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Modulation of Antioxidant Compounds in Organic vs Conventional Fruit (Peach, *Prunus persica* L., and Pear, *Pyrus communis* L.)

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Despite the increasing interest in organic products, knowledge about how different levels of fertilization affect nutritionally relevant components is still limited. The concentration of polyphenols and the activity of polyphenoloxidase (PPO), together with the content in ascorbic acid, citric acid, and α- and γ-tocopherol, were assayed in conventional and organic peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams). 2-Thiobarbituric acid reactive substances and the tocopherolquinone/α-tocopherol ratio were used as markers of oxidative damage in fruits. A parallel increase in polyphenol content and PPO activity of organic peach and pear as compared with the corresponding conventional samples was found. Ascorbic and citric acids were higher in organic than conventional peaches, whereas α-tocopherol was increased in organic pear. The concentration of oxidation products in organic samples of both fruits was comparable to that of the corresponding conventional ones. These data provide evidence that an improvement in the antioxidant defense system of the plant occurred as a consequence of the organic cultivation practice. This is likely to exert protection against damage of fruit when grown in the absence of pesticides.

KEYWORDS: Organic fruit; polyphenols; polyphenoloxidase; tocopherols; TBA-RS

INTRODUCTION

In the recent years, the growing consumer's awareness of health and safe-controlled foods, together with environmental protection plans, have determined an increase in the areas assigned to organic cultivations as an alternative to the conventional agricultural practices in western countries.

According to EC Regulation (Verordnung (EWG) 24/6/91 no. 2092 and updatings), plant organic products are considered those produced without the use of chemically synthetic pesticides and largely without the addition of readily soluble mineral fertilizers.

Although food products from organic origin are believed to be healthier than the corresponding conventional foods, clear experimental evidence supporting this assumption is still lacking and assessment of the nutritional potential of these products requires further research (*29*).

Among the difficulties in obtaining comparable data on organic vs conventional food products is the selection of neighboring farms and fields so that a close comparison of products grown in a same area is achieved, thus ruling out any effect of climate and soil conditions on differences observed in food properties.

In the frame of a national (MiPAF) project aimed at the characterization of organic plant food, we tried to identify compounds of possible use in the distinction between organic and conventional products. Because of the paucity of information about the chemical composition of organic foods, endogenous markers of organic production are not available so far.

Phenolic compounds are likely to be involved in the plant defense mechanism by acting as a chemical barrier to invading phytopathogens (*13*, *18*). Oxidation of phenolics to quinones by polyphenoloxidase (PPO), followed by polymerization to dark pigments, helps the plant to repair the injured surface, as does fibrin blood clot formation in humans (*13*). Moreover, polymerization of polyphenols increases toxicity of the compounds to phytopathogens. Indeed, modifications in the activity of enzymes related to the antioxidant endogenous defense system of the plant (PPO, superoxide dismutase, catalase, and glutatione peroxidase) have been observed to precede damage symptom expression (*10*, *25*): a higher activity of superoxide dismutase and peroxidases has been measured in tomato plants infected by nematodes (*30*). In line with these findings, modulation of the level of these compounds by pesticides (herbicides, insecticides, and fungicides) has been observed (*1*, *8*, *19*).

The antioxidant profile has been recognized to represent an important parameter to predict the impact of food on human health, and it also affects the shelf life of the products. Indeed, the oxidative deterioration of food is a major cause of loss of

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Table 1. PPO Activity and Total Polyphenol Content of Conventional and Organic Peach and Pear Samples[a]

sample	PPO activity (unit min^{-1}/100 g f.w.)[b]			total polyphenols (mg tannic acid/100 g f.w.)
	caffeic acid	chlorogenic acid	catechol	
		Peach		
conventional	2451.9 ± 126.4	2053.2 ± 145.0	nd[c]	21.3 ± 1.6
organic	2174.5 ± 198.2	2655.3 ± 171.2*	nd	29.0 ± 1.2**
		Pear		
conventional	674.2 ± 50.5	959.1 ± 100.9	557.1 ± 143.2	58.4 ± 2.0
organic	865.1 ± 43.8*	3020.7 ± 235.4***	401.4 ± 110.3	64.5 ± 1.5*

[a] Values are the average of at least six determinations ± SD. [b] Conventional vs organic: significantly different; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. [c] nd, not detectable.

nutritional quality as well as a cause of concern for food safety. Therefore, in this study, the concentration of polyphenols and the activity of PPO, together with the content in ascorbic acid, citric acid, and α- and γ-tocopherol (TH), were assayed in conventional and organic peaches and pears. 2-Thiobarbituric acid reactive substances (TBA-RS) and the tocopherolquinone (TQ)/α-TH ratio, widely used as markers of oxidative damage in foods (*11*, *12*, *15*, *26*), were also compared in conventionally and organically grown fruits.

