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Cocomerina pear: an old and rare fruit with red pulp. Analysis of phenolic content and antioxidant/anti-inflammatory capacity

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ABSTRACT

The study evaluated the phenolic content, antioxidant and anti-inflammatory activity (1,1-diphenyl-2-picrylhydrazyl, DPPH, and 5'-lipoxygenase assay, respectively), and oxygen radical absorbance capacity (ORAC), in Cocomerina pear, an old and rare variety of *Pyrus communis*. There are two cultivars of this pear that ripen in August and October, both of which are nowadays cultivated only in a small area of central Italy. Significant differences in polyphenolic content were observed between these cultivars, with a 30–40% increase in the late ripening cultivar relative to the early ripening one. Anthocyanin concentration was also strongly affected by the period and stage of maturation. In particular, it was 126-fold higher in fruits that ripen in October as compared to those that ripen in August. Ripe fruits of the late cultivar also exhibited higher antioxidant and anti-inflammatory activities. These findings suggest that this ancient pear variety should be recovered and considered in the framework of biodiversity conservation.

Pera 'Cocomerina': un frutto antiguo y raro de pulpa roja. Análisis del contenido de compuestos fenólicos y capacidad antioxidante/anti-inflamatoria.

RESUMEN

El estudio evalúa el contenido de compuestos fenólicos, antioxidantes (1,1-diphenyl-2-picrylhydrazyl, DPPH), la actividad anti-inflamatoria (ensayo de la 5'-lipoxigenasa) y la capacidad de absorción del radical oxígeno (ORAC) en la pera Cocomerina, una variedad antigua y rara de *Pyrus communis*. Existen dos cultivares de esta pera, de maduración en Agosto (cultivar precoz) y de maduración en Octubre (cultivar tardía) y actualmente son cultivadas sólo en una pequeña zona de Italia central. Se detectaron diferencias significativas en contenido de polifenoles entre los cultivares, con un 30 a 40 % de aumento en la cultivar tardía respecto a la cultivar precoz. La concentración de antocianinas también resultó fuertemente afectada por el período y por el estado de maduración. En particular, fue 126 veces más alto en frutos que maduran en Octubre que en aquellos que maduran en Agosto. La fruta madura del cultivar tardío en general mostró mayor actividad antioxidante y de la actividad anti-inflamatoria. Nuestros resultados sugieren que esta variedad antigua de pera debe ser recuperada y además ser considerada en el marco de la conservación de la biodiversidad.

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Introduction

In recent years, the interest in the so-called ancient fruits has greatly increased. These fruits, whose memory is partially or completely lost, are not easy to find, since they are grown only in limited areas, and little is known of their nutritional properties and secondary metabolites content. Many species belong to the genus *Pyrus* whose fruits are known to be an important source of polyphenolic compounds, which possess multiple biological properties and play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes (Dai & Mumper, 2010; Giomaro et al., 2014). Therefore, screening the fruits of these plants on the basis of their antioxidant capacity could serve both to highlight their properties and for biodiversity conservation (Büchs, Harenberg, Zimmermann, & Weiß, 2003).

Pears (*Pyrus communis* L.) are one of the most frequently consumed fruits. For centuries they have been used also as a pharmaceutical supplement in folk medicine to treat respiratory diseases (Li, Gao, Huang, Zhang, & Guo, 2011). In

addition, they have important roles in providing taste characteristics, such as subtle aroma, sweetness, and crispness (Li et al., 2011). Studies carried out on different pear cultivars showed that this fruit, besides being an important source of monosaccharides, minerals, and fiber, also contains various bioactive compounds, including leucocyanidins, catechin, epicatechin, chlorogenic acid, quercitrin, quercetin, caffeic acid, and coumarylquinic acid (Li et al., 2011, 2012; Manach, Scalbert, & Morand, 2004). In particular, the total antioxidant capacity of the different cultivars was consistent with the total phenolic and flavonoid contents (Li et al., 2011). The phenolic composition of fruits may contribute to color and flavor, and their qualitative and quantitative differences appear as a function of the species, degree of ripening, and storage (Spanos & Wrolstad, 1992; Spanos, Wrolstad, & Heatherbell, 1990). Some horticultural pear cultivars contain relevant amounts of polyphenols, such as chlorogenic acid, flavan-3-ols, and arbutin (Escarpa & Gonzalez, 2000), which are related to pulp browning (Hamazu & Hanakawa, 2003).

