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Efficiency of DPPH and FRAP assays for estimating antioxidant activity and separation of organic acids and phenolic compounds by liquid chromatography in fresh-cut nectarine

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Abstract

Nectarine contain relevant amounts of antioxidants, including hydroxycinnamic acids, flavan-3-ols, flavonols, anthocyanins, procyanidins, and carotenoids. The aim of this research was to compare the efficiency of DPPH and FRAP assays to estimate antioxidant activities contents in nectarine minimally processed. Moreover, separation of organic acids, phenolic compounds and carotenoids were done by HPLC. Nectarine fruits 'R48' cv. were hand harvested, with 80% of their reddish coloration. The following treatments were applied: Control; 1% citric acid + 1% calcium chloride as anti-browning (AB) agent; 4 KJ m^{-2} UV-C radiation; 8 KJ m^{-2} UV-C; 12 KJ m^{-2} UV-C ; AB + ≅ 95 kPa O_2 (High O_2); AB + 4 KJ m^{-2} UV-C + High O_2; AB + 8 kJ m^{-2} UV-C + High O_2 and AB + 12 KJ m^{-2} UV-C + High O_2. We determined antioxidant activity to DPPH; antioxidant activity to FRAP; total phenols and HPLC-DAD analyses. In general the DPPH showed higher efficiency of antioxidant concentration in comparison with FRAP in minimally processed nectarines. There was clear trend in phenolic content in fresh-cut nectarine the treatment AB + 8 kJ m^{-2}UV-C + High O_2. The 22 minimally processed nectarine phenolic compounds were studied and quantified by HPLC, being detected: Hydroxycinnamates, flavonols, and anthocyanins. These results allow to conclude that the most abundant compounds found in this study of fresh-cut nectarine were chlorogenic acid, caffeic acid and ellagic acid with HPLC chromatograms were recorded at 280 nm, quercentin 3-galactoside, quercetin 3- Xyloside at 340nm and Cyanidin 3- glucoside at 510nm.

Keywords: *Prunus persica;* polyphenols; flavonols; anthocyanins*; HPLC*.
Abbreviations: DPPH_2,2- diphenyl-1-picrylhydrazyl; FRAP_ferric reducing antioxidant power; HPLC_liquid chromatography; UV-C_Ultraviolet radiation; AB_antibrowning; High O_2_≅ 95 kPa O_2; $CaCl_2$_ calcium chloride.

Introduction

Fact observed in the evolution of diet is the search for healthy food and quality, highlighting the increased consumption of fruits and vegetables. We observe today that the foods are no longer considered mere sources of nutrients, but also a source of quality of life, health, prevention and longevity. In this context, functional foods represent a promising market and constantly growing, which has aroused the interest of the scientific community. The importance of consuming fruits as sources of compounds with antioxidant activity has been suggested by different research groups. These compounds include flavonoids, anthocyanins, ascorbic acid, carotenoids, tocopherols (Gil et al., 2002; Cevallos-Casals et al., 2005). Nectarine contain relevant amounts of antioxidants, including hydroxycinnamic acids, flavan-3-ols, flavonols, anthocyanins, procyanidins, and carotenoids, which are mainly located in the skin (Tomas-Barberan et al., 2001; Gil et al., 2002). Antioxidants play a very important role in the body defense system against reactive oxygen species (ROS). The ROS are the harmful byproducts generated during normal cell aerobic respiration (Gutteridge and Halliwell, 2000). In addition, different environmental stress factors such as pollution, drought, temperature, excessive light intensities and nutritional limitation are able to increase the production of ROS (Arora et al., 2002; Rijstenbil, 2002). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Halliwell, 1996). Many of these

compounds are known antioxidant, which when present at high levels compared with a compound oxidisable significantly retard or inhibit oxidation of these compounds (Chen et al., 2012). Oxidative stress, caused by an imbalance between antioxidant systems and production of oxidative compounds (free radicals, ROS) is apparently associated with various diseases of multifactorial nature, especially the various types of cancer, cardiovascular diseases and inflammatory disorders. The mechanisms by which these pathologies develop generally involve oxidative changes considered critical molecules, including proteins, carbohydrates, nucleic acids, in addition to the substances involved in the modulation of gene expression and inflammatory responses (Kawanishi et al., 2002; Laguerre et al., 2007).

