at least 1.4, defining Spinoso sardo as long shape type, as reported by Mauromicale and Ierna (2000). Data collected on globe artichoke organs demonstrated that they reacted in a different way to photoperiodic regimes. For example, the floral stem diameter (FS) of the control exceeded that of the 15 and 17 h (Ph15 and Ph17) by 24.66% and 28.59%, respectively (Table 1). Photoperiod is known to control the vegetative and reproductive growth of plants. In artichoke, the photoperiod increased the time from emergence to harvesting (days after emergence, DAE, expressed in days). In plants exposed to Ph15 and Ph17, the photoperiod increased DAE by 42 and 54 days in respect to the control (Table S1). This effect could be important since it might allow an extension of the marketing season. In many species, the effect of photoperiod on vegetative growth may be further influenced by temperature (Keatinge et al., 1998, Bradford et al., 2010). In this research, the extension of the vegetative growth was mainly attributable to the effect of photoperiod, since, as shown in Table S1, the average temperatures calculated during 10 days before

**Table 1.** Effect of photoperiodic treatments: control (natural light conditions) and 15–17 h conditions (Ph15 and Ph17, respectively) on head length (HL), head diameter (HD), head length/diameter ratio (HL/D), and floral stem diameter (FS).

| Treatment | HL (mm) | HD (mm) | HL/D | FS (mm)[x] |
|---|---|---|---|---|
| Control | 94.6 ± 7.0 | 65.5 ± 3.6 | 1.4 ± 0.1 | 25.7 ± 2.6a |
| Ph15 | 92.6 ± 5.6 | 63.4 ± 5.2 | 1.5 ± 0.1 | 19.3 ± 1.0b |
| Ph17 | 87.1 ± 9.0 | 59.5 ± 7.5 | 1.5 ± 0.1 | 18.3 ± 2.3b |
| *F*-ratio | n.s.* | n.s.* | n.s.* | 0.18 |
| P-value | | | | 0.0002 |

* n.s.: not significant.
[x] Means with different letters within the same column are statistically different by Fisher's least significant difference (LSD) ($P < 0.05$).

harvesting in the control and Ph17 were similar, whereas the DAE was significantly different.

### 3.2. Plant carbon (C), nitrogen (N), and C/N ratio

C accumulation was significantly affected by the photoperiodic conditions, the portion of plant considered, and their interaction (Table 2). Data on C concentration demonstrated that not all tissues respond to the photoperiodic treatments in the same way (Table 2). In the control and Ph17, the highest C concentration in IB and OB was observed, whereas in the Ph15, no significant differences were observed among plant organs, although a slight increase in the accumulation of C occurred in IB and LF. It has been reported that photoperiod influences C transport in plant tissues (Kelly and Davies, 1988). In peas, for instance, long-day light treatments induced a larger transport of $^{14}C$ from leaves to reproductive organs, with the highest percentage in the apical buds, compared to the short-day condition. The highest content of C in head organs, found both in the control and Ph17-treated plants, could be explained by considering head tissues as preferential sink organs for C compounds. In Ph15 head tissues, the photosynthetic rate could play a role in the source-sink plant system (Wang et al., 2003). The partitioning of photoassimilates was less efficient than in the control and Ph17. Previous studies conducted in cucumber and tomato (Robbins and Pharr, 1987; Shishido et al., 1990) showed that the increase of day length can significantly influence the export of photoassimilates from source to sink (from leaves to plant accumulation organs). In other species, such as soybean, the C content in the leaves is inversely related to day length. In Spinoso Sardo, beside the photoperiod, other environmental factors could play a significant role in translocation and accumulation of photoassimilates.

In the control, Ph15-, and Ph17-treated plants, N concentration was highest in LP and lowest in FS

**Table 2.** Effect of photoperiodic treatments on the concentration (%) of C, N and on the C/N ratio in outer and inner bracts (OB, IB), rosette and peduncle leaves (LR; LP), receptacle (R), and floral stem (FS).

