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Evaluation of the quality and antioxidant capacity of woodland strawberry biotypes in Sicily

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Abstract

In Sicily, the woodland strawberry grows wild in forest glades in the Madonie and Nebrodi mountains and on Mount Etna. In this region, the main cultivated clone is Fragolina di Ribera, named after the towns where the crop originally developed. The cultivated woodland strawberry is different from its wild counterparts not only in vegetative vigour and size, but also in organoleptic quality. Fragolina di Ribera has always been described with sensory analysis as one of the best Sicilian berry. This study was carried out in Sicily and compared two June-bearing *Fragaria vesca*: Fragolina di Ribera and Fragolina di Maletto, and an everbearing variety Regina delle Valli, in order to determine the production, quality and nutraceutical characteristics of the fruit. Research results provided useful, more detailed information on those fruit compounds with nutritional and health benefits and the June-bearing Fragolina di Ribera was found not only to produce highly sweet, bright red fruits, but also fruits with high antioxidant capacity and high ascorbic acid, polyphenol and anthocyanin levels.

Key words: *Fragaria vesca*, quality, nutraceutical compounds.

Introduction

Two types of *Fragaria vesca* are grown in Sicily. The first is the wild, woodland, June-bearing type which grows in forest glades, alongside walls, in semi-shaded areas and at the edge of meadows or by streams in the Madonie and Nebrodi mountains and on Mt. Etna. Successful fruit production depends on the balance between sun and moisture. The plant is highly stoloniferous, producing medium-small fruits (approx. 1 g) in a limited production season. Fruits, produced from a single flowering (June-bearing genotype), ripen between spring and summer. Although highly aromatic and pleasant tasting, low yields per hectare and a limited production season have reduced cultivation to only 15 hectares, located mostly in the Provinces of Agrigento and Catania. Strawberry production is sold for fresh consumption on the local Sicilian and Central-Southern Italian markets, where the fragrance and quality of the small fruits are highly appreciated. The multiple year (2-3 years) strawberry planting systems are set up in winter on raised beds or ridges and the runner plants are set out in the quincunx order at a density of 4-5 plants/m^2. The crop is allowed to grow in matted rows using the plant's natural capacity to form runners capable of covering the whole area within the same year of planting. Average fruit production varies from 4-6 t/ha [4]. Currently there are two biotypes in Sicily: Fragolina di Ribera and Fior di Noto, both named after the town in which it was cropped for the first time (Agrigento and Siracusa). Another cultivation area is on the slopes of Etna in the town of Maletto, Province of Catania, where the strawberries are grown from runner plants gathered directly from the underwood.

The second type is the everbearing woodland strawberry, a recent introduction to cropping systems in Sicily. It was introduced at the end of the last century and two cultivars are grown: Alpine and Regina delle Valli. These cultivars are gradually taking the place of the June-bearing woodland strawberry due to better unit yields and a longer harvesting calendar, not withstanding the short shelf life and a fragrance incomparable to that of the endemic Sicilian type. There are currently 40 hectares of everbearing woodland Regina delle Valli strawberry being grown, 15-18 ha of which are being grown in a soilless system in the Marsala area (Trapani) and the remainder on the slopes of Etna. The cultivation system is set up at the end of summer and production starts 2-3 months after planting and continues for 8-10 months. Yields can reach up to a kg of fruit per plant at a density of 2-3 plants m^{-2} [5].

The June-bearing woodland strawberry has distinct organoleptic properties, such as fragrance, taste and colour, recognized by the consumer and is perceived as natural and beneficial to health [11]. The fruits are rich in fibre, bioactive compounds such as vitamin C and polyphenols, which affect both the sensory-organoleptic properties and nutritional values [2, 8]. It has recently been shown that the total antioxidant capacity (TAC) of the strawberry is normally 2-10 times higher than in apples, pears, grapes, tomatoes, oranges and kiwi fruit [13].

The above factors have increased the need to carry out research into the health promoting properties and factors affecting the quality of this Rosaceae species, with particular attention given to the antioxidant capacity.

Antioxidant compounds include a vast range of chemical elements, which, although only occurring in limited quantities compared to an oxidizable substrate, have a significant delaying or preventative action on the oxidation of the substrate. Antioxidants are of primary interest to scientific research as many of these molecules have been shown to exert a positive effect on cell damage caused by free radicals and, therefore, possess chemoprotective properties against many diseases affecting human health [3].

