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Total Antioxidant Capacity and Total Phenolics Content of *Phyllostachys* Taxa Shoots

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Abstract

Total phenolic content (TP) and total antioxidant capacity (AC) were analysed in shoots of *Phyllostachys aureosulcata* (PA), *P aureosulcata* f. *aureocaulis* (PAA), *P. aureosulcata* f. *spectabilis* (PAS), *P. bissetii* (PB), *P. flexuosa* (PF), *P. humilis* (PH), *P. iridescens* (PI), *P. nigra* var. *nigra* (PNN), *P. nigra* var. *henonis* (PNH), *P. mannii* (PM), *P. sulphurea* var. *sulphurea* (PSS), *P. violascens* (PVI), *P. viridiglaucescens* (PVG), *P. vivax* f. *aureocaulis* (PVA), collected on four harvest dates. Both TP and AC were determined following three processing methods, fresh, boiled and pickled in shoots of PF. Comparative study of TP and AC in the above *Phyllostachys* species shoots has not been reported before. The highest TP (1,227.6 μg GA/ml) and AC (154.0 μg AA/ml) values were measured in fresh shoots and the lowest in pickled ones. The highest values of TP were measured in the case of PA (1,321.95 μg GA/ml). The other taxa followed in decreasing order: PF, PVI, PI, PAA, PB, PAS, PNN, PNH, PM, PH, PSS and PVA. The highest AC values were obtained in the case of PI (184.24 μg AA/ml). The other taxa followed in decreasing order: PA, PF, PSS, PNN, PNH, PVG, PB, PAA, PAS, PV, PVA, PM and PH. The highest TP values were measured in taxa harvested on the first collection date and the values consequently decreased in taxa collected at later harvest dates. Our findings suggest that the earlier harvest date, through the influence of lower temperatures, could enhance the phytochemical content of bamboo shoots.

Keywords: bamboo, Bambusoideae, olericulture, Poaceae, vegetable

Introduction

Phyllostachys species are temperate bamboos of *Poaceae, Bambusoideae,* Shibataeinae, predominantly native of subtropical to warm temperate areas of China and have long been introduced to Korea (Ohrnberger, 2002). *Phyllostachys* species are of great agricultural importance in the Far East, primarily in forestry, as the culms are used in wood industry (Kleinhenz and Midmore, 2001) and harvested when developing young shoots for fresh market or processed vegetables (Chongtham *et al.*, 2011; Choudry *et al.*, 2012). Because of their aesthetic qualities, they are also planted in gardens and parks as ornamentals (Ohrnberger, 2002).

Bamboos have long been used in Chinese and Korean traditional medical pharmacology (Hong *et al.*, 2010). Bamboo shoots contain dietary fiber (Park and Jhon, 2009), protein, carbohydrates, amino acids, minerals (Chongtham *et al.*, 2011), flavonoids, phenolic compounds (Park and Jhon, 2010) and sterols (Lu *et al.*, 2009). Shoot or culm extracts showed high antioxidant (Choi *et al.*, 2008; Lee *et al.*, 2008; Li *et al.*, 2013), anti-inflamatory (Chae *et al.*, 2010; Park and Lee, 2012), anti-hypertensive (Kim *et al.*, 2008), antibacterial (Kim *et al.*, 2011) and anti-malassezia (Lee *et al.*, 2010) properties. Shoot or culm extracts have also been shown to have immuno-stimulating (Kweon *et al.*, 2003), anticarcinogenic properties (Lu *et al.*, 2010) and also anti-apoptic effects in ischemic injury treatment (Hong *et al.*, 2010). Other beneficial health effects include the use as a source of dietary fiber for improving lipid profile and bowel function (Park and Jhon, 2009), as a therapeutic agent for

