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Evaluation of the use of reactive oxygen species (ROS) generated through oxyion® technology in strawberry (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier cv. Monterrey) storage

Evaluación del uso de especies reactivas de oxigeno (ERO) generadas a través de la tecnología oxyion® en el almacenamiento de fresa (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier cv. Monterrey)

Rafael Andrés Ramírez- Alvarado[1]*, Alejandro Jiménez[1], Claudia Patricia Pérez- Rodríguez[2] and Laura Alejandra Restrepo-Flórez[3]

[1]Universidad Nacional Abierta y a Distancia UNAD. Bogotá D.C., Colombia. [2]Faculty of Engineering. Universidad Nacional de Colombia campus Bogotá. Bogotá D.C., Colombia. [3]Sepa Consultores S.A.S. Bogotá D.C., Colombia. Author for correspondence: raramireza@unal.edu.co

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Abstract

Reactive oxygen species (ROS) play a key role in oxidative stress processes at the biological level. In most cases the presence of these chemical species is undesirable due to the impact they have on tissues and cellular structures, however, their effects can be used to control the incidence of microorganisms responsible for deterioration processes in fruits and vegetables. In the present study the feasibility of combining low temperature storage with the presence of reactive oxygen species generated using Oxyion® technology to control the deterioration process in strawberry (*Fragaria X ananassa*) was studied. The treatments used were as follows: control storage (4°C ± 2°C without Oxyion®) and ROS storage (4°C ± 2°C with Oxyion®), for two product categories according to weight and maturation state according to NTC 4103 (ICONTEC, 1997). The variables were monitored with measuring points at 1, 3, 5, 7, 10 and 15 days after harvest in percentage of loss of mass, soluble solids, respiratory intensity, acidity, resistance, color and ethylene and significant interactions among variables. At the conclusion of the study, Oxyion® technology was found to have statistically significant differences compared to control, and have allowed to less weight loss, higher resistance and lower exogenous ethylene production, extending the life of strawberry cv. Monterrey in a 40% during storage, additionally the losses by action of microorganisms present in strawberries surface were reduced considerably, generating a positive precedent in the processes of storage and conservation of fruits for Colombia.

Keys words: Food technology; keeping quality; oxidative stability; oxidative stress; postharvest conservation, postharvest physiology; shelf life.

Resumen

Las especies reactivas de oxigeno (ROS) tienen un papel primordial en los procesos de estrés oxidativos a nivel biológico. En la mayoría de los casos la presencia de dichas especies químicas es indeseable debido al impacto que tienen sobre tejidos y estructuras celulares, sin embargo, sus efectos pueden ser empleados para controlar la incidencia de microorganismos responsables de procesos de deterioro en frutas y hortalizas. En el presente estudio se estudió la viabilidad de compaginar el almacenamiento a baja temperatura con la presencia de especies reactivas de oxigeno generadas empleando la tecnología Oxyion® para el control del proceso de deterioro en fresa (*Fragaria X ananassa*). Los tratamientos empleados fueron almacenamiento control (4°C± 2°C sin Oxyion®) y almacenamiento ROS (4°C± 2°C con Oxyion®), para dos categorías del producto de acuerdo con el peso y estado de maduración según la NTC 4103 (ICONTEC, 1997). Las variables fueron monitoreadas con puntos de medición en 1, 3, 5, 7, 10 y 15 días después de cosecha en porcentaje de pérdida de masa, sólidos solubles, intensidad respiratoria, acidez, resistencia, color y etileno. Adicionalmente se analizaron los efectos y las interacciones significativas entre variables. Al concluir el estudio, se determinó que la tecnología Oxyion® presentó diferencias estadísticamente significativas en comparación con el control, e indujo a una menor pérdida de peso, mayor resistencia y menor producción exógena de etileno, prolongando la vida útil de la fresa cv. Monterrey en un 40% durante almacenamiento, adicionalmente las pérdidas por acción de microorganismos presentes en la superficie de las fresas se redujeron de forma considerable, generando un precedente positivo en los procesos de almacenamiento y conservación de frutas para Colombia.

Palabras clave: Conservación poscosecha; estabilidad oxidativa; estrés oxidativo; fisiología poscosecha; mantener la calidad; tecnología de alimentos; vida útil.