It would seem that the beneficial health effects linked to strawberry consumption are determined by a combination of antioxidant compounds which, when acting together, provide antioxidant activity which is far greater than the sum of the antioxidant activity of each single compound.

Results obtained on large fruit strawberries show that there is great genetic variability between cultivars and growth environment regarding polyphenol, anthocyanin and ascorbic acid levels [1].

In litterature, it was found that in wild species of *Fragaria (F. vesca)* there were more phenolic compounds than in the strawberry cultivars [10].

The ecotype Fragolina di Ribera has always been described with sensory analysis, but nobody analysed chemically the bio-active compounds. The aim of the study was to analyse the biologically active substances contained in this biotype in comparation with another June-bearing biotype and an everbearing variety.

Materials and Methods

The study took place during the agricultural year 2010-2011 at the University of Palermo experimental farm at Sciacca, Agrigento. Two types of *Fragraria vesca* were compared:

A: June-bearing with two biotypes - an endemic woodland strawberry which grows in the Sicilian underwood and the Ribera woodland strawberry.

B: Everbearing woodland strawberry Regina delle Valli, which grows wild throughout the whole of North Italy.

The tests were carried out in open field on loamy soils with a sub-alkaline reaction of pH 8.2 and 8.1% active limestone. The soils had a good level of exchangeable K_2O (620 ppm), phosphorus (60 ppm), total nitrogen at 1.7 ‰ and organic matter.

The planting system was set up in September 2011 for the everbearing woodland strawberry using fresh runner plants at a density of 2.5 plants m^{-2}. The June-bearing woodland strawberry system was set up in January 2011 using fresh plants at a density of 5 plants m^{-2}. The cropping technique used was one that is commonly employed throughout Sicily [6]. A randomized block design was adopted with plots of 10 m^2 and 4 replications.

The fruits of the different woodland strawberry biotypes in the study were harvested at two different times (May and June). A representative sample of ripe fruits was collected per plot (250 g), repeated 4 times, in order to carry out quality analysis at harvest.

Protocols for physical-chemical quality analysis of the biotypes, based on MIPAAF Directory of recommended fruit-bearing varieties 'strawberry' [9] required data on the following parameters: Marketable fruit yield, average fruit weight, surface colour, flesh firmness, refractometric dry residue, titratable acidity, total antioxidant capacity, vitamin C, total anthocyanins and total polyphenols.

Statistical analysis: Results of the quality and production parameters were subjected to statistical elaboration using the biotype as a source of variation, through analysis of the variant with SAS ANOVA procedure and the Tukey's test for the mean separation.

Determination of marketable fruit production and average marketable fruit weight: The weight of the marketable fruit production was determined for each plot and harvest in order to calculate the total marketable production. The weight of 100 marketable fruits randomly chosen was measured for each plot and harvest in order to calculate the weighted average fruit weight.

Determination of flesh firmness: Flesh firmness was measured (g) with a hand-held penetrometer TR model with a 6 mm flat tip probe. The probe was pressed with constant force to a depth of 1 cm, in order to overcome resistance from skin elasticity.

Determination of refractometric dry matter and total acidity: Total soluble solids (°Brix) were measured with a digital refractometer (mod. Atago DBX 55) on the juice of each sample.

Total acidity (in meq kg^{-1} of juice) was determined by means of a digital titrator, using sodium hydroxide 0.1 N NaOH with bromothymol blue as an indicator of the solution colour change (pH 7.8). Levels were determined on 10 g of juice.

Determination of colorimetric properties: The colour of the epidermis was measured on two opposite sites with a Minolta automatic reflectance colorimeter (measurement/illumination area 8 mm), which measured the coordinates L* (lightness), a* (redness) and b* (yellowness). The Chroma index was calculated using the coordinates a* and b* ($[(a^2+b^2)]^{1/2}$).

Determination of nutraceutical fruit properties: On a sample of 250 g fruit harvested from each plot in the month of May/June and frozen to -20°C, the following nutraceutical analyses were carried out.

Vitamin C: L-ascorbic acid content (vitamin C) was determined colorimetrically on juice diluted in water (1:9). A Merck digital RQflex was employed using the Reflectoquant system and the principle of reflectance. The equipment measures reflected light on a strip containing molybdophosphoric acid (yellow) which, when immersed in the analyte juice, is reduced to phosphomolybdenum (blue) in the presence of ascorbic acid. Quantification is based on the difference in intensity of emitted and reflected light based on colour variations on the test strip. Results are expressed in mg kg^{-1} of fresh weight.