A high dietary intake of flavonoids and stilbenes obtained by consuming vegetables and especially fruits, such as apple, plum, pear, and peach, was shown to reduce serum triglyceride concentrations and TG/HDL-C ratios in Chinese adult female subjects (Li et al., 2013).

These results are in agreement with the inverse association of fruit (particularly apples and pears) and green leafy vegetable consumption with stroke risk (Larsson, Virtamo, & Wolk, 2013).

In the present article, we analyzed the polyphenol, flavonoid, and anthocyanin content in fruit extracts of *P. communis* var. Cocomerina, a sweet and very fragrant pear, so called because of the red color of the pulp reminiscent of watermelon. This ancient variety is cultivated at present only in the Apennine area of Romagna and Tuscany (Italy). There are two cultivars of this pear: an early-ripening (ER) one whose fruits are harvested in August, and a late-ripening (LR) cultivar whose fruits are harvested in October (Biscotti, Guidi, Forconi, & Piotta, 2010). The postharvest durability of fruits is very short and they are traditionally eaten fresh or used to make jams. The *in vitro* radical scavenging and anti-inflammatory activities were also evaluated. The aim of our study was, in fact, to investigate the nutritional value of this ancient variety as a source of antioxidants.

Materials and methods

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 (43°46'34.21"N; 12°1'44.36"E), either in August (ripe fruits of the ER cultivar) or in September and October (unripe and ripe fruits, respectively, of the LR cultivar). The ripe fruits of the ER cultivar exhibited a greenish-brown skin and a coarse slightly aromatic white/pink pulp; in ripe fruits of the LR cultivar the skin was brownish-red and the pulp was red, crisp, sweet, and sour. By contrast in the unripe fruits of this cultivar the skin was green with red streaks and the pulp was hard and pink.

Extracts preparation

Pears (whole fruit), deprived of the core, were cut into pieces and extracted with 80% ethanol (40:100, w:v) three times, 5 min each, in a mixer on ice. The homogenates were heated at 90°C for 10 min, then filtered and centrifuged at 10,000 g (Giomaro et al., 2014). The supernatants were collected and maintained at -20°C until use.

Determination of total phenolics

Total phenolic compounds were determined colorimetrically using the Prussian Blue method (Hagerman & Butler, 1994) with slight modifications. Aliquots of supernatants were withdrawn, taken to a final volume of 1 mL with distilled water, added with 60 µL of 0.1 M $\text{FeNH}_4(\text{SO}_4)_2$, and then incubated for 20 min at room temperature. Afterwards, 60 µL of 8 mM $\text{K}_3\text{Fe}(\text{CN})_6$ were added and, after 20 min of incubation at room temperature, absorbance was read at 720 nm (Jasco V-530 spectrophotometer, Tokyo, Japan) and phenolic concentration was calculated by comparing sample absorbancies with a standard quercetin curve.

Total flavonoids, flavones, and flavonols determination

Total flavonoids content was measured according to the method of Liu et al. (2002) with slight modifications. Extracts (0.1 mL) were added with 0.5 mL of distilled water and 30 µL of 5% NaNO_2 , incubated for 6 min at room temperature and then added with 150 µL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. After 5 min of incubation, samples were added with 0.5 mL of 1 M NaOH and taken to the final volume of 1 mL with distilled water. Absorbance was read at 510 nm. A standard curve was prepared with quercetin.