Introduction

Strawberry (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier) is notable for its content of vitamin C, tannins, flavonoids, anthocyanins, catechin, quercetin and kaempferol, organic acids being the citric which occurs in higher concentration followed by malic acid, oxalic, salicylic and ellagic and minerals (K, P, Ca, Na and Fe), in addition to pigments and essential oil (Pinto, Lajolo & Genovese, 2008). These compounds present in strawberry have a powerful antioxidant power that used as additives in foods could help to reduce the risk of cardiovascular events, improve vascular endothelial function and reduce the risk of thrombosis (Giampieri, Alvarez-Suarez, Mazzoni, Romandini, Bompadre, Diamanti, Capocasa, Mezzetti, Quiles, Ferreiro, Tulipani & Battino, 2013).

Despite its benefits, strawberry has a relatively short post-harvest life of 3-5 days in refrigerated conditions at 3°C to 4°C, which can be influenced and reduced by the action of environmental pathogens, especially fungi and bacteria. In this way, there is a need to evaluate technologies that increase the shelf life of the product, such as Oxyion®.

This technology is usually applied in isolated and refrigerated rooms in which the circulating air is subjected to a controlled electric reaction process that produces filtrations, which facilitate the production of reactive oxygen species (ROS) that are emitted through the tube diffuser of the equipment. The term reactive oxygen species, is used to include the hydrogen peroxide molecule, which is not a free radical, according to theoretical definition, but its chemical properties are similar to those of superoxide, and can easily form the hydroxyl radicals that are very reactive (Halliwell, 2006). In the beginning, chain reactions requires energy and subsequent steps that are exothermic happen spontaneously, with enzymes participation, oxygen can receive an excess of energy and produce an oxygen singlet ($1O_2$), which is a highly reactive molecule when compared to O_2, the singlet can transfer its energy to other biological molecules or react with itself, producing endoperoxides or hydroperoxides (Vranová, Inzé & Van Breusgem, 2002).

H_2O_2 is a moderately reactive molecule and can diffuse at some distance from the point of production, H_2O_2 can inactivate enzymes by oxidizing thiol groups, for example, enzymes from the Calvin cycle, Cu-Zn superoxide dismutase and Fe-superoxide dismutase can be inhibited by H_2O_2. The hydroxyl radical (OH.) that is formed from H_2O_2 is the most reactive, because the cells do not have an enzymatic mechanism to eliminate them, OH- radicals can react with all biomolecules and excess production that leads to cell death (Halliwell, 2006).

In this sense, Oxyion® technology performs a sanitizing effect, which have allowed to reduce the process of fungal reproductive structures formation, inhibit the development of bacteria, added to its antagonistic effect with ethylene molecule, produced during fruit maturation process. The present study seeks to determine the impact that involves the presence of reactive oxygen species (ROS) generated through Oxyion® technology in strawberry fruits storage at 4°C in order to provide new alternatives for post-harvest management of strawberry.

Material and methods

Plant material

Plant material used was supplied by Mancera Strawberries Company located in the municipality of Suesca, 59 km north of Bogotá, Cundinamarca. Strawberry crops are located at altitude of 2600 m.a.s.l., with an average annual temperature of 14° C, minimum night temperature average of 5°C and maximum dayly temperature average of 24°C. For the development of experimental design, a previous statistical survey was carried out on strawberry fruits classified into two categories as follows: category 1 = 13.5g to 19.5g and category 2 = 20.5g to 26.5g. Experimental design consisted of a group which was subjected to storage in a cold room at a temperature of 4°C ± 2°C, for a period of 15 days with the presence of an Oxyion® device operating at 50% power. In another cold room, products were stored under same conditions without the presence of the generator equipment of reactive oxygen species as control treatment. The evaluated parameters were tested on samples randomly selected from boxes of the same batch. Temperature and relative humidity of the rooms was monitored through humidity and temperature sensor with built-in EXTECH® datalogger, every 30 minutes during the storage time as shown in Figure 1.

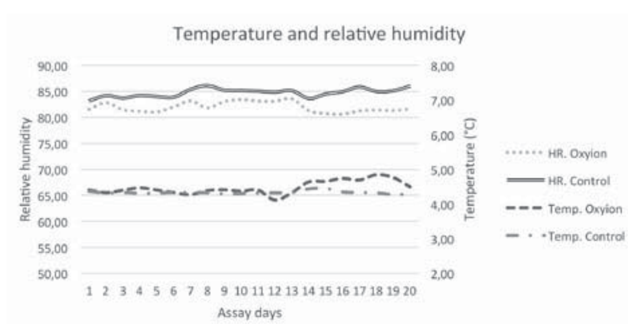

Figure 1. Temperature and relative humidity for control room and room with Oxyion® device.

