DR. WILMAN CARRILLO (Orcid ID : 0000-0002-8517-0134)

Article type : Original Article

Effect of modified atmosphere packaging (MAP) in the antioxidant capacity of arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) fruits from Ecuador

Wilma Llerena[1,4], Iván Samaniego[2], Monserrat Navarro[3], Jacqueline Ortíz[1], Ignacio Angós[1,3], Wilman Carrillo[1*]

[1]Universidad Técnica de Ambato (UTA), Facultad de Ciencia e Ingeniería en Alimentos y Biotecnología, Av. Los Chasquis y Rio Payamino, 1801334, Ambato, Ecuador.

[2]Instituto Nacional de Investigaciones Agropecuarias (INIAP), Estación Experimental Santa Catalina, Quito, Ecuador.

[3]Universidad Pública de Navarra (UPNA), Departamento de Agronomía, Biotecnología y Alimentación, Edificio Los Olivos, Calle Cataluña, s/n, 31006 Pamplona, España.

[4] Universidad Técnica Estatal de Quevedo, Facultad de Ciencias Pecuarias, Ingeniería en Alimentos, Km 7 1/2 vía Quevedo-El Empalme, 120313 Los Ríos, Ecuador.

*Corresponding author: Wilman Carrillo, PhD. Adress: Av. Los Chasquis y Rio Payamino. Ambato-Ecuador. Telephone: +539980281086. Email: wi.carrillo@uta.edu.ec

ABSTRACT

This study aimed to determine the effects of the modified atmosphere packaging (MAP) in the antioxidant capacity (AC) of arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.). AC was evaluated by ABTS and DPPH methods. Fruits were cold stored using three MAPs (MAP 1:2.5%2.5% O_2: 5.0% CO_2); (MAP 2: 2.5% O_2: 2.5% CO_2) and (MAP 3: 80% O_2: 10% CO_2) for 1, 4, 7 and 10 days. ABTS showed the highest AC values with respect to DPPH. Arazá fruit AC was correlated with total flavonoids content (TFC) (r> 0.8360) and total polyphenols content (TPC) (r> 0.7252). Naranjilla fruit (r> 0.8188) and tree tomato 2.5% O_2: 2.5% CO_2 (r> 0.7365), TPC and TFC showed significant correlations with the DPPH. When using the canonical correlation analysis, it was observed that TPC is responsible for AC of the three fruits, showing strong correlations (0.9716) with DPPH.

Keywords: *antioxidants capacity, anthocyanins, carotenoids, flavonoids, modified atmosphere packaging*

Practical Applications

The present study has as practical applications to provide knowledge on the cold storage of araza *Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) fruits. It also describes the effect of modified atmosphere packaging (MAP) on the antioxidant capacity of the three fruits studied. This study provides knowledge about biological properties of these fruits during cold storage with modified atmosphere.

1. INTRODUCTION

Consumers demand a high amount of fresh fruit for its nutritional value, quality, and healthy properties. The consumption of fruits is highly recommended because through its consumption, the human body gets an important content of vitamins, minerals, fiber, tocopherols and polyphenols (Rico, Martin-Diana, Barat, and Barry-Ryan, 2007; Llerena et al., 2019; Canova, Bobbio and Manganelli, 2020; Deng, Mai and Niu, 2020). It is known that eating habits can have an influence for the prevention of metabolic diseases such as hypertension, diabetes, and obesity. Various studies have described many polyphenols compounds found in foods as fruits, vegetables, tea, and seeds. These polyphenols compounds can prevent damage caused by oxidative stress such as damage cell and damage in DNA (Zhu et al., 2019). Epidemiological studies suggest that there is a correlation between the high consumption of phenolic compounds in the diet and the reduction of cardiovascular disease risks. Probably the main antioxidant activity that has been associated with polyphenols is the ability to scavenge free radicals (Hu et al., 2020).

Arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) are a native crop of South America, belonging to the Myrtaceae and Solanaceae family (Espin et al.,2016; Llerena et al., 2019). These fruits have a high content of chlorophyll, polyphenols, carotenoids, flavonoids, and anthocyanins. The antioxidant activity of different extracts obtained from arazá, naranjilla and tree tomato using the ABTS, FRAP, DPPH and ORAC methods has been previously described (Contreras-Calderon et al., 2011). A direct correlation between polyphenol content and antioxidant activity has been established depending on the variety studied (Zebrowska et al., 2019). The antioxidant capacity adds a high quality to foods characterized with this biological property. The antioxidant capacity can increase their protection against lipid oxidation (Nardini and Garaguso, 2020). The phenolic composition can help to prevent several determined diseases. Diet food can help to prevent diseases such as hypertension, hypercholesterolemia, diabetes, obesity (Grosso et al., 2017).

Fruits and vegetables after harvesting begin to lose quality due to various chemical and enzymatic reactions that occur inside them during the technological and the storage processes. In addition, damage caused by cuts and handling during processing accelerates the deterioration of fruits causing economic losses to the food industry (Belay, Caleb, and Opara, 2019; Siddiq, Auras, Siddiq, Dolan, and Harte, 2020). The stress produced in the fruit by cuts or manipulation during processing can activate different defense mechanisms involved in the synthesis and/or degradation of antioxidant compounds such as polyphenols (Belay, Caleb, and Opara, 2019).

Currently, the food industry has a high interest in minimizing the packaging of processed foods and seeks to innovate in new quality products. Industry searches new products safe for the consumer free of chemical treatments (Opara, Hussein, Caleb, and Mahajan, 2015). Modified atmosphere in packaging (MAP) with the combination of cold storage, is a useful tool to preserve the quality and shelf life of fresh fruits and vegetables (Ma, Zhang, Bhandari, and Gao, 2017; Yousuf, Qadri, and Srivastava, 2018; Ozturk et al., 2019). MAP helps to extend the shelf life of fruits studied because a reduction in the respiratory rate of the fruit is achieved. MAP also prevents contamination during storage (Jo, An and Lee, 2014; Pace, Capotorto, Cefola, Minasi, Montemurro, and Carbone, 2020). In addition, MAP has a combination of gases in the free space of the packaging that allows to reduce physiological processes, oxidative processes, and the growth of microorganisms such as bacteria and fungi. Important parameters for the quality of the fruit such as pH, vitamin C content, sugars, fatty acids, tocopherol and polyphenols can be affected by MAP treatment (Ozturk et al., 2019; Boerzhijin, Makino, Hirai, Sotome, and Yoshimura, 2020). MAP can be an effective tool to increase certain compounds with interest for the shelf life of foods such as the content of polyphenols, carotenoids and flavonoids (Pinela et al, 2018). MAP can help the conservation of antioxidant capacity of fruits stored for a long time. The aim of the study was to evaluate the effect of MAP treatments in the AC of the Arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) Ecuadorian blackberry variety.

