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Fresh Juices of Cocomerina Pear an Ancient and Rare Fruit with Red Pulp: A New Source of Polyphenols for Human Health

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Abstract

The aim of this study was to focus our attention on two ecotypes of an ancient variety of Pyrus communis recently rediscovered whose main feature is the reddish and/or red color pulp. There are two ecotypes of this pear that ripen in August and October, both of which are nowadays cultivated only in a small area of central Italy. Since it is known that the juices obtained by cold pressing maintain unaltered nutritional properties and are easy to prepare, it seemed interesting to examine, in both the ecotypes, the polyphenols, flavonoids and anthocyanins content. A greater concentration of these secondary metabolites in late type pear juice correlated with antioxidant and anti-inflammatory activity evaluated by DPPH, ORAC and 5I-Lipoxygenase assay, respectively, was shown. We can confirm that the fruits of cocomerina pear can be considered a new source of antioxidant and anti-inflammatory compounds. In fact, even the fresh juices, when compared to the extracts studied in our previous work, have shown antioxidant and anti-inflammatory properties. The results obtained allow us, therefore, to assume that the introduction of fresh juices obtained from this rediscovered variety of pear, may have positive implications for population health benefit.

Keywords: Fruits; Vegetables; Diets; Flavonoids; Cocomerina

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Introduction

Diets rich in fruits and vegetables can prevent various diseases [1-3]. Recent investigations have shown that fruits are a natural source of dietary fiber, trace elements, and antioxidant compounds and that diets rich in fruits positively influence plasma lipid levels and antioxidant capacities in experiments on laboratory animals [4-7]. In 2003, Leontowicz et al. [8] investigated whole traditional fruits and their influence on lipid metabolism in rats. In addition, some authors have demonstrated that the peels of fruits possess a higher content of bioactive substances than peeled fruits [9,10]. However, the fear of pesticides prevents most fruit consuming people to eat whole fruits. In a previous work [11] the authors decided to study separately the bioactive compounds in apple and pear peel and pulp and their influence on plasma lipids and antioxidant potential in rats nourished by cholesterol-rich diets. They have concluded that in apple and pear peel the contents of dietary fiber, phenolic acids, flavonoids, and total polyphenols are significantly higher than in pulp. Previous studies have correlated a high intake of fruit and vegetables with a lower incidence of chronic diseases such as cancer, diabetes, cardiovascular and neurovegetative impairments. It is also well established that these health benefits are, in part, attributed to the antioxidant capacity derived from the phenolic compounds present in edible plants [12,13]. In addition fruits, salads and fresh vegetables provide nutrients and vitamins. Pear (*Pyrus* sp.) is a major fruit crop of temperate regions with an expanding cultivation.

As medicinal fruit, pear is considered very important. In fact this genus includes 25 Euroasiatic species. This fruit has astringent and febrifugal properties. The leaves contain glucosids with antiseptic properties for the urinary tract and the fruits contain phenolic compounds including flavonoids, flavones, isoflavones, flavonones, anthocyanins and catechins which have strong antioxidant capacities [13]. Thus, it is important to characterize the beneficial phytonutrients present in these foods and the mechanisms responsible for these effects [14]. Pear flavonoids

contribute to the fruit color and pathogen defense, and are health-beneficial ingredients of the fruits.

Diets alternative to the Mediterranean diet take into account the considerations described above, and propose dietary intake of so-called "live" juices in “raw-food diet”. These juices are obtained by using special new generation extractors that operate at a low number of revolutions and utilize cold pressing providing a juice in which the same nutrients contained in fruits remain unchanged. In fact, recent studies have found that people who regularly consume fresh juices receive numerous health benefits such as increased longevity and decreased risk of cancer and heart disease [15]. Other studies have shown that the latter consideration is valid not only for special diets; in fact, many researchers have shown that unpasteurized juices, obtained from fresh raw vegetables are needed as a complement to any diet. When a person follows or indulges in an irregular diet, these juices are vital, because they provide the body with live elements and vitamins that are deficient in cooked and processed foods [16].