Percentage of weight loss

The percentage of weight loss was determined by gravimetric analysis using digital analytical balance. Percentage of weight loss was calculated using Equation 1.

$$\%\ \text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Equation 1

Content of soluble solids

To determine soluble solids, fruit juice was extracted from three strawberry samples in a mortar, then filtered using 20 to 25 micron filter paper and measured in a Hanna™ digital refractometer, using distilled water as a target.

Respiratory intensity

In the determination of respiratory intensity a Vernier breatharometer LABQUEST™ was used. An initial calibration was made bringing the equipment to 380 ppm of CO_2, for 2 minutes, strawberry samples were taking into account their weight in grams and the occupied volume, subsequently, they were placed into a closed chamber of the equipment and measurement is carried out. To determine the respiratory intensity (ratio of CO_2 in kilograms of strawberry per hour) equation 2 is used.

$$IR = \frac{mgCO_2}{kg\ strawberry * h} = \frac{3{,}6 * (V1 - V2) * y}{m}$$

Equation 2

Titratable acidity

Titratable acidity of strawberry fruits was measured using the standard method 942.15 (37.1.37) of the AOAC (1996) determination for titratable acidity and using Equation 3.

$$\%\ \text{Titratable acidity} = \frac{\text{V(NaOH)} * \text{N(NaOH)} * 0.064\text{g(meq} - \text{g acid)} * 100}{\text{W (weight of sample)}}$$

Equation 3

Resistance to cutting or deformation

Fruit resistance was measured using a Bertuzzi™ hand-held penetrometer following the methodology established by Sams (1999). For resistance determination, the instrument was calibrated to zero, strawberry sample was taken from swollen thalamus and it was approached directly to penetrometer between the thumb and forefinger of the hand, after positioning fruit tip, pressure was applied progressively until it entered the tip in the pulp of strawberry.

Color determination

Color was determined using a PCE-RGB portable colorimeter, and later conversion using color conversion software RGB-CIELAB®, the results were expressed in luminosity L *. Calibration was initially performed with the white standard and subsequently, strawberry samples were taken from bulging thalamus, approaching them directly to the white light diodes of the equipment and taking the measurement, waiting until the spectral measurement was finished and RGB values were recorded.

Ethylene production

Ethylene production in strawberry fruits was determined with the portable ethylene detector SKY2000. The equipment was previously calibrated for 120 seconds to the environment, a sample of strawberry was taken and placed into an airtight container with the opening to introduce the equipment and ethylene concentration is taken after 30 seconds. The measured ethylene values were reported in ppm.

Statistic analysis

In the present assay, a factorial design with two factors and two levels was used in a type 2^2 arrangement with replicated measurements over time, the following factors being studied:

Strawberries stored in cold room without Oxyion® (Control): Categories 1 and 2

Strawberries stored in cold room with Oxyion® (with technology treatment at 50% of total power of the equipment): Categories 1 and 2.

Data were analyzed using SAS statistical software version 9.2. ANOVA analysis of variance was performed. To identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$. Tukey's multiple range test was used to compare the mean values of treatments.

Results

Fruit conservation

Control treatment maintained its physical characteristics until 5 days in storage at 4°C ± 2°C (USDA, 2016). However, after 7 days, strawberry fruits showed signs of deterioration in fruit quality, which is easily identifiable by hyphae growth on strawberries surface.

In addition, there was less fruit deterioration subjected to the Oxyion® technology evaluated on the tenth day of storage compared to control. During this study was possible to demonstrate the presence of fungi in two treatments, however, the appearance of them originated first in control treatment around day 7, while in strawberry fruits treated with Oxyion® they appeared on day 15 of storage, generating a protection window of 53.3% compared to control.

It is important to mention that diseases presented in starwberry fruits were mostly associated with fungi such as gray mold caused by *Botrytis cinerea* Pes. ex Fr, anthracnose, caused by different species of Colletotrichum spp., dry rot by *Rhizoctonia solani* Kühn and rots by Rhizopus spp., Mucor spp, Sphaerotheca spp. and *Aspergillus* spp. According to the above mentioned, in strawberry fruits treated with Oxyion® and significantly had achieved a decreasing in the percentage of weight loss, reaching a reduction of 14.48% ± 1.14. However, control fruits suffered an estimated superior weight loss of 21.54% ± 1.56, highlighting that values with the greatest difference were obtained after 15 days of storage (Figure 2).

