https://dx.doi.org/10.22161/ijaers.5.7.34 ISSN: 2349-6495(P) | 2456-1908(O)

Physical-Chemical Properties of Strawberry Pseudofruits Submitted to Applications of Zinc Oxide Nanoparticles

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Abstract—*Strawberry cultivation is appreciated in many countries because of the fruit's well-defined, attractive and nutritional sensorial characteristics. As such, it is of great commercial value. The flavor and nutritional properties of the fruit are characteristics that have been developed and gaining importance, both in breeding programs and in productive systems. In this sense, this work proposes the application of nanotechnology for the improvement of the physicochemical characteristics of strawberry crops, with the main objective of analyzing the influence on nutritional performance of different fruit treatment doses with zinc oxide nanoparticles. The physicochemical analyses of the strawberry pseudofruitswere carried out from November to June 2016. The experimental design was in randomized complete blocks, in a factorial scheme, with 7 replicates. The collected data were submitted to analysis of variance with the F-test and the differences between means were compared by the Tukey test (P≤0.05).The treatment process of the strawberry crop with zinc oxide nanoparticles was shown to be efficient for anthocyanin nutrients and soluble solids. The method for analyzing vitamin C, which consisted in freezing the raw material, was ineffective, probably because of the reduction of ascorbic acid levels by the freezing and crystallization of the sample. Climatic factors significantly influence the nutritional composition of anthocyanins and soluble solids. The application of nanoparticles at 100% of the recommended dose proved to be more effective than zinc oxide in its natural form in increasing the soluble solids values.*

Keywords— ***Nanotechnology. Physicochemical properties. Pseudofruits. Strawberry. Nutrition***.

I. INTRODUCTION

Strawberry cultivation is appreciated in many countries because of its attractive and well-defined sensory and nutritional characteristics, which gives it great commercial value. The quality of foods consumed today, both regarding physicochemical and nutritional aspects, has raised great concern, mainly because certain foods are related to the prevention or control of certain diseases because of the presence of bioactive compounds [1].

The physicochemical and nutritional quality of strawberries is genetically determined and may be influenced by the cultivation environment as a function of such edaphoclimatic factors as light, temperature and relative air humidity, types of agricultural cultivation systems, fertilization, soil type, ripeness at harvest, storage, transport and packaging conditions [2].

As such, several factors should be considered to improve the quality characteristics, because combining quality and high productivity results in the best consumer products and is an important stimulus to the producer. Nutrition and fertilization stand out among these factors. One of the most important nutrients required for the growth and development of strawberries is Zinc. It should be noted that, in recent years, technological innovations in agriculture are mostly geared to reductions in production costs, increases in productivity, improvements in the final products and in yields [3].

In this context, some practices still need to be better studied and improved, such as the use of the science of nanotechnology in agribusiness.

For there are great opportunities for research and innovation in associating this field with nanotechnology. In particular, improvements can be obtained in the physicochemical characteristics of oleraceous crops with nanoparticles containing the elements that are natural nutrients of the plant, such as zinc oxide nanoparticles. In this sense, this study proposes to apply nanotechnology for the improvement of the physicochemical characteristics of the strawberry crop, with the main objective of analyzing the influence on nutritional performance of different fruit treatment doses of zinc oxide nanoparticles.

II. MATERIALS AND METHODS

This study was conducted in a plastic greenhouse at the seedling nursery of the Universidade Comunitária da Região de Chapecó in the municipality of Chapecó. The greenhouse, set up in the north-south direction, has a structure of galvanized iron and an arch cover with low density polyethylene (LDPE) with a thickness of 150 µm. The local climate is of the Cfa type in the Köeppen classification, characterized as subtropical with well distributed rainfall in the summer [4]. The chemical analysis of the substrate of the bagsto which the strawberry pseudofruitswere transplanted was performed in September 2015 at the Soil Laboratory of Epagri-Cepaf in Chapecó, following the methods proposed by Tedesco et al. (1995) [5].