In Europe, including Italy, researchers are focusing their attention on the ancient varieties of genus *Pyrus,* studying the various beneficial properties to human health and thus contributing to the recovery and value of these old varieties. This is especially important because, most of the old varieties of fruit studied have shown an excellent resistance to pathogens which normally attack the varieties most traded. We can assume that this feature typical of ancient indigenous fruits may contribute to their re-evaluation and help to limit the use of synthetic pesticides thereby contributing to the welfare of the surrounding community and human health [17-19].

For the above reasons, therefore, in this preliminary study, we are interested in the so-called "fresh" juice obtained from an ancient variety of pear whose peculiarity is that it has red pulp, rich in anthocyanins. *Pyrus communis* var. cocomerina is an ancient pear, sweet and very fragrant; the few remaining trees are scattered in the countryside of Emilia Romagna and, to a lesser extent, of Tuscany and Marche. The few specimens have come to us through the work of local propagators. The main feature is the pink/red flesh colour that increases with the degree of ripeness. There are two ecotypes of this fruit: the early-type with fruits that ripen in August, and the late-type with fruits that ripen in October.

To our knowledge, in the literature there are no studies concerning pear with red pulp because, contrary to the apple, until now, pears with this peculiarity have not been not identified. In our study, for the first time, the total content of polyphenols, flavonoids and anthocyanins was evaluated; the in *vitro* antioxidant and anti-inflammatory activity of the juice was also examined.

Methods

Plant material

Fruits of *Pyrus communis* var. cocomerina were harvested in Ville di Monte Coronaro (FC, Italy), a locality situated at 850 m above sea level. The fruits were collected in two different periods: in summer at the end of August (early-type) and at about the second week of November (late-type). For both types, fruits that had reached full maturity were used.

Preparation of juices

For each experiment, ripe fruit was used (for a weight of about 50 g). The ripe fruits were washed and extracted in a Versapers slow juicer, generally used by raw food advocates, in which the extraction rate corresponds to 80 revolutions per minute. This extractor is equipped with a mechanism completely different from the other juice extractors and/or from the normal centrifugal extractors because the juice is obtained by squeezing rather than by grinding or centrifugal force. The juice sample was immediately used for different experiments.

Determination of total phenolics

Polyphenols compounds were determined colorimetrically using the Prussian blue method [20]. With slight modifications. Aliquots of the sample were made up to 1 mL with distilled water; after adding 60 µL of 0.1 M $FeNH_4(SO_4)_2$, they were incubated for 20 min at room temperature. Subsequently, 60 µL of 8 mM $K_3Fe(CN)_6$ were added to the sample, and after 20 min at room temperature the optical density of the mixture was determined at 720 nm (Jasco V-530 spectrophotometer, Tokyo, Japan). A standard curve was prepared with quercetin.

Total flavonoid, flavone and flavonol determination

Total flavonoid content was measured according to the method of Bucchini et al. [20] with slight modifications. The assay mixture contained: 0.1 mL of extract, 0.5 mL of distilled water and 30 µL of 5% $NaNO_2$. After 6 min, 150 µL of 10% Al $Cl_3 \cdot 6H_2O$ were added and the mixture was allowed to stand for another 5 minutes. Immediately after the incubation, 0.5 mL of 1 M NaOH was added and the final volume of assay (1 mL) was reached with distilled water. The solution was well mixed and the absorbance was measured at 510 nm. A standard curve was prepared with quercetin.

Flavones and flavonols were determined according to Popova et al. [21]. Briefly, 40 L of the extract, 0.4 mL methanol and 20 L 5% $AlCl_3$ were mixed and the volume was made up to 0.54 mL with methanol. After 30 minutes at room temperature the absorbance at 425 nm was detected. Quercetin was used as the reference standard.