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atopic dermatitis (Qi *et al.*, 2009) and against allergic airway diseases including asthma (Ra *et al.*, 2010). It also has been used for the prevention of hypercholesterolemia (Cho *et al.*, 2009; Ham *et al.*, 2009), high blood pressure, pulmonary inflammation and lung injury (Chae *et al.*, 2010) and to relieve lipotoxicity (Cho *et al.*, 2009). The study of Kim *et al.* (2012) has evaluated the nutritional components and antioxidant activities of ojuk (*Phyllostachys nigra*) shoot and leaf water extracts prepared by traditional tea manufacturing processes compared with green or mate tea. Their results indicated that the ABTS and DPPH free radical scavenging activities of shoot tea was seven fold higher compared to green or mate tea. Zhang *et al.* (2011) has compared the effects of different cooking methods on antioxidant activity and nutrient components in *Phyllostachys praecox* shoots. They found that compared to the traditional boiling, stir-frying or steaming did not decrease the antioxidant capacities or total phenolic content of bamboo shoots. The measured differences in total phenolic content were not significant. Yoo and Chung (1999) examined the changes in chemical properties of bamboo shoots during the manufacture of pickles. They measured significantly lower moisture, crude protein and fat, tannin and ascorbic acid content in the bamboo shoot pickles. Kim *et al.* (2012) studied the shelf-life of pickles processed from maengjong bamboo (*Phyllostachys pubescens*). High temperatures (35 or 45 °C) had a negative effect on sensory characteristics, while shelf-life at 20 °C was determined to be 14 months.

The present study aimed to investigate the total phenolics content and antioxidant activity of different edible *Phyllostachys* bamboo taxa shoots, including the effect of three preparation methods, namely fresh, boiled or pickled shoots.

Materials and methods

Plant material and sample preparation

Five shoots per each sample of similar size, diameter and position under the canopy were randomly collected when they emerged from the soil surface on four harvest dates in 2011. The following 14 *Phyllostachys* taxa were collected: on 22nd of April: *P. aureosulcata* (PA), *P. flexuosa* (PF), *P. iridescens* (PI), *P. violascens* (PVI), on 29th of April: *P aureosulcata* f. *aureocaulis* (PAA), *P. aureosulcata* f. *spectabilis* (PAS), *P. bissetii* (PB), *P. viridiglaucescens* (PVG), on the 13th of May: *P. humilis* (PH), *P. nigra* var. *nigra* (PNN), *P. nigra* var. *henonis* (PNH), *P. mannii* (PM), and on the 27th of May: *P. sulphurea* var. *sulphurea* (PSS), *P. vivax* f. *aureocaulis* (PVA), plants growing in the Botanical Garden of Szent István University, Gödöllő (47°35'38" N latitude, 19°22'09" E longitude and 242 m altitude). Samples were collected in four repetitions per taxa.

Processing of Phyllostachys shoots

The collected shoots were washed, the culm sheaths were removed and the remaining shoots were cut into small pieces and grounded. 5 g of samples were infused with 100 ml of boiling water. The aqueous extracts were stored at room temperature for 24 h. After centrifugation (13,000 rpm, 10 min) the supernatant was stored in a refrigerator at -20 °C, for 48 hours, until the analyses. For the comparison of fresh shoots with boiled and pickled shoots, the above procedure was also carried out in the case of boiled (10 min at 100 °C water temperature) or pickled (boiled for 10 min in a mixture of 200 ml 20% acetic acid, 200 g sodium chloride, 400 g saccharose and 500 ml of water, placed in glass vessels sealed with lids and gradually allowed to cool) shoots of *P. flexuosa*.

Determination of total phenolics content

The total amount of soluble phenols (TP) was determined using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1965) and determined spectrophotometrically at 760 nm. The content of soluble phenols was calculated from a standard curve obtained by different concentrations of gallic acid (GA) and given in μg GA/ml.

Determination of total antioxidant capacity by FRAP assay

The total antioxidant capacity (AC) related to ascorbic acid was determined spectrophotometrically using the FRAP (Ferric Reducing Antioxidant Power) method according to Benzie and Strain (1996). It is based on the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at low pH. This reduction was monitored by measuring the absorption change at 593 nm. Results were expressed as μg equivalents of ascorbic acid (AA).

Measurement of environmental parameters and irrigation

Air temperature (°C) and precipitation (mm) were recorded during the course of the experiment. Air temperature was measured six times per hour by a meteorological instrument. Potential evapotranspiration (ET_0) was calculated according to Helyes and Varga (1994) and Helyes *et al.* (2013) for other vegetable crops; the amount of daily water demand was calculated based on weather forecasting data of the Hungarian Meteorological Service (http://www.met.hu/idojaras/elorejelzes) from daily average temperature (in °C) divided by five and was expressed in millimetre:

Every week temperature averaged over 2-3 day intervals was used to calculate the daily potential evapotranspiration (ET_0). The amount of irrigation was calculated by ET_0 for the

$$I_d = \left(\frac{T_{\min} + T_{\max}}{2}\right) / 5$$

forecasting period corrected by the amount of precipitation. If precipitation covered the irrigation demand until the next irrigation date there was no irrigation, but if it was less than irrigation demand, the respective amount of ET_0 was supplied. Irrigation was given by overhead sprinklers from April 1st till the end of October by the calculated amount of water on every Monday, Wednesday and Friday morning.