| Photoperiod[x] | Plant part | C (%)[z] | N (%)[z] | C/N ratio[z] |
|---|---|---|---|---|
| Control | OB | 40.3 ± 0.7 | 2.1 ± 0 | 19.5 ± 0.3 |
| | IB | 41.4 ± 0.5 | 2.1 ± 0.1 | 19.4 ± 1.1 |
| | R | 40.6 ± 0.3 | 2.4 ± 0.1 | 17.0 ± 0.5 |
| | FS | 37.4 ± 0.1 | 1.5 ± 0.1 | 24.7 ± 1.0 |
| | LR | 35.7 ± 0.9 | 2.5 ± 0.2 | 14.5 ± 0.7 |
| | LP | 35.8 ± 1.1 | 2.6 ± 0.2 | 13.7 ± 0.9 |
| Ph15 | OB | 37.1 ± 2.6 | 2.1 ± 0.1 | 17.7 ± 1.5 |
| | IB | 39.9 ± 0.2 | 2.4 ± 0.1 | 16.8 ± 0.7 |
| | R | 37.9 ± 0.3 | 2.7 ± 0.1 | 14.3 ± 0.2 |
| | FS | 37.2 ± 0.1 | 1.6 ± 0.1 | 23.1 ± 0.2 |
| | LR | 36.5 ± 0.6 | 3.2 ± 0.2 | 11.6 ± 0.5 |
| | LP | 38.8 ± 1.9 | 3.9 ± 0.6 | 10.0 ± 0.7 |
| Ph17 | OB | 39.9 ± 0.1 | 2.5 ± 0.2 | 15.2 ± 0.4 |
| | IB | 40.5 ± 0.3 | 2.3 ± 0.1 | 17.3 ± 0.4 |
| | R | 36.6 ± 0.7 | 2.2 ± 0.1 | 16.4 ± 0.4 |
| | FS | 37.8 ± 0.5 | 1.7d ± 0 | 22.5 ± 0.9 |
| | LR | 36.2 ± 0.2 | 2.4 ± 0.1 | 14.9 ± 0.6 |
| | LP | 38.0 ± 1.2 | 2.9 ± 0.2 | 13.3 ± 1.3 |
| Probability level of significance (ANOVA)[y] | | | | |
| Photoperiod (A) | | .0121 | < .0001 | < .0001 |
| Plant organ (B) | | < .0001 | < .0001 | < .0001 |
| A × B | | < .0001 | < .0001 | .0015 |
| LSD | | 0.77 | 0.16 | 0.63 |

[x] Photoperiodic treatments: control (natural conditions), Ph15 (15 h), and Ph17 (17 h).
[y] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and plant organs (B) as factors.
[z]Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.

(Table 2). Bendevis et al. (2014) reported a differential N distribution on quinoa tissues as a consequence of photoperiodic conditions. The reduction of day length increases and extends the leaves' photosynthetic activity, improving the plant's growth and yield (Thomas et al., 1981). In Spinoso Sardo, day length seems to be not the only factor influencing the partitioning of photosynthates. The increased N concentration observed in LR and LP in the Ph15 could be influenced by temperature. Average, minimum, and maximum temperatures of Ph15, measured 10 days before harvest, were always higher compared to

those of the control and Ph17 (Table S1). The C/N ratio was affected by photoperiod and was different among the plant tissues analyzed (Table 2). The ratio is minimum in leaves and maximum in FS. The C/N ratio in plants subjected to photoperiodic treatment was significantly lower than that in the control, in all plant organs.

Globe artichoke is an important component of the Mediterranean diet. Recently, its by-products (leaves and OB) have been appreciated for their nutraceutical and energetic value, thus increasing its economic importance. Due to the economic relevance of the exploitation of by-products, the effect of photoperiod was studied both in edible (IB, R, and FS) and inedible (LR, LP, OB) plant tissues (Table 3). The inedible tissues had a significantly higher C content than the edible organs. The day length did not play a significant role in C distribution. A similar trend was observed for the N content, which is in general higher in the inedible parts compared to the edible tissues. The C/N ratio, also, is significantly influenced by day length. The Ph15 and Ph17 edible tissues showed a higher level of C/N compared to the inedible organs. An opposite trend was shown in the control treatment. This could be because of the contribution of FS tissue, which was considered as an edible part. Considering that the FS represents an edible organ only in a few Italian regions, the C, N, and C/N contents were explored also considering the FS as an inedible portion of artichoke. As expected, the mean percentage of C and N in inedible parts increased in all treatments, while it decreased in edible tissue (Table S2). Overall, the photoperiod, edibility, and photoperiod × edibility have a similar statistic trend as revealed in Table 3. The principal differences considering FS as edible (Table 3) or inedible tissue (Table S2) are associated with the C/N ratio. In this case, a few observations can be made: in natural day length, edible and inedible tissues showed no significant differences; whereas Ph17 caused a significant increase in C/N ratio.