Determination of total pear juice anthocyanins

The total anthocyanin content of the apple extracts was measured using the differential pH method reported by Elisia et al. [22] and Tzulker et al. [23]. Two aliquots of juice (sample) were dissolved separately in potassium chloride buffer (KCl 0.025 M, pH 1.0) and sodium acetate ($CH_3COONa\ 3H_2O$, 0.4M, pH 4.5). The absorbance measurements of the samples were read at 510 and 700 nm against a control cell containing solvent instead of sample (in the same quantity). The absorbance (A) of the diluted sample was then calculated as follows:

$A=(A_{510\ nm}-A_{700\ nm})_{pH\ 1.0}-(A_{510\ nm}-A_{700\ nm})_{pH\ 4.5}$

The monomeric anthocyanin pigment concentration in the original sample was calculated as reported by Elisa et al. and Tzulker et al. [22, 23].

Determination of antioxidant activity

Scavenging capacity of stable free radicals (DPPH$^{\cdot}$): Radical scavenging activity was measured spectrophotometrically [20]. This method is based on the reduction of an ethanolic solution of 1,1-dipheny l-2-picrylhydrazyl (DPPH). The extract was added to 1.5 mL of 100 µM DPPH (ethanolic solution). The decreasing absorbance at 517 nm was recorded after about 30 min at room temperature and the percent decrease (corrected for the control, without addition of antioxidant agents) was taken as an index of the antioxidant capacity. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as positive control. Results were expressed as the amount of the extract that caused 50% decrease of the initial DPPH$^{\cdot}$ concentration (EC_{50}).

Oxygen radical absorbance capacity (ORAC): The original method of Cao et al. [24]. was used with slight modifications. Fluorescein (3^{I},6^{I}-dihydroxy-spiro[isobenzofuran-1[3*H*],9^{I}[9*H*]-xanthen]-3-one) was used as fluorescent probe. The final reaction mixture for the assay (1 mL) was prepared as follows: 825 µL of 0.05 µM fluorescein sodium salt in 0.075 M sodium phosphate buffer, pH 7.0, 100 µL of properly diluted sample in 0.075 M sodium phosphate buffer, pH 7.0. The reaction was started with 75 µL of 5.55 mM AAPH and fluorescence was measured every 10 sec using a JASCO FP-6200 spectrofluorometer at 485 nm excitation, 520 nm emission. The area under the curve (AUC) of fluorescence decay was proportional to the antioxidant capacity of the sample, and a comparative evaluation with Trolox was performed.

Determination of "in vitro" anti-inflammatory activity

5^{I}-Lipoxygenase assay: Inhibition of 5^{I}-lipoxygenase activity was assayed spectrophotometrically according to the method of Sud'Ina [25], modified by Bucchini et al. [20]. The assay mixture (1 mL) contained: 0.1 mM linoleic acid, the sample (or the same quantity of solvent as reference) and 50 mM sodium phosphate, pH 6.8. This mixture was maintained at 23°C for about 10 min. Subsequently, 0.18 µg mL^{-1} of commercial 5-lipoxygenase was added to the mixture and the formation of hydroperoxides from linoleic acid was observed spectrophotometrically at 235 nm at 23°C. BHT and caffeic acid, were used as positive controls. Results are expressed as the amount of extract that caused 50% inhibition of lipoxygenase activity (IC_{50}).

Statistical analysis*:* All data are the mean of triplicate analyses carried out on three different juices of each sample. Statistical analysis was performed with the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). Analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used to assess significant differences between samples.

Results and Discussion

Determination of polyphenols and flavonoids

In the literature there are many works that focus on drinks and juices obtained from fruits. It has been shown that they all contain polyphenolic compounds that contribute to their health benefits. Lugasi and Hovari, in a 2003 work, [26] studied red wines, white wines, lagers, stouts, fruit juices and vegetable juices and proved that, among alcoholic beverage, the highest polyphenol content was found in red wines (1720 mgL^{-1}). In fruit juices the total polyphenol concentration ranged from 5680 mgL^{-1} (elderberry fruit juice) to 159 mgL^{-1} (pineapple juice). The juices of different cultivars of pear showed a polyphenols content which varied between 150 mgL^{-1} and 541 mgL^{-1}. The pear juices studied in our laboratory demonstrated, according to the data above, a total polyphenols content ranging from 190 mgL^{-1} (pear cocomerina early) to 320 mgL^{-1} (pear cocomerina late). The total polyphenol content of the juice obtained from "cocomerina" pear, when compared to the mg of dry weight, comes to 0.29 mg and 0.17 mg, respectively, in the juice of the late and early ecotypes, highlighting a concentration difference of 41% between the two types.

