Scientia Horticulturae 176 (2014) 311–318

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Altitude effects on fruit morphology and flour composition of two chestnut cultivars

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ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 2 July 2014
Accepted 3 July 2014
Available online 5 August 2014

Keywords:
Castanea sativa Mill.
Elevation
Fruit characteristics
Proximate analysis
Amino acids
Fatty acids

ABSTRACT

Environmental conditions may significantly affect both the chemical composition and the morphological parameters of chestnuts. The aim of this work was the evaluation of altitude effects on flour chemical composition and on fruit morphological parameters of different chestnut cultivars (Luetta e Leccardina), grown at two different altitude levels (700 and 1000 m.a.s.l.). In particular, crude protein, crude fat, amino acid and essential fatty acid content were determined on flours. The two genotypes were also studied from a morphological standpoint, by a biometric and descriptive evaluation of fruits at the different altitudes. Compositional and morphological data were statistically evaluated by ANOVA. The altitude influence on chestnut fruit and flour was ascertained; besides significant differences between the two cultivars, equally significant were the differences within each cultivar when plants grown at different altitudes were compared.

1. Introduction

The chestnut is present in Italy in all regions within a range of altitude levels, depending on latitude. This condition favored the formation of a rich diversity of cultivars, differing for a number of characters, particularly those concerning the fruit, but also for the traits involved in plant tolerance to biotic and abiotic stress.

The fruit of chestnut has been of great importance from the alimentary standpoint through centuries, but in the past century its importance has gradually decreased; however, in recent years an upswing of consumption, of both the fresh and transformed fruit, has been observed.

Chestnut fruit is interesting from a nutritional point of view: besides being a good source of starch (>70%), it has a good content of proteins (2–4%), fats (2–5%), and fair amounts of minerals, vitamins and fiber. A great deal of research is carried out on the chemical composition of the chestnut fruit, addressing a number of different aspects: alcaloid content (Hiermann et al., 2002), sugars (Míguez Bernárdez et al., 2004), fatty acid (Borges et al., 2007), polyphenols (Vekiari et al., 2008), modifications of starch structure and digestibility after cooking (Pizzoferrato et al., 1999), heat effects on starch, sugars and fatty acids composition, and on fruit quality (Künsch et al., 2001).

The morphological features and quality of the product of a given cultivar depend on two main components, genotype and environment. Each cultivated genotype represents a definite agrarian variety (cultivar, cv.); studies made by Künsch et al. (2001) and by Míguez Bernárdez et al. (2004) indicate that the genetic component determines the chemical composition of the fruit, and in some instances it is possible to discriminate the various cultivars according to chemical values. As concerns the environmental component, few studies are available on the variation of morphological characters with reference to environment variables. Garcea et al. (2005) evaluated shoot growth of some chestnut cultivars at three different altitudes, finding significant differences within the same cultivar. Dinis et al. (2011) found ecophysiological changes in *C. sativa* cv. Judia grown at different altitudes; in this case, differences were noticed with regard to fruit size, which was smaller at higher elevations, although the extent of the difference depended on the year. No research is available on *Castanea* concerning changes of fruit or flour chemical composition with changing environment; such studies, however, exist for other plant species, such as sunflower (Sobrino et al., 2003). On this specie it appears that seed lipid composition varies with latitude and altitude.

The aim of this research was to evaluate how altitude may influence chemical composition of flours and fruit morphology of two chestnut cultivars (Luetta and Leccardina), grown in North-West

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http://dx.doi.org/10.1016/j.scienta.2014.07.008
0304-4238/

Italy (Ceno and Taro valleys). In particular, great attention was given to the lipid- and nitrogen-containing fraction, on account of the relevant technological role. Moreover, the antioxidant fraction was also considered, in regard of the protective role toward oxidative degradation played along the flour shelf life. Finally, the volatile profile was also described, since very little is known about this fraction in chestnut flour, so far.

2. Materials and methods

2.1. Study area and cultivar choice

The research was carried out in the municipality of Albareto (Parma, Italy); two chestnut groves were selected, the first in Folta of Albareto (700 m.a.s.l.), the second in Breda of Albareto (1000 m.a.s.l.). Two cultivars were chosen: Luetta and Leccardina. These cultivars are widely cultivated in the Valleys of the rivers Ceno and Taro, province of Parma (Bagnaresi et al., 1977; Beghè et al., 2013). Both are early ripening, but their agronomical and carpological features are distinct: Leccardina has a small fruit and is suitable for both fresh consumption and transformation (flour); Luetta produces a medium size fruit, less suited to flour production. Four plants of each cultivar, homogeneous as concerns age, size, exposure and agronomical conditions, were selected in the two groves.

2.2. Fruit morphological characterization

The description of the fruits of the two cultivars was made according to the methodology proposed by Bellini et al. (2007)

Plant material was sampled in September 2008, 2009 and 2010. On each plant the sampling comprised 20 fruits, and samples were taken from all sides of the trees. The samples were taken from the median zone of fruiting shoots, avoiding misshapen or abnormally small or big fruits (with reference to the average of fruit population).

