Agronomic, Nutraceutical and Organoleptic Performances of Wild Herbs of Ethnobotanical Tradition

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Despite the growing interest for wild herbs as nutraceutical food there is a lack of information on how to grow them. Ten wild herbs were collected in natural and/or anthropized environments and assessed for their agronomic performance as fresh-cut (or ready-to-eat) leafy vegetables and their nutraceutical and organoleptic attributes. Seed dormancy prevented acceptable germination in many species. However, a physiological seed treatment (soaking with sodium hypochlorite followed by incubation for 3 mo at 4°C in sand moistened with potassium nitrate solution) allowed satisfactory germination, usually above 80%. Cultivation in alveolar containers produced highly diversified fresh-cut productivity (250-550 $g \cdot m^{-2}$), lower than that of lettuce (*Lactuca sativa* L.; >900 $g \cdot m^{-2}$) grown as a reference fresh cut green vegetable. Antioxidant power was often much greater in wild herbs (20.0 to 62.0 mmol $Fe^{2+} \cdot kg^{-1}$ FW) than in lettuce (21.0 mmol $Fe^{2+} \cdot kg^{-1}$ FW). Evaluation of the sensory profile indicated that softness and sweet taste of lettuce were generally preferred to the more robust flavors of wild herbs. Hardness and bitter taste produced a poor appreciation of most wild herbs. However, exceptions were evidenced due to characteristics of spiciness [*Alliaria petiolata* (M.Bieb) Cavara & Grande]

and/or crunchiness (*Silene vulgaris* [Moench] Garcke). Frequent distrust for most herbs was expressed as an example of food neofobia that generally occurs for unknown bitter flavors. Most of the wild herbs were not suitable as fresh-cut leafy vegetables, but some species could be ingredients for mixed products with better flavor and health properties.

Keywords: food biodiversity, human health, new crops, sensory profiles

Ethnobotanical traditions are an important guide in regard to ancient foods (Łuczaj et al., 2012) that could become examples of so-called “novel foods”. An emerging need for new food, able to fit organoleptic acceptance, and nutraceutical functionality (Hussain et al., 2015), has expanded the agronomic horizon from traditional crops to wild species that are now available almost exclusively from collection in natural ecosystems.

Wild herbs play a crucial role not only for their important gastronomic emotionality, due to old recipes, but for increasing scientific evidences of their nutraceutical properties. The most studied parameter is antioxidant power that was high in several traditional food herbs (Trichopoulou et al., 2000; Wojdyło et al., 2007) as well as in wild and/or cultivated edible flowers (Benvenuti et al., 2016). Wild plants evolved a wide array of secondary metabolites with strong antioxidant activity, which facilitate essential interactions with the biotic and abiotic environment, including chemical defense against herbivores and pathogens (Neilson et al., 2013). This marked ability has increased interest toward wild germplasm for production of plant-derived antioxidants in food (Gülçin, 2012). The antioxidant power of several wild herbs constituents is responsible for their anti-aging activity (Ferrari, 2004). Antioxidant compounds are able to improve human health by acting as anticancer molecules (Greenlee et al., 2012) and against cardiovascular (Kris-Etherton et al., 2002) and degenerative diseases (Ames et al., 1993).

Ancient ethnobotanical gastronomy is based on many wild herbs (Tardio et al., 2006), which continue to be an integral part of the Mediterranean diet (Vasto et al., 2014). Consequently, there is an increasing need to investigate beneficial effects of wild herbs (Trichopoulou et al., 2006) associated with their high nutraceuticals (Vardavas et al., 2006) and antioxidants (Vanzani et al., 2011) contents. Wild herbs could be psychologically perceived as a sort of organoleptic expression of a friendly rural environment, contributing to emotional well-being. Since rural tradition is now almost disappeared due to the declines of the rural population, levels of appreciation that these often unfamiliar foods may have in urban populations is unpredictable. Consequently, nutraceutical investigations should include sensory profiles of herbs and their real appreciation.

The sensory profile of wild herbs has received limited study (Jiand et al., 2014) and its evaluation is often hindered by the so-called neofobia generated by new flavors (Pliner and Hobden, 1992). In children, who usually do not have any food taste imprinting, unfamiliar wild herbs flavors could induce picky/fussy-eating behavior (Dovey et al., 2008).