**Table 3.** Effect of photoperiodic treatments on tissue concentration (%) of C, N and on the ratio C/N in edible and inedible globe artichoke organs.

| Photoperiod[x] | Edibility | C (%)[z] | N (%)[z] | C/N ratio[z] |
|---|---|---|---|---|
| Control | Edible | 20.2 ± 2.1 | 2.3 ± 0.1 | 8.7 ± 0.7 |
| | Inedible | 53.5 ± 1.7 | 3.2 ± 0.3 | 16.6 ± 2.0 |
| Ph15 | Edible | 20.8 ± 2.3 | 1.1 ± 0.2 | 18.3 ± 1.1 |
| | Inedible | 53.9 ± 1.4 | 4.4 ± 0.3 | 12.4 ± 0.6 |
| Ph17 | Edible | 19.0 ± 0.8 | 1.0 ± 0 | 19.5 ± 0.5 |
| | Inedible | 54.5 ± 0.5 | 3.5 ± 0 | 15.4 ± 0.3 |
| Probability level of significance (ANOVA)[y] | | | | |
| Photoperiod (A) | | n.s.* | .0016 | .0002 |
| Edibility (B) | | < .0001 | < .0001 | n.s.* |
| A × B | | n.s. | < .0001 | < .0001 |
| LSD | | 1.78 | 0.16 | 1.1 |

[x] Photoperiodic treatments: control (natural conditions), Ph15 (15 h), and Ph17 (17 h).

[y] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and edibility (B) as factors.

* n.s.: not significant.

[z]Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.

### 3.3. Lignin content

High variability in lignin content was found among globe artichoke tissues and photoperiodic treatments (Figures 1a and 1b). In control plants, the lignin content ranged from 0.4 g/100 g DW in R to 2.8 g/100 g DW in LR with no statistical differences among head tissues (R, OB, IB), FS, and LR. In the Ph15, LR and LP had a significantly higher lignin content as compared to head organs and FS. Similarly, the lignin content of LP of the Ph17 stood out with respect to the other plant tissues. In general, the two extended day length conditions highlighted a wider range of lignin compared to the control conditions. Overall, its mean was higher in the Ph15 (1.41 g/100 g DW) and lower in the control (0.91 g/100 g DW) (data not shown). Although the pattern of lignin was different among the three treatments, a few common trends were observed. Lignin was predominant in leaves of the three treatments; in particular, it was significantly higher in LR in the control compared to the Ph15 and Ph17. LP lignin contents of the Ph15 and Ph17 groups were significantly higher than that of the control group. IB and R of the Ph17 treatment showed a significantly higher lignin content compared to that of the control (Figure 1b). Despite the role of lignin in plant development and response to stress, its content could represent an antiquality trait for food industry. Lignin is not directly associated with fruit or plant taste, but its high content is not appreciated by consumers because it increases the toughness of plant tissue, reducing its marketability (Lattanzio, 2003). As reported by Amthor (2003), the biosynthesis of lignin is a high-energy-consuming pathway requiring a large amount of carbon. In the Ph15, the less efficient translocation of photoassimilates could have induced a reduction of C compounds available for lignin production, which was then overcome in the Ph17. Little information is available on the role of day length in accumulation of secondary metabolites in plants, and even less data are available for lignin concentration in different plant tissues. Photoperiodic conditions influenced the content of lignin only in IB, R, LR, and LP (Figures 1a and 1b). Significant differences observed in IB were negligible, showing very little impact of day length on increasing the proportion of lignin in globe artichoke tissues. Similar to artichoke, in *Dactylis glomerata* L. and in *Bromis inermis* Leyss, day lengths had very little influence