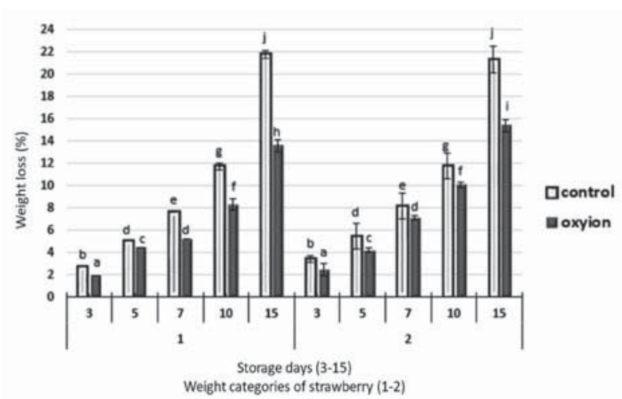

Figure 2. Percentage of weight loss compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Percentage of weight loss

Treatments present significant differences with respect to controls. Strawberries subjected to the Oxyion® technology presents on the seventh day of storage average values of 6.15% ± 0.79 compared to control that presents 7.93% ± 1.59, situation in which is considered that strawberry fruit has already lost its commercial quality (Figure 3).

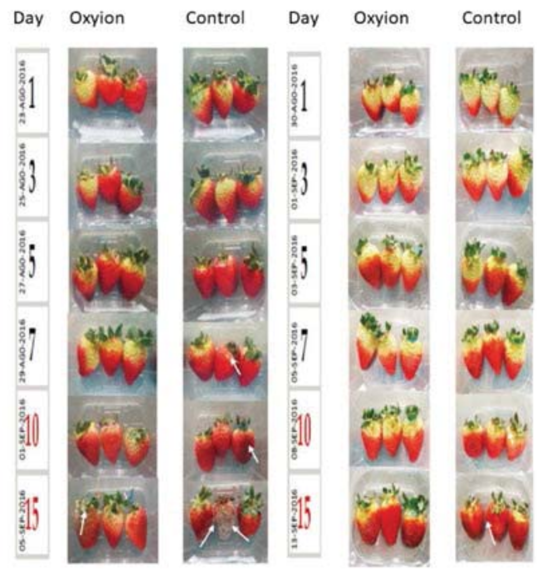

Figure 3. Comparison between treatments with Oxyion® and controls during evaluation process.

In Figure 3, can be observed how strawberry fruits under treatment with ROS do not show signs of deterioration with the same speed as control fruits.

Total soluble solids

When comparing the behavior of soluble solids variable in the values with strawberries of two treatments, there were no significant differences (α> 0.05), the reported values were found in an adequate range, however, in Figure 4, we can appreciate that in two treatments until day 7 present a similar behavior, but from that day on control strawberries can be observed a decrease until day 15, this can be caused by dehydration and fungi that attacked starwberry fruits.

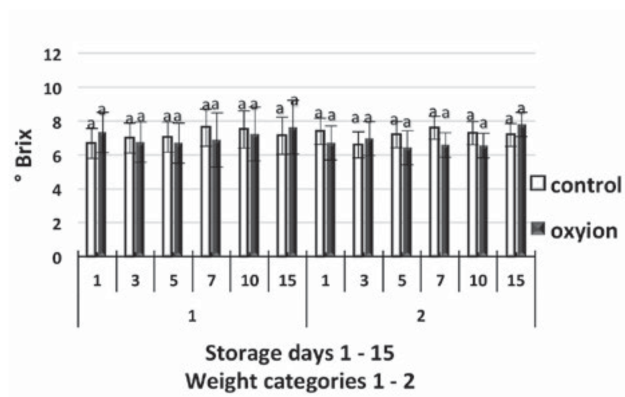

Figure 4. Soluble solids compared by strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Respiratory intensity

Significant differences were determined (**α**> 0.05). The highest respiratory peak was observed in strawberry control fruits on day 1 of storage, a decreasing in the general respiratory intensity was identified on days 10 and 15 of storage in strawberry fruits treated with Oxyion® technology. In fact, on days 3, 5 and 7 there was an increasing in the respiratory intensity of strawberry fruits treated with Oxyion, a situation that generates dispersion during data analysis.