Phenolic compounds are aromatic metabolites of plants secondary metabolism that have a common structure with an aromatic ring with at least one hydroxyl group, which provides the ability to neutralize reactive species, helping the body to protect itself from oxidative stress (Wojdyto et al., 2009). Additionally, phenols contribute to fruits' color and taste and have been described as possessing anticarcinogenic and antimutagenic activity (Al-Duais, 2009; Gorinstein et al., 2009). Various studies have shown that phenolic compounds have high antioxidant potential, resulting in a beneficial effect to human health (Vijaya Kumar Reddy et al., 2010).

There are several methods to determine antioxidant activity of fruits and vegetables, also known as bioactive substances. However, from the biochemical point of view there is no way to select the most efficient way to determine those compounds that can be influenced by several factors, such as the solvent employed for the determination, time, extraction temperature and the nature the plant.

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995; Gil et al., 2002), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999; Guo et al., 2003; Jimenez-Escrig et al., 2001).

DPPH method consists in determining the ability to capture free radical DPPH by antioxidants. The free radical 2,2-diphenyl-1-picrilhidrazina presents a maximum absorbance at 515 nm. After addition of the antioxidant, produces a decrease in absorbance proportional to the concentration and the antioxidant activity of sample (Brand-Williams et al., 1995).

Pulido et al. (2000) describe the method FRAP (Ferric Reducing Antioxidant Power)- developed as an alternative to determine the iron reduction in biological fluids and aqueous solutions of the pure compounds. The method can be applied not only for the study of antioxidant activity in extracts of food and beverage, but also for the study of antioxidant efficiency of pure substances, with results comparable to those obtained with other more complex methods.

The aim of this research was to compare the efficiency of DPPH and FRAP assays to estimate antioxidant activities contents in nectarine minimally processed and the separation of organic acids, phenolic compounds and carotenoids by liquid chromatography.

Results and discussion

The concentration of antioxidant by the FRAP method has been decreasing over the period of storage for all treatments with UV-C radiation and other treatments being around 16.82 to 5.95 mg/100gAA (Figure 1). In contrast, Costa et al. (2006) and González-Aguilar et al. (2007) found that UV-C radiantion treatment increased total antioxidants in broccoli and fresh-cut mango. Andrade-Cuvi et al. (2011) checking the effect of the antioxidants in red pepper affected by UV-C Treatments at storage, overall, the present work shows that exposure to 10 kJ/m^{-2} UV-C radiation in red bell peppers do not cause marked modifications in DPPH radical scavenging capacity or AA content.

The anti-browing (AB) treatment was increased the antioxidant concentration over the period up to seven days Storage (13.5mg/100AA) in comparison with control (7.09mg/100gAA) (Figure 1).

The treatments combined with high concentration of oxygen was increased the content of antioxidant up to 7 days of storage. The highest value was found with treatment with AB + ≅ 95 kPa O_2 (High O_2) (16.62 mg/100gAA) and the lowest value was reached with treatment AB + 12 KJ m^{-2} UV-C + High O_2 (12.01mg/100gAA) (Figure 1).

Antioxidant activities agreed with the results of concentration of polyphenols of this study (Figure 3) and according to Gil et al., (2002). There is a high correlation high correlation (R40.9; p≤0.05) between antioxidant activities as determined by FRAP assays and phenolic contents in nectarines.

It can be observed in Figure 2 an increase in the concentration of antioxidant by DPPH at the fourth day of storage in which the treatment with antibrowning (AB) (16.42mg/100gAA) showed the highest, followed by treatment with 12 KJ m^{-2} UV-C (15.87mg/100g AA) and 8KJ m^{-2} UV-C (14.68mg/100gAA). Lavelli et al (2009) show that the quality of nectarine was affected by storage with antioxidant activity from 1.8 ± 0.2 mmol TE/kg.

Treatments with high concentration of oxygen showed an increase in antioxidant activity until 7 days of storage, the values were around 17.51 to 16.31mg/100gAA by the DPPH methodology. Treatment with AB + 12 KJ m^{-2} UV-C + High O_2 presented the highest value 17.51mg/100gAA (Figure 2). The minimally processed nectarine had higher antioxidant activity in DPPH method, in agreement with Sawai et al., (2005), DPPH has been widely used in the analysis of reaction mechanisms of polyphenolic compounds with free radicals. An advantage of this method is that the free radical is stable and is commercially available, which prevents its generation by different ways, and ease of use.

For treatments with UV-C, control and antibrowning (AB), the concentration of polyphenols was increased to 7 days of storage. The treatment UV-C and control have the lowest level (18.46mg/100g) but the treatment with antibrowning (AB) presented the highest content (22.16mg/100g). These results show a correlation with results obtained for antioxidant activity determined by DPPH and FRAP method in this work (figure3).