2. MATERIALS AND METHODS

2.1 Plant Material

Arazá (*Eugenia stipitata* McVaugh) clone 003 was obtained of the Coca city, Orellana province in the Amazonian region, in Ecuador. Naranjilla (*Solanum quitoense* Lam.) quitoense 2009 variety was obtained of the Chaco city, Napo province, Amazonian region. Tree tomato (*Solanum betaceum* Cav.) were obtained of Patate city, Tungurahua province in the Andean region, Ecuador.

2.2 Physiochemical properties analysis of fruits

The fruits were cleaned and washed, weighed, measured their degree of maturity, luminosity, fastness, brightness, chroma, pH, soluble solids and acidity was determined follow the Ecuadorian INEN (2015) standard. The fruits were collected hand-harvest and separated from the peduncles, transported in cardboard boxes (40 cm x 40 cm) previously disinfected with ethanol (70%) until

used in the assays with the MAPs and antioxidant activity. The boxes were transported in cold using a portable fridge at 10°C.

2.3 Postharvest preservation by MAP

For gas combinations, a KM100-3 Flow gas mixer (Witt Gasetechnik; Germany) was used, using food grade gas cylinders at oxygen (99.5% quality), and carbon dioxide (99.9% quality), acquired from Linde Ecuador. The generated gas mix was stored in a 10 L stainless steel pressure tank manufactured by (Witt Gasetechnik, Germany). The gas was distributed through a pneumatic fitting system consisting of a Harris type double stage pressure regulators, flow control valves, shut-off valves, reductions, quick connections and Teflon, polyurethane and polyamide pipes (Angós, 2008).

Each gas combination was compared against the control, the atmospheric air, injected into the storage chambers by means of an oil-free air compressor (Marathon Electric, Mexico), with an approximate flow rate of 450 mL/h. Prior to entering the storage chambers, all gases were passed through a scrubber, where they were bubbled through water to humidify the incoming mixture into the system until reaching a relative humidity of 90-95%. The process scheme is shown in Figure 1 for treatments with both MAPs and control-air of arazá, naranjilla and tree tomato. Arazá fruit (12 °C), naranjilla fruit (6 °C) and tree tomato (4 °C) were treated with MAPS for 1, 4, 7 and 10 days.

2.4 Total polyphenols content (TPC)

TPC was determined using the method described by Samaniego et al. (2020). The sample lyophilized was dissolved in methanol: water: formic acid (70:30:0.1 *v:v:v*). The sample were mixed using Vortex Mistral 4600 (Multi-Mixers; Illinois, USA). Then, the sample was subjected to an ultrasonic bath Cole-Parmer 8892-MTH (Cole-Parmer, Illinois, USA) and then centrifuged. A part of the sample was mixed with the reagent Folin-Ciocalteu and Na_2CO_3 at 20%. The absorbance was measured using a spectrophotometer (Shimadzu UV-VIS 2600 Kyoto, Japan) at a wavelength of 760 nm. Gallic acid was used as standard (0 to 100 ppm) for triplicate in three days ($n=9$). The curve of calibration was stablished ($y = 0.0117x + 0.0789$, $R^2 = 0.9995$). TPC was expressed in terms of mg of gallic acid equivalents GAE/100 g of dry weight (DW) sample.

2.5 Total flavonoids content (TFC)

TFC was determined according to the method described by Zhishen et al (1999). The sample was dissolved in methanol: water: formic acid (70: 30: 0.1, v: v: v). An aliquot of the supernatant was reacted with sodium nitrate, aluminum chloride and sodium hydroxide. The absorbance of the mixture was measured with the help of a Shimadzu UV-VIS 2600 spectrophotometer (Shimadzu, Kyoto, Japan) at 490 nm. The absorbance was measured at 430 nm. TFC was determined using a calibration curve of quercetin ($y = 0.0032x + 0.0669$, R^2=0.9998) for triplicate in three days (*n=9*). The results were expressed as mg quercetin/100 g dry weight (DW).

2.6 Total carotenoids content (TCC)

TCC determination was performed following the method described by Llerena (2019). The lyophilized sample of tree tomato was started by weighing in a gram of calcium chloride and a solution of hexane, acetone, ethanol and BHT in proportion 50: 25: 25: 0.1 (v: v: v: v) , to carry out the extraction. The mixture was stirred in a vortex (Mistral 4600, Multi-Mixers; Illinois, United States), and centrifuged. Finally, the extract was passed to a glass cell, to read the absorbance at 490 nm on a Shimadzu UV-VIS 2600 spectrophotometer (Kyoto, Japan).

The method was validated by performing a series of extractions to determine the precision of the method with 6 repetitions and for the accuracy different extraction cycles were performed to obtain 100% of the content of the bioactive component of the sample.

2.7 Total anthocyanins content (TAC)

The TAC determination was performed following the method described by Llerena et al. (2019). The lyophilized sample of tree tomato was made by weighing. Then, a pH 1.0 buffer solution (0.2 N HCl and 0.2 N NaCl) was added to carry out the extraction. The mixture was stirred in a vortex (Multi-Mixers; Illinois, United States), taken to an ultrasonic bath (Cole-Parmer 8892-MTH; Illinois, United States) and centrifuged. The same procedure was used considering that the pH 4.5 buffer (1M CH_3COONa and 0.1M HCl) was used. Finally, the extract was passed to a glass cell, to read the absorbance at 510 nm and 700 nm, respectively in a Shimadzu UV-VIS 2600 spectrophotometer (Kyoto, Japan). The TAC was calculated by absorbance differences of pH 1.0 minus pH 4.5, as described below:

$$\boldsymbol{A = [(A_{510} - A_{700})\mathbf{pH1.0} - (A_{510} - A_{700})\mathbf{pH4.5}]}$$

The method was validated by carrying out a series of extractions to determine the precision of the method with 6 repetitions for pulp and 5 repetitions for shell; and for accuracy, different extraction cycles were performed to obtain 100% of the content of the bioactive component of the sample.

2.8 Preparation of samples fruits

At each time assayed, the breathing chambers of D1R1 and D1R2 to D10R1 and D10R2 were removed from the refrigerated storage cold. The samples from each vessel was taken to perform the antioxidants tests (ABTS and DPPH assays). The skin of the fruits was separated from the pulp. The pulp was cut into slices, and pulp and shell were placed separately in hermetically sealed sleeves, then frozen at -80 °C and finally lyophilized to be stored until the respective analysis.

2.9 ABTS assay

The extracts obtained of pulp and the edible portion of arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) were treated with MAPs and then used to evaluate their antioxidant activity using the ABTS method described by Piñuel et al. (2019a). Trolox standard was used as the reference standard (0-800 µmol Trolox/L). The calibration curve was stablished at ($y = 0.0007x + 0.0671$, $R^2 = 0.999$). All assays were made six times (*n=6*). The results obtained were expressed as µmoL Trolox Equivalents TE/g sample, dry weight (DW).