In this sense, is defined that respiratory process in strawberry fruits is variable, with peaks and subsequent drops throughout storage (Figure 5), until a large peak at the end occurs. Technology application had no effect on respiration index.

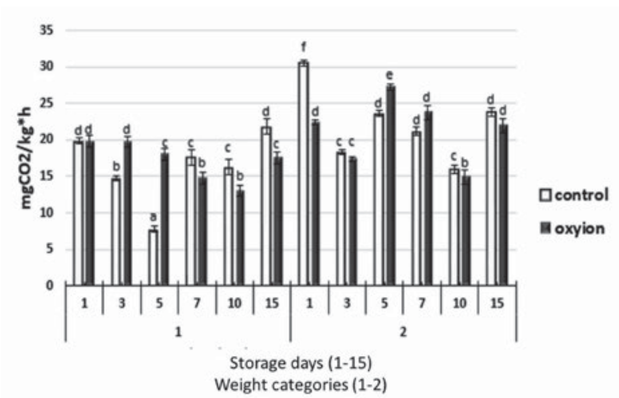

Figure 5. Respiratory intensity behavior, compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Total titulable acidity

Acidity values in two treatments did not show significant differences (α> 0.05), subsequently, the percentage of change that was measured in acidity (Figure 6) coincides with the values reported for soluble solids, being higher the correlation coefficients between two analyzes.

Fruit firmness

The effects of treatments indicated that resistance was significantly different in terms of treatments and categories, Tukey test (α <0.05), showed that Oxyion® treatment had greater resistance with mean values 1.80 ± 0.75 kg compared to resistance of the control 1.53 ± 0.32 kg after 7 days of storage and 1.06 ± 0.57 kg (Oxyion®) versus 0.10 ± 0.37 kg (Control) after 15 days, average values can be seen in Table 1.

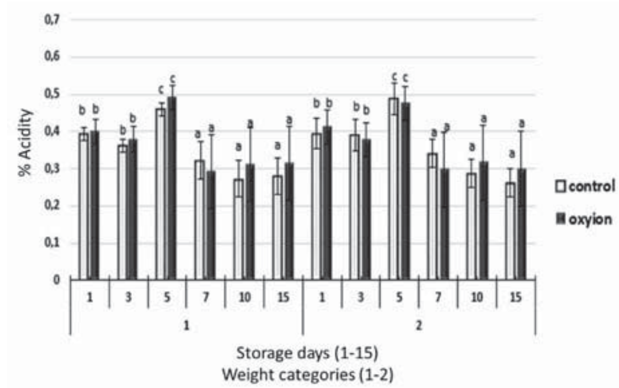

Figura 6. Acidity variation compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Table 1. Average values of the effect of treatments on the resistance

Day	Control		Oxyion	
	Mean value	Standard deviation	Mean value	Standard deviation
1	2.15 (ef)	0.29	1.95 (e)	0.25
3	1.84 (d)	0.19	1.92 (d)	0.28
5	1.77 (d)	0.21	1.83 (d)	0.2
7	1.53 (cd)	0.32	1.80 (d)	0.35
10	1.21 (bc)	0.15	1.81 (d)	0.22
15	0.10 (a)	0.07	1.06 (b)	0.27

A decreasing in resistance after day 10 can be affected by phytopathogens that attack mainly in the senescence state of strawberry fruits, in spite of this, Oxyion® treatment represents a lower loss of resistance in the last days and presents a lower incidence of affection by microorganisms as can be shown in Figure 7.

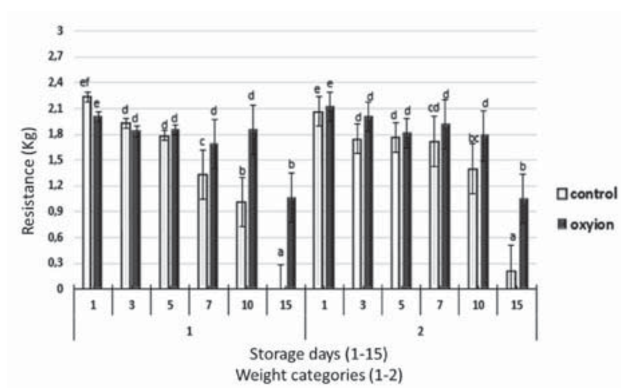

Figure 7. Effect of the treatments on fruit firmness, compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Color variation per treatment

In this parameter it is important to mention that strawberries stored had achieved an opaque color, being a useful indicator of this darkening the color parameter L * (Figure 8).