EXPERIMENTAL PROCEDURES

Investigations occurred over a period of 3 years (1998−2000). Peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams) fruits, either organically (according to EU Regulation) or conventionally grown on tilled soil (of the same age, 5 years), were obtained from the experimental orchard of the Istituto Sperimentale per la Frutticoltura (Ciampino, Rome). Under the denomination of organic fruits are marked all of those products that are produced under controlled cultivation conditions in line with the provisions of the EC Regulation on organic farming (Verordnung (EWG) 2092/91 and updatings) as well as with related national laws. These fruits, unlike conventional fruits, are produced without the aid of chemically synthetic pesticides and largely without the use of soluble mineral fertilizers, within a diverse range of crop rotation and extensive soil tillage. Fruits were harvested at commercial maturity (at the end of June and August for peach and pear, respectively) and were immediately sent to the Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione for analyses. No physical defects or signs of pathogen contamination and no significant differences in size and color between conventional and organic products were evidenced (*23*). On arrival, part of the fruits was homogenized and analyzed for ascorbic and citric acids and part was frozen at −40 °C. Water content of the fruits was 88.6 and 88.0%, for conventional and organic peaches, respectively, and 82.3 and 83.1% for conventional and organic pears, respectively. Total polyphenols were assayed by the method of Joslyn (*2*).

The extraction of PPO from peach fruits was performed using the method of Kader et al. (*17*). A 150 g sample of frozen (−40 °C) fruits was homogenized with 200 mL of ice-cold (−18 °C) solution of pure acetone/water/Triton X-100 (80:19:1, v/v) in a Waring (Blendor) CB6 24CB blender (15 500*g*/min). The resulting homogenate was left at −25 °C during 1 h and then filtered under vacuum through a glass filter on a Buchner funnel (G-3); the residue was washed several times using 250 mL of ice acetone (−18 °C) until a white powder was obtained. The powder was dried (2 h) at room temperature. Aliquots of powder (0.02 g/mL) were suspended in 0.005 mol/L sodium phosphate buffer, pH 7.0, containing 0.005 mol/L cysteine and stirred at 4 °C for 2 h. The suspension was centrifuged at 2000 rpm for 20 min (4 °C), and the supernatant was recovered. This procedure was repeated twice, and the supernatants were collected and centrifuged at 12 000 rpm for 15 min (4 °C); collected supernatants were finally used as crude extracts for activity assays.

The extraction of PPO from pears was performed using the procedure of Gauillard and Richard-Forget (*14*), with some modifications. Crude samples were homogenized for 3 min with a Ultra Turrax T25 (I-Ka-Labor Technik) blender in 0.05 mol/L sodium phosphate buffer at pH 6.5, containing 0.02 mol/L ascorbic acid and 2% PVPP (polyvinylpolypyrrolidone) as a phenolic scavenger. The homogenates were stirred overnight at 4 °C. The suspensions were centrifuged (2200 rpm, 15 min, 4 °C), and supernatants were recovered. Further extraction of the sediments was carried out in the same buffer by stirring in an external ice bath. The supernatants were collected and centrifuged (14 000 rpm, 15 min, 4 °C), and the recovered supernatants were used for PPO activity assays.

PPO activity was tested toward caffeic acid, chlorogenic acid, and catechol. For activity assay, 50−200 μL of enzyme extracts was incubated in the specific solution buffer (0.05 mol/L sodium acetate buffer at pH 4.0 and 0.05 mol/L sodium phosphate buffer at pH 6.5, for PPO obtained for peaches and pears, respectively).

The total reaction mixture (1.0 mL) contained complementary amounts of enzyme extract and buffer at which 100 μL of 1 mmol/L substrate solutions—caffeic acid, chlorogenic acid, or catechol (Sigma Chemical Co., St. Louis, MO), dissolved in the specific buffers used for activity assays—were added. The increase in absorbance at 400 nm for chlorogenic acid or at 420 nm for caffeic acid and catechol was followed for at least 3 min at 25 °C with a spectrophotometer Beckman DU640 (Beckman Instruments, Fullerton, CA).