Wild plant collection is not sustainable in the long term from an ecological point of view, as it involves potential biodiversity loss, and can supply only small local markets (Hanlidou et al., 2004). Significant cultivation of nutraceutical herbs on a commercial scale will be necessary. Furthermore, it is important to recognize that nutraceutical herbs, potentially new crops, will arouse strong interest for consumers, especially when grown with organic cropping systems since they could improve human health.

Little information is available about agronomic productivity of these emerging crops. A problem restricting cultivation of the herbs is seed dormancy (Baskin and Baskin, 2004), which can be overcome with specific pre-germination treatments (Benvenuti and Pardossi, 2016).

Popular wild herbs, which are components of the ethnobotanical tradition in the Mediterranean region, were investigated to determine: i) germination and emergence performance, ii) agronomic productivity, iii) antioxidant power, and iv) acceptance through organoleptic sensory profiles.

Materials and Methods

A number of wild herbs of ancient ethnobotanical tradition (Pieroni, 2000 and 2001) were identified in various locations of Tuscany, Italy. Ten species were selected among those commonly used as food in the folk tradition (Table 1). These species have a similar growing period (winter-spring). All species are typically eaten raw. Mature seed (achenes in the case of Asteraceae and Dipsacaceae families) were collected in summer-autumn 2009, when plants were senescencent. Seed were cleaned, dried in room with low relative humidity, and kept in glass containers at 20°C.

To overcome dormancy, at 3-6 months following collection seed were soaked in 50% sodium hypochlorite for 30 min, carefully washed in tap water, placed in Petri dishes on sand moistened with 0.1% potassium nitrate (KNO_3) solution, and stored at 4°C in the dark for 3 mo (Benvenuti and Pardossi, 2016). In spring 2010, treated, and untreated, seed were sown in alveolar polystyrene trays (52×32 cm, 160 holes, 5 cm depth), filled with a peat-perlite substrate (1:1 v/v). One seed per hole was sown on the surface and covered with a 1 mm substrate layer. Emergence (defined as cotyledons appearance) was evaluated daily until no further emergence occurred within a 1 week period. Mean emergence time (MET) was calculated according to

Benvenuti and Pardossi (2016). For each species, 3 replicates (3 trays) were used. During the experiment, the temperature ranged from 5-10°C (night) to 20-25°C (day).

Each species was established in spring 2011 in alveolar trays, as previously described, to evaluate productivity. Missing plants were replaced by seedlings of the same species from additional containers. The growing substrate was enriched with 3 $g \cdot L^{-1}$ of controlled-release fertilizer (Osmocote Plus Organics® 13.1N-2.3P-4.0K, Everris, Australia). Daily irrigation was with an over-head (boom) with 2 $L \cdot m^{-2}$ at each application. Greenhouse climatic conditions were similar to those of the previous year. Lettuce, cv. Lollo, was grown as a reference crop. Plants of all species were harvested 30 days after emergence. To synchronize harvest, slow-emerging species were sown earlier than fast-emerging ones, so that species achieved the same cotyledon phenological stage at the same time. At harvest, plants were cut about 0.5 cm above the substrate surface and immediately weighted. After weighing, fresh plant biomass was placed in plastic containers the same as those used for sale packets, on absorbent paper at the bottom to prevent leakage due to guttation. The packs were immediately placed in refrigerator bags, stored at -80°C and analyzed within 3-4 weeks from collection.

Antioxidant power was expressed on a fresh weight (FW) basis. Samples (0.5 g) were extracted with 5 mL methanol for 12 h at 4°C. Antioxidant activity was determined spectrophotometrically on extracts after proper dilution by the FRAP (ferric ion reducing antioxidant power) assay following Benzie and Strain (1996). A calibration curve was prepared with increasing concentrations of ammonium iron (II) sulfate (reagent grade, Sigma Aldrich, Saint Louis, MO).

A sensory panel used fresh-cut biomass obtained in Spring 2011. Seventy-five free-tasters (35 males, 40 females, mean age 36 years) were recruited by advertisement to students, teachers, and other staff at the Department of Agriculture, Food and Environment, University of Pisa.

The test was based on previous studies on taste evaluation performed on vegetables (Zhao et al., 2007) and edible flowers (Benvenuti et al., 2016). Leaves of wild herbs and lettuce were tasted over unsalted crackers, with only a few drops of extra virgin olive oil with a delicate flavor. After a careful evaluation of perceived flavors, the tasters filled out a questionnaire.