Flavones and flavonols content was determined according to Popova et al. (2004). Briefly, 40 µL of the extract were added with 0.4 mL methanol and 20 µL 5% AlCl_3 and taken to a final volume of 1 mL with methanol. After 30 min of incubation at room temperature the absorbance was read at 425 nm and flavones and flavonols concentration was calculated from a standard curve of quercetin.

Determination of anthocyanins

Anthocyanin content was determined by the differential pH method according to Elisia, Hu, Popovich, and Kitts (2007).

Determination of antioxidant activity

Scavenging capacity of stable free radicals (DPPH assay)

Radical scavenging activity was measured spectrophotometrically according to the method of Mellors and Tappel (1966) based on the reduction of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Different volumes of sample extracts were added to 1.5 mL of 100 µM DPPH ethanolic solution. Absorbance at 517 nm was recorded after 30 min at room temperature in the dark. The percent decrease in absorbance (corrected against the blank) was indicative of the degree of scavenging of the DPPH radical, i.e., 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 concentration (EC_{50}).

Oxygen radical absorbance capacity (ORAC)

The original method of Cao, Alessio, and Cutler (1993) was used with slight modifications. Fluorescein (3',6'-dihydroxy-spiro[isobenzofuran-1[3H],9[9H]-xanthen]-3-one) was used as fluorescent probe. Sample extracts (100 µL) were added to 825 µL of 0.05 µM fluorescein sodium salt in 0.075 M sodium phosphate buffer, pH 7.0. The reaction was started by adding 5 µL of 5.55 mM 2,2'-azo-bis-(2-methylpropionamide) dihydrochloride and fluorescence was measured every 10 s using a JASCO FP-6200 spectrofluorimeter (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. Final ORAC values were expressed in µM Trolox equivalents (TE) per g dry weight (DW).

Determination of anti-inflammatory activity

5'-lipoxygenase assay

Inhibition of 5'-lipoxygenase activity was assayed spectrophotometrically according to the method of Holman, modified by Sud'ina et al. (1993). The reaction mixture contained

0.1 mM linoleic acid, sample extract, and 50 mM sodium phosphate, pH 6.8. After 10 min of incubation at 23°C, hydroperoxides ($0.18 \mu\text{g mL}^{-1}$) of commercial 5-lipoxygenase were added to the mixture and the formation of linoleic acid was determined spectrophotometrically (A_{235}) at 23°C. Butylated hydroxytoluene (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 extracts 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

This study provides data on the content of bioactive phytochemicals in Cocomerina pear and highlights differences between early- and late-ripening cultivars. As shown in Table 1, fruits of the LR cultivar had higher levels of polyphenolic compounds (0.85 and $1.02 \text{ mg g}^{-1} \text{ DW}$ in unripe and ripe fruits, respectively), while in ripe fruits of the ER cultivar it was significantly lower ($0.61 \text{ mg g}^{-1} \text{ DW}$). With regard to flavonoids, the highest amount was observed in unripe fruits of the LR cultivar ($7.76 \mu\text{g g}^{-1} \text{ DW}$); in ripe fruits of both the ER and LR cultivar it was quite similar (Table 1). The highest level of flavones and flavonols was detected in ripe fruits of the ER cultivar (Table 2). A positive correlation was observed between the level of these compounds and the maturation stage as indicated by the 50% increase observed in LR cultivar fruits harvested in October as compared with the ones harvested in September. By contrast, dihydroflavonol content was higher in the late cultivar as compared with the early one (47% and 58% in unripe and ripe fruits, respectively) (Table 2).

Since both the skin and pulp of these pear cultivars are differently colored, the anthocyanin content was also evaluated. Anthocyanins are responsible for most of the red, blue, and purple colors of fruits, vegetables, flowers, and other plant tissues or products. They are particularly abundant in berries, red grapes, purple sweet potato, red cabbages, and red wine (Mazza & Miniati, 1993). Epidemiological studies indicate that the consumption of products rich in anthocyanins (such as red wine and several species of berries) is associated with a lower

Table 1. Polyphenol, flavonoid, and anthocyanin content in Cocomerina pear fruits at different stages of maturation. Different letters in a column indicate significant differences ($p < 0.05$).