Statistical analysis

Results were expressed as the average plus/minus standard deviations. The data were analysed by two-factor analysis of variance (ANOVA) with repetitions and the means separated using the LSD test at p=0.05. Regression analysis was performed using Statistica 9 software.

Results and discussions

Effect of different preparation methods on total phenolic content and antioxidant capacity in Phyllostachys flexuosa

Fig. 1 shows that higher TP values were measured in fresh shoots, compared to boiled or pickled shoots. Significantly different, the highest value of 1,227.6±75.5 µg GA/ml for TP was measured in the case of fresh shoots, which also gave similarly the highest measured value of 154.0±13.8 µg AA/ml) in the case of AC compared to boiled or pickled shoots. The second highest TP (21% lower than fresh shoots) and AC values (22% lower than fresh shoots) were measured in the case of boiled shoots (964.5 ± 44.9 µg GA/ml and 120.1 ± 11.2 µg AA/ml), and both parameters were significantly higher than values measured in pickled shoots. Significantly, the lowest TP (65 and 55% lower than fresh or boiled shoots respectively) and AC (93 and 91% lower than fresh or boiled shoots) values were measured in the case of pickled shoots (431.7 ± 44.5 µg GA/ml and 10.4 ± 1.6 µg AA/ml). The obtained results are comparable to those of Zhang *et al.* (2011) who have also found that compared to fresh shoots, boiled bamboo shoots had 10% and 23% lower TP and AC values respectively. Chung and Kim (2009) have examined the antioxidant activities of garlic (*Allium sativum* L.) stems and garlic bulbs according to processing methods and have also found the lowest total phenolic content and antioxidant activity in pickles. The same was true for pickled *Agaricus bisporous* compared to the fresh product (Ganguli *et al.*, 2007).

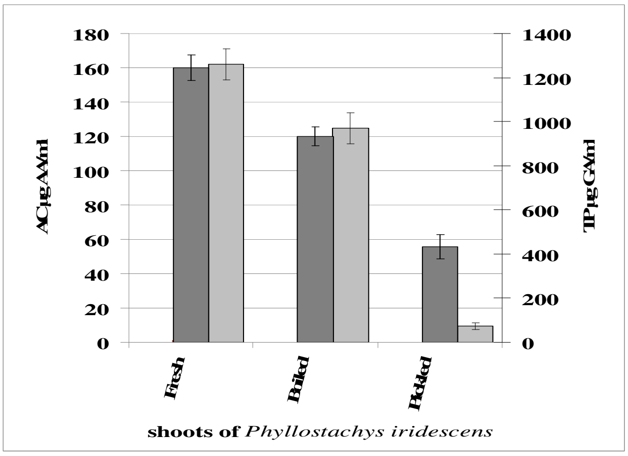

Fig. 1. Total phenolics content (TP) (µg GA/ml) and antioxidant capacity (AC) (µg AA/ml) of fresh, boiled and pickled shoot extracts of *Phyllostachys flexuosa* (n = 4, ±SD). White and grey columns refer to antioxidant capacity and total phenolic content, respectively

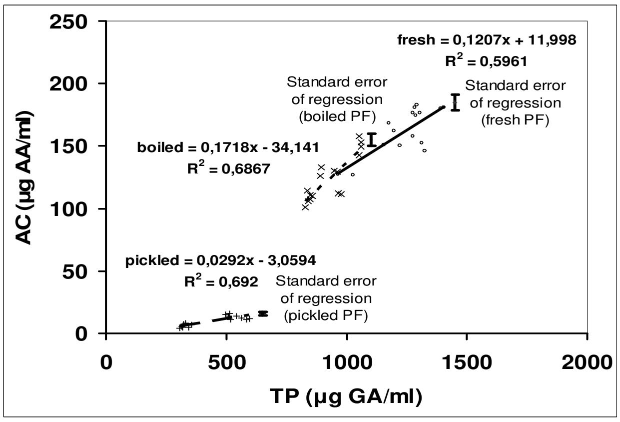

Fig. 2. Correlation between total antioxidant capacity (AC) (µg AA/ml) and total phenolic content (TP) (µg GA/ml) in fresh, boiled and pickled shoots of *Phyllostachys flexuosa* (p=0.01). Solid, dotted and dashed lines refer to fresh (o), boiled (X) and pickled (+) shoots respectively