For treatments with high concentration of oxygen, checks shown in figure 3, had decreased during the storage period for the concentration of polyphenols. The treatment AB + 12 kJ m^{-2} UV-C + High O_2 presented the lowest concentration (13.93 mg/100g) during the days of storage and the

Table 1. Initial characterization of whole nectarine fruit.

Weight g	VD cm	LD cm	L*	Chrome	Hue angle º Hue	TA Citric acid/100g	SSC ºBrix	pH
183.74	6.89	7.06	85.94	9.38	23.65	0.085	11.85	3.70

VD: Vertical diameter; LD: Longitudinal diameter; L*: Luminosity; TA: Titratable acidity; SSC: Soluble solids content.

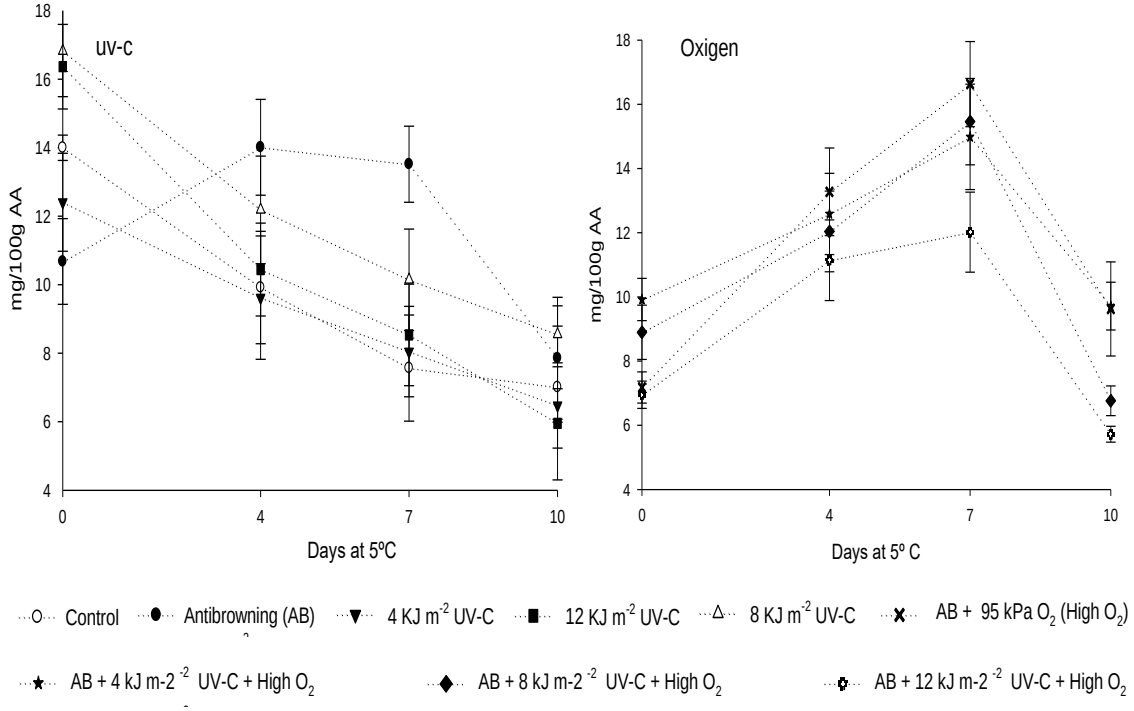

Fig 1. Antioxidant activities determined by the FRAP in nectarine minimally processed storage at 5ºC.