One unit of activity of PPO is defined as the amount of enzyme that caused an absorbance increase of 0.001 unit min^{-1} (initial rate) in the condition of the assay (*17*). The results are reported as enzymatic activity (UE) in 100 g of fresh sample.

Ascorbic and citric acids were determined by high-performance liquid chromatography (HPLC) after extraction in 5% *m*-phosphoric acid (*20*). The HPLC system consisted of a Waters (Milford, MA) 510 pump, a Waters 996 detector, and a Hypersil ODS C_{18} column (4.6 mm × 250 mm) (Waters). After separation, ascorbic and citric acids were detected by UV absorption at 254 and 220 nm, respectively. Ascorbic acid and citric acid concentration were calculated on the basis of a calibration curve and expressed as milligrams per 100 g fresh sample.

α- and γ-TH were assayed after HPLC separation on a Nova-Pak C_{18} column (3.9 mm × 150 mm) (Waters). The elution of the complex was monitored by a spectrofluorimetric detector (model RF-551, Shimadzu, Kyoto, Japan), set at $\lambda_{EX} = 295$ nm and $\lambda_{EM} = 330$ nm. The TQ amount, expressed as percent of α-TH content, TQ/TH × 100, was used to evaluate the extent of lipid oxidation, according to Fedele and Bergamo (*12*).

TBA-RS were determined by both the distillation (spectrophotometric) method of Tarladgis et al. (*27*) and by HPLC (*3*). In the latter case, the malondialdehyde (MDA)−TBA complex was fractionated on a Nova-Pak C_{18} column and monitored by a spectrofluorometric detector ($\lambda_{EX} = 515$ nm; $\lambda_{EM} = 543$ nm). MDA concentration was calculated on the basis of a calibration curve and expressed as nanomoles per 100 g fresh sample.

Analyses were performed at least in triplicate on fruit samples of the different harvests and average values ± standard deviations are reported. Data were subjected to analysis of variance. The significance of the differences between means was estimated by Student's *t*-test.

RESULTS AND DISCUSSION

PPO activity and total polyphenol content of peach and pear samples are presented in **Table 1**. As already reported (*14*) for either fruit, the highest enzymatic activity was found toward

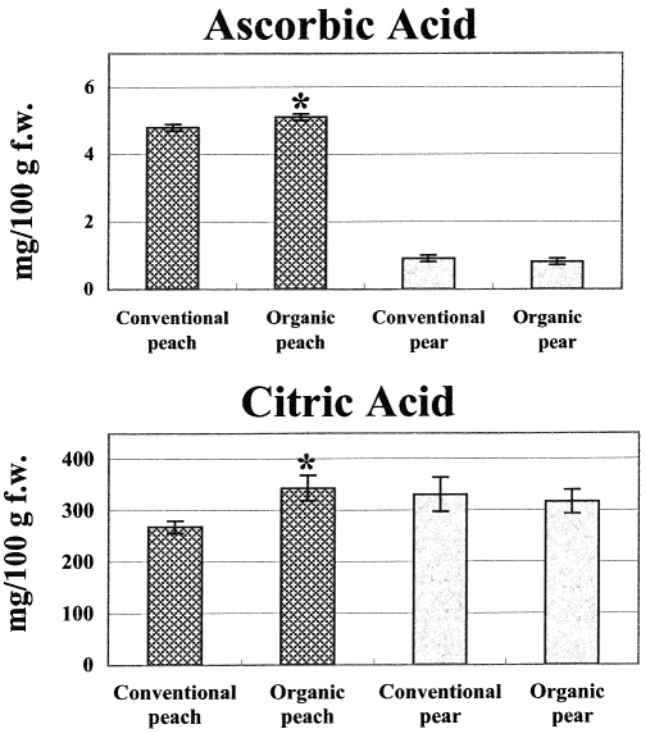

Figure 1. Ascorbic acid and citric acid content of conventional and organic peach and pear samples. Values are the mean ± SD of three replicates. *Conventional vs organic: significantly different ($P < 0.05$).

caffeic and chlorogenic acids, whereas it was very low or undetectable when assayed toward catechol. PPO activity toward chlorogenic acid of peach samples and toward both caffeic and chlorogenic acids of pear samples was found to be significantly increased in the organic as compared with conventional fruits. A parallel increase in polyphenol content of organic peaches and pears as compared with the corresponding conventional samples was observed (**Table 1**). Therefore, the increase in polyphenols of plants, described by some authors (*8*, *19*, *24*) when grown in the absence of pesticides, was confirmed in this study. Thus, it was likely that modifications in phenolic metabolism occurred as a consequence of the organic cultivation practice.