The organoleptic characteristics spiciness, sweetness, softness, crunchiness, and bitterness were included in the evaluation and expressed in a scale of 1 to 100. The sensory profile was presented as spider plots (Johansson et al., 1999). A synthetic evaluation, scale 1-10, was used to establish degree of overall appreciation of each species.

Experiments were replicated 3 times using a completely randomized design. After the test of variance homogeneity, angular values from arcsine transformation of percentage data (seedling emergence), or untransformed data (mean emergence time, fresh-cut productivity and antioxidant power), were subjected to ANOVA. If an interaction was significant it was used to explain the data. If an interaction was not significant means were separated with Tukey's test. Statistical analysis was performed using the CoHort software (ver. 6.4, Minneapolis, MN).

Results

Crop and treatment, and their interaction, affected emergence; only crop affected emergence time (Table 2). Seed emergence varied by species (Table 3). Untreated seed had a relatively high emergence (>60%) in *Sonchus oleraceus* L., *Silene vulgaris* (Moench) Garcke, *Taraxacum officinale* Weber and *Picris echioides* L. Suboptimal emergence (<50%) was for untreated seed of *Campanula rapunculus* L., *Hyoseris radiata* L., *Scabiosa columbaria* L., *Leontodon tuberosus* L., *Papaver rhoeas* L. and in *Alliaria petiolata* (M.Bieb.) Cavara &

Grande, which exhibited long seed dormancy as expressed by time to emergence. For *S. columbaria*, *S. vulgaris* and *S. oleraceus* emergence time was relatively short; it was longer (>2 mo) for *A. petiolata*).

Cold stratification removed seed dormancy in all species except *P. rhoeas* and *S. columbaria*; in those, the rate of emergence was less than in *L. tuberosus*, *A. petiolata*, *H. radiata* and *P. echioides*, *S. oleraceus*, *S. vulgaris* and *T. officinale* (Table 3). Emergence time was least for *S. columbaria* and longest for *A. petiolata* (Table 4) which was slightly affected by seed treatment. Only *A. petiolata* and *P. rhoeas* showed a significant increase in emergence velocity.

Biomass production of the wild herbs and lettuce 1 mo after emergence varied (Fig. 1). Lettuce had higher productivity than the wild herbs. The most productive herbs were *S. columbaria*, *T. officinale*, *S. oleraceus*, *H. radiata* and *P. echioides*. In these production was about one-half fresh biomass of that of lettuce. Lower productivity occurred for *S. vulgaris*, *A. petiolata*, *L. tuberosus*, *C. rapunculus* and *P. rhoeas*.

Antioxidant capacity did not differ in lettuce, *S. vulgaris*, *C. rapunculus*¸ *P. echioides*, *A. petiolata* and *P. rhoeas*, and was higher in *L. tuberosus*, *S. columbaria*, *T. officinale*, *S. oleraceus* and *H. radiata*. Among species, *H. radiata* had an antioxidant capacity that was almost 3-fold higher than in lettuce (Table 5).

For the organoleptic assessment: *S. columbaria*, *P. echioides*, *L. tuberosus*, *T. officinale*, *H. radiata*, *C. rapunculus* and *S. oleracea*, were appreciated less than lettuce (Table 5). In contrast, *P. rhoeas*, *S. vulgaris* and *A. petiolata* received higher appreciation scores than lettuce (Table 4), although these herbs were unknown to the tasters (data not shown). In addition to this synthetic assessment it was important to investigate sensory components (positive or negative) perceived by tasters allowing for information to be developed about their organoleptic characteristics.

The sensory profile of each species perceived by tasters for spiciness, sweetness, softness, crunchiness and bitterness of the herbs varied (Fig. 2). The high evaluation score of lettuce (Table 5) was associated with a sensory profile where softness and sweetness predominate along with crunchiness; spiciness and bitterness was not appreciated. The low appreciation of *S. columbaria* (Table 4) appears to be due to a pronounced bitter taste and toughness, indicating leaves were considered hard to chew. Similarly, *P. echioides* and *L. tuberosus* received low appreciation scores as a result of excessive bitterness. Leaves of *T. officinale* and *H. radiata*, were perceived as bitter, but had higher appreciation scores probably due to their softness. Bitter taste was appreciated in *S. oleraceus*, which was likely off-set with the perception of softness. The good appreciation of *C. rapunculus* and *S. vulgaris* was likely due to pronounced sweetness, softness and crunchiness. This last feature probably played a role in evaluation of *S. vulgaris*. Bitterness was positively appreciated when combined with softness and spiciness as for *P. rhoeas* and *A. petiolata*, which was the most appreciated herb.