2.10 DPPH assay

The extracts obtained of pulp and the edible portion of arazá (*Eugenia stipitata* McVaugh), naranjilla (*Solanum quitoense* Lam.) and tree tomato (*Solanum betaceum* Cav.) were treated with MAPs and then used to evaluate the antioxidant activity using the DPPH method described by Piñuel et al. (2019b). Trolox standard was used as the reference standard (0-800 µmol Trolox/L) and the calibration curve was stablished ($y = 0.0013x + 0.007$, $R^2 = 0.999$). All assays were made six times (*n=6*). The results obtained were expressed as µmoL TE/g sample, dry weight (DW).

.

2.11. Statistical analysis

The results of this study were expressed as mean ± standard deviation (SD). Significant statistical differences between treatments was evaluated by one-way ANOVA followed of Tukey´s test. Statistica version 10 software for Windows (StatSoft, Paris, France) was used. Two independent

experiments (MAPs) were performed with six separate measures (ABTS/DPPH) for all assays (*n=12*).

3 Results and Discussion

3.1 Physicochemical analysis of araza, naranjilla and tree tomato

Before subjecting the fruits to MAP treatments, the fruits were selected according to their physicochemical characteristics that allow determining their degree of maturity. Aspects such as weight, length, diameter, color, tone, and maturity index were determined. The fruit of the arazá in its maximum degree of maturity presented physicochemical values as a weight of 102.35 g, length of 5.54 cm, a diameter of 6.77 cm, tone of 55.65 h° , chroma of 87.91 c* and a maturity index of 2.20 MI. Naranjilla fruit present a weight of 139.83 g, length of 5.90 cm, diameter of 6.37 cm, tone of 81.23 h°, chroma of 64.41 c* and a maturity index of 3.54 MI. Tree tomato present a weight of 152.25 g, length of 8.54 cm. diameter of 6.26 cm, tone of 49.78 h°, chroma of 36.66 and a maturity index of 5.88 MI.

3.2 Phytochemical composition of arazá, naranjilla and tree tomato

TPC, TFC and TCC were measured in pulp from arazá and naranjilla. TPC, TFC, TCC and TAC were measured in pulp from tree tomato. Table 1 shows the results of phenolic composition. The values of TPC found were 1569.60, 775.31, 704.60 mg GAE/100 g, DW of sample, respectively. Arazá fruit present a TPC higher value than naranjilla and tree tomato. Some fruits are considered super fruits for their high content of polyphenols, flavonoids, carotenoids, anthocyanins, vitamins, and other phytocomponents. To be considered as a superfruit, TPC is expressed as mg GAE/100 g, with values between 113.02 to 1620.00 mg GAE/100 g (Chang, Alasalvar, & Shahidi, 2019). Our results show that arazá fruit could be considered a superfruit because its TPC content was of 1569.60 mg GAE/100 g DW. The TFC content was of 600.72 (mg catechin/100 g DW) and present a TCC value of 31.00 (µg β-carotene/g DW).

3.3 Antioxidant capacity of arazá, naranjilla and tree tomato treated with MAP

Table 1 shows the arazá, naranjilla and tree tomato AC evaluated using the ABTS and DPPH methods before treatment with MAPs (day 0). AC by ABTS method of arazá fruit present a high value of 758.22 (µmol TE/100 g DW). Arazá fruit present a high value of AC by DPPH method

with 392.10 (μmol TE/100 g DW) followed of tree tomato with a value of AC by ABTS of 161.04(μmol TE/100 g DW) and AC by DPPH method of 47.82 (μmol TE/100 g DW).

3.4 Evaluation of effect of MAPs in AC of arazá, naranjilla and tree tomato

Figure 2A shows the results of the MAP effect [2.5%O_2 : 5.0% CO_2] on AC evaluated with ABTS for the Arazá fruit. AC of the fruit treated with MAPs for 1 (926.15 μmol TE/100 g DW) and 4 (925.38 μmol TE/100 g DW) days of storage were higher than for zero days storage. The application of MAP produces an effect on the arazá AC. AC of the fruit treated with MAP was higher than the AC of the fruit treated with air. The AC by ABTS values obtained from the MAP treatment show that during the treatment period the AC of the fruit is maintained until day 4 of the test, showing a slight decrease at 7 and 10 days of storage cold. On the contrary, AC of the control treatment was lower on the first day of the test and reached its maximum value at 4 days, to subsequently decrease. MAP vs Control presented statistical differences ($p < .05$) in the storage times of 1 and 10 days. Between days 4 and 7 no significant differences were observed ($p < .05$).

Figure 2A shows the arazá AC treated with MAP measured by DPPH. A similar behavior to the one registered by ABTS was observed. AC of the fruit treated with MAP was higher than the AC of the fruit treated with air. The highest AC by DPPH method values were observed on day 4 of treatment and a slight decrease was observed on days 7 and 10. When the AC by DPPH method data from MAP were compared to the control, significant differences were observed ($p < .05$) during days 1, 4 and 7.

González-Aguilera et al. (2010) have described that most of the post-harvest treatments used to extend the useful life of the fruit, involve the alteration of its natural conditions. The fruits suffer from biotic stress. For these authors, the use of MAPs can affect the metabolic activity of the fruit and modify some biochemical processes, causing the activation of its antioxidant system. MAP would increase the content of phenolic compounds. This increase could be associated with the higher AC of the arazá stored under MAPs compared to the control sample.

Mashabela, Mahajan, and Sivakumar (2019) have described the effect of MAP films on the composition of biocompounds and the AC evaluated by FRAP and ABTS on fresh-cut cauliflower. They found that the samples treated with MAP in Polypropylene increased their

content of TPC, TFC, AC by FRAP method and AC by ABTS method during days 4 and 8 stored at 5 °C.

Figure 2B shows the results of AC by the ABTS method of naranjilla treated with MAP [2.5%O_2 : 5.0% CO_2] at 6 °C for 1, 4, 7 and 10 days. It was observed that the AC by the ABTS method treated with MAP presented lower values than the AC by the ABTS method of the control group. No significant differences ($p < .05$) were observed between the times tested for the MAP group or for the control group. No significant differences were observed when the AC by ABTS treated with MAP values are compared with the AC by ABTS-control values. Figure 2B shows the AC by DPPH results of MAP-treated orange and control during 1, 4, 7 and 10 days of storage at 6 °C. AC by DPPH-control presented higher values than AC by DPPH method treated with MAP.