As far as ascorbic and citric acids are concerned, their content was increased in the organic as compared with conventional peach but not pear fruit samples (**Figure 1**). In the latter, ascorbic acid content of either conventional or organic samples was very low.

Available data on the effect of the cultivation system on ascorbic acid content of vegetable products gave controversial results even for a similar product, i.e., potatoes (*29*).

Browning in fruit is initiated by oxidation of phenolics by PPO. The quinones produced quickly polymerize to form insoluble melanins (*21*). Because ascorbic acid is a powerful reducing agent, coupled oxidation between PPO-generated *o*-quinones and ascorbic acid can occur, giving the regenerated phenol and dehydroascorbic acid, thus preventing the formation of pigments (*24*). Stability of *o*-quinones is variable, depending on the phenolic compound they originated (*29*), and this affects consumption of ascorbic acid by the reaction.

Vitamin E is an essential nutrient that exerts its basic biological activity by scavenging peroxyl radicals (*5*): among the eight naturally occurring THs, isomers α and γ are the most abundant in vegetable foods.

Analysis of α- and γ-TH in the samples indicated no relationship between TH content and production system: indeed,

Table 2. α- and γ-TH, TQ, and TQ/α-TH Ratio of Conventional and Organic Peach and Pear Samples[a]

	μg/100 g f.w.			
	α-TH	γ-TH	TQ	TQ/α-TH × 100
		Peach		
conventional	0.65 ± 0.02	0.46 ± 0.01	1.80 ± 0.27	276.92 ± 51.21
organic	0.57 ± 0.01*	0.37 ± 0.01*	1.34 ± 0.03*	235.09 ± 9.49
		Pear		
conventional	0.58 ± 0.03	0.68 ± 0.09	1.43 ± 0.37	246.55 ± 52.45
organic	0.71 ± 0.05*	0.68 ± 0.15	2.0 ± 0.10	281.70 ± 20.49

[a] Values are the average of three replicates ± SD. Conventional vs organic: significantly different, *$P < 0.05$.

significantly higher α- and γ-TH contents were measured in conventional than in organic peach samples (**Table 2**). On the other hand, a higher amount of α-TH was found in organic pears than in the conventional ones.

During the early phase of lipid oxidation, α-TH oxidation occurs leading to the formation of a number of byproducts (*16*), and among them, α-TQ is the most abundant (*4*).

The TQ/α-TH ratio was recently used as a lipid oxidation index in various foods (*28*), such as meat and dairy foods (*11*, *12*). Therefore, TQ concentration was also measured in organic and in conventional fruits to evaluate the degree of lipid oxidation.

As shown in **Table 2**, despite a higher concentration of TQ in conventional than organic peaches, no significant differences in the TQ/α-TH ratio between conventional and organic samples were evidenced.

There are no literature data concerning the effect of ripening and of postharvest storage on α-TH oxidation. However, data obtained in this study, indicating the association between α-TH and TQ level, may suggest the involvement of fruit ripening on TQ yield.

Quantification of TBA-RS of fruit samples by either the spectrophotometric method or the HPLC analysis is shown in **Figure 2**.

Despite its widespread use, the TBA test has been criticized for its low specificity, when performed by spectrophotometric detection, because of the presence of interfering substances that may lead to an overestimation of the MDA−TBA adduct (*15*). Besides MDA, TBA has been reported to react in a similar way with other products of lipid oxidation (alkanals, alkenals, and dienals) and with substances other than lipid oxidation products, especially sucrose.

However, conventional and organic products of either species analyzed in this study were previously found to display a similar sucrose concentration (*6*) that was far lower than that reported to give significant interference in the TBA assay (2.5−10 mM) (*9*). Therefore, correction for the presence of sucrose, suggested in the case of measurement of TBA-RS of lipid oxidation in sugar-rich plant tissues (*9*), was not necessary.