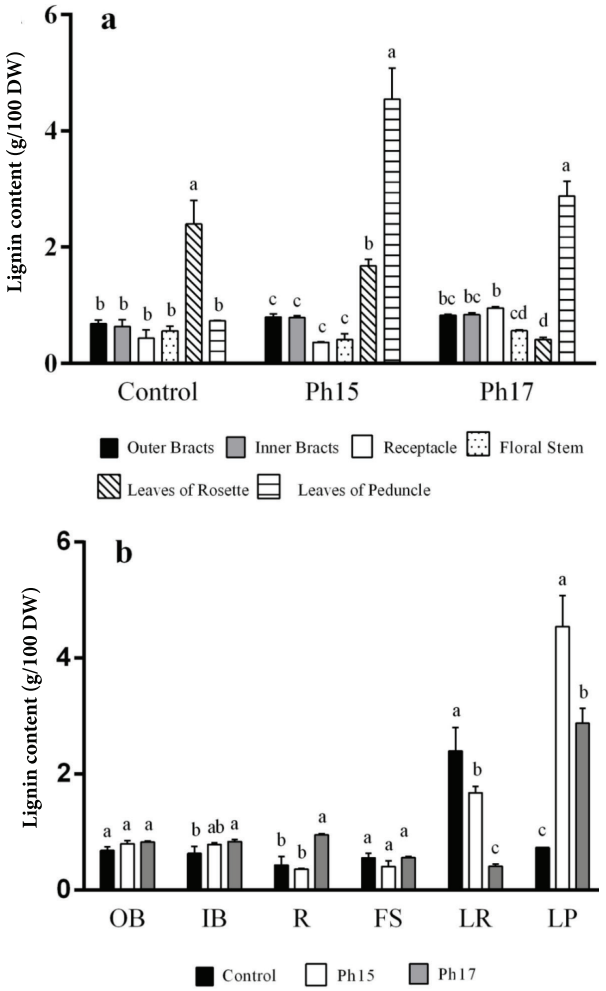


**Figure 1.** Lignin assay in artichoke. a) Control, photoperiod 15 and 17 (Ph15, Ph17) were compared for lignin content in the 6 plant tissues studied: leave of peduncle and rosette (LP, LR), inner and outer bracts (IB, OB), floral stem (FS), and receptacle (R). Statistical analysis was performed comparing tissue responses inside each treatment (control, Ph15, and Ph17). b) Comparison of lignin content in the three day length conditions tissue by tissue. Different letters between each column and treatment indicate statistically significant differences between means ($P < 0.05$; Tukey–Kramer test).

on lignin content (Bowman and Law, 1964). For the food industry, a low lignin content represents an interesting trait, particularly in R and FS, which are appreciated as fresh and minimally processed produce. Lignin content of inedible tissues was significantly higher than that of edible parts in all treatments (Table 4). Photoperiodic treatments significantly affected lignin content; the Ph15 revealed the lowest lignin content in edible tissue (0.08 g/100 g DW), while the Ph17 registered the highest content (0.13 g/100 g DW). In addition, the interaction between photoperiod

**Table 4.** Lignin content in edible (inner bract, receptacle, and floral stem) and inedible (outer bracts, leaves of peduncle, and rosette) tissues.

| Treatment[x] | Edibility | Lignin (g/100 g DW)[z] |
|---|---|---|
| Control | Edible | 0.10 ± 0.1 |
| | Inedible | 0.69 ± 0.9 |
| Ph15 | Edible | 0.08 ± 0.1 |
| | Inedible | 0.86 ± 1.0 |
| Ph17 | Edible | 0.13 ± 0.1 |
| | Inedible | 0.44 ± 0.8 |
| Probability level of significance (ANOVA)[y] | | |
| Photoperiod (A) | | .0181 |
| Edibility (B) | | < .0001 |
| A × B | | .0037 |
| LSD | | 0.26 |

[x] Photoperiodic treatments: control (natural conditions), Ph15 (15 h) and Ph17 (17 h).
[y] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and edibility (B) as factors.
[z]Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.

and edibility was significant (Table 4). A similar trend was found considering the FS as inedible part (Table S3). The general higher level of lignin found in inedible tissues in the Ph15 could be useful for the reuse of globe artichoke wastes. Raw materials with lignin residuals are appreciated for fermentative ethanolification (Hamelinck et al., 2005). Several environmental factors could affect lignin biosynthesis. A partial role could be ascribed to temperatures during head development. Inedible tissues in the Ph15 showed the highest lignin content. These plants, in the 10 days before harvesting, experienced environmental temperatures higher than those of the other two treatments (Tables 4 and S3). Further investigations will aim at exploring the role of temperature on lignin deposition and biosynthesis.