2.3. Flour monovarietal production

In September 2008 and 2009, for each chestnut cultivar, 5 kg of fresh fruits were used for flour production. In the year 2010 it was not possible to produce a sufficient quantity of flour because the damage caused by *Dryocosmus kuriphilus* Yasumatsu heavily reduced production.

Fruit drying, for each cultivar, was carried out in a traditional dry kiln. These buildings, little two-floor cabins (metato), have a square or rectangular plan, are built in local stone, and have a slab stone roof. In the ground floor heating is produced by a fire made with wood and scrap chestnut. In the first floor, homogeneous layers of chestnuts are laid for drying on a rack. During drying, which continues for 30 days, fruits are turned over several times and temperature (40 °C) is daily controlled to keep it constant.

The samples were milled used a cereal mill in CRA (Consiglio per la Ricerca e la sperimentazione in Agricoltura) – Istituto Sperimentale per la Cerealicoltura, Fiorenzuola d'Arda (Piacenza, IT).

2.4. Chemical characterization

2.4.1. Proximate analysis

The chemical characterization of the analyzed cultivars was made in stages. Each analysis was repeated three times and data was expressed on dry matter, determined by desiccation in stove at 110 °C for 12 h until constant weight.

2.4.1.1. Extraction and quantification of total carbohydrates. Determination of sugars was performed by the Lane and Eynon volumetric method (AOAC 930.15); 0.5 g of sample were digested with 10 ml of HCl 6 N for 17 min at 68 °C. Quantitative determination was calculated with Lane and Eynon tables (AOAC 930.15), whose values were corrected with the exact title of Fehling reagent.

2.4.1.2. Extraction and quantification of crude fat. Total fat determination was performed with an acid hydrolysis method followed by extraction with a Soxhlet apparatus for 70 min using diethilic ether as the extraction solvent. The residue obtained was dried for 1 h 30 min at 101 °C ± 2 °C, until constant weight, according to the acid hydrolysis method.

2.4.1.3. Extraction and quantification of total protein. Each sample was analyzed in triplicate for total nitrogen by Kjeldahl method in combination with a copper catalyst using a block digestion system Foss Tecator 2006 Digestor (Höganäs, Sweden) and a Foss 2200 Kjeltec AutoDistillation unit (Foss Tecator). The percentage of nitrogen was transformed into protein content by multiplying the total nitrogen by a conversion factor of 5.30, specific for chestnut fruit (McCarty and Meredith, 1988).

2.4.2. Fatty acid (FA) profile

The fatty acid profile of each sample was determined in triplicate by GC/MS analysis after trans-esterification to FA methyl esters (EU 2568/91). The results were reported as relative percentage, calculated on the chromatographic area of each peak and expressed on dry matter content. GC/MS analysis was performed by a Hewlett Packard 5890 separation system (GMI Inc., Minneapolis, USA), equipped with a Hewlett Packard 5971 single quadrupole mass spectrometer with an electronic impact source (GMI Inc., Minneapolis, USA). Chromatographic conditions were the following: the column was a 250 mm × 2.5 mm i.d., 250 nm f.t., Carbowax; the injection volume was 1 μl; gradient elution was performed using helium as carrier gas: initial conditions at 80 °C, 0–3 min isothermal step at 80 °C, 3–16 min linear gradient to 210 °C, 16–21 min isothermal step at 210 °C (total analysis time: 21 min); injector temperature, 220 °C, source block temperature, 230 °C. MS detection was performed using a full scan mode from 50 to 500.

2.4.3. Dietary fats and antioxidant activity

2.4.3.1. Dietary fats. According to the results of the fatty acid profile, the quantities were evaluated of saturated fatty acid (SFA), cis-monounsaturated fatty acids (MUFA), cis-polyunsaturated fatty acids (PUFA) and the MUFA/PUFA ratio.

2.4.3.2. DPPH radical scavenging activity. Each flour sample (0.1 g) was added with a 5 ml water:methanol solution (70:30, v/v) and magnetically stirred for 1 h. After centrifugation, the supernatant (1 ml) was diluted with methanol (4 ml). The chestnut extracts (0.2 ml) were then mixed with 2 ml of methanol and 1 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The antioxidant capacity was then reported as Trolox Equivalent Antioxidant Capacity (TEAC) (Thaipong et al., 2006). All the data are expressed on dry matter content.

2.4.4. Amino acidic profile

Each sample was analyzed in triplicate for the determination of free amino acids. In particular, after Soxhlet defatting, an aliquot of the resulting powder (0.2 g) was extracted by magnetically stirring for 20 min with 5 ml bidistilled water added with 0.75 ml trifluoroacetic acid and 0.05 ml internal standard solution (D,L *nor*-leucine 50 mM in water). The extract was then centrifuged for 3500 rpm at