2.1 Environmental Control and Management

Data was collected on temperature, relative air humidity, wind speed and the Lux index using a digital Termo-Higro-Anemometer, model THAL-300, installed at a height of 1.20m in the inner part of the protected environment. The measurements were carried out in two periods of the day, in the morning (09:00AM) and afternoon (16:00PM). The monthly temperature, relative air humidity and Lux means were taken for these periods.

2.1.1 Treatments

The treatments of the experiment were allocated into two factors:

Factor A referring to the applied doses, namely: recommended zinc dose (RD), which was composed of N: 9%; - P: 48%; - K: 9%; Mg: 0,5%; - B: 0.02%; - Cu: 0.05%; Mn: 0.05%; - Mo: 0.02%; - Zn: 0.01%.; 50% of Nano Zn equivalent to the Zn RD; and 100% of Nano Zn equivalent to the Zn RD;

Factor B referring to the harvest period:

1st Period (29/10/2015 to 14/12/2015); 2nd Period (15/12/2015 to 21/01/2016); 3rd Period (22/01/2016 to 18/02/2016); 4th Period (19/02/2016 to 21/03/2016); 5th Period (22/03/2016 to 17/06/2016).

2.1.2 Experimental Design

The experiments used a factorial (3 x 5), randomized complete block design (RCB), with factor A (fertilizer) and factor B (harvest period) and 7 repetitions. Each plot was made up of four plants along the line. Based on the number of bags and plants, an outline was made to demonstrate the treatments of each plot.

2.1.3 Performance of the Experiment

The seedlings of the strawberry cultivar San Andreas were acquired on August 29, 2015, and the transplant in to the bags was performed on August 31, 2015, at 15:30. The bags with seedlings measured 1.35m in length and 0.29m in width. The phytosanitary treatments were performed according to the needs of the respective crops through daily observations.

The irrigation was performed manually with distilled water and with the aid of a 60ml syringe, applying 50ml per plant. Depending on the environmental conditions (temperature and relative air humidity), the procedure was performed one or two times per day. It should be noted that the nursery has an automatic irrigation system in the inner part of the greenhouse using sprinklers, but it does not have a standardized frequency.

The zinc oxide nanoparticles used in this work were provided by the Kher Chemical Research and had an average particle size of 25nm and a degree of purity of 99.5%. The nanoparticles were weighed weekly in Unochapecó's chemical and food science laboratories at the concentrations of 100%, 50% of nano-zinc as recommended dose (RD) of zinc oxide, with the values of: 0.0017g of nanozinc at 100% and 0.00085g of nanozinc at 50%.

For the application, 1.3ml (measured in a 3ml syringe) of fertilizer free of Zn, 200ml of distilled water, the masses of 100%, 50% of nanozinc and the zinc oxide solution were mixed in a 200ml beaker. The components were weighed using a precision scale. Subsequently, the fertilizer without the Zn nutrient was mixed with the nanoparticles at the doses of 50 and 100% and then placed in agitators just before the application, allowing for the homogenization of the nanoparticles at the time of application.

The pseudofruits for analysis were selected considering the viable and completely healthy ones. As such, strawberries of different sizes and weight were chosen. Pseudofruits harvested throughout the plot were picked when 3/4 of them were in the dark red stage. The pseudofruitswere frozen immediately after harvesting, from the beginning to the end of the crop cycle.

After the selection and removal of the peduncles, the samples were washed with deionized water and placed in a small plastic bag with the identification of the period, plot, quantity and treatment. Afterwards, the samples were stored in a freezer at a freezing temperature. The pseudofruits were removed from the freezer on the day prior to the laboratory analyses and put in a refrigerator for defrosting. They were then crushed in a Britânia Black Pus mixer and immediately submitted to analysis at ambient temperature (±25ºC). The physicochemical analyses of the pseudofruits from the strawberry cultivar of the San Andreas species submitted to the application of ZnO nanoparticles were carried out in the Chemistry and Food Sciences laboratories of the Universidade Comunitária da Região de Chapecó