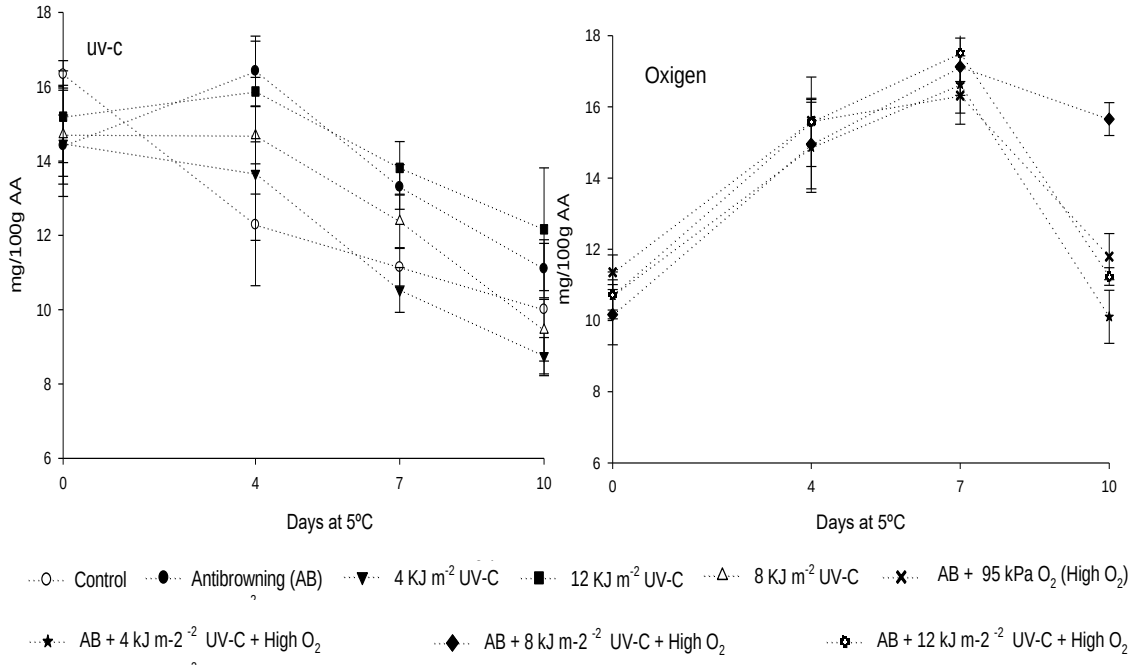

Fig 2. Antioxidant activities determined by the DPPH in nectarine minimally processed storage at 5ºC.

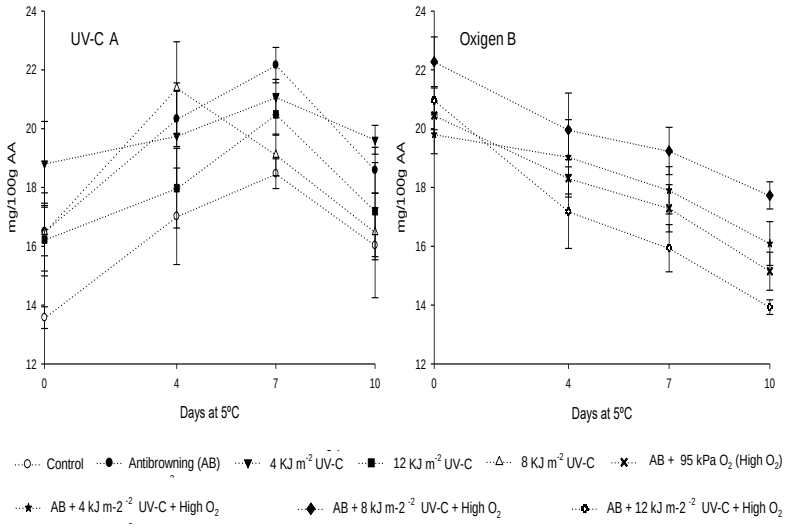

Fig 3. Total phenolics contents in nectarine minimally processed storage after 10 days at 5ºC.

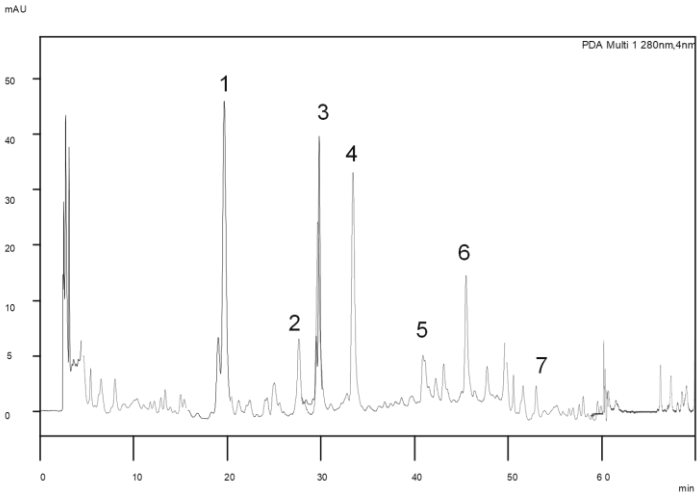

Fig 4. HPLC chromatogragrams of cv. R48 extracts recorded at 280 nm, (1) chlrogenic acid, (2) ferulic acid, (3) caffeic acid, (4) ellagic acid, (5) ác. *p*- coumaric, (6) fumaric acid, (7) sinap acid.