### 3.4. Total polyphenol, flavonoid content, and antioxidant activity

This study provided a quantitative evaluation of the major classes of secondary metabolites of Spinoso Sardo artichoke. The plant tissues exhibited a high variability ($P < 0.0001$) in the content of secondary metabolites (Table 5). Even the photoperiodic conditions ($P < 0.005$) and the "photoperiod × plant organ" interaction ($P < 0.0001$) played a significant role in the concentrations of TF and TP, and in AA. TP content was higher in the control (average: 25.9 mg GA/g DW) than that in the Ph15 (average: 17.8 mg GA/g DW) and in the Ph17

**Table 5**. Total Polyphenols (TP) (mg GA/g DW), antioxidant activity (AA) (µmol trolox/g DW), and total flavonoids (TF) (mg CE/gDW) of different plant parts in relation to the photoperiodic treatment used (control, photoperiod 15 h, Ph15; photoperiod 17 h, Ph17).

| Treatment | Tissue | TP[y] | AA[y] | TF[y] |
|---|---|---|---|---|
| Control | OB | 19.8 ± 1.3 | 145.5 ± 11.5 | 17.7 ± 1.2 |
| | IB | 58.6 ± 3.9 | 290.7 ± 6.9 | 50.1 ± 4.8 |
| | R | 18.3 ± 1.4 | 109.7 ± 10.8 | 15.4 ± 2.0 |
| | FS | 22.3 ± 1.7 | 114.2 ± 6.3 | 21.8 ± 1.4 |
| | LR | 18.3 ± 1.5 | 109.6 ± 7.3 | 12.2 ± 1.8 |
| | LP | 18.3 ± 1.7 | 108.9 ± 2.9 | 15.0 ± 1.3 |
| Ph15 | OB | 11.6 ± 0.2 | 56.9 ± 8.9 | 11.5 ± 1.7 |
| | IB | 26.2 ± 1.2 | 223.2 ± 7.8 | 42.8 ± 3.8 |
| | R | 17.4 ± 1.3 | 172.3 ± 3.7 | 14.7 ± 0.7 |
| | FS | 19.5 ± 0.7 | 173.9 ± 7.1 | 30.8 ± 5.6 |
| | LR | 13.8 ± 0.9 | 103.6 ± 1.5 | 16.2 ± 1.5 |
| | LP | 18.5 ± 1.1 | 82.2 ± 7.3 | 12.7 ± 0.6 |
| Ph17 | OB | 15.9 ± 0.8 | 74.6 ± 5.2 | 11.9 ± 1.6 |
| | IB | 28.0 ± 1.1 | 251.1 ± 23.2 | 46.5 ± 7.6 |
| | R | 19.9 ± 1.6 | 145.1 ± 7.2 | 16.7 ± 0.2 |
| | FS | 19.5 ± 0.4 | 91.8 ± 9.2 | 13.7 ± 1.1 |
| | LR | 10.3 ± 0.1 | 68.2 ± 6.7 | 10.2 ± 1.2 |
| | LP | 11.3 ± 0.7 | 90.8 ± 7.0 | 10.5 ± 0.9 |
| Probability level of significance (ANOVA)[x] | | | | |
| Photoperiod (A) | | .0007 | < .0001 | .0007 |
| Plant organs (B) | | < .0001 | < .0001 | < .0001 |
| A × B | | < .0001 | < .0001 | < .0001 |
| LSD | | 1.15 | 7.34 | 2.37 |

[x] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and plant organs (B) as factors.
[y] Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.
Leaf or rosette and peduncle (LR; LP), floral stem (FS), outer and inner bract (OB, IB); receptacle (R).

(17.5 mg GA/g DW) treatments (means calculated from Table 5). IB in all photoperiodic treatments showed the highest value, as previously reported by Lombardo et al. (2012) in Violetto di Sicilia clones. In the Ph15 and Ph17 treatments, TP content was lower by about 50% compared to that of the control. The other plant tissues were slightly influenced by day length with comparable TP values among treatments. Similar to TP, AA presented a high variability among the photoperiodic conditions studied. It was on average higher in the control (146.4 µmol trolox/g

**Table 6.** Effect of photoperiod treatments on total polyphenols (TP) (mg GA/g DW), antioxidant activity (AA) (µmol trolox/g DW), and total flavonoids (TF) (mg CE/g DW) in globe artichoke grown under natural (control), 15 h (Ph15), and 17 h (Ph17) day-lengths.

| Treatment | Edibility[z] | TP[y] | AA[y] | TF[y] |
|---|---|---|---|---|
| Control | Edible | 30.5 ± 2.1 | 165.5 ± 16.3 | 26.8 ± 1.2 |
| | Inedible | 23.4 ± 0.7 | 148.2 ± 8.0 | 17.4 ± 1.6 |
| Ph15 | Edible | 13.5 ± 0.7 | 121.0 ± 9.8 | 20.0 ± 2.2 |
| | Inedible | 18.4 ± 0.8 | 114.3 ± 3.9 | 18.6 ± 2.1 |
| Ph17 | Edible | 14.3 ± 0.9 | 99.7 ± 7.0 | 16.1 ± 1.7 |
| | Inedible | 15.0 ± 0.2 | 95.3 ± 8.0 | 13.7 ± 1.5 |
| Probability level of significance (ANOVA)[x] | | | | |
| Photoperiod (A) | | < .0001 | < .0001 | < .0001 |
| Edibility (B) | | n.s.* | n.s.* | .0004 |
| A × B | | < .0001 | n.s.* | .0058 |
| LSD | | 1.01 | 8.24 | 1.54 |