Total anthocyanins: Total anthocyanin content, mg PGN kg^{-1} fresh weight, was determined colorimetrically by a pH-differential method [14]. The extract was obtained by placing 2 g of frozen strawberry at -20°C in 10 ml of methanol solution HCl (99.9:0.1 v:v), left overnight at +4°C and filtered through a Whatman #1 filter paper. The extract was left to react with a buffer solution pH 1.0 (KCl 0.025 M) and pH 4.5 (0.4 M sodium acetate). The spectrophotometric readings were carried out at wavelengths of 510 and 700 nm.

Absorbance readings were converted to mg pelargonidin-3-glucoside (PGN)/100 g fresh weight using the following formulas:

Anthocyanins mg = (A*MW*DF*100)/ε*L

Where: A= $[(A_{510}\ A_{700})_{pH1.0}\ (A_{510}\ A_{700})_{pH4.5}]$
MW = molecular weight of the reference anthocyanin, which for strawberry is pelargonidin- 3- glucoside = 433.2 g/mol.
DF = dilution factor of sample = 2.
ε = molar extinction coefficient of pelargonidin - 3- glucoside = 31,600 L cm^{-1}.
L = cuvette length used in the spectrophotometer = 1 cm.

The amount of anthocyanin (mg) obtained was divided by actual weight of analysed strawberries (2 g) and multiplied by 100 to obtain mg PGN/100 g fresh weight.

In order to carry out analyses for the determination of antioxidant capacity and total polyphenols, an extract was obtained by placing 10 g frozen fruit at -20°C in 50 ml of methanol and water (80% v/v).

Total polyphenols: Spectrophotometric determination was carried out using with a Genesys 10 UV spectrometer at a wavelength of 750 nm using the Folin-Ciocalteau method [12]. Quantification was performed using a calibration curve of gallic acid and results were expressed as mg gallic acid equivalent mg GAE g^{-1} fresh weight.

Total antioxidant capacity: The total antioxidant capacity (TAC) was defined using the TEAC (Trolox® equivalent antioxidant activity) essay. Spectrophotometric determination was carried out using a Genesys 10 UV spectrometer at a wavelength of 734 nm and the method by Gao *et al.* [7]. This method is based on the decolorization of a blue ABTS+ [diammonium salt, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation solution, which, in the presence of antioxidant molecules, is reduced to ABTS+ non-radical cation and converted back to its colourless form.

Antioxidant capacity was determined by comparing the data with that of a standard solution containing ABTS and Trolox®. The data were expressed in mmol Trolox® equivalent (TE) g^{-1} fresh weight.

Results and Discussion

Regina delle Valli obtained the highest total marketable production with the biggest fruits (Fig. 1, Table 1) while Fragolina di Ribera produced the sweetest fruits with the highest anthocyanin content and high total antioxidant capacity, polyphenol and ascorbic acid content, but without significative difference at the ANOVA with Fragolina of Maletto (Table 2, Figs 2 and 3).

The highest total marketable production m^{-2} was obtained by the cv. Regina delle Valli with a yield of 1,810 g m^{-2}, appreciably higher than the June-bearing woodland strawberry, which produced an average yield of only 603 g m^{-2}. A significant statistical difference was found between the two types of woodland strawberry whereas no difference was found between the two biotypes of June-bearing woodland strawberry (Table 1).

The highest average marketable fruit weight was obtained with the biotype Regina delle Valli (1.18 g), followed by the biotype Fragolina di Ribera (1.03 g) and Fragolina di Maletto (1.01 g).

The highest values for flesh firmness throughout the harvesting period were obtained with the June-bearing biotypes with an average of 140 g. Regina delle Valli biotypes produced fruits with low firmness which, coupled with high temperatures and light intensity, did not exceed 100 g (Table 1).

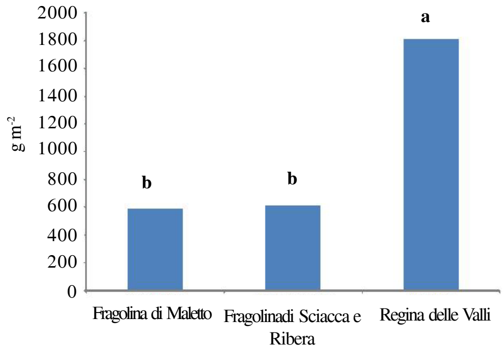

Figure 1. Total marketable fruit production of woodland strawberry in Sicily.
Figures followed by different letters differ by P≤0.05.