Discussion

Most of the species had long seed dormancy but this is not surprising because seed dormancy and the consequent unsynchronized emergence allows survival in erratic environmental conditions (Allen and Mayer, 1998). Only *S. vulgaris*, *T. officinale*, *S. oleraceus* and *P. echioies* had >50% emergence and no herb reached 80%, which is considered the minimum for seed certification and trade of many vegetable crops (ISTA, 1999). In the case of *A. petiolata*, seed emergence was practically absent without cold stratification. Seed emergence of food herbs must be improved to overcome their dormancy (Ladizinsky, 1987) and induce a high and synchronized emergence.

Specific seed treatments (chemical, physiological or physical) on food herbs have efficacy to improve emergence (Benvenuti and Pardossi, 2016). All species were subjected to an opportune treatment-sequence, allowing achievement of a fully satisfactory effect, since emergence was >80% in 8 species, and only slightly lower in *P. rhoeas* and *S. columbaria*. The most striking result concerned *A. petiolata* since the seed treatment (chemical-physiological) allowed a change from almost absolute dormancy to emergence beyond 80%. The plant *A. petiolata* is a deep-dormant species (Baskin and Baskin, 1992) and its invasiveness in forest environments (Anderson et al., 1996) is mediated by loss of dormancy during the most favorable periods, as typically occurs in wild species (Allen and Meyer, 1998).

Dormant seed, due to physiologically dormant embryos (Baskin et al., 2002), of *P. rhoeas* exhibited good emergence after seed treatment. However, treatment sped up emergence of only *A. petiolata*, *L. tuberosus* and *P. rhoeas.* Emergence time was particularly long (about 2 mo) in untreated seed of *A. petiolata* but was reduced to 5 days in treated seed. Mean emergence time of most species was similar to many common vegetables, ranging between 1 and 2 weeks.

One of the most effective treatments to accelerate emergence involves use of gibberellic acid (Miransari and Smith, 2014). In the present work this compound was not employed, as use of synthetic substances (actually available even from natural origin (Shukla et al., 2005) but of probable uneconomic application) is not allowed for eventual organic cropping systems.

Minimally-processed horticultural products are cleaned, washed, cut and packaged soon after harvest (Degl'Innocenti et al., 2007) making them suitable to be used as ready to eat food (Watada et al., 1996). Lettuce had superior yield compared to undomesticated species. Although productivity of the food herbs was 25-50% compared to lettuce, the market of the wild species appears to be linked to richness in secondary metabolites with a high antioxidant power. Wild herbs contain large amounts of antioxidant secondary metabolites (Vanzani et al., 2011) and a great antioxidant power was also found in the wild herbs.

The comparison between wild and domesticated species indicates that plant productivity and antioxidant power were inversely related, in that contents of secondary metabolites typically decreased during domestication (Herms and Mattson, 1992). For this reason, genetic improvement of herb productivity decreases their antioxidant power and does not appear a useful strategy. Rather, small quantities of herbs could be employed to improve the flavor and the antioxidant power of other green vegetables in fresh-cuts mixtures.

The bitter-tasting *L. tuberosus*, *H. radicata*, *P. echioides*, *S. columbaria* and *T. officinale* were less appreciated, albeit to a different extent, by tasters. Elimination, or reduction, of the bitter flavor was an objective during domestication of the wild *L. serriola* L. to obtain the widely cultivated *L. sativa* (De Vries, 1997). Bitterness of leaves of many wild species likely evolved as a survival strategy for rejection by mammals (Glendinning, 1994) even if it was found dose-dependent in humans (Drewnowski and Gomez-Carneros, 2000). Reducing the bitter flavor during plant domestication (Meyer et al., 2012) implies reducing potential health benefits (Schmidt et al., 2008). Food neophobia (Pliner et al., 1993; Barrena and Sánchez, 2013) seems to be directly related to bitter taste, especially in children (Coupland and Hayes, 2014). Dilution of bitter herbs with lettuce could reduce the gap between novel and familiar foods. The crunchiness of *S. vulgaris*, the spiciness of *P. rhoeas* and *A. petiolata*, and the softness of *T. officinale* or *C. rapunculus* could all contribute to organoleptic improvement of a vegetable mixture.