[x] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and edibility (B) as factors.
* n.s.: not significant.
[y] Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.
[z] Edible (inner bracts, stem, and receptacle) and inedible (outer bracts, leaves of peduncle, and rosette) tissues were studied.

of DW) compared to that in the Ph15 (135.3 µmol trolox/g of DW) and Ph17 (120.3 µmol trolox/g of DW) treatments (means were calculated from Table 5). IB showed the highest AA activity among all treatments. These data differ from Lombardo et al. (2012), where AA activity was evaluated in different cultivars of artichoke. The different experimental conditions applied and the different genetic material could explain the apparent discrepancy between the two studies. Like TP and AA, TF was significantly affected by photoperiod showing the highest average value in the control (22.0 mg CE/g DW) and the lowest content in the Ph17 (18.3 mg CE/g DW). Leaves (LR and LP), FS, bracts (IB and OB), and R had significantly different TF contents (Table 5). In the control, TF ranged from 12.2 mg CE/g DW to 50.1 mg CE/g DW in LR and IB, respectively. IB, in all day length conditions, showed the highest value. The Ph15 and Ph17 treatments reduced TF by about 15% and 7%, respectively, compared to the control. These data are comparable to the TF level found by Dabbou et al., (2016) in Tunisian globe artichoke cultivars. As expected, a strongly significant positive correlation was found among TP, AA, and TF (data not shown). The high content of TP, TF, and AA in globe artichoke parts, such as IB, FS, and R represent an important nutritional aspect for fresh

consumption (Ceccarelli et al., 2010). Whereas, the low TP content in plants grown under Ph15 and Ph17 conditions could be useful in food processing since it is associated with reduced browning during transformation, handling, and storage (Lattanzio, 2003). Recently, globe artichoke leaves, OB, and FS are largely used as polyphenolic and food additive sources by pharmaceutical and animal food industries (Lattanzio et al., 2009). For this reason, the contents of TP, AA, and TF were analyzed in edible and inedible tissues as well. Photoperiod plays a significant role ($P < 0.0001$) in metabolite contents of edible and inedible organs (Table 6). The extension of day length caused a significant decrease in TP content and AA in the treated plants in both edible and inedible organs. The control showed a significant major content of TP and TF in the edible parts compared to the inedible tissues (Table 6). A comparison between edible and inedible plant tissues in the Ph15 revealed a significantly higher TP content in inedible tissues than in edible tissues, and no significant differences of AA in all photoperiodic conditions. Ph17 is the only photoperiodic condition that did not show any statistically different content within the three secondary metabolites tested (Table 6). A different picture was found when FS was considered as inedible plant part (Table S4). In this case, FS significantly influenced TP, TF, and AA in the edible and inedible organs. Photoperiod, as well as edibility, and their interaction significantly differ in TP, TF, and AA. In addition, as expected, the content of TP, TF, and AA in inedible tissues was significantly higher compared to that of their edible fraction. Considering that globe artichoke head is the principal product for fresh consumption and food industry, TP, AA, and TF were evaluated only in heads from the different photoperiodic treatments (Figure 2). Photoperiod significantly influenced the TP content in the whole globe artichoke head (Figure 2). The higher value (19.1 mg GA/g DW) was detected in the control, while the lower value was found in the Ph15. A similar trend was observed for AA. In the control (110.9 µmol trolox/g of DW), AA was significantly higher than that in the Ph15 (87.3 µmol trolox/g of DW) and Ph17 (93.9 µmol trolox/g of DW). By contrast, TF content in globe artichoke head was not influenced by photoperiod.