Table 1. Production and quality characteristics of the fruits of woodland strawberry biotypes in Sicily.

Variety /Biotype	Marketable fruit production, g m^{-2}	Weighted average fruit weight, g	Fruit flesh firmness, g	Surfice fruit color	
				L	Chroma index
Fragolina di Maletto	592b	1.01b	132a	32.2a	32.4a
Fragolina di Ribera	614b	1.03b	149a	31.2a	30.2b
Regina delle Valli	1810a	1.18a	100b	27.6b	32.5a

Figures followed by different letters differ by P≤0.05.

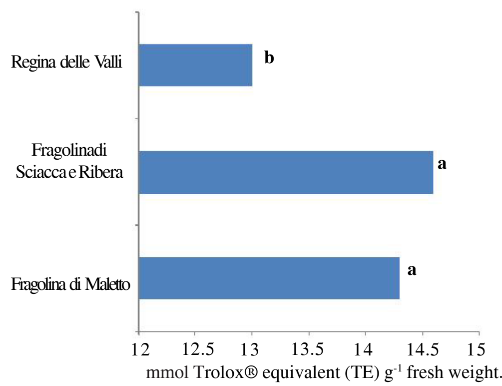

Figure 2. Total antioxidant capacity of woodland strawberry biotypes in Sicily. Figures followed by different letters differ by P≤0.05.

The June-bearing woodland strawberry from Sciacca produced fruits with the highest total soluble solids content (10.8°brix), followed by the biotype Regina delle Valli (10.5°brix) and Fragolina di Maletto (10.2°brix).

The most acidic fruits were produced by Fragolina di Maletto (21.2 meq kg^{-1}), followed by Fragolina di Sciacca (19.5 meq kg^{-1}) and Regina delle Valli (18.7 meq kg^{-1}).

The fruits produced by Fragolina di Maletto were a red-orange colour, much brighter than the Regina delle Valli, whose fruits were also red but not so bright. Fruits of the Sciacca woodland had a very intense, bright red colour (Table 2).

Achenes on the June-bearing *Fragaria vesca* fruits were slightly protruding, whereas on the Regina delle Valli the achenes tended to be slightly sunken into the flesh; a characteristic which makes handling during harvesting and transport more difficult, leading to bruising.

Table 2. Chemical and nutraceutical analysis of woodland strawberry biotypes in Sicily.

Variety /Biotype	Total soluble solids, °brix	Titratable acidity, meq kg^{-1}	Ascorbic acid, mg kg^{-1}	Total Antioxidant Capaxity, umolTE g^{-1}	Total Polyphenols, mg GAE g^{-1}	Anthocyanins, mg PGN kg^{-1}
Fragolina di Maletto	10.2b	21.2a	58a	14.3a	4.6a	25.7b
Fragolina di Ribera	10.8a	19.5b	64a	14.6a	4.7a	31.7a
Regina della Valli	10.3b	18.7b	32b	13.0b	3.8b	20.5c

Figures followed by different letters differ by P≤0.05.

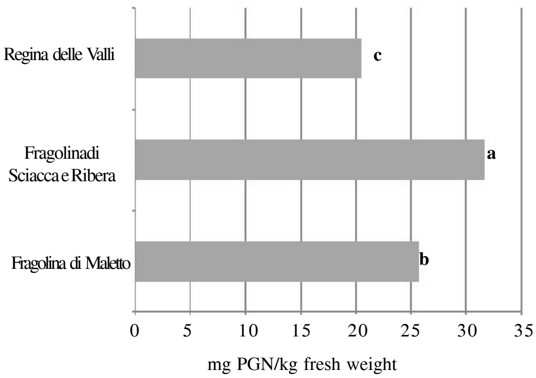

Figure 3. Total anthocyanin content of woodland strawberry biotypes. Figures followed by different letters differ by P≤0.05.

Regarding the ascorbic acid content in the biotype fruits, significant differences were found: Average levels of 62 mg kg^{-1} were recorded in the June-bearing woodlands but half that quantity in the Regina delle Valli. Total antioxidant capacity differed in the two types of woodland strawberry, with the June-bearing producing average values of 14.4 µmolTE kg^{-1} compared to 13.0 µmolTE kg^{-1} for the everbearing types (Fig. 2).