Figure 2C shows the results obtained from AC by ABTS method of tree tomato fruit treated with MAP [2.5% O_2: 2.5% CO_2] and control for 1, 4, 7 and 10 days stored at 4 °C. It was observed that the fruit treated with MAP presented lower AC by ABTS method values than AC by ABTS-control. In the case of AC by DPPH method treated with MAP, higher values were observed than those obtained by AC by DPPH-control. No significant differences were found at $p<.05$ when MAP was compared to control, nor when the different times of each group were compared (Figure 2C).

Furthermore, the AC of tree tomato was evaluated under MAP treatment [80% O_2: 10% CO_2] for 1, 4, 7 and 10 days of storage at 4 °C. AC by ABTS method treated with MAP presented lower values than the control group (Figure 2D). AC by DPPH method treated with MAP showed an increase in activity on day 7 of storage at 4 °C (Figure 2D). No significant differences were observed at $p < .05$.

3.5 Analysis of correlation of Pearson of phenolic components and AC

To establish the relationship between the content of phenolic compounds and the AC/MAP [2.5% O_2: 2.5% CO_2] of arazá measured by ABTS and DPPH, a Pearson correlation analysis was performed (Table 2).

Table 2 shows that the arazá AC (ABTS and DPPH) showed a positive and statistically significant correlation ($p < .05$) with TPC and TFC; however, TCC showed negative correlations against this parameter. The highest correlation coefficients were associated with the AC values measured by ABTS (r = 0.95 and r = 0.96), followed by DPPH (r = 0.837 and r = 0.72).

Arazá fruit has a high TPC content. For this reason, its AC presents a direct correlation with the hydrophilic fraction of the bioactive compounds in the fruit, which includes polyphenols, flavonoids, and water-soluble vitamins. The high correlation (r close to 1) in ABTS and the DPPH associated with these compounds is due to their different redox characteristics (Contreras-Calderón et al., 2011; Cuellar, Ariza, Anzola, and Restrepo, 2013).

In the naranjilla fruit, it was observed that the highest AC values of the naranjilla corresponded to the measurements made by ABTS. However, when determining the Pearson's correlation coefficients (Table 2), it was observed that there is a significant correlation ($p < .05$) associated with AC by DPPH method.

There is a very high correlation between the TPC content (r = 0.9109) and AC by DPPH method of the fruit. Although somewhat lower, a very high correlation associated with TCC and TFC of the fruit can be seen (Table 2). Vasco, Ruales, and Kamal-Eldin (2008) report that the high correlation of AC by DPPH method, indicates that the antioxidants of naranjilla have a high antiradical efficiency. According to Mertz et al. (2009) the antioxidant and pro-oxidant activity of naranjilla is the result of the interaction of its free water-soluble components (organic acids, vitamin C), some water-soluble phenolic compounds and carotenoids, confirming the results obtained in this study.

As in the naranjilla, it has been observed that the phenolic components of the tree tomato are highly correlated with AC by DPPH method (Table 2), being statistically significant for TAC (r=0.793), TFC (r = 0.736) and TPC (r = 0.8767). However, the highest AC values were those recorded by ABTS.

Pearson's correlation analysis for the tree tomato case was also performed when the fruit was treated with MAP [80% O_2: 10% CO_2]. Table 2 shows that the AC by ABTS method presented statistically significant correlations ($P < 0.05$) with the content of TAC (r = 0.7259), TFC (r = 0.7944) and TPC (r = 0.7259). Despite its high degree of correlation (r = 0.7010), TCC did not show a significant effect on the AC of the fruit. In contrast, AC by DPPH method did not show a statistically significant correlation ($p<.05$) with any of the bioactive compounds in this fruit.

The results obtained demonstrate that the type of modified atmosphere that is applied for the post-harvest treatment of tree tomato can affect the phenolic components and the AC of the fruit. When applying classic atmospheres (2.5% O_2: 2.5% CO_2), it was observed that there is a high correlation between these components and the AC by DPPH method. Atmospheres with 80% O_2: 10% CO_2 are correlated with AC by ABTS method.

According to Zheng et al. (2003), in blueberry the AC (ORAC) is due to the polyphenols present in the fruit, since they are considered as strong antioxidant agents.

3.6 Analysis of correlation canonical

When performing the Pearson (r) correlation analysis, it was observed that depending on the type of fruit, there is a greater or lesser correlation of the different groups of antioxidant compounds with the AC by ABTS method or AC by DPPH method values, and in some cases there is no significant correlation with one of the methods used. Therefore, it was considered necessary to evaluate the effect of the antioxidant content of the three fruits on the AC determined by the two methods used (ABTS and DPPH) simultaneously. For this, a canonical correlation analysis (Figure 3) was used, the objective of which is to search for relationships between two groups of variables; the first group of variables considered are the antioxidants of the fruits and the second group, the one formed by the results of the antioxidant capacity. Figure 3 shows a high correlation between the content of total antioxidants (TAC, TCC, TFC and TPC) and the AC measured by ABTS and DPPH. The first canonical correlation found is statistically significant (p = 0.000), with a magnitude of 0.9716; considering himself particularly strong. The associated canonical equations (Equation 6 and 7) were expressed as follows:

$$AC=-0.0096\cdot TAC-0.1794\cdot TCC-0.1492\cdot TFT+0.8014\cdot TPC \quad \text{Ec 6}$$

$$TA=0.1253\cdot ABTS+0.8867\cdot DPPH \quad \text{Ec 7}$$

The coefficients of the canonical equations show how the variable called total antioxidants (TA) is fundamentally defined by the total polyphenol content of the fruits, while the variable antioxidant capacity (AC) is defined by the determinations obtained with the DPPH method.

Based on the AC of the fruits, two groups can be distinguished. The first group is made up of the arazá whose AC is mainly due to the TPC content. The second group is made up of naranjilla and tree tomato. This could be because the arazá is a fruit that has been recognized for its high AC. It has been observed in *in vitro* tests that it is effective in the elimination of radicals, neutralization of the harmful action of reactive species and transfer of hydrogen radicals, presenting high AC values by different methods: ABTS (435.31 µM Trolox/g), FRAP (245.67 µM Trolox/g), ORAC (260.76 µM Trolox/g) and TEAC (118.53 µM Trolox/g) (Bakkali, Ruiz-Larrea, and Ruiz-Sanz, 2010; Contreras-Calderón et al., 2011; Lizcano,). According to Neri-Numa et al. (2013) and Barros,

Andrade, Denadai, Nunes, and Narain, (2017) this AC of the fruit is due to the presence of high contents of gallic acid, ascorbic acid, myricetin, quercetin, cinnamic acid and traces of vanillic acid, kaempferol and naringenin.

The second group of fruits is composed of naranjilla preserved in MAP with 2.5% O_2: 5.0% CO_2 and tree tomato preserved in classic atmospheres (2.5% O_2: 5.0% CO_2) rich in oxygen and CO_2 (80% O_2 : 10% CO_2) presented a lower canonical correlation with their AC than the arazá. Vasco, Ruales and Kamal-Eldin (2008) indicate that the low antiradical efficiency and the low AC of these fruits may be because their phenolic compounds are weak antioxidants or are linked to other molecules such as carbohydrates, which considerably reduce their AC.

