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The influence of seasonality on total fat and fatty acids profile, protein and amino acid, and antioxidant properties of traditional Italian flours from different chestnut cultivars

Lucia Morrone, Chiara Dall'Asta*, Annalisa Silvanini, Martina Cirlini, Deborah Beghè, Andrea Fabbri, Tommaso Ganino

Department of Food Science, University of Parma, Parco Area delle Scienze, 59/a, 43124 Parma, Italy

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ABSTRACT

Chestnut (*Castanea sativa* Mill.) is an important fruit crop, representing a fundamental economic resource in rural areas. The consumption of chestnut fruit has increased in recent years, after decades of neglect due to high prices and loss of food traditions. Nevertheless, chestnut is a precious food, since it is a source of starch, essential fatty acids, essential amino acids, antioxidants and vitamins. Moreover, chestnut flour can be used in gluten free formulations. The aim of this work was to evaluate by proximate analysis the fatty acids and amino acids profile and the antioxidant capacity of nine native cultivars from Emilia Romagna region, for two consecutive years. By using 2-way ANOVA analysis considering the factors "*C. sativa* cultivar" and "harvesting year" it was possible to state the non-dependence of carbohydrates on these two factors. On the contrary, the variability of crude fat, crude proteins, free amino acids profile and total antioxidant capacity depend both on cultivar and harvesting year. Chestnut flours are a good source of oleic and linoleic acids: their levels, together with that of palmitic acid, are affected by the cultivar; stearic and linolenic acid variability is instead due to the harvest year. The nutritional data from this research can provide useful information for the bakery industry, in view of the new trend of functional products able to merge tradition and innovation.

1. Introduction

The chestnut (*Castanea sativa* Mill.) belongs to the Fagaceae family, like other genera such as *Castanopsis, Fagus, Lithocarpus, Northofagus* and *Quercus* (Bounous, 2002). The genus *Castanea* includes 12 species, among which *C. sativa* Mill. is the most important (Ribeiro et al., 2007).

In 2012 the world production of chestnuts was estimated to be close to 2 million tons, China being the main producer, with almost 83% of the total production, followed by the Republic of Korea (3.5%) and by Turkey, Bolivia and Italy (all below 3%) (FAOSTAT, 2014). Chestnut production is a firm tradition in Italian rural districts, both as a source of income for the mountain farmer and as a high environmental value crop thanks to its role in soil protection. This crop in Italy is presently far from being economically competitive, because of the neglect it received in the past owing to several pathologies such as *Phytophthora cambivora* (Petri) Buisman (ink disease) and *Cryphonectria parasitica* (Murr.) Barr. (chestnut blight), as well as to rural depopulation (Adua, 1999).

More recent years have witnessed an important recovery in the chestnut market thanks to a renewed interest of consumers and food supply industry towards traditional products, whose use can be easily readdressed towards innovation. This interest arises from many factors, including the contribution to agricultural diversification and the improvement in the use of land, the economic potential and the opportunity to provide diet diversification.

A new push to the market has been given to the use of chestnut flour as substitute of cereal flours in bakery goods with different or enhanced nutritional attributes (i.e. gluten-free products), on account of the high nutritional, technological and organoleptic properties (Sacchetti et al., 2004; Demirkesen et al., 2010; Di Monaco et al., 2010; Moreira et al., 2011; Dall'Asta et al., 2013; Rinaldi et al., 2015). Chestnut flour presents, indeed, high quality proteins with essential amino acids (4–7%), a relatively high amount of sugars (20–30%), starch (50–60%), dietary fiber (4–10%); and low amount of fat (2–4%), most of them unsaturated (Demirkesen et al., 2013). In addition, it is a good source of phenolic compounds, oligominerals and vitamins, mainly group B

* Corresponding author. Tel.: +39 0521 905431.
E-mail address: chiara.dallasta@unipr.it (C. Dall'Asta).

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(De Vasconcelos et al., 2010a). As gluten-free products often lack of micronutrients such as folates and iron, and dietary fiber (Moroni et al., 2009), the interest in the use of chestnut flour to replace more common ingredients such as rice or maize has strongly grown in the last few years. Furthermore, due to the peculiar composition, chestnut flour may contribute to enhance color and aroma in gluten-free bakery products, especially when sourdough-based process is used (Cirlini et al., 2012; Aponte et al., 2013).