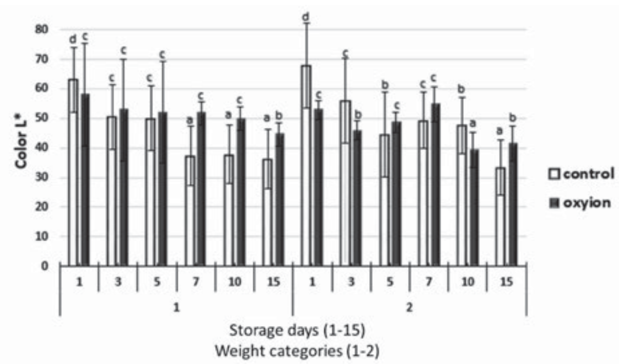

Figure 8. Analyzed parameter color values L * compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Ethylene production

Significant differences were found among treatments, due to first 5 days of storage did not show any ethylene value measured in ppm in each two treatments, while at 15 days there was a drastic increasing in 2.26 ± 0.31 ppm in strawberries category 1 control and 1.46 ± 0.17 ppm strawberries treated with Oxyion® category 1 (Figure 9), it is important to note that there was a decreasing in ethylene production with Oxyion® treatment in senescence period.

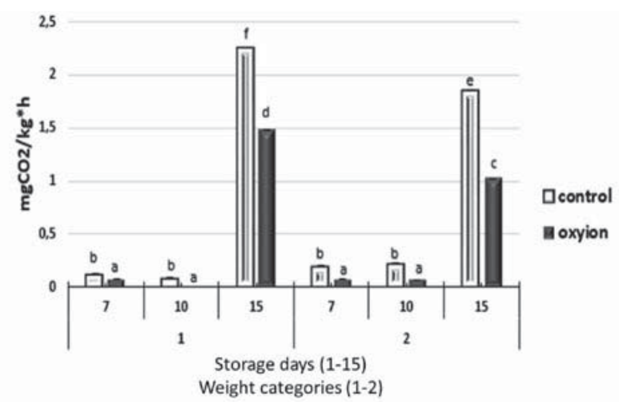

Figure 9. Ethylene values compared to strawberry weight categories subjected to two types of storage.

Equal letters indicate that there are no statistically significant differences.

Discussion

Fruit conservation

Under storage conditions, strawberries are very susceptible to be affected by fungi that deteriorate the external fruit tissue, generating loss of firmness and loss of water and its mass is significantly decreased (Yahia, 2009).

Total soluble solids

The lack of significant differences among treatments may be related to non-accumulation of starch by strawberries during their development, which present their characteristic of sweet taste due to sucrose breakdown, accumulating during plant development stage. For that reason it must be harvested in ripening stage or very close to its full maturity so that its flavor is acceptable by consumer (Thompson, 2014).

Respiratory intensity

Fruit behavior is in agreement with the reported by Jenks & Bebeli (2013) and Merchante, Vallarino, Osorio, Aragüez, Villarreal, Ariza, Martínez, Medina-Escobar, Civello, Fernie, Botella & Valpuesta (2013), who indicate that non-climacteric fruits exhibits a gradual decreasing in their respiration and rate, which is variable according to storage temperature. Another definition indicates that non-climacteric fruits are those that do not show extensive ripening after being harvested and whose respiratory pattern could change after this stage.

pH and acidity

An acceptable flavor in strawberries requires a minimum of solids of 7% and/or a maximum of titratable acidity of 0.8% (Rahman, Moniruzzaman, Rashid, Sarkera & Hurshid, 2016). Nutritional value of strawberries is given by their content of ascorbic acid (Vitamin C), since they are considered a good source of this content (Skender, Ajdinović & Bećirspahić, 2015). This content varies between 26-120 mg.100g^{-1} of fruit (Rahman *et al.*, 2016). Total content of ascorbic acid depends on fruit cultivar and is affected by several factors as follows: is susceptible to degradation in the presence of light and oxygen and to internal enzymatic activity, which occurs in internal fruit tissue when undergoes mechanical damage. It is affected by temperature, humidity, acidity, since the acids regulate cellular pH and can influence the appearance of pigments inside the fruit and finally is also affected by the degree of maturity in strawberry fruit harvest (Skender, Ajdinović & Bećirspahić, 2015), as well as the conditions between harvest and consumption, temperatures during transport and differences between growth areas and seasons.