It can not be expected that wild food herbs could have productivity comparable to domesticated crops. After overcoming problems of seed dormancy through appropriate treatment, wild species may become new crops for novel foods. Low productivity, and often too pronounced flavors, of several wild herbs, which hinder their exclusive consumption, could be solved by qualitatively improving the already familiar mixed fresh cuts (Rico et al., 2007). Use of organic cropping could be an important tool to attract consumers by limiting the natural distrust for unknown foods. Food herbs could have a crucial role from a gastronomic point of view, and in terms of human health and psychological awareness of a sustainable environment.

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Table 1. Botanical characteristics and habitat of germplasm collection of the wild herbs used.

Scientific name	Botanic family	Life form	Chorology[b]	Typical habitat	Locality of seed collection[c]	Geographical coordinates
Alliaria petiolata (M.Bieb.) Cavara&Grande	Brassicaceae	H[a]	EA	Deciduous forest	Calci	43° 75’ N, 10° 53’ E
Hyoseris radiata L.	Asteraceae	H	SM	Dry meadow	Asciano	43° 74’ N, 10° 43’ E
Leontodon tuberosus L.	Asteraceae	H	SM	Dry meadow	Agnano	43° 73’ N, 10° 47’ E
Papaver rhoeas L.	Papaveraceae	T	EM	Agroecosystem	Vecchiano	43° 78’ N, 10° 39’ E
Picris echioides L.	Asteraceae	T	EM	Dry meadow	Asciano	43° 74’ N, 10° 43’ E
Campanula rapunculus L.	Asteraceae	H	EA	Wood borders	Agnano	43° 73’ N, 10° 49’ E
Scabiosa columbaria L.	Dipsacaceae	H	EA	Road sides	Agnano	43° 73’ N, 10° 50’ E
Silene vulgaris (Moench) Garcke	Caryophyllaceae	H	P	Wood borders	Calci	43° 74’ N, 10° 54’ E
Sonchus oleraceus L.	Asteraceae	T	C	Urban meadows	Asciano	43° 75’ N, 10° 43’ E
Taraxacum officinale Weber	Asteraceae	H	C	Wet meadow	Agnano	43° 73’ N, 10° 49’ E

[a] H = Hemicryptophyte; T = Terophyte.
[b] EA = Euroasiatic; EM = Euri-Mediterranean; SC = Subcosmopolitan; SM = Steno-Mediterranean; P = Paleotemperate; C = Cosmopolitan.
[c] all localities are within 10 km from Pisa, Tuscany, Italy.

Table 2. ANOVA for effects of crop and measurement time in relation to treatment for emergence percent and average emergence time.

Source	Emergence	Average emergence time
Crop (C)	**	*
Measurement in relation to time of treatment (M)	**	ns
Interaction C × M	**	ns

ns, *, ** not significant or significant at P<0.05 or P<0.01, ANOVA.

Table 3. Final percent and average emergence time of herbs before and after seed treatment.

Crop	Measured in relation to treatment	Emergence (%)
Alliaria petiolata	Before	1.5
	After	81.2**
Hyoseris radiata	Before	38.2
	After	84.3
Leontodon tuberosus	Before	28.7
	After	80.5**
Papaver rhoeas	Before	23.3
	After	72.4**
Picris echioides	Before	62.2
	After	86.6**
Campanula rapunculus	Before	44.3
	After	90.2**
Scabiosa columbaria	Before	33.3
	After	78.3**
Silene vulgaris	Before	78.3
	After	92.4*
Sonchus oleraceus	Before	73.3
	After	90.4*
Taraxacum officinale	Before	78.2
	After	94.5*

ns, *, ** not significant or significant at $P<0.05$ or $P<0.01$ between values in a crop by treatment group, Tukey's test.

Table 4. Average emergence time of herbs.

