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Health properties of fresh apple purees obtained from fruits of ancient local genotypes

Abstract

The consumption of ready-to-eat products is increasing, and consumers demand tasty products with nutritional properties similar to those of fresh produces. Apple fruits and their derivatives are known to provide bioactive substances that bring benefits to the human organism; however, the chemical composition varies among cultivars. This study analyzed some main properties of fresh homogenates obtained from fruits of two ancient local apple genotypes (*Malus domestica* Borkh.), i.e. Limoncella and Sergente, typical of a mountain area of Southern Italy, compared to those of a renowned cultivar taken as a reference, i.e. Golden delicious. All the fruits were produced in the same orchard. The analytical results highlighted that Limoncella and Sergente homogenates have better nutritional properties than those of Golden Delicious. Between the two local accessions, Sergente showed a tendency for a higher total organic acid and polyphenol content and for a greater antioxidant capacity in the fresh homogenate of fruit.

Keywords: Limoncella, Sergente, sugars, organic acids, ascorbic acid, total polyphenols, antioxidant activity

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Damiano Antoniciello, Patrizio Limosani, Gabriella Massenzio, Laura de Palma
Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Italy

Correspondence: Laura de Palma, Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Italy, Tel +39 320 4394555

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Introduction

Consumers increasingly demand ready-to-eat products, including those made from fruits, as long as they are convenient and tasty and also have nutritional characteristics similar to those of fresh products. Apples provide dietary fiber and chemical compounds which act as antioxidants, including organic acids and polyphenols. Studies have shown that apple consumption reduces cholesterol and the risk of cardiovascular disease, diabetes and some types of cancer.[1] The positive effects related to the consumption of apple and its derivatives increase if these products contain both solid and liquid parts are used, such as apple puree, which can be used as a dessert as well as semi-finished products to obtain nectars, thick juices, apple sauces, baby food, etc.[2]

Fruit chemical composition shows important changes among apple cultivars,[3] thus it should be analyzed to choose genotypes able to give a high nutritional contribute. Fruits of local ancient genotypes adapted to difficult agro-environmental conditions are often endowed with high nutritional properties and antioxidant capacity, which are largely related to an intense production of phenolic compounds as a response to abiotic and biotic stressors.

The aim of the present study was to analyze some main properties of fresh purees obtained from fruits of two ancient local apple genotypes typical of a mountain area of South Italy, in order to explore the potentiality of these apples to produce health ready-to-eat derived products.

Materials and Methods

Plant material and fruit sampling

The study was conducted on the fruits of two minor local apple accessions (*Malus domestica* Borkh.), Limoncella and Sergente, typical of central-southern Italy, and on fruits of Golden delicious, one of the most commercially grown apple cultivar around the world, taken as a reference. All the fruits were produced by trees grown at an adult orchard of Calitri farm sited in the Panni countryside (41°13'25" N – 15°15'03" E, 446 m a.s.l.), in the southern part of Monti Dauni (South Italy, Apulia region, Foggia province), an area covered by the SNAI's (National Strategy for Internal Areas) policy and characterized by a hot arid summer climate. The orchard is cultivated in dry soil adopting low-input methods, green manure of spontaneous herbs, organic fertilization. Trees are grafted on MM.111 stock and trained to palmette system. Samples of 50 fruits per genotype were collected by various portions of three trees per genotype. All the fruits were harvested on October 20th 2024 and stored at temperature of 10 °C and 85% relative humidity.

Preparation of fruit puree

After 15 days of storage, three replicates of 10 fruits per genotype were taken at overnight, then rapidly private of stalk, skin and seeds, cut in small pieces and cold-homogenized at 1800 rpm x 3 min (AstorMixer Power, Astori Tecnica srl, Poncarale, BS, Italy) by setting equipment at the temperature of 2 °C to minimize oxidations.

Contents of total soluble solids, total organic acids, ascorbic acid

Fifty milliliters of homogenized were centrifuged at 4500 rpm for 15 min at 10 °C. The supernatant was separated, filtered and used for the further analyses. The total soluble solid concentration (TSS, °Brix) was assessed using a digital refractometer (Digital wine VM-7, Atago, Japan). The amount of the two main sugars, *i.e.* glucose plus fructose, was determined using an enzymatic kit (Exacta+Optech Labcenter SpA, Prospero, MO, Italy) and an automatic multiparametric analyzer (Miura One, Biogamma, Italy). The same method was applied to assess the total organic acid concentration as a sum of the main acids (malic, citric, lactic, tartaric and ascorbic). The concentration of L-ascorbic acid was also separately considered, given its recognized high contribution to antioxidant activity.