Tabla 1. Contenido de polifenoles, flavonoides y antocianinas en frutos de pera Cocomerina durante la maduración. Letras diferentes en una columna indican diferencias significativas ($p < 0,05$).

Stage and cultivar	Polyphenols $\text{mg g}^{-1} \text{ DW}$	Flavonoids $\mu\text{g g}^{-1} \text{ DW}$	Anthocyanins $\mu\text{g g}^{-1} \text{ DW}$
Ripe fruits (ER cv.)	0.61 ± 0.09^a	5.52 ± 0.03^a	0.45 ± 0.05^a
Unripe fruits (LR cv.)	0.85 ± 0.11^b	7.76 ± 0.36^b	24.37 ± 3.11^b
Ripe fruits (LR cv.)	1.02 ± 0.13^b	6.33 ± 0.36^b	56.54 ± 5.89^c

Table 2. Flavones, flavonols, and dihydroflavonols content in Cocomerina pear fruits at different stages of maturation. Different letters in a column indicate significant differences ($p < 0.05$).

Tabla 2. Contenido de flavonas, flavonoles y dihidroflavonoles en frutos de pera Cocomerina durante la maduración. Letras diferentes en una columna indican diferencias significativas ($p < 0,05$).

Stage and cultivar	Flavones and flavonols $\mu\text{g g}^{-1} \text{ DW}$	Dihydroflavonols $\mu\text{g g}^{-1} \text{ DW}$
Ripe fruits (ER cv.)	0.06 ± 0.007^a	2.5 ± 0.27^a
Unripe fruits (LR cv.)	0.02 ± 0.003^b	4.7 ± 0.41^b
Ripe fruits (LR-cv.)	0.04 ± 0.003^a	5.9 ± 0.47^b

risk of cardiovascular diseases (Jennings et al., 2012; Johnson et al., 2015; Wallace, 2011). In addition, anthocyanins display strong free radical scavenging and antioxidant activities, and inhibitory effects on the growth of some cancer cells (Kang, Seeram, Nair, & Bourquin, 2003; Liu et al., 2002). A strong difference in anthocyanin content was observed in Cocomerina pear between fruits of the two cultivars at the same ripening stage (Table 1). In fact, anthocyanin content was 126-fold higher in the LR cultivar than in the ER one. This dramatic difference could depend upon the different seasonal period of ripening (summer in the ER cultivar and autumn in LR cultivar). In fact, peel color development is enhanced by low temperature, which increases the quantities of anthocyanins or carotenoids present (Koshita, 2015) as a positive mechanism against environmental stress factors (Chalker-Scott, 1999). In unripe fruits of the LR cultivar, anthocyanin content was significantly lower than in ripe fruits indicating a positive correlation between anthocyanin synthesis and fruit ripening. Moreover, it was 54-fold higher than in the peel of ripe fruits of the ER cultivar suggesting a strong localization in fruit skin and confirming that ripening at a later period positively affected the accumulation of these compounds.

Phenolic compounds are known to perform strong antioxidant and free radical scavenging activities (Dai & Mumper, 2010). The radical scavenging activity of fruit extracts was evaluated using the DDPH test and the results are shown in Table 3. The ethanolic extracts of unripe and ripe pears of the LR cultivar showed a significantly higher radical scavenging activity ($EC_{50} = 1.82 \text{ mg DW mL}^{-1}$ and $EC_{50} = 2.02 \text{ mg DW mL}^{-1}$, respectively) than ripe fruits of the ER cultivar ($EC_{50} = 3.22 \text{ mg DW mL}^{-1}$). However, when these values were compared with the reference standard, their activity was negligible, although there was a linear correlation

Table 3. *In vitro* radical scavenging capacity and inhibitory effect on 5'-lipoxygenase activity and ORAC activity in Cocomerina pear fruits at different stages of maturation. Different letters in a column indicate significant differences ($p < 0.05$ in DPPH and ORAC assay; $p < 0.001$ in 5'-lipoxygenase assay).