3.7 Antioxidant capacity of portion edible of araza, naranjilla and tree tomato

Table 3 presents the AC values expressed in mg of Trolox/100g fresh weight (FW) of edible portion of arazá, naranjilla and tree tomato preserved in MAPs during a period of 10 days at 4 °C.

The results of AC de arazá obtained in this study are comparable with the values published by other authors (Lizcano, Bakkali, Ruiz-Larrea, and Ruiz-Sanz2010; Contreras-Calderón et al., 2011; Neri-Numa et al., 2013). In this fruit it has been determined that its fresh consumption can provide an AC between 137.66 and 505.59 mg Trolox/100g FW, depending on the method used for its measurement (ABTS, FRAP, ORAC, TEAC, among others). Therefore, the values obtained in ABTS (192.53 mg Trolox/100g FW at the end of the MAP treatment period (2.5% O_2: 5.0% CO_2) were very close to those reported by these authors; however, AC by DPPH method (726.26 mg Trolox/100g FW is higher.

When adjusting the values of the CA of naranjilla on a wet basis, it was observed that its contribution can be very similar to the one of the arazá. In an edible portion of 100 grams of this fruit you can obtain between 80.09 and 410.48 mg Trolox/100g FW (Vasco, Ruales and Kamal-Eldin, 2008; Mertz et al., 2009; Contreras-Calderón et al., 2011). As in arazá, the values obtained will depend on the method used for its determination (ABTS, DPPH, FRAP and ORAC). In this fruit, MAP (2.5% O_2: 5.0% CO_2) also allowed to preserve the AC of the naranjilla since the values obtained are within the range obtained by these authors for ABTS (113.71 mg Trolox/100g FW) and DPPH (357.32 mg Trolox/100g FW).

In tree tomato it was observed that when adjusting the results of the AC of the fruit on a wet basis, its value increased. Thus, the AC by the methods of ABTS (168.61 and 171.32 mg Trolox/100g FW) and DPPH (521.84 and 454.35 mg Trolox/100g FW) for fruits preserved in atmospheres with

2.5% O_2: 2.5% CO_2 and 80% O_2 : 10% CO_2; respectively; showed AC values within the ranges shown (45.07 to 606.95 mg Trolox/100g FW) by Espin et al. (2016), Mertz et al. (2009), Schotsmans (2011) and Vasco, Ruales and Kamal-Eldin, (2008) for purple tree tomato of different origins and cultivars, measured by ABTS, DPPH, FRAP, ORAC and AA-TROLOX. Finally, it can be concluded that the application of MAPs for the conservation of tropical fruits (arazá and naranjilla) and Andean (tree tomato) allows to maintain their antioxidant properties.

4. CONCLUSIONS

The climacteric fruits such as the arazá and naranjilla responded better to the treatments with MAPs than tree tomato. Due to its physiological characteristics, this fruit continues its breathing process after harvest. AC by the ABTS method and AC by the DPPH method of naranjilla fruit increased after MAP treatment at 6 °C. The highest AC values obtained in the three fruits corresponded to the results obtained by ABTS.

During the MAPs treatment period, the arazá presented a higher AC by the ABTS method and AC by the DPPH method than naranjilla and tree tomato. Pearson's correlation analyzes showed that AC was related to DPPH assays. The arazá fruit presented statistically significant correlations in ABTS and DPPH. The canonical correlation analysis showed that the TPC content is the main responsible for the AC of the three fruits, showing a strong correlation between the antioxidant content and the AC of arazá, naranjilla and tree tomato. Fruit treatment with MAPs can be used to preserve the AC at different storage times under cold.

ACKNOWLEDGEMENTS

The authors wish to thank Jonathan Sánchez and Colegio Padre Miguel Gamboa (El Coca, Ecuador) for growing and free supplying the Araza fruits, SENESCYT for their contribution through the Project Prometheus Grant awarded to Dr. Angós and the funding from the Dirección de Investigación y Desarrollo (DIDE) of the Technical University of Ambato through its open call for research projects, and Fundación Carolina for the financial support of MSc. Llerena during her stage at the Public University of Navarre.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article

REFERENCES

Angós, I. (2008). Empleo de atmósferas ricas en CO_2 y altas concentraciones de O_2 en la mejora de las características de calidad de patata mínimamente procesada del cv. Monalisa almacenada en refrigeración. (Doctoral Thesis), 2008. Universidad Pública de Navarra, Pamplona, Spain.

Barros, R. G. C., Andrade, J. K. S., Denadai, M., Nunes, M. L., & Narain, N. (2017). Evaluation of bioactive compounds potential and antioxidant activity in some Brazilian exotic fruit residues. *Food Research International, 102*, 84-92. https://doi.org/10.1016/j.foodres.2017.09.082

Belay, Z. A., Caleb, O. J., & Opara, U. L. (2019). Influence of initial gas modification on physicochemical quality attributes and molecular changes in fresh and fresh-cut fruit during modified atmosphere packaging. *Food Packaging and Shelf Life*, *21,* 100359. https://doi.org/10.1016/j.fpsl.2019.100359

Boerzhijin, S., Makino, Y., Hirai, M. Y., Sotome, I., & Yoshimura, M. (2020). Effect of perforation-mediated modified atmosphere packaging on the quality and bioactive compounds of soft kale (*Brassica oleracea* L. convar. *acephala* (DC) *Alef. var. sabellica* L.) during storage. *Food Packaging and Shelf Life*, *23*, 100427. https://doi.org/10.1016/j.fpsl.2019.100427

Canova, L., Bobbio, A., & Manganelli, A. M. (2020). Predicting fruit consumption: A multi-group application of the Theory of Planned Behavior. *Appetite*, *145*, 104490. https://doi.org/10.1016/j.appet.2019.104490

Chang, S. K., Alasalvar, C., & Shahidi, F. (2019). Superfruits: Phytochemicals, antioxidant efficacies, and health effects A comprehensive review. *Critical Reviews in Food Science and Nutrition*, *59*, 1580-1604. https://doi.org/10.1080/10408398.2017.1422111

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., & García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International, 44*(7), 2047-2053. doi: https://doi.org/10.1016/j.foodres.2010.11.003

Cuellar, F. A., Ariza, E., Anzola, C., & Restrepo, P. (2013). Research of antioxidant capacity of araza (Eugenia stipitata Mc Vaugh) during the ripening. *Revista Colombiana de Química, 42*, 21-28.