Preparation of extract for analyses of phenolic content and antioxidant activity

Five grams of homogenized were added of 20 mL of a hydroalcoholic solution (methanol:water, 80:20 v/v) adjusted to pH 2 with 85% orthophosphoric acid. The suspension was macerated for

30 min in the dark and then centrifuged at 4500 rpm for 15 min at 10 °C. The supernatant was separated, filtered and used for the further analyses.

Total polyphenol content

It was determined using the method described by Singleton and Rossi,[4] using 0.1 ml of extract, a few milliliters of distilled water and 1 mL of Folin-Ciocalteu reagent. After approximately 2 minutes, 4 mL of 10% sodium carbonate were added; the solution was made up to volume of 20 mL with distilled water. A "blank" was prepared following the same procedure (starting with distilled water instead of the extract). The flasks were placed in the dark for 90 min, after which the readings were taken with a spectrophotometer (Shimadzu UV-1700 PharmaSpec, Shimadzu Corporation, Japan) at a wavelength of 750 nm. A calibration curve of gallic acid as a standard (0-2500 mg/L) was used for quantification (R^2=0.998). The total polyphenol content was measured in milligrams of gallic acid equivalent per gram of fresh weight of homogenate.

Antioxidant activity

It was evaluated using the Trolox Equivalent Antioxidant Capacity (TEAC), based on the ability of antioxidant molecules to capture free radicals; it uses $ABTS^{\bullet+}$ as a radical, a green-blue chromophore with absorption at 734 nm.[5] A 0.2 mL sample was taken after appropriate dilution; 2 mL of chromogen (whose absorbance was previously measured) were added. After a reaction time of 15 minutes, the spectrophotometric reading was performed at 734 nm; ethanol was used as a "blank". The decrease in absorbance was converted in equivalent micromoles of Trolox (an antioxidant compound widely adopted as a standard) by a linear regression line, and measured as grams of fresh weight of homogenized.

Statistical analysis and data presentation

For each parameter analyzed, the differences among genotypes were tested by ANOVA, after having verified the normality of the data by Shapiro-Wilk test, and tested the homogeneity of variance by Levene's test. For the parameters that showed significant differences among the means, these latter were separated by Tukey test at $p \leq 0.05$.

To better highlight the differences of the local genotypes compared to the commercial cultivar taken as a reference, the final results were reported as a percentage of the reference cultivar (Golden delicious = 100%).

Results and discussion

The chemical parameters of fresh apple purees from the two local accessions showed values similar to each other and quite distant from those found in the reference cultivar, except for the total soluble solid concentration (Figure 1a), which was the same in all three genotypes, i.e. about 20 °Brix. The sugar content, assessed as the sum of the glucose plus fructose, was lower in the homogenate obtained from the two local accessions compared to that of Golden delicious (Figure 1b), although all had a same total soluble solid content: the difference was -33% for Limoncella and -24% for Sergente. From these results, the purees derived from fruits of these two latter genotypes appeared more suitable from a dietary point of view when a moderate carbohydrate intake is recommended. In apple, fructose is the most abundant sugar followed by glucose, sucrose and finally sorbitol, in decreasing order;[6] in some historical apples it has been found that the quantity of glucose and fructose can exceed that of sucrose by ten times.[7] In this study, the amount of fructose was found to be 4 times higher than that of glucose in the reference cultivar, and 7-8 times higher in the two local accessions. Since fructose has a sweetening power almost double that of glucose,[8] Limoncella and Sergente purees, despite their lower content of these two sugars compared to Golden delicious, can be as sweet as the puree obtained from this latter cultivar.

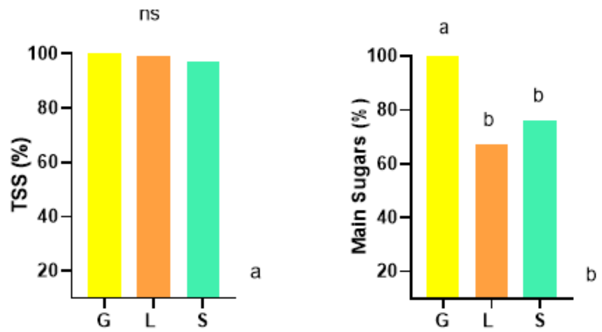

Figure 1 Total Soluble solid concentration (a) and main sugar content (b, fructose plus glucose) in fresh fruit homogenates obtained from two local apple accession (Limoncella=L and Sergente=S) expressed as percentage of the reference cultivar Golden delicious (G) detected in the 2024 year (different letters indicate significant differences at $p \leq 0.05$; mean separation by Tukey test).