Tabla 3. Capacidad captadora *in vitro* de radicales, efecto inhibitor sobre de la actividad de la lipoxigenasa y la actividad ORAC en frutos de pera de Cocomerina durante la maduración. Letras diferentes en una columna indican diferencias significativas ($p < 0,05$ en DPPH y ORAC ensayo; $p < 0,001$ en 5'-lipoxigenasa ensayo).

Stage and cultivar	DPPH assay $EC_{50} \text{ mg DW mL}^{-1}$	Lipoxygenase assay $\mu\text{C}_{50} \mu\text{g DW mL}^{-1}$	ORAC $\mu\text{mol TE g}^{-1} \text{ DW}$
Ripe fruits (ER cv.)	3.22 ± 0.04^a	3.90 ± 0.40^a	97.30 ± 8.52^a
Unripe fruits (LR cv.)	1.82 ± 0.02^b	0.13 ± 0.02^b	83.34 ± 9.12^a
Ripe fruits (LR cv.)	2.02 ± 0.03^b	0.11 ± 0.02^b	137.83 ± 14.52^b

between antiradical activity and polyphenolic ($0.9818 < r^2 < 0.9979$) or anthocyanin content ($0.9818 < r^2 < 0.9992$).

In a previous study, Li et al. (2011) reported that extracts of some commercial Chinese pear cultivars performed *in vivo* anti-inflammatory activity since they inhibited xylene-induced ear edema and enhanced vascular permeability in mice. Here the anti-inflammatory activity of fruit extracts was evaluated *in vitro* by the 5'-lipoxygenase assay. Ethanolic extracts obtained from ripe and unripe fruits of the LR cultivar drastically inhibited this enzyme activity ($IC_{50} = 0.11 \mu\text{g DW mL}^{-1}$; $IC_{50} = 0.13 \mu\text{g DW mL}^{-1}$) (Table 3). They were 35 and 52 times more effective than BHT and caffeic acid, respectively. Moreover, their effect was significantly different ($p < 0.001$) compared with that of extracts from ER fruits ($IC_{50} = 3.90 \mu\text{g DW mL}^{-1}$).

In the last decade, the introduction of antioxidant compounds in diet has become increasingly important due to the growing awareness about their health-promoting properties. To evaluate the total antioxidant capacity of food products, the ORAC assay is widely employed since this method is highly specific and capable of responding to a greater number of antioxidant compounds (Zulueta, Esteve, & Frigola, 2009). As shown in Table 3, the highest antioxidant activity was detected in ripe fruits of the LR cultivar (ca. 30% and 40% more than in ripe fruits of ER cultivar and unripe fruits of LR cultivar, respectively). It was significantly ($p < 0.05$) higher than that of unripe fruits of the same cultivar, even though the results obtained with the DPPH and 5'-lipoxygenase tests showed similar activities. Interestingly, all the Cocomerina pear extracts examined showed an antioxidant activity that was comparable to or higher than that detected in some commercial pear cultivars (Kevers et al., 2007; Kevers, Pincemail, Tabart, Defraigne, & Dommes, 2011). Only cultivar Durondeau, in fact (Kevers et al., 2011), showed a higher antioxidant capacity as compared with that detected in ripe fruits of the LR cultivar ($4251 \mu\text{mol TE } 100 \text{ g}^{-1}$ fresh weight vs. $3378 \mu\text{mol TE } 100 \text{ g}^{-1}$ fresh weight).

Conclusions

In conclusion, the data reported here indicate that this ancient pear, endemic of the Apennines of central Italy, is a rich source of antioxidants with relevant amounts of phenolics and flavonoids. In particular, the anti-inflammatory activity of the late-ripening cultivar characterized by a red pulp, together with its high antioxidant capacity (as demonstrated by the ORAC assay), could offer diverse opportunities for nutraceutical and functional food applications. Moreover, its production and commercialization as a functional food could represent an incentive for its recovery and biodiversity conservation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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