Crop	Avg. emergence time (days)
Alliaria petiolata	57.8 *
Hyoseris radiat	10.6 ns
Leontodon tuberosus	16.0 ns
Papaver rhoeas	14.0 *
Picris echioides	11.6 ns
Campanula rapunculus	12.2 ns
Scabiosa columbaria	8.2 ns
Silene vulgaris	10.0 ns
Sonchus oleraceus	10.2 ns
Taraxacum officinale	12.4 ns

ns, * not significant or significant at $P<0.05$.

Table 5. Antioxidant power (mmol Fe^{2+} kg^{-1} FW) and synthetic taste evaluation (1-10 scale) of wild herbs and lettuce.

Crop	Antioxidant power (mmol $Fe^{2+}\cdot kg^{-1}$ FW*)	Tasting evaluation (1-10 scale)
Alliaria petiolata	25.1 [de**]	8.5 [a]
Campanula rapunculus	20.8 [e]	7.4 [ab]
Hyoseris radiata	62.0 [a]	7.1 [ab]
Lactuca sativa	21.0 [e]	8.0 [a]
Leontodon tuberosus	38.1 [cd]	6.5 [c]
Papaver rhoeas	31.2 [d]	8.2 [a]
Picris echioides	23.6 [e]	6.2 [c]
Scabiosa columbaria	48.7 [bc]	5.2 [d]
Silene vulgaris	20.0 [e]	8.3 [a]
Sonchus oleraceus	52.6 [ab]	7.5 [ab]
Taraxacum officinale	51.6 [ab]	7.0 [bc]

* Fresh Weight

** Values in columns followed by the same letter are not significantly different, $P<0.05$ (Tukey's test).

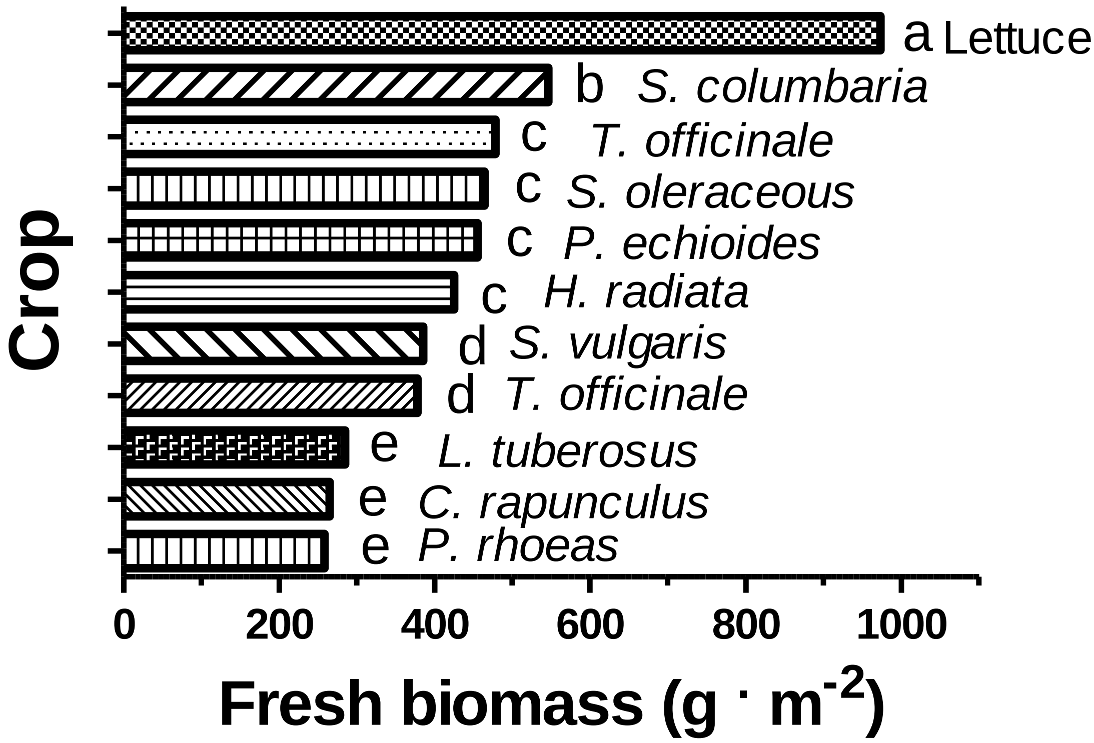

Figure 1. Fresh biomass production of wild herbs and lettuce. Values of bars followed by the same letter are not significantly different, P <0.05 (Turkey test).