To the best of our knowledge, the effect of photoperiod has never been studied in Spinoso Sardo globe artichoke. This study provides a wide overview of the influence of the extension of day length on agronomic traits, C and N contents, and the production of secondary metabolites in artichoke. Our results demonstrated that day length influences the physiological development of specific globe artichoke organs, such as the FS. The extension of photoperiod to Ph15 and Ph17, in fact, significantly reduced the floral stem in these two treatments compared to natural conditions. In addition, the C and N content and C/N ratio presented a different distribution among plant tissues. Lignin content, TP, AA, and TF contents were all significantly affected by day length. An interesting aspect of future research will be to explore the effects of other common factors (temperature, air humidity, crop management practices, etc.) on head morphology and secondary metabolites contents. These results are useful

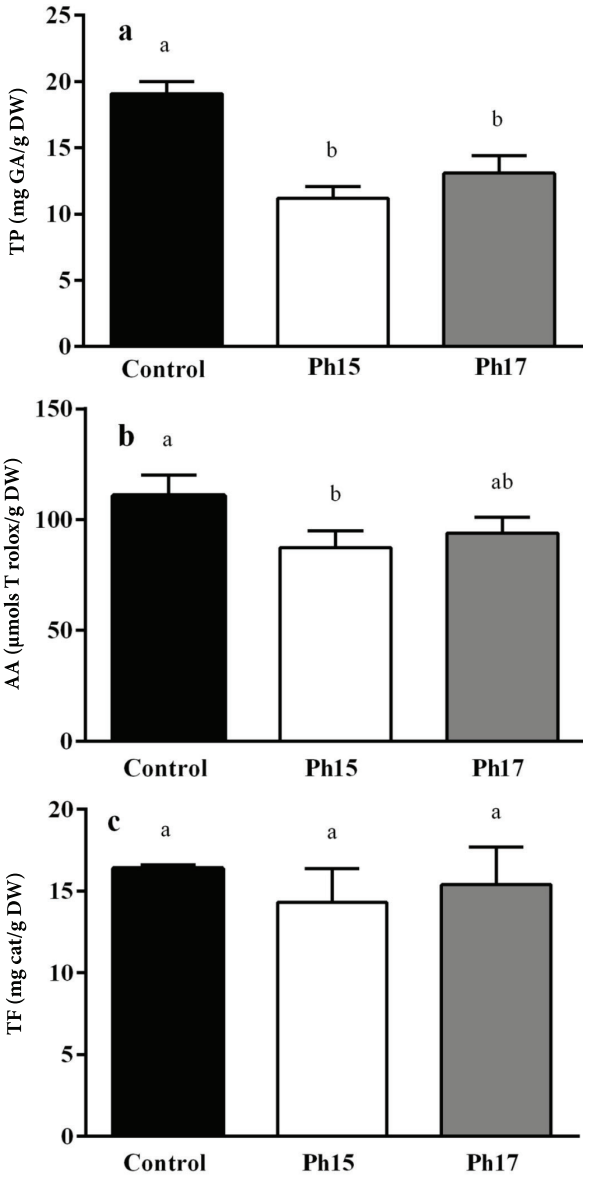


**Figure 2.** Total polyphenolic content (TP), antioxidant activity (AA), total flavonoids (FL) of the whole globe artichoke head in relation to the photoperiodic condition used: control; photoperiod 15 h (Ph15) and photoperiod 17 h (Ph17). A different letter within each column indicates statistically significant differences between means ($P < 0.05$).

for food and other industries that require a specific characteristic on the final product destination. The evaluation of secondary metabolites content (lignin, phenols, flavonoids, and antioxidants) in all plant tissues could give a second life to the nonfood material, optimizing crop utilization.

**Acknowledgments**
The authors gratefully thank Prof. Francesco Giunta for his agronomical advice and scientific suggestions, Gavina Serra and Mario Antonello De Roma for technical support.

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## SUPPORTING INFORMATION

**Table S1.** Harvest time expressed as days after emergence (DAE) and its characterization in terms of difference in photoperiod between control and extended treatments 15 h (Ph15), 17 h (Ph17).

| Treatment | DAE | Day length difference (h) | T during 10 days before harvest (°C) | $T_{min}$ during 10 days before harvest (°C) | $T_{max}$ during 10 days before harvest (°C) |
|---|---|---|---|---|---|
| Control | 139b | 0.0 | 9.3 | 3.5 | 15.1 |
| Ph15 | 181a | 5.4 | 11.8 | 6.2 | 17.3 |
| Ph17 | 193a | 6.4 | 7.7 | 3.6 | 11.7 |

Average temperature (T), minimum and maximum temperatures ($T_{min}$ and $T_{max}$, respectively) collected during 10 days before harvest.
Probability level of significance (ANOVA) for DAE $P < .001$.
Means with different letters within the same column are statistically different by Fisher's least significant difference (LSD) ($P < 0.05$).