Since total antioxidant capacity and total phenolic content followed a similar trend (Fig. 1) in fresh, boiled and pickled shoots of *Phyllostachys flexuosa*, it was investigated the correlation between AC and TP with the different processing methods. There was a significant correlation (p=0.01) between AC and TP values in all cases, as shown in Fig. 2. These correlation results were in agreement with those of Park and Jhon (2010), Zhang *et al.* (2011) and Li *et al.* (2013) who have also found a significant correlation between bamboo shoot total phenolics content and antioxidant activity.

Total phenolic content and antioxidant capacity in the shoots of different Phyllostachys taxa

Total phenolic content

The results regarding total phenolic content are shown in Fig. 3 for all *Phyllostachys* taxa. The highest values of TP were measured in the case of *P. aureosulcata* (1,321.95 ± 74.49 µg GA/ml), *P. flexuosa* (1,306.05 ± 65.25 µg GA/ml, 1% lower than PA) and *P. violascens* (1,270.51 ± 12.89 µg GA/ml, 4% lower than PA), but the difference between these was not significant. The fourth highest value was measured in *P. iridescens* (1,217.96 ± 26.02 µg GA/ml, 8% lower than PA) which was significantly lower than the previous taxa. The fifth highest value characterized *P. aureosulcata* f. *aureocaulis* (1,208.74 ± 50.63 µg GA/ml, 9% lower than PA), closely followed by *P. viridiglaucescens* (1,203.84 ± 92.78 µg GA/ml, 9% lower than PA) and *P. bissetii* (1,193.81 ± 14.35 µg GA/ml, 10% lower than PA) and *P. aureosulcata* f. *spectabilis* (1,187.77 ± 101.31 µg GA/ml, 10% lower than PA). The difference between these taxa was not significant. The ninth highest value was obtained from *P. nigra* var. *nigra* (1178.17 ± 69.45 µg GA/ml, 11% lower than PA) followed by *P. nigra* var. *henonis* (1,130.49 ± 66.45 µg GA/ml, 15% lower than PA). There was no significant difference between PNN and PNH and neither previous taxa except for PA, PF and PVI. The next lower value was that of *P. mannii* (1,082.41 ± 93.39 µg GA/ml, 18% lower than PA) which was significantly lower only than the first four. The next lowest value was measured in *P. humilis* (983.18 ± 33.66 µg GA/ml, 26% lower than PA) which was significantly lower than all previous taxa except for PM. The next lowest TP value was given by *P. sulphurea* var. *sulphurea* (970.14 ± 62.69 µg GA/ml, 27% lower than PA) which was significantly lower than all previous taxa except for PM and PH. The lowest value of TP was measured in *P. vivax* f. *aureocaulis* (826.22 ± 64.75 µg GA/ml, 38% lower than PA) which was significantly lower than TP contents in all other taxa. The obtained results are in agreement with those of Jin and Yuan (2012) and Li *et al.* (2013) who have also found that *P. aureosulcata* f. *spectabilis* is a species which contains high levels of flavonoids and phenolic acids, but they used ethanolic extracts in their experiments. There is no comparative study of TP in the above *Phyllostachys* species shoots in the available literature, except for that of Jin and Yuan (2012) who have made an intra-specific comparison of *P. aureosulcata* and *P. edulis* cultivars (forms).

Total antioxidant capacity

Results for AC in the case of all taxa are shown in Fig. 3. The highest values were obtained in the case of *P. iridescens* (184.24 ± 3.75 µg AA/ml), *P. aureosulcata* (177.99 ± 10.27 µg AA/ml, 3%