The results of the spectrophotometric determination of TBA-RS were compared with those of HPLC analysis of the MDA−TBA adduct (**Figure 2**). In both cases, no significant differences in this parameter between fruits obtained with the two different cultivation systems were found. Although the data obtained by the two different methods presented a similar trend, a higher concentration of TBA-RS was measured by the spectrophotometric rather than the HPLC method, in agreement with the lower specificity of the former as compared with HPLC determination (*15*). Indeed, the sample preparation procedure

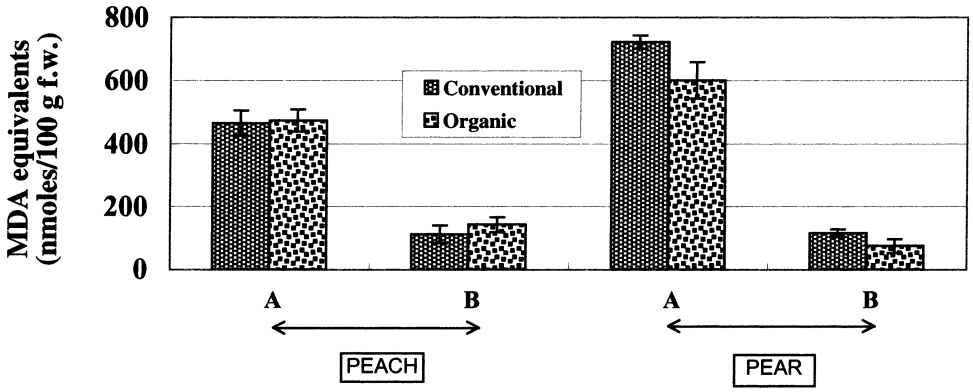

Figure 2. TBA-RS concentration (MDA equivalents) of conventional and organic peach and pear samples measured by (A) spectrophotometric-TBA assay and (B) HPLC. Values are the mean ± SD of three replicates.

employed for the MDA assay by HPLC allowed prevention of TBA-RS formation from aldehydes other than MDA (*3*).

Because plant tissues develop complex systems that regulate buildup of oxidation products, a similar trend for all of the parameters examined in the different fruits was unlikely. The increase in ascorbic acid content observed in organic vs conventional peach (**Table 2**) is consistent with a general improvement in the antioxidant system developed by the plant in organic fruits. However, even in fruits where it is known to be present in a high concentration, such as apples, ascorbic acid has been reported to represent only a minor part of the total antioxidant activity, polyphenols being the major antioxidant compounds (*22*).

Several studies demonstrated that the total amount of phenolics, as well as the proportion of the various components, are subjected to considerable variations among varieties and species, as well as during development and storage of fruits and vegetables, thus affecting resistance against external injuries. Among phenolic acids, chlorogenic acid, an ubiquitous phenolic compound that represents the majority of total hydroxycinnamoyl derivatives in pears (*1*), has been shown to possess a strong fungistatic activity (*18*). This is in agreement with the increased activity of PPO toward chlorogenic acid measured in organic as compared with conventional fruits (**Table 1**).

Most of studies on the mechanism conferring plant resistance reported that an increase in both the phenolic compounds and the activity of phenoloxidase (and oxidative enzymes in general) is associated with an improvement of the resistance against phytopathogens and herbivorous animals (*18*, *25*). Indeed, these oxidases have been found to inhibit polygalacturonase of the pathogen, and increased susceptibility usually follows decrease in oxidative enzymes with postharvest age (*25*). As a confirmation of a protective role of polyphenols and PPO-catalyzed polymerization reactions in helping wound sealing in injured tissues in plants (*13*), an increase in the activity of both PPO and phenylalanine ammonia lyase—an enzyme involved in the biosynthetic pathway of phenolic compounds—has been registered at the site of injury to fruits and vegetables (*21*).

Although lipid peroxidation is known to be an important factor in the development of fruit tissue senescence, a correlation among postharvest storage disorder development, TBA-RS, peroxides, and activity of oxidative enzymes was not always evidenced. Scald resistant apples were reported to accumulate more TBA-RS and peroxides during storage than did scald susceptible varieties (*10*), thus indicating that the appearance of symptoms of senescent breakdown in plant tissue is a complex phenomenon, depending on a balance of the activity of several enzymes and compounds controlling the increase of peroxidation products.

Whatever the cellular processes underlying the accumulation of TBA-RS in organic and conventional peach and pear, the results of PPO activity and of quantification of polyphenols strongly suggest that metabolic changes, resulting in an improvement in the antioxidant defense system of the plant, occurred as a consequence of the organic cultivation practice. Indeed, organic and conventional fruits presented a similar level of oxidation products.

The impact of these changes in composition on health needs further studies to be assessed. However, such changes might exert protection against oxidative damage, which is responsible for development of postharvest disorders during storage of organic plant products, similar to what has already been suggested in the case of biodynamic apples (*7*). Moreover, parameters that are significantly increased in organic samples may be chosen as markers for the characterization of organic vs conventional products.

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