A subclass of polyphenols is represented by flavonoids that are of significant importance in plant metabolism; in addition, flavonoids determine additional characteristics that are of particular significance in fruits. The color is mainly determined by the presence of anthocyanins but also from other classes of flavonoids (e.g., flavonols) that act as their copigments [13]. Pears contain different classes of flavonoids that contribute to the color, the quality of the fruit and the plant resistance [27-28]. Based on these data it seemed interesting to first assess the concentration of these compounds in our two "raw" juices **(Figure 1a).** The data are shown in Fig.1a and demonstrate that the greatest amount of these compounds is present in the late "cocomerina" pear juice with values of 0.13 mg compared with 0,022 mg in the early (values referred to g dry weight). It is interesting to note that flavonoids account for about half of total polyphenols in the juice of the late type while in that of early type represent only 13%.

Determination of anthocyanins

The presence of polyphenols in fruit juices is largely influenced by genetic factors and environmental conditions, but the variety and degree of maturity determine the major quantitative differences in the phenolic profile. In the case of our pear, for both juices, we also evaluated the antocyanin content. The results are shown in **Figure 1b.**

Even for the antocyanins content, the obtained values confirm the greater presence of these compounds in the late pear juice. This parameter is certainly related to the intense red color of the pulp, this feature is important because it might be a marker for the characterization and differentiation of the juices obtained from the two different cultivars. In fact, the late pear juice looks

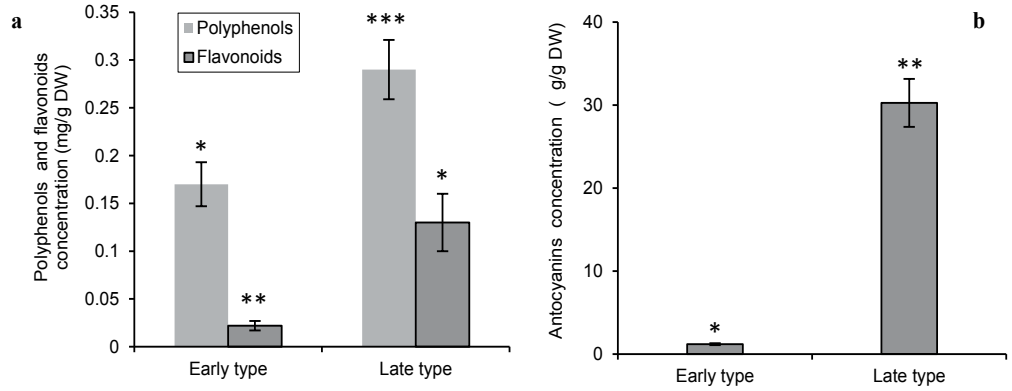

Figure 1 Polyphenol, flavonoid (a) and anthocyanin (b) content in "cocomerina" pear juice. Each value is the mean ± S.D. of five independent determinations. Different symbol indicate significant differences (P<0.05).