Wild herbs

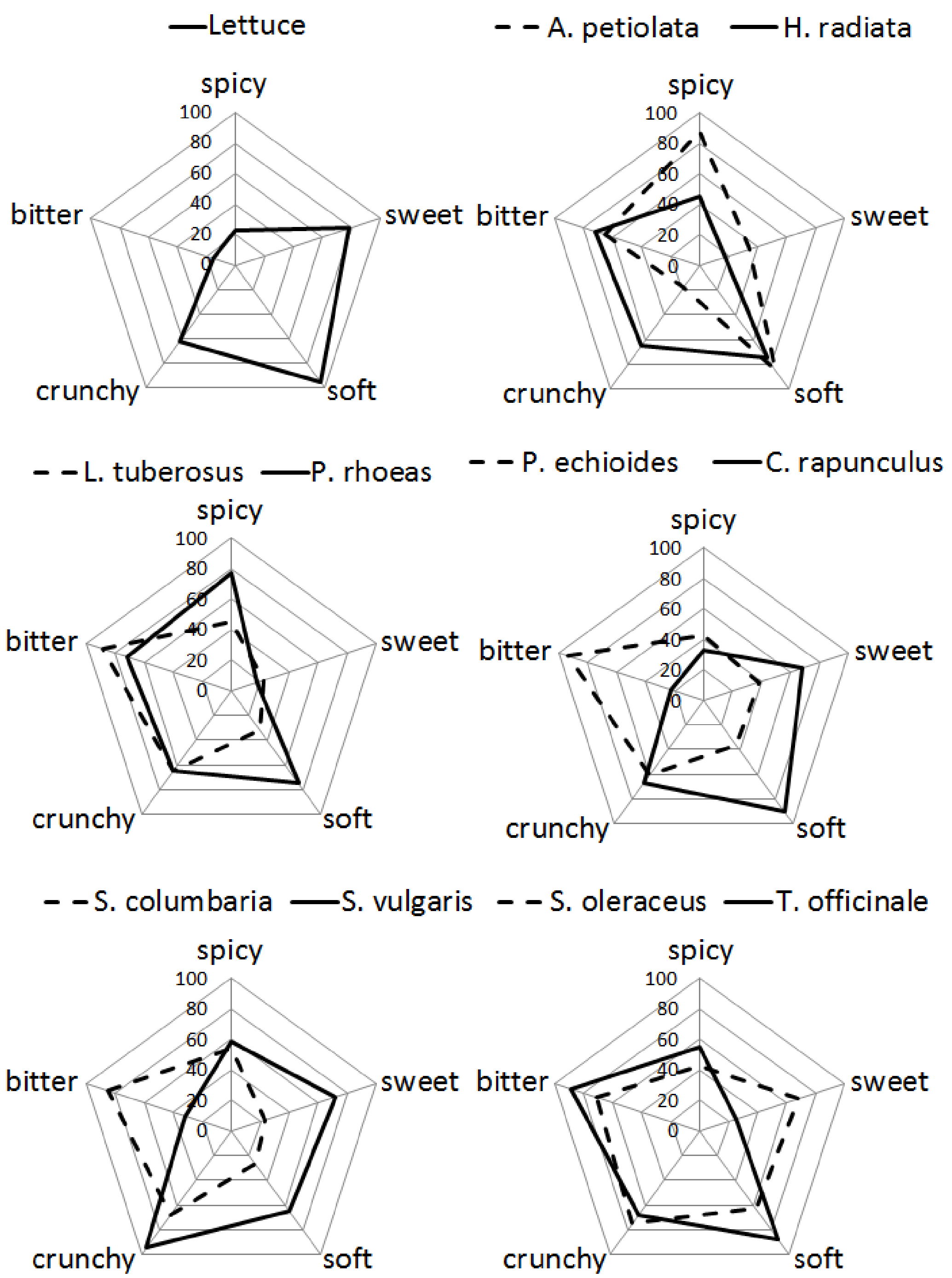

Figure 2. Sensory profile as percent (0-100 on vertical bar) of evaluation of spicy, sweet, soft, crunchy and bitter sensory of wild herbs and lettuce by the taster panel.