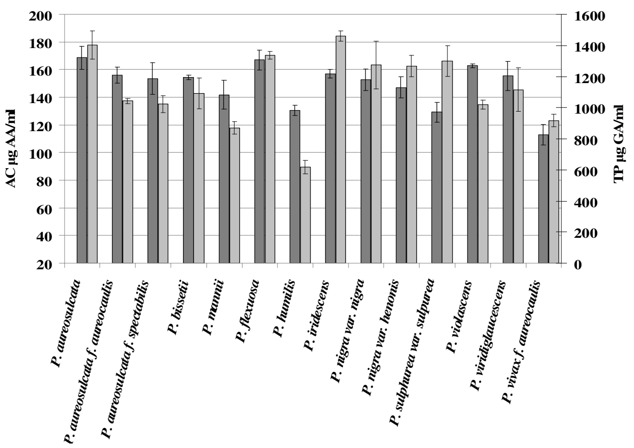

Fig. 3. Total phenolics content (TP) (µg GA/ml) and antioxidant capacity (AC) (µg AA/ml) of shoot extracts of different *Phyllostachys* taxa (n = 4, ±SD). White and grey columns refer to antioxidant capacity and total phenolic content, respectively

lower than PI) and *P. flexuosa* (170.42 ± 3.06 µg AA/ml, 8% lower than PI), but the difference between them was not significant. The fourth highest AC value was measured in *P. sulphurea* var. *sulphurea* (166.18 ± 11.09 µg AA/ml, 10% lower than PI) which was significantly lower only than the first taxa. The fifth highest value was given by *P. nigra* var. *nigra* (163.39 ± 17.18 µg AA/ml, 11% lower than PI), followed by *P. nigra* var. *henonis* (162.73 ± 7.87 µg AA/ml, 11% lower than PI) and the difference was significant only compared to PI. The difference between the two *P. nigra* varieties was not significant. *P. viridiglaucescens* (145.42 ± 15.78 µg AA/ml, 21% lower than PI) and *P. bissetii* (142.93 ± 10.97 µg AA/ml, 23% lower than PI) produced the seventh and eighths highest values. There was no significant difference between the two taxa, whereas their values were significantly lower than the first 3 taxa. These were followed by *P. aureosulcata* f. *aureocaulis* (137.45 ± 1.93 µg AA/ml, 25% lower than PI) and *P. aureosulcata* f. *spectabilis* (135.24 ± 6.16 µg AA/ml, 27% lower than PI), but there was no significant difference between the two *P. aureosulcata* forms, while they significantly differed from the first six taxa. The next lowest value of AC was measured in *P. violascens* (134.77 ± 3.22 µg AA/ml, 27% lower than PI) which was only significantly different from the first six taxa. *P. vivax* f. *aureocaulis* (123.15 ± 4.65 µg AA/ml, 33% lower than PI) produced the next lowest AC value, which was significantly lower than all previous taxa. *P. mannii* (117.83 ± 4.57 µg AA/ml, 36% lower than PI) gave the next lowest value and was significantly different from all previous taxa except for PVA. The lowest AC value was measured in *P. humilis* (89.63 ± 4.94 µg AA/ml, 51% lower than PI) which was significantly lower than all the other taxa. *Phyllostachys aureosulcata* was among the taxa producing the highest antioxidant capacity which is in agreement with the findings of Jin and Yuan (2012) and Li *et al.* (2013). In the available literature there is no comparative study of AC in the above *Phyllostachys* species shoots, except for the intra-specific comparison of *P. aureosulcata* and *P. edulis* forms (cultivars) by Jin and Yuan (2012).

The results of TP with AC in shoots of the different taxa were compared and regarding the measured values from the highest to the lowest, the taxa could not be ranked in the same order. This could possibly be explained by the fact that not only TP constitutes to AC in bamboo shoots (Zhang *et al.*, 2011), but there is possibly inter-specific differences in other phytochemical contents, including L-ascorbic acid (Park and Jhon, 2010; Zhang *et al.*, 2011) and polysaccharides (Zhang *et al.*, 2011), even though this has not been reported for *Phyllostachys* taxa and should be the target of further investigation.