**Table S2.** Percentages of Carbon (C), Nitrogen (N), and the ratio C/N in globe artichoke under different photoperiodic treatments (control, Ph15, Ph17).

| Treatment | Edibility | C (%) | N (%) | C/N ratio |
|---|---|---|---|---|
| Control | Edible | 10.0 ± 2.3 | 0.6 ± 0.2 | 16.0 ± 0.8 |
| | Inedible | 56.1 ± 1.3 | 3.6 ± 0.4 | 15.9 ± 2.4 |
| Ph15 | Edible | 10.3 ± 2.4 | 0.7 ± 0.2 | 15.2 ± 0.4 |
| | Inedible | 57.4 ± 0.7 | 4.4 ± 0.3 | 13.1 ± 1.0 |
| Ph17 | Edible | 8.5 ± 0.7 | 0.5 ± 0.1 | 16.7 ± 0.3 |
| | Inedible | 57.4 ± 1.0 | 3.6 ± 0.1 | 16.0 ± 0.3 |
| Probability level of significance (ANOVA) | | | | |
| Photoperiod (A) | | n.s.* | .0168 | .0117 |
| Edibility (B) | | < .0001 | < .0001 | n.s.* |
| A × B | | n.s.* | < .0494 | n.s.* |
| LSD | | 1.28 | 0.20 | 0.92 |

Means separation was performed by Fisher's least significant difference (LSD) procedure ($P < 0.05$) and the LSD value is provided.
* n.s. indicates not significant data.
Globe artichoke tissues were grouped as edible (inner bracts and receptacle) and inedible parts (outer bracts, leaves of peduncle, leaves of rosette, and floral stem). Data are expressed as mean of three replicates.

**Table S3.** Lignin content in edible (inner bract and receptacle) and inedible (outer bracts, leaves, and floral stem) tissues. ANOVA results (P value) are also indicated.

| Treatment | Edibility | Lignin[y] |
|---|---|---|
| Control | Edible | 0.11 ± 0.01 |
| | Inedible | 2.23 ± 0.14 |
| Ph15 | Edible | 0.13 ± 0.02 |
| | Inedible | 2.71 ± 0.46 |
| Ph17 | Edible | 0.24 ± 0.06 |
| | Inedible | 1.49 ± 0.60 |
| Probability level of significance (ANOVA)[x] | | |
| Photoperiod (A) | | .0301 |
| Edibility (B) | | < .0001 |
| A × B | | .0097 |
| LSD | | 0.26 |

[x] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and edibility (B) as factors. [y] Means separation was performed by Fisher's least significant difference (LSD) procedure (P < 0.05) and the LSD value is provided.

**Table S4.** Effect of photoperiod treatments (Ph15; Ph17; control) on total polyphenols (TP), antioxidant activity (AA), and total flavonoids (TF) production in globe artichoke plants.

| Treatment | Edibility | TP[y] (mgGA/g DW) | AA[y] (µmol trol./g DW) | TF[y] (mg CE/g DW) |
|---|---|---|---|---|
| Control | Edible | 14.1 ± 1.0 | 73.5 ± 7.1 | 11.9 ± 0.1 |
| | Inedible | 29.7 ± 1.1 | 180.6 ± 10.5 | 23.6 ± 2.2 |
| Ph15 | Edible | 8.0 ± 0.7 | 71.7 ± 8.3 | 11.2 ± 1.6 |
| | Inedible | 23.9 ± 1.1 | 163.7 ± 9.5 | 27.4 ± 2.6 |
| Ph17 | Edible | 8.7 ± 0.9 | 73.5 ± 4.9 | 12.2 ± 1.8 |
| | Inedible | 20.6 ± 0.7 | 121.5 ± 9.6 | 17.6 ± 1.7 |
| P level of significance (ANOVA)[x] | | | | |
| Photoperiod (A) | | < .0001 | .0002 | .0040 |
| Edibility (B) | | < .0001 | < .0001 | < .0001 |
| A × B | | < .0037 | .0002 | .0009 |
| LSD | | 0.76 | 6.95 | 1.49 |

[x] The ANOVA table shows the results of a two-way ANOVA performed using photoperiod (A) and edibility (B) as factors.
[y] Means separation was performed by Fisher's least significant difference (LSD) procedure (P < 0.05) and the LSD value is provided.
Edible (inner bract and receptacle) and inedible (outer bract, floral stem, leaves of peduncle, and rosette) tissues were studied.