Deng, S., Mai, Y., & Niu, J. (2019). Fruit characteristics, soluble sugar compositions and transcriptome analysis during the development of Citrus maxima "seedless", and identification of SUS and INV genes involved in sucrose degradation. *Gene*, 689, 131-140. https://doi.org/10.1016/j.gene.2018.12.016

Gonzalez-Aguilera, G. A., Villa-Rodrigueza, J. A., Ayala-Zavalaa, J. F., & Yahia, E. M. (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Review. Trends in Food Science & Technology, 21*(1), 475-482. https://doi.org/10.1016/j.tifs.2010.07.004

Grosso, G., Micek, A., Godos, J., Pajak, A., Sciacca, S., Galvano, F., & Giovannucci, E. L. (2017). Dietary flavonoid and lignan intake and mortality in prospective cohort studies: Systematic review and dose-response meta-analysis. *American Journal of Epidemiology*, *185*(12), 1304-1316. https://doi.org/10.1093/aje/kww207

Hu, J., Wang, Z., Tan, B. K., & Christian, M. (2020). Dietary polyphenols turn fat "brown": A narrative review of the possible mechanisms. *Trends in Food Science & Technology.97,* 221-232. https://doi.org/10.1016/j.tifs.2020.01.013

Instituto Ecuatoriano de Normalización (INEN). (2009). Frutas Frescas *Tomate de árbol, requisitos* (Vol. NTE INEN 1909: 2009, pp. 12). Quito, Ecuador: Norma Técnica Ecuatoriana.

Jo, Y. H., An, D. S., & Lee, D. S. (2014). Active air flushing in a sensor-controlled fresh produce container system to maintain the desired modified atmosphere. *Biosystems Engineering*, 125, 122-127. https://doi.org/10.1016/j.biosystemseng.2014.07.003

Lizcano, L. J., Bakkali, F., Ruiz-Larrea, M. B., & Ruiz-Sanz, J. I. (2010). Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chemistry*, *119*, 1566-1570. https://doi.org/10.1016/j.foodchem.2009.09.043

Llerena, W., Samaniego, I., Angós, I., Brito, B., Ortiz, B., & Carrillo, W. (2019). Biocompounds Content Prediction in Ecuadorian Fruits Using a Mathematical Model. *Foods*, *8*(8), 284. https://doi.org/10.3390/foods8080284

Ma, L., Zhang, M., Bhandari, B., & Gao, Z. (2017). Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends in Food Science & Technology*, *64,* 23-38. https://doi.org/10.1016/j.tifs.2017.03.005

Mashabela, M., Mahajan, P. V., & Sivakumar, D. (2019). Influence of different types of modified atmosphere packaging films and storage time on quality and bioactive compounds in fresh-cut cauliflower. *Food Packaging and Shelf Life*, *22*, 100374. https://doi.org/10.1016/j.fpsl.2019.100374

Mertz, C., Gancel, A.-L., Gunata, Z., Alter, P., Dhuique-Mayer, C., Vaillant, F., Perez, A. M., Ruales, J., & Brat, P. (2009). Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits. *Journal of Food Composition and Analysis, 22*(5), 381-387. doi: https://doi.org/10.1016/j.jfca.2008.06.008

Nardini, M., & Garaguso, I. (2020). Characterization of bioactive compounds and antioxidant activity of fruit beers. *Food Chemistry*, *305*, 125437. https://doi.org/10.1016/j.foodchem.2019.125437

Neri-Numa, I. A., Carvalho-Silva, L. B., Morales, J. P., Malta, L. G., Muramoto, M. T., Ferreira, J. E. M., de Carvalho, J. E., Ruiz, A. L. T. G., Maróstica Junior, M. R., & Pastore, G. M. (2013). Evaluation of the antioxidant, antiproliferative and antimutagenic potential of araçá-boi fruit (Eugenia stipitata Mc Vaugh Myrtaceae) of the Brazilian Amazon Forest. *Food Research International, 50*(1), 70-76. https://doi.org/10.1016/j.foodres.2012.09.032.

Opara, U. L., Hussein, Z., Caleb, O. J., & Mahajan, P. V. (2015). Investigating the effect of perforation and storage temperature on water vapour transmission rate of packaging film; experimental and modelling approaches. *Wulfenia Journal*, *22,* 498–509.

Ozturk, A., Yildiz, K., Ozturk, B., Karakaya, O., Gun, S., Uzun, S., & Gundogdu, M. (2019). Maintaining postharvest quality of medlar (*Mespilus germanica*) fruit using modified atmosphere packaging and methyl jasmonate. *LWT- Food Science and Technology*, *111,* 117-124. https://doi.org/10.1016/j.lwt.2019.05.033

Pace, B., Capotorto, I., Cefola, M., Minasi, P., Montemurro, N., & Carbone, V. (2020). Evaluation of quality, phenolic and carotenoid composition of fresh-cut purple Polignano carrots stored in modified atmosphere. *Journal of Food Composition and Analysis*, *86,* 103363. https://doi.org/10.1016/j.jfca.2019.103363

Pinela, J., Barros, L., Barreira, J. C., Carvalho, A. M., Oliveira, M. B. P., Santos-Buelga, C., & Ferreira, I. C. (2018). Postharvest changes in the phenolic profile of watercress induced by

post-packaging irradiation and modified atmosphere packaging. *Food Chemistry*, *254,* 70-77. https://doi.org/10.1016/j.foodchem.2018.01.181

Piñuel, L., Boeri, P., Zubillaga, F., Barrio, D. A., Torreta, J., Cruz, A., ... & Carrillo, W. (2019b). Production of White, Red and Black Quinoa (*Chenopodium quinoa* Willd Var. Real) Protein Isolates and Its Hydrolysates in Germinated and Non-Germinated Quinoa Samples and Antioxidant Activity Evaluation. *Plants, 8,* 257. https://doi.org/10.3390/plants8080257

Piñuel, L., Vilcacundo, E., Boeri, P., Barrio, D. A., Morales, D., Pinto, A., ... & Carrillo, W. (2019a). Extraction of protein concentrate from red bean (*Phaseolus vulgaris* L.): Antioxidant activity and inhibition of lipid peroxidation. *Journal of Applied Pharmaceutical Science, 9,* 1-14. https://doi.org/10.7324/JAPS.2019.90804

Rico, D., Martin-Diana, A. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology*, *18,* 373-386.

Samaniego, I., Espín, S., Quiroz, J., Ortiz, B., Carrillo, W., García-Viguera, C., & Mena, P. (2020). Effect of the growing area on the methylxanthines and flavan-3-ols content in cocoa beans from Ecuador. *Journal of Food Composition and Analysis, 88,* 103448. https://doi.org/10.1016/j.jfca.2020.103448

Schotsmans, W.C. (2011). Tamarillo (*Solanum betaceum* Cav.). In Woodhead Publishing Limited (Ed.), *Postharvest biology and technology of tropical and subtropical fruits* (pp. 427-442).