Influence of shoot phenology and temperature on total phenolic content

Comparative study of total phenolics content in the above *Phyllostachys* species shoots has not been reported before. When comparing TP values of the different taxa shown and the initial harvest dates, the highest TP values were measured in the taxa harvested on the first collection date and the values consequently decrease in taxa collected at later harvest dates, with the lowest values in taxa collected at the last harvest date. Because of our findings that there was a clear decreasing trend in TP values measured in the successively later harvested bamboo taxa shoots, it was examined the possible environmental cause of this phenomenon. Since the plants were irrigated, it was assumed that water supply did not play a role in the phenomenon. Besides the obvious genetic differences between the taxa in the capacity of phenylpropanoid synthesis reported for other plants by Prohens *et al.* (2007) or other phytonutrients reported by Yuan *et al.* (2009), temperature seemed to be another factor which is known to influence the synthesis of phenolic compounds (Janda *et al.*, 1999; Pál *et al.*, 2013). Therefore, daily maximum and minimum temperature (°C) values for the examined harvest period, from April to May, are shown on Fig. 4. During April daily maximum values (were between +10 °C and +23 °C) started to rise one week prior to the first harvest date (22nd of April), while daily minimum values (were between +1 °C and +6 °C) did not alternate much and remained more or less constant. Maximum values (were between +14 °C and +25 °C) levelled off with larger fluctuation between 23rd of April to 10th of May, while daily minimum values (were between +5 °C and +10 °C) gradually increased until the second harvest date (29th of April). Daily minimum and maximum values increased again during the days preceding the third harvest (13th of May). From the 17th of May temperatures started to rise again and the highest daily minimum and maximum temperatures (+15 °C and +29 °C respectively) were recorded during the last week of May. Generally, either the daily minimum or daily maximum temperature ranges rose gradually between the harvest dates, during the examined April-May period. None of the previous reports (Jin and Yuan, 2012) monitored environmental parameters and their possible effects on phytonutrient content of bamboo shoots in the examined *Phyllostachys* taxa. Thus, examining reports on other plants, the current results could be explained by the findings of Oh *et al.* (2009) who have found that low temperatures caused an increase in total phenolics content in lettuce. The same was reported by Zobayed *et al.* (2005) for *Hypericum perforatum*. Also Ni *et al.* (2013) found that in leaves of bamboo *Indocalamus latifolius,* altitudinal variation also caused an increase in total phenolic and flavonoid contents and antioxidant activity. Our findings suggested that besides the genetic influence on the capacity of phenylpropanoid biosynthesis and on specific shooting times of bamboo taxa, the earlier harvest date through the influence of lower temperatures could enhance the phytochemical content of bamboo shoots intended as vegetables. But further intra-specific experiments,

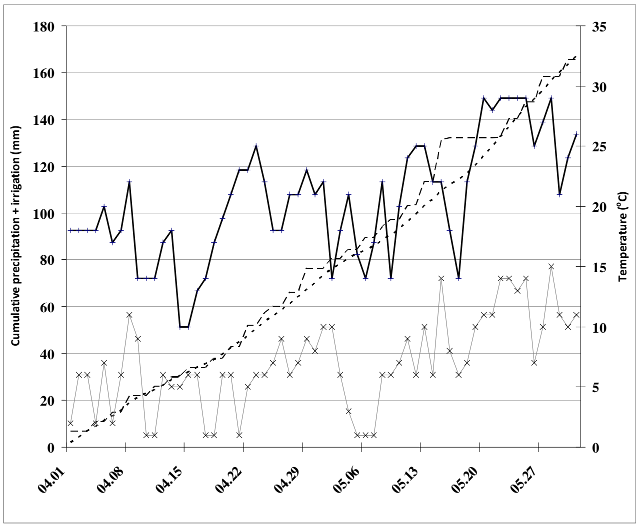

Fig. 4. Change of daily minimum, maximum air temperature (°C), cumulative precipitation plus irrigation (mm) and cumulative potential evapotranspiration (mm) during the harvest period (April-May). Grey line (X), black line (+), dotted line, dashed line represents minimum temperature, maximum temperature, cumulative potential evapotranspiration, cumulative precipitation plus irrigation respectively

involving more frequent harvest dates with regards to phytochemical content of bamboo shoots, need to be carried out to underline this.

Conclusions

When comparing the processing methods, the highest TP and AC values were obtained in the case of fresh shoots of *Phyllostachys iridescens* compared to boiled or pickled shoots, while the lowest TP and AC values were measured in the case of pickled shoots. There was a significant correlation ($p=0.01$) between AC and TP values in fresh, boiled and pickled shoots of PF. The inter-/intra-specific comparison showed that the highest TP and AC were measured in the case of PA, PF, PVI, PI and PI, PA, PF, PSS, respectively. The highest TP values were measured in taxa harvested on the first collection date and the values subsequently decreased in taxa collected at later harvest dates. Based on the current findings, it can be concluded that besides the genetic factors influencing shoot phenology and capacity of phenylpropanoid biosynthesis, through the influence of lower temperatures the earlier harvest date possibly also influenced the phytochemical content of bamboo shoots.

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