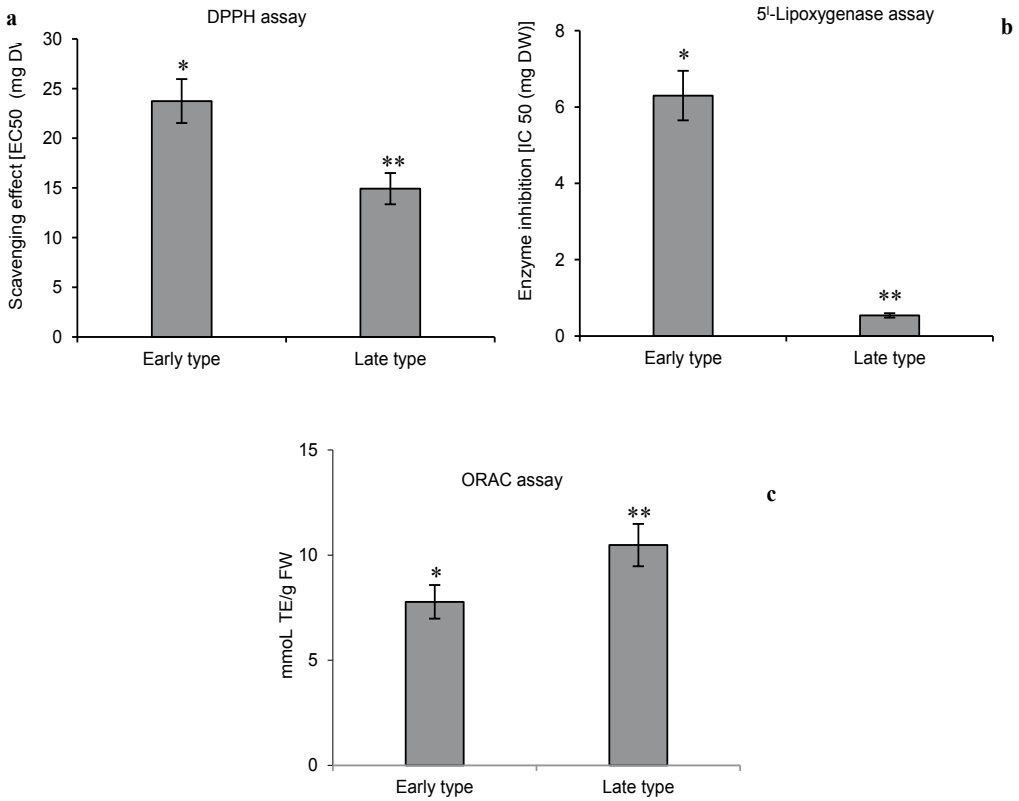

Figure 2 Scavenging capacity (a), 5l-lipoxygenase activity inhibition (b) and ORAC capacity (c) of "cocomerina" pear juices. Each value is the mean ± S.D. of five independent determinations. Different symbol indicate significant.

appears red while that of the early pear is visually similar to the juice obtained from other varieties of pears with white flesh. However, it should be emphasized that even in limited quantity, anthocyanins are also present in the early pear juice and this is the peculiarity that differentiates the juice of the latter from the juice obtained from other cultivars of white pulp pears.

The presence of anthocyanins in other cultivars of pear with white pulp was only evidenced in the extracts obtained from pears which had a variously red-colored exocarp. Dussi and Sugar [29] have characterized and quantified the anthocyanin concentration in the peel of the cultivar "Sensation Red Bartlett" showing that the concentration was related to the chromaticity values of the fruit. In this work the pigments were analyzed by HPLC technique and the authors have shown that in the extracts obtained from the peel, anthocyanins were present in an amount of 68.3 μg g^{-1} dry weight, and then, at an early superficial analysis,

greater than the values we obtained from the late cultivar (30 µg g^{-1} dry weight). This evidence should not be misleading as two basic factors are to be considered: the first, very important, is that the authors had performed an extraction with organic solvents then brought to dryness; the second is that the extracts were obtained from fruits with a typically intense red-coloured exocarp.

Therefore, in agreement with Dussi and Sugar [29], we also detected that in the late pear, the anthocyanin content represented 23% of flavonoids contrary to what was shown in the early pear in which anthocyanins accounted for only 6% of the total flavonoids content.