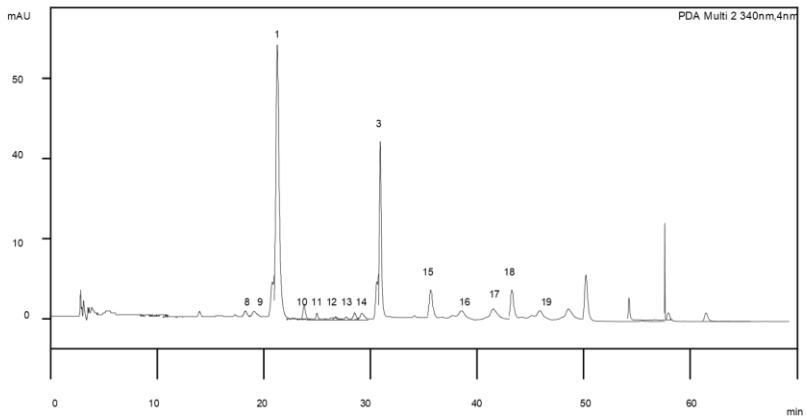

Fig 5. HPLC chromatograms of cv. R48 extracts recordes at 340 nm, (8) catequin, (9) procyanidin B4, (10) Procyanidin trimer, (11) procyanidin A Type dimer, (12) epicatechin, (13) procyanidin A type dimer, (14) procyanidin dimer, (15) quercetin 3- galactoside, (16) quercetin 3- glucoside+ quercetin 3- rutinosidade, (17) quercetin pentosyle-pentoside, (18) quercetin 3- xyloside, (19) quercetin 3-rhamnoside.

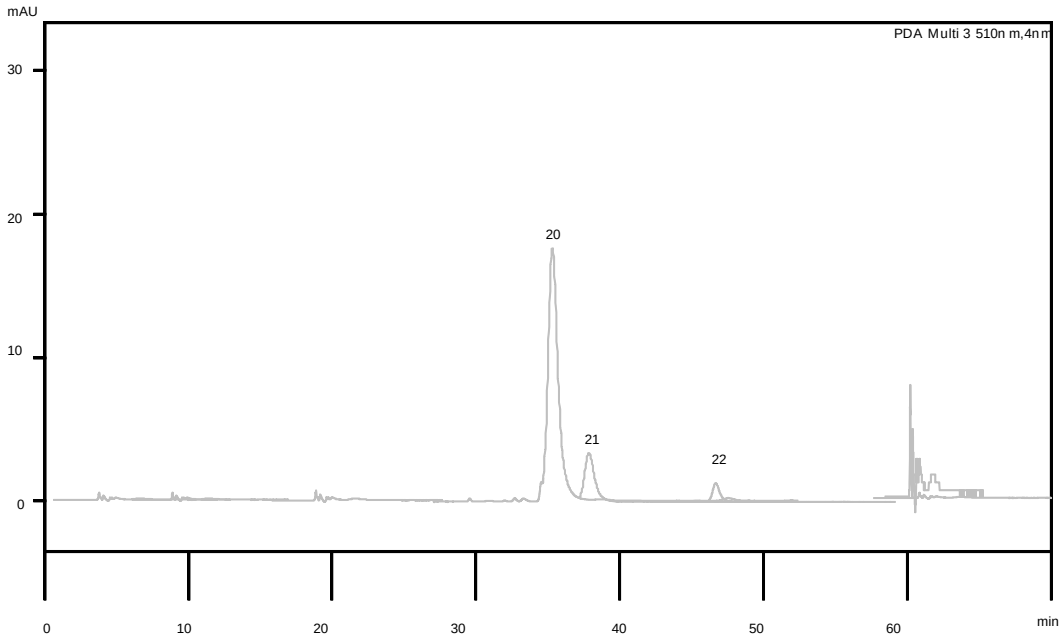

Fig 6. HPLC chromatograms of cv. R48 extracts recorded at 510 nm, **(20)** Cyanidin 3- glucoside, **(21)** Cyanidin 3- rutinoside, **(22)** Cyanidin 3- acetylglucoside.

treatment AB + 8 kJ m^{-2}UV-C + High O_2 had the highest concentration (17.72 mg/100g).

It was hoped that the antioxidant capacity reflects the phenolic compounds (total phenols and flavonoids) found in nectarine minimally processed and stored for 10days at 5 ° C, however was not observed in this study. Probably the observed antioxidant activity is not attributed to these compounds, but other substances such as carotenoids (β-carotene), vitamins and minerals that also have the ability to eliminate reactive oxygen species.

Acording to Lavelli et al (2009), the antioxidant activity was linearly correlated to ascorbic acid content. This correlation is consistent with the prevalence of ascorbic acid in the nectars, compared to other antioxidants. In contrast, Previous studies demonstrated that the antioxidant activity of different peach and nectarine fruits, evaluated as both the ability to scavenge the DPPH radical and the ferric reducing capacity, is correlated to total phenolic content, whereas no correlation exists with the ascorbic acid and carotenoids contents (Gil et al., 2002). In this study, ascorbic acid probably responsible for the antioxidant activity of nectarine minimally processed.

In general, the DPPH showed higher concentration of antioxidant with a range of 16.93 to 8.75mg/100gAA in comparison with FRAP, with a range of 16.8 to 5.95mg/100gAA. Since these values for the two methods within a range established by Gil et al., 2002, which was the ranges of AA contents (mg/100g) were 4.8 to 13.2 and the ranges of phenolic contents (mg/100 g) were 14 to 102 in nectarines.

To identify the different phenolics, a number of markers were available, including hydroxycinnamic acid derivatives, flavan-3ols, flavonols and anthocyanins. In this study were identified 22 different phenolic compounds in fresh-cut nectarine. Although the study of phenolic compounds, and specifically phenolic acids, is considered to be most interested in finding linked to most biological phenomena, botanists, genetic and taxonomic. It is difficult to estimate its content quantitative in plant tissues absolutely, due to the wide variety of metabolic processes in the formation of phenolic substances (Evaristo and Leitão, 2001).

The HPLC-DAD analyses showed that the quantification for hydroxycinnamates, flavonols, and anthocyanins was quite good, especially in the specific wavelengths for the different compound types (280 nm for hydroxycinnamates (Figure 4), 340 nm for flavonols (Figure 4) and 510 nm for anthocyanins (Figure 5). Typically, each species is associated with a particular most important class of polyphenols, whose content increases with age and vary with the vegetative growth of the plant absoluto (Evaristo and Leitão, 2001). The cultivar used in this work is the cycle later (June), which would justify the amount of the compounds found. In addition, the application of UV-C radiation and O_2 concentrations as high conservation treatment in minimally processed nectarines.

Compounds found at high concentration in nectarine were chlorogenic acid, caffeic acid and ellagic acid in chromatograms were recorded at 280 nm (Figure 4). Tomas-Barberan et al., (2001) analyzing phenolic compounds in peach, nectarine, and plum cultivars, have identified 25 phenolic compounds by HPLC-DAD-ESIMS. Fattouch et al. (2008), analyzed the profile of polyphenols and activities antioxidant and antimicrobial pulps and peels of apples, pears and quince,found that chlorogenic acid was the major phenolic compound in three pulps from fruits. This later was also observed in this work. Caffeic and chlorogenic acids have been reported as good free radical scavengers (Arrua et al., 2010).

We also found that the study synaptic acid, ferulic acid and p-coumaric acid in chromatograms were recorded at 280 nm. According to Wanasundara et al. (1994) synaptic acids, ferulic and p-coumaric are more active antioxidants than

acid derivatives benzoic, such as protocatechuic acid, vanillic and syringic. This is due to the double bond present in the molecule of derivative cinnamic acid, which participates in the stability of the radical by resonance shift of unpaired electron, while the benzoic acid derivatives do not exhibit this characteristic, this provavelmete acids justified the antioxidant activity found in this study.

Analyses allowed the identification of the 12 flavonols: Catequin, procyanidin B4, procyanidin trimer, procyanidin A type dimer, epicatechin, procyanidin A type dimer, procyanidin dimer, quercetin 3- galactoside, quercetin 3- glucoside + quercetin 3- rutinosidade, quercetin pentosyle-pentoside, quercetin 3- xyloside e quercetin 3- rhamnoside by HPLC chromatograms at 340 nm (Figure 5). The Flavonoids pigments in fresh-cut nectarine may be related to the chemical structure of the flavonoids, because the presence of water in the solution of the solvent used in this work, probably increased cell permeability and facilitated interactions of hydrophobic compounds. Polyphenolic compounds have been largely studied as antioxidant compounds and its dietary ingest have shown protective effect against diseases such as coronary heart (Engler and Engler, 2006).

The anthocyanin pigments identified in fresh-cut nectarine by HPLC chromatograms in 510 nm were (20) Cyanidin 3-glucoside, (21): Cyanidin 3- rutinoside, (22): Cyanidin 3-acetylglucoside (Figure 6). According to the results found by Tomas- Barberan et al., (2001) the main pigment was identified as cyanidin-3- glucoside and the minor one was identified as cyanidin- 3-rutinoside.

There are some methods described for analysis of phenolic compounds by gas chromatography, based on its polarity characteristics. However, there is a need for a systematic investigation of sample preparation and determination of phenolics in foods, quantification is complete, individually and / or group or class of phenolic compounds

Materials and methods

Plant materials

Nectarine fruits 'R48' cv. were hand harvested in a commercial farm from Frutas Esther S.A. located in Cieza (Murcia) on the southeastern Mediterranean area of Spain. Immediately after harvesting the fruits were transported about 80 km in an air conditioned car to the Pilot Plant of the Technical University of Cartagena and stored at 0-1ºC and 90% HR. The next day the nectarine was minimally processed as described below.

The nectarine fruits were carefully selected to obtain uniformity in the whole lot. All fruits with lesions or cracks were discarded and only intact fruits were processed. The physical characteristics of nectarines were monitored in a sample of 20 whole fruits, randomly selected (Table 1).

Treatments

The UV-C equipment used consisted of two banks of 15 stainless steel reflectors, each with unfiltered germicidal emitting lamps (TUV 36W/G36 T8, Philips, Eindhoven, and The Netherlands). The treatment chamber was covered with a protective reflecting inner layer that enhanced homogeneous distribution of the emitted light and allowed indirect illumination of practically all sides. In order to determine the UV-C radiation intensity of the lamps a VLX 254 radiometer (Vilber Lourmat, Marne la Vallée, France) was used. The applied UV-C intensity was calculated as the mean of 18 UV-C readings on each side of the net. Thus both sides received the same UV-C intensity. The UV-C light intensity was kept constant and the applied dose was varied by altering the exposure time at the fixed distance (Artés-Hernández et al. 2009).

For every treatment and immediately after the application of the UV-C radiation, 200 g of nectarine pieces were placed in PP baskets of about 750 mL capacity and thermally sealed at the top with a BOPP film of 30 µ thickness (for the treatments in air to generate a passive MAP) and with a BOPP of 50 µ (for high O_2 treatments by injecting O_2 in each basket to reach about 95 kPa O_2 in order to generate an active MAP).

The following treatments were applied: Control; 1% citric acid + 1% calcium chloride as antibrowning (AB) agent (Then, the pieces were immersed in an anti-browning solution (1% citric acid and 1% $CaCl_2$) for 10 min); 4 KJ m^{-2} UV-C radiation; 8 KJ m^{-2} UV-C radiation; 12 KJ m^{-2} UV-C radiation; AB + ≅ 95 kPa O_2 (High O_2); AB + 4 KJ m^{-2} UV-C radiation + High O_2; AB + 8 kJ m^{-2} UV-C radiation + High O_2 and AB + 12 KJ m^{-2} UV-C radiation + High O_2 (to reach about 95 kPa O_2). Three replicates of one basket per processing treatment and storage duration (0, 4, 7 and 10 days) were prepared. All baskets were then stored at 5ºC in the dark.

Antioxidant activity determinations to DPPH

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol. Fresh nectarine samples (1.0 g) were extracted in 99.8% methanol (3 mL) and centrifuged at 6,000 × g (Hettich Zentrifugen Mikro 220R) for 10 minutes at 5 °C. Supernatant aliquots (21 µL) and DPPH solution (194 µL) were stored in the dark for 30 minutes were introduced to LE-8404-PLATE_UV, then the absorbance was taken at 517 nm using the spectrophotometer. A negative control was prepared with 194 µL DPPH in 21 µL methanol, in order to observe the DPPH radical decay against the sample antioxidant capacity. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mg/100g AA fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

Antioxidant activity determinations to FRAP

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300mM acetate buffer (3.1 g C2H3NaO2 _ 3H2O and 16mL C2H4O2), pH 3.6, 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40mM HCl, and 20mM FeCl3 _ 6H2O solution. The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution, and 2.5mL FeCl3 _ 6H2O solution and then warmed at 37 ºC before using. Fresh nectarine samples (1.0 g) were extracted in 99.8% methanol (3 mL) and centrifuged at 6,000 × g (Hettich Zentrifugen Mikro 220R) for 10 minutes at 5 °C. Fruit extracts (6 µL) were allowed to react with 198 µL of the FRAP solution in LE-8404-PLATE_UV for 10 min in the dark condition. Readings

of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mg/100g AA fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Determinations of total phenols

Analysis of total phenols was performed in accordance with the Folin-Ciocalteu spectrophotometric method (Singleton Jr. and Rossi, 1965). Fresh nectarine samples (1.0 g) were extracted in 99.8% methanol (3 mL) and centrifuged at 6,000 × g (Hettich Zentrifugen Mikro 220R) for 10 minutes at 5 °C. Fruit extracts (19.2 µL) were allowed to react with 29 µL of Folin–Ciocalteau reagent and after 3 minutes in LE-8404-PLATE_UV, saturated solution of Na2CO3 was added, and the reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 750 nm and the results were expressed in mg phenols $100g^{-1}$ dry mass.

Extraction of phenolic compounds

The frozen fruit material (5g) was homogenized in a Polytron (1 min on ice) with 10 mL of extraction solution (water/methanol 50:50 containing 2 mM NaF to inactivate polyphenol oxidases and prevent phenolic degradation due to browning). Homogenates were kept in ice until centrifuged (11500 rpm, 15 min, 2-5 °C, 16000g); the supernatant was recovered carefully to prevent contamination with the pellet, and the volume was measured. A portion 1 ml of this extract was filtered through a 0.45 µm filter (Osmonics/MSI Cameo Nylon Filters, Fisher Scientific, Los Angeles, CA) and directly analyzed by HPLC after a period not exceeding 24 h. This extraction procedure recovers 85% of the total soluble anthocyanins, 86% of the hydroxycinnamates and flavonols, and 92% of the procyanidins present in the nectarine.

HPLC-DAD analyses

Samples of 50 µL of extracts were analyzed using an HPLC system (Hewlett-Packard 1050 pump) coupled with a photodiode array detector (DAD) (Series 1040M, Series II) and an autosampler (Series 1050), operated by HP ChemStation software. A reversed-phase C18 Nucleosil column (150 x 4.6 mm i.d.; particle size 5 µm) (MetaChem Technologies, Inc. Torrance, CA) with a guard column containing the same stationary phase (Safeguard holder 5001-CS) was used. Four pumps (A, B, C, and D) were used for mixing the mobile phase to avoid pressure fluctuations due to the mixing of methanol (MeOH) in water. Formic acid (5%) was added to both water and methanol to increase peak resolution before preparing the following mobile phases: 95% water + 5% methanol (A); 88% water + 12% MeOH (B); 20% water + 80% MeOH (C); and MeOH (D). All solvents were HPLC grade. Elution started with 100% A, which remained isocratic until 5 min. A gradient was then used to reach 100% B at 10 min, held isocratic for 3 more minutes. From 13 to 35 min a linear gradient was used to reach 75% B and 25% C, and then 50% B and 50% C at 50 min, and 100% C at 52 min, then maintained isocratic until 57 min. The column was then washed with 100% D at 60 min. The flow rate was 1 mL/min and chromatograms were recorded at 510, 340, and 280 nm. These later were used for analysis of the phenolic compounds of UVC treatment, and treatment AB + 8 kJ m^{-2}UV-C + High O_2 which had the highest concentration of total phenols (17.72 mg/100g).

Identification and Quantification of Phenolic Compounds

The phenolic compounds in fruit extracts were identified by chromatographic comparisons with authentic markers. Individual anthocyanins were quantified by comparisons with an external standard of cyanidin 3-rutinoside (Apin Chemicals Ltd., Oxon, UK) at 510 nm; flavonols as quercetin 3-rutinoside at 340 nm; hydroxycinnamic acid derivatives as chlorogenic acid at 340 nm; and flavan 3-ols as catechin at 280 nm (all these markers were from Sigma, St. Louis, MO).

Statistical analysis

The statistical design was completely randomized design with nine treatments and four times of assessment, and using three replicates per treatment. All data were statistically analyzed by an ANOVA with F test. Analyses were done in the software SISVAR (Ferreira, 2011).

Conclusion

In general the DPPH showed higher efficiency of antioxidant concentration in comparison with FRAP in minimally processed nectarines. There was clear trend in phenolic content in fresh-cut nectarine with treatment AB + 8 kJ m^{-2}UV-C + High O_2. The 22 phenolic compounds of nectarine were studied and quantified by HPLC. Hydroxycinnamates, flavonols, and anthocyanins were detected. These results allow to conclude that the most abundant compounds found in this Study of fresh-cut nectarine were chlorogenic acid, caffeic acid and ellagic acid with HPLC chromatograms recorded at 280 nm, quercentin 3-galactoside, quercetin 3-Xyloside at 340nm and Cyanidin 3- glucoside at 510nm.

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