While chemical composition of chestnut fruits has been widely studied and recently reviewed (Borges et al., 2008; De Vasconcelos et al., 2010a; Barreira et al., 2009a, 2009b; Pereira-Lorenzo et al., 2006; Neri et al., 2010), studies have been performed also taking into consideration the effects of industrial processing (De Vasconcelos et al., 2010a–2010c), and the technological applications of chestnut flours (Sacchetti et al., 2004; Demirkesen et al., 2010; Di Monaco et al., 2010; Moreira et al., 2011; Dall'Asta et al., 2013; Rinaldi et al., 2015).

Italian chestnuts are commonly considered of high quality, as shown by the number of cultivars and flours thereof awarded with protected geographical indication (PGI) or protected denomination of origin (PDO). As the domestic production has dropped dramatically, reaching the minimum production of 18,000 t in 2014, prizes have shot up, leading to a strong increase in imports from China (67,800 t in 2014) (FAOSTAT, 2014). Due to this trend, frauds due to misuse of low quality Chinese varieties as Italian are increasing. In this frame, the characterization of minor local cultivars and their repositioning on the market is a challenge.

Chestnut flour has been for centuries the mainstay of diets within mountain population of Taro and Ceno valleys (North-West Italy, Emilia Romagna region, Parma province), but little is known about the ecotypes from that area (Beghè et al., 2013), and in particular flour features have been overlooked. The present work, part of a wider research aimed at the valorization and retrieval of local chestnut ecotypes from Taro and Ceno valleys, deals with the evaluation of the chemical composition of flours obtained from nine local cultivars, harvested for two consecutive years. The identification of distinctive features can be useful to outline the best technological exploitation of each flour obtained from a single cultivar, based on its peculiar composition, and may provide key information for authentication.

2. Materials and methods

2.1. Plant material and growth conditions

The chestnut cultivars studied were chosen according to their presence in the above mentioned territory. The cultivars were Ampollana, Gursona, Leccardina, Luetta, Lusetta, Massese, Mondadì, Perticaccia and Preila. For all these cultivars the DNA analysis had been previously made in order to unequivocally identify the clones (Beghè et al., 2013).

The chestnut fruits were harvested in the second decade of October at the same phenological stage (cupula opening) for each cultivar, for two consecutive seasons (2009 and 2010). Fruit drying was carried out in a traditional dry kiln (metato) as described in Cirlini et al. (2012). During drying the fruits are turned over several times and kept at a constant temperature of 40 °C for 30 days. The samples, after the drying process and the mechanical cleaning (removal of shell and episperm), were milled utilizing a cereal mill in a CRA Institute (Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Istituto Sperimentale per la Cerealicoltura, Fiorenzuola d'Arda—Piacenza, IT). After milling the diameter of the flour particles ranged from 16.5 to 91.1 μm. The chestnut flours produced were stored in glass at −80 °C to preserve the flour properties, until analyses were carried out.

The climatic conditions of the growing area were characterized by maximum and minimum temperatures and daily rainfall, as given in Fig. 1. Daily rainfall and temperature data were collected from the Regional Agency for Prevention and Environment (ARPA) of Emilia-Romagna region located at 597 m a.s.l., longitude 9.732836° and latitude 44.633788°.

2.2. Chemical analyses

2.2.1. Proximate analysis

The chemical characterization of flours obtained from the considered cultivars was made in stages. Each analysis was repeated three times and data was expressed on dry matter, determined by desiccation in stove at 105 °C for 12 h until constant weight (AOAC, 2006).

Extraction and quantification of total carbohydrates: determination of sugars was performed by the Fehling method (AOAC, 1990), 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 (1923) tables, and the resulting values were corrected with the exact title of Fehling reagent.

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 diethyl 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 (AOAC, 2000).

Extraction and quantification of total protein: each sample was analyzed in triplicate for total nitrogen by the Kjeldahl method in combination with a copper catalyst using a block digestion system Foss Tecator 2006 Digestor (Höganäs, Sweden) and a Foss 2200 Kjeltec Auto Distillation unit (Foss Tecator) (AOAC, 1994). The percentage of nitrogen was transformed into protein content by multiplying the total nitrogen by a conversion factor of 5.30, specific for chestnut (Meredith et al., 1988).

2.2.2. 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 (EC Regulation 256/91, 1991), as already reported by Silvanini et al. (2014) The results have been 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 Carbowax 250 mm × 2.5 mm i.d., 250 nm f.t. (Supelco, Bellefonte, PA), 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.

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.

2.2.3. Total antioxidant capacity

DPPH (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) radical scavenging activity: the extraction of the hydrofilic antioxidants was performed from 0.1 g of chestnut flour with a 5 mL water:methanol solution (70:30, v/v). The solution, after been magnetically stirred for 1 h, was filtered and the hydro-alcoholic