The absence of significant differences between acidity and pH values in measurements indicates that treatment with Oxyion® does not generate a negative impact on organoleptic properties for the case of strawberry, demonstrating that this treatment is safe with respect to sensory fruit quality.

Fruit firmness

In accordance with Sams (1999), the resistance includes mechanical properties (hardness, elasticity and viscosity), geometric (size and shape) and chemical properties (water content and fats). Alterations in texture normally occur during growth and development of horticultural products. Involves genetically programmed alterations in the structure of cell wall and other physiological factors in plant development. Biochemical alterations in cell wall occur in cellulose, pectin and hemicellulose components, due to the action of pectic enzymes, especially polygalacturonase (PG) and pectin-methylesterase (PME). In the case of fruits such as strawberry, the resistance of cultivars 'Sweet charlie', 'Big Bear' and 'Chandler' decreases as the fruit enters into its period of senescence in storage conditions.

The positive effect on firmness parameter in strawberry fruits shows that Oxyion® technology for the generation of reactive oxygen species helps to maintain the strawberry fruits quality, decreasing the loss of firmness, which is a measurement parameter of the senescence speed in strawberry fruit (Pattee 2014).

Response of L* parameter

Color tonality in strawberry fruits had achieved a decreasing in storage conditions as days go by, this situation originates because strawberry fruit loses its natural turgor, loses its characteristic brightness which is reflected in a decreasing in L* parameter, which measures the brightness. This parameter generally indicates fruit freshness or absence of water loss. The results obtained are consistent with the study carried out by Costa, Duarte, Puschmann & Finger (2011), who demonstrated that strawberries with pre-cooling for 6 hours presented a dark color, less bright and with a lower L * value with an average value ranged from 31 to 28.

Despite the loss of fruit brightness, when comparing starwberry fruits with and without treatment, can be observed that fruits stored under the action of ROS have a higher L * parameter and had achieved an increasing in their quality compared to similar control fruits.

Response in ethylene production

Studies have shown that very small amounts of ethylene are generated due to low concentration of ACC synthase found in strawberry fruits (Perkins, Huber & Brecht, 2008). In addition, ABA (abscisic acid) controls certain physiological or developmental functions in normal plant development stages, in parallel recent studies have revealed that this hormone is also active in response to pathogen-dependent biotic stress. It has been proposed that small amount of ethylene produced during the senescence process of strawberry fruits could be enough to trigger some of the physiological processes related to fruit senescence (Trainotti, Pavanello & Casadoro, 2005). In fact, the presence of type II-type ethylene receptors encoded by the FaEtr2 gene (AJ297513) in strawberry fruit has been observed (Perkins, Huber & Brecht, 2008; Leshem & Pinchsov, 2000; Merchante *et al.*, 2013).

In addition, three different strawberry genes encoding ethylene receptors have been cloned (Trainotti, Pavanello & Casadoro, 2005) and whose expression is induced by this hormone differentially during fruit senescence (Trainotti, Pavanello & Casadoro, 2005), which could indicate a possible role of ethylene in strawberry senescence and this could mean that a significant decreasing in the expression of this hormone will generate a less marked fruit aging, which implies a maintaining in fruit quality for a longer shelf-life. Ethylene measurements in samples of strawberries stored under the effect of Oxyion® technology generate less ethylene, thus ensuring a higher fruit quality compared to control.

Conclusions

The results obtained show that Oxyion® technology with 50% of maximum power, prolongs the shelf-life and maintains strawberry fruit quality, ensuring the technology efficiency until 7 days of storage at 4°C ± 2°C, temperature usually used by producers in the region. The weight loss parameter was positively affected by the use of Oxyion® technology. Fruit weight did not decrease in the same percentage as control strawberries, maintaining a higher quality.

Like weight loss parameter, the other parameters measured in the study (acidity, pH, ethylene concentration, respiration rate, color, firmness) showed that the presence of reactive oxygen species generated from Oxyion technology ® generates a positive impact on the average fruit quality compared to control fruits stored under the same conditions.

The reactivity and characteristics of the reactive oxygen species ensure that the use of said technology will not generate the production of undesirable by-products in strawberry fruits, increasing their competitiveness with respect to strawberries that use other forms of shelf life extension.

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