The June-bearing woodland strawberry produced the highest levels of total polyphenols, with average values of 4.6 mg GAE g^{-1}, higher than the Regina delle Valli fruits (3.8 mg GAE g^{-1}).

The highest total anthocyanin content was found in the fruits of the Fragolina di Ribera at 31.7 mg PGN kg^{-1}, higher than the Fragolina di Maletto at 25.7 mg PGN kg^{-1}. These levels were even lower in the Regina delle Valli woodland at 20.5 mg PGN kg^{-1} (Table 2, Fig. 3).

Conclusions

Tthe results of this paper provide further important information on those elements which provide health promoting and nutritional activities. We can conclude that the June-bearing woodland strawberry from Ribera, as well as producing highly sweet and brightly coloured fruits also has high levels of ascorbic acid, anthocyanins, antioxidant capacity and total polyphenols substances which give added value to the fruits, already highly appreciated for their organoleptic properties.

References

[1]Bacchella, R., Testoni, A. and Loscalzo, R. 2009. Valutazione del potenziale antiossidante di varietà commerciali di fragola. Atti del VII Convegno Nazionale - La Fragola Presente e Futuro, pp. 292-293.

[2]Battino, M., Beekwilder, J., Denoyes Rothan, B., Laimer, M., McDougall, G. J. and Mezzetti, B. 2009. Bioactive compounds in berries relevant to human health. Nutrition Reviews **67**(suppl. 1):S145-S150.

[3]Brighenti, F., Valtueña, S., Pellegrini, N., Ardigò, D., Del Rio, D., Salvatore, S., Piatti, P. M., Serafini, M. and Zavaroni, I. 2005. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity c-reactive protein in adult Italian subjects. Br. J. Nutr. **93**:619-625.

[4]Caruso, P. and D'Anna, F. 1994. Ricerche sulla pacciamatura e sulla densità d'impianto della fragolina (*Fragaria vesca*). Atti delle II giornate Scientifiche SOI, pp. 22-24.

[5]D'Anna, F., Moncada, A. and Nicosia, M. 2000. Esperienze sulla coltura della fragola fuori suolo in Sicilia. Atti del Convegno SOI, pp. 28-30.

[6]D'Anna, F., Moncada, A., Caracciolo, G., Prinzivalli, C. and Leonardi, C. M. 2009. Evoluzione e prospettive della fragolicoltura siciliana. Atti del VII convegno nazionale - La fragola presente e futuro, pp.22-31.

[7]Gao, X., Ohlander, M., Teppsson, N., Bjork, L. and Trajkovski, V. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. Journal of Agricultural Food Chemistry **48**:1485-1490.

[8]Hannum, S. M. 2004. Potential impact of strawberries on human health: A review of science. Crit. Rev. Food Sci. **44**:1-17.

[9]Lovati, F., Nuzzi, M., Avitabile Leva, A., De Colellis, G., Testoni, A., Magnani, S. and Lucchi, P. 2000. Valutazione della qualità delle fragole in post-raccolta. Frutticoltura n°12:36-41.

[10]Milivojevic, J., Maksimovic, V., Nikolic, M., Bogdanovic, J., Maletic, R. and Milatovic, D. 2011. Chemical and antioxidant properties of cultivated and wild Fragaria and Rubus berries. Journal of Food Quality **34**:1-9.

[11]Penarrieta, J. M., Alvarado, J. A., Bergenstahl, B. and Akesson, B. 2009. Total antioxidant capacity and content of phenolic compounds in wild strawberries (*Fragaria vesca*) collected in Bolivia. International Journal of Fruit Science **9**:344-359.

[12]Proteggente, R., Pannala, A. S., Pagana, G., Van Buren, L., Wagner, E., Wiseman, S., Van De Put, F., Dacombe, C. and Rice Evans, C. A. 2002. The antioxidant activity of regularly consumed fruit and vegetable reflects their phenolic and vitamin C composition. Free Radical Research **36**(2):217-233.

[13]Wang, H., Wao, G. and Prior, R. L. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. **44**:701-705.

[14]Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Smith, D. M. and Sporns, P. 2005. Handbook of Food Analytical Chemistry. Wiley Press, 351 p.