Antioxidant activities

Radical scavenging effect (DPPH assay): The bioactive compounds in juices and vegetables are adept donors of hydrogen in the presence of radical DPPH. The remarkable ability to donate hydrogen was observed by Lugasi and Hovari [26] in the juice of elderberries, currants, plums, and red grapes, different varieties of pears with white flesh and a mix of fruit juices. The EC_{50} values of different fruit juices in this work ranged from 2.7 (elderberry fruit juice) and 915 microliters (pineapple juice); in particular, regarding the pear cocomerina juice, the results showed values of 61.2 microliters. In this context, the ability of our juices to reduce the DPPH radical was particularly significant, when compared with the data listed above. In fact, the study of the juice obtained by late cultivar showed an EC_{50} of 13.3 microliters. In **Figure 2a** the data obtained by DPPH assay are shown. The results are expressed as EC_{50} (mg dry weight), showing an antioxidant activity greater than 37% for the late (14.93 mg dry weight) compared to early cultivar (23.74 mg of dry weight) **(Figure 2a).**

Inhibition of the enzyme 5'-lipoxygenase (5-LOX); *in vitro* anti-inflammatory effect*:* For completeness of data, we decided to evaluate the inhibition of 5-lipoxygenase enzyme activity exerted by our two samples to evaluate their anti-inflammatory activity. In fact, the 5-LOX is an enzyme that catalyzes the biosynthesis of leukotrienes: proinflammatory mediators involved in various inflammatory processes. Our data showed that the juice of both types of pear "cocomerina", exerts a good anti-inflammatory activity with IC_{50} values corresponding to: 0.54 g dry weight for the late cultivar and 6.3 g dry weight for the early cultivar. The results are shown in **Figure 2b**: in the late pear an inhibitory activity greater than 91% has been highlighted on the enzyme compared to that of the early pear. This percentage difference is also evident in the flavonoid and anthocyanins content. This, while requiring further evaluations and studies, allows us to hypothesize the involvement of such compounds in the inhibition of 5'-LOX, something that does not occur or occurs to a lesser extent with regard to the reduction of DPPH.

ORAC assay (oxygen radical absorbance capacity): The fresh juices of pear "cocomerina" have also shown a good antioxidant capacity when it was valued by ORAC (oxygen radical absorbance capacity). This method, is considered as a reference technique for the measurement of antioxidant food and supplements. The evaluation of the antioxidant capacity of a sample with this method is important and suggested by many authors [30] as this is correlated with the inhibition of the oxidation of PUFA and, therefore, is necessary when foods must be considered for their health properties.

The results obtained are in agreement with those obtained by other authors such as Ehlenfeldt and Prior [14], Monagas et al. [31] and Zulueta et al. [32]. The ORAC values of fruits belonging to different cultivars of blueberries ranged between 4.3 and 31.3 equivalent of Trolox/g of fruits and 12.44 and 0.88 equivalent of Trolox/g of fruits for other fruit juices such as strawberries, red grapes, kiwi, white grape, banana, apple, tomato and melon. The "cocomerina" pear juice, when compared with the juices used in the works mentioned above, have ORAC values of 10.48 and 7.78 equivalent of Trolox/g fruits for the late type and the early-type, respectively. The greater antioxidant activity was detected for the pear cocomerina juice late-type and is shown in **Figure 2c**.

Conclusions

The results, while still in the preliminary phase, have shown that the fresh juice (expecially the "cocomerina" pear late type juice) is particularly rich in polyphenols, anthocyanins and flavonoids and consequently showed a significant *in vitro* antioxidant and anti-inflammatory activity when compared with other fruit juice. Our results confirm the importance of further enhancing the fruit trees which seem to have forgotten, taking into account that often the old varieties are the most suitable for agriculture with low environmental impact.

It is evident that with the changes in climate, plants with great adaptability and characterized by high resistance are needed to counteract the biotic (caused by fungi, bacteria, nematodes and various insects) and abiotic stress (dependent on water availability and water, light and temperature quality). These characteristics have been demonstrated for all "forgotten fruits" already studied. Based on the above considerations, the recovery, enhancement and encouragement to cultivate this fruit are desirable to help meet the increasing need to eat healthy foods, free from damage and harmful organisms. In the future, we will have to face a new study in order to definitively identify the compounds biologically active of "cocomerina" pear. In addition, the bioactivity of this pear "cocomerina" juice, will should be tested with *in vivo* model.

Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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