The total amount of organic acids was much higher in the homogenate of Limoncella and Sergente, in the order of +70% and +100%, respectively, compared to that of Golden delicious (Figure 2a). It is known that 90% of the total acidity of apple fruits derives from malic acid. Organic acids give "freshness" and flavor to fruit pulps and their derivatives; however, malic acid can also impart astringent sensations. Organic acids contribute to the antioxidant activity of fruits and their processed products;[9] among the single acids, particular importance is attributed to the ascorbic acid, the pure form of vitamin C, since it has a strong effect as a free radical scavenger and activator of antioxidant systems.[10,11] It is often integrated into commercial foods as an anti-browning agent and protector of sensory and nutritional properties.[12] Limoncella and Sergente homogenates were found richer in L-ascorbic acid than the Golden delicious one, in the order of +40% and +30%, respectively (Figure 2b).

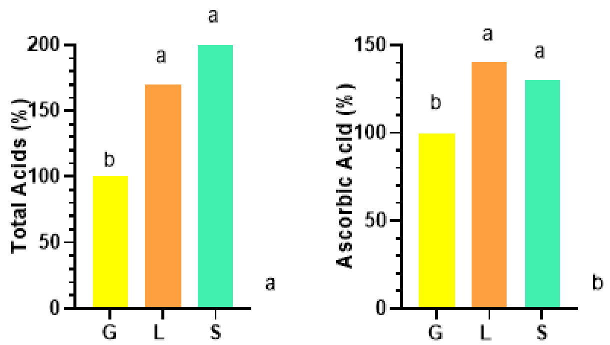

Figure 2 Total content of organic acid (a) and L-ascorbic acid (b) in fresh fruit homogenates obtained from two local apple accession (Limoncella=L and Sergente=S) expressed as percentage of the reference cultivar Golden delicious (G) detected in the 2024 year (different letters indicate significant differences at $p \leq 0.05$; mean separation by Tukey test).

The total polyphenol content of the homogenates of Limoncella and Sergente apples were 60% and 80% higher than that of Golden delicious (Figure 3a). In apple fruits, the following phenolic classes are mostly represented in decreasing order: flavanols, hydroxycinnamic acids, dihydrochalcone derivatives, flavonols, and small changes are found in fruit processed products.[13] Flavanols have been indicated

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as the most active phenolic group in determining apple antioxidant capacity, but the analyses of several genotypes have showed that it is the complex of total polyphenol compounds that generally achieves the strongest correlation with the antioxidant activity.[14] In the present study, the homogenates obtained from Limoncella and Sergente fruits had antioxidant activity respectively 20% and 50% greater than the homogenate obtained from Golden delicious (Figure 3b).

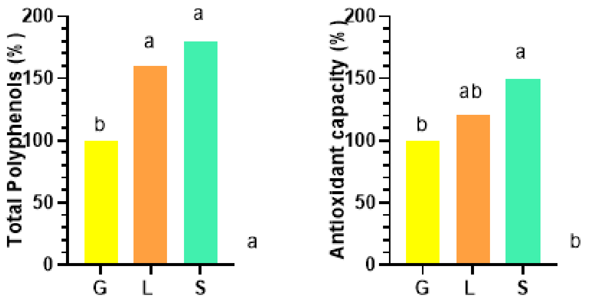

Figure 3 Total polyphenol content (a) and Antioxidant capacity (b) in fresh fruit homogenates obtained from two local apple accession (Limoncella=L and Sergente=S) expressed as percentage of the reference cultivar Golden delicious (G) detected in the 2024 year (different letters indicate significant differences at $p \leq 0.05$; mean separation by Tukey test).

Conclusions

The analytical results obtained in this study highlighted that the fruits of the local ancient apple genotypes Limoncella and Sergente are provided of better nutritional traits than those of the renowned cultivar Golden delicious assumed as a reference; therefore, they showed to have interesting potentialities to produce healthy derivative ready-to-eat products as demanded by consumers. Further experiments are currently underway on these apple matrices; however, the result already obtained encourages the valorization of these two traditional accessions through the technological transformation of their fruits.

As for the comparison between the two local genotypes, the fresh homogenates obtained from their fruits showed quite close analytical values and no statistical differences between them; however in this trial, that derived from Sergente tended to develop a higher antioxidant capacity than that from Limoncella. This result is in line with the tendency detected in Sergente homogenate towards a higher content of organic acids and total polyphenols, and suggests to focus special attention on this genotype for the production of processed apple products.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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