Siddiq, R., Auras, R., Siddiq, M., Dolan, K. D., & Harte, B. (2020). Effect of modified atmosphere packaging (MAP) and NatureSeal® treatment on the physico-chemical, microbiological, and sensory quality of fresh-cut d'Anjou pears. *Food Packaging and Shelf Life*, *23,* 100454. https://doi.org/10.1016/j.fpsl.2019.100454

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry, 111*, 816-823. doi: https://doi.org/10.1016/j.foodchem.2008.04.054

Yousuf, B., Qadri, O. S., & Srivastava, A. K. (2018). Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: A review. *LWT-Food Science and Technology, 89,* 198-209. https://doi.org/10.1016/j.lwt.2017.10.051

Żebrowska, J., Dyduch-Siemińska, M., Gawroński, J., Jackowska, I., & Pabich, M. (2019). Genetic estimates of antioxidant properties in the conventionally and in vitro propagated

strawberry (*Fragaria* × *ananassa* Duch.). *Food chemistry*, *299,* 125110. https://doi.org/10.1016/j.foodchem.2019.125110

Zheng, Y., Wang, C. Y., Wang, S. Y., & Zheng, W. (2003). Effect of High-Oxygen Atmospheres on Blueberry Phenolics, Anthocyanins, and Antioxidant Capacity. *Journal of Agricultural and Food Chemistry, 51*, 7162-7169. https://doi.org/10.1021/jf030440k

Zhishen. J., Mengcheng. T. & Jianming. W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry. 64*(4). 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zhu, M., Huang, Y., Wang, Y., Shi, T., Zhang, L., Chen, Y., & Xie, M. (2019). Comparison of (poly) phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. *Food chemistry*, *271,* 425-432. https://doi.org/10.1016/j.foodchem.2018.07.151

Figures and Tables

Figure 1. Diagram of treatment MAP1 [2.5% O_2:5.0% CO_2], MAP2 [2.5% O_2: 2.5% CO_2] and MAP3 [80% O_2:10% CO_2] used to arazá, naranjilla and tree tomato fruits from Ecuador. D1R1, D4R1, D7R1 and D10R1 (Day 1, 4, 7 and 10 of the assay 1). D1R2, D4R2, D7R2 and D10R2 (Day 1, 4, 7 and 10 of the assay 2). TR (respiratory rate). Storage cold: Arazá 12°C, naranjilla 6°C and tree tomato 4°C.

Figure 2. Effect of MAPs in the AC by ABTS and DPPH of arazá, naranjilla and tree tomato . A) Analysis of arazá fruit with MAP [2.5% O2: 5.0% CO2]. MAP (•) green point, B) Analysis of naranjilla with MAP [2.5% O2: 5.0% CO2]. MAP (•) yellow point, C) Analysis of tree tomato with MAP [2.5% O2: 2.5% CO2]. MAP (•) red point. D) Analysis of tree tomato with MAP [80% O2: 10% CO2]. MAP (•) red point and control-air (•) black point.

A, B: significant differences between MAPs and control

a, b, c: significant differences between time of storage (1, 4, 7 and 10 days)

Figure 3. Analysis of correlation canonical of arazá, naranjilla and tree tomato. MAP1 [2.5% O_2:5.0% CO_2], MAP2 [2.5% O_2: 2.5% CO_2] and MAP3 [80% O_2:10% CO_2].

Table 1.

Determination of total polyphenol content (TPC), total flavonoids content (TFC), total carotenoids content (TCC) and total anthocyanins content (TAC) in araza, naranjilla and tree tomato fruits and Antioxidant activity of arazá, naranjilla and tree tomato using ABTS and DPPH methods before treatment with MAPs.

Results of phenolic components were expressed as mean ± standard deviation (*n=9*).
Results of antioxidant activity were expressed as mean ± standard deviation (*n=6*). Different letters represent statistical different with (*P<0.05)* by One-way ANOVA of column results.

Table 2. Analysis of coefficient of correlation of Pearson (r) between antioxidant capacity (ABTS and DPPH) and phenolic component of arazá and naranjilla and tree tomato treated with MAPs.
* Significant statistical (*p<.05*) at a confidence level of 95%. MAP 1 [2.5% O_2: 5.0% CO_2], MAP 2[2.5% O_2: 2.5% CO_2] and MAP 3 [80% O_2: 10% CO_2].

Table 3. Antioxidant capacity of edible portion of arazá, naranjilla and tree tomato fruits.

Results were expressed as mean ± standard deviation (*n=6*). Different letters represent statistical different with (*p<.05)* by One-way ANOVA of column results.

Table 1. Determination of total polyphenol content (TPC), total flavonoids content (TFC), total carotenoids content (TCC) and total anthocyanins content (TAC) in araza, naranjilla and tree tomato fruits and Antioxidant activity of arazá, naranjilla and tree tomato using ABTS and DPPH methods before treatment with MAPs.

	Antioxidant capacity (µmol TE/g)±SD		**Phenolic components**			
Sample	**ABST**	**DPPH**	**TPC (mg GAE/100 g DW)**	**TFC (mg catechin/100 g DW)**	**TCC (µg β-carotene/g DW)**	**TAC (mg Cy-3-glu/100 g DW)**
Arazá	758.22 ± 5.01^{c}	392.10 ± 9.67^{c}	1569.60 ± 3.31	600.72 ± 22.25	31.00 ± 0.22	N.D
Naranjilla	76.40 ± 1.33^{a}	21.26 ± 1.35^{a}	775.31 ± 18.26	991.57 ± 20.24	34.08 ± 2.42	N.D
Tree tomato	161.04 ± 8.48^{b}	47.82 ± 2.94^{b}	704.60 ± 3.18	237.86±7.88	31.27 ± 0.19	344.19 ± 3.31

Results of phenolic components were expressed as mean ± standard deviation (*n=9*).

Results of antioxidant activity were expressed as mean ± standard deviation (*n=6*). Different letters represent statistical different with (*P<0.05)* by One-way ANOVA of column results.

Table 2. Analysis of coefficient of correlation of Pearson (r) between antioxidant capacity (ABTS and DPPH) and phenolic component of arazá and naranjilla and tree tomato treated with MAPs.

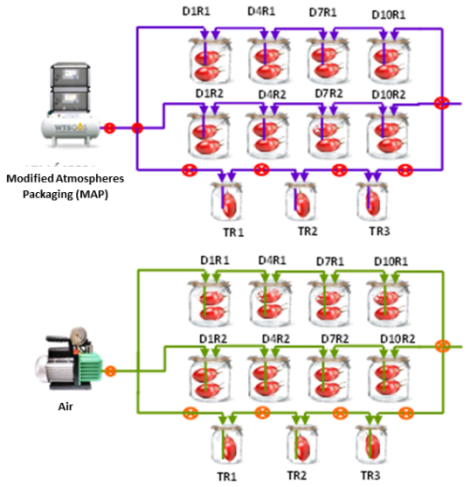
Fruits	Treatment	Biocompounds	Coefficient of correlation of Pearson (r)	
			ABTS	DPPH
Arazá	MAP 1	TCC	-0.4686	-0.5580
		TFC	0.9507*****	0.8360*
		TPC	0.9637*****	0.7252*
Naranjilla	MAP 1	TCC	0.3166	0.8258*
		TFC	-0.4945	0.8188*
		TPC	0.1661	0.9109*
Tree tomato	MAP 2	TAC	0.2252	0.7930*
		TCC	0.5641	0.6589
		TFC	0.2412	0.7365*
		TPC	0.1463	0.8767*
Tree tomato	MAP 3	TAC	0.7259*	0.5429
		TCC	0.7010	0.2120
		TFC	0.7944*	0.2024
		TPC	0.7259*	0.5429

* Significant statistical (*P<0.05*) at a confidence level of 95%. MAP 1 [2.5% O_2: 5.0% CO_2], MAP 2[2.5% O_2: 2.5% CO_2] and MAP 3 [80% O_2: 10% CO_2].

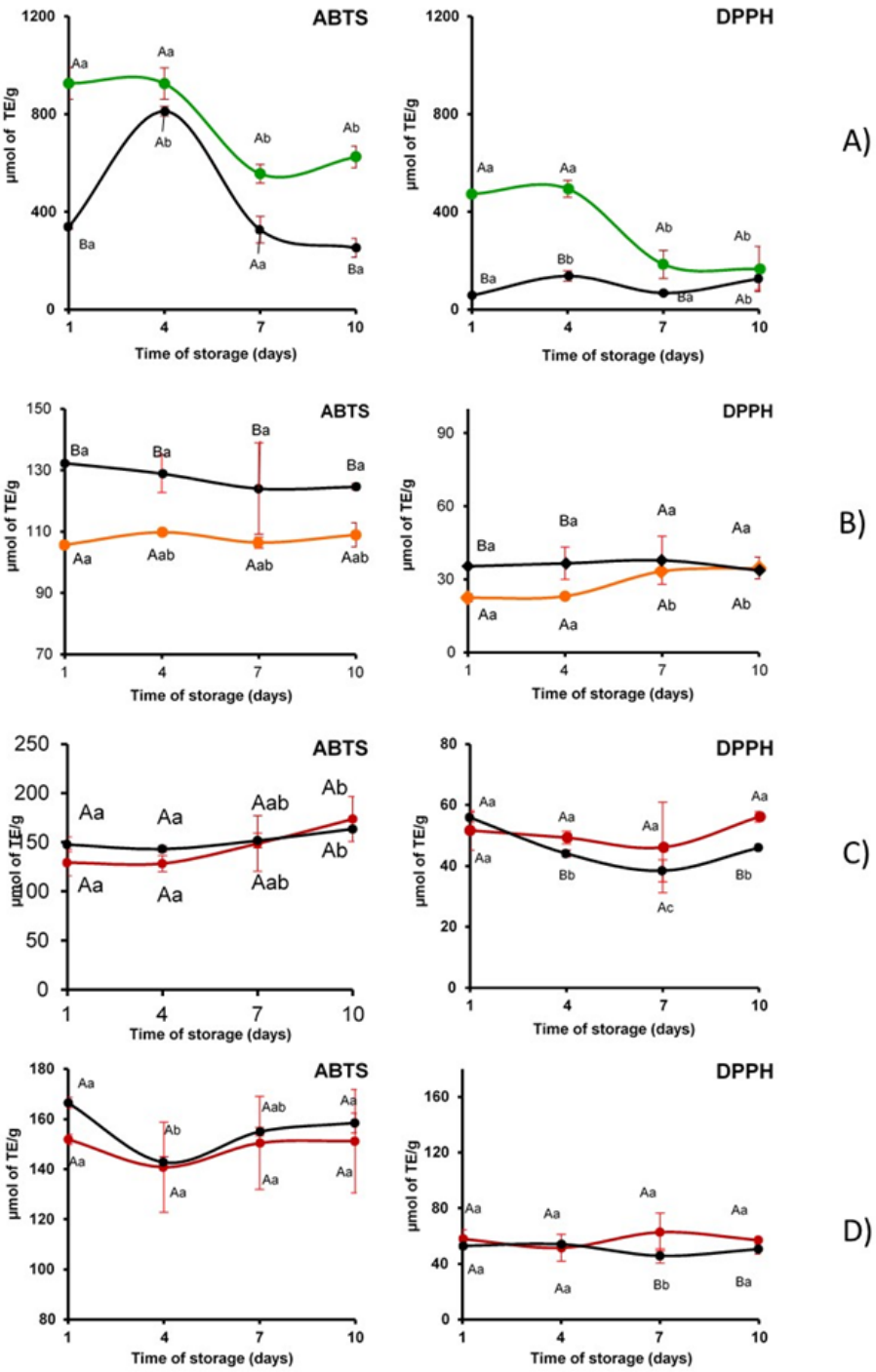
Table 3. Antioxidant capacity of edible portion of arazá, naranjilla and tree tomato fruits.

Time of storage (Days)	Antioxidant capacity (mg of TE/100g FW)							
	Arazá ($2.5\%O_2$: $5.0\%CO_2$)		Naranjilla ($2.5\%O_2$:$5.0\%CO_2$)		Tree tomato ($2.5\%O_2$: $2.5\%CO_2$)		Tree tomato ($80\%O_2$:$10\%CO_2$)	
	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH
1	549.16	1075.67	73.60	346.51	155.22	388.72	174.38	456.40
4	574.49	1074.77	75.68	360.10	148.08	385.69	154.93	423.10
7	214.93	645.97	109.34	348.96	138.42	447.01	188.68	452.04
10	192.53	726.26	113.71	357.32	168.61	521.84	171.32	454.35

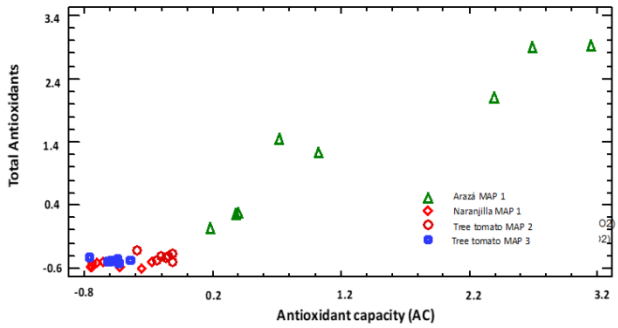
Results were expressed as mean ± standard deviation ($n=6$). Different letters represent statistical different with ($P<0.05$) by One-way ANOVA of column results.



jfpp_14757_f1.tif



jfpp_14757_f2.tif



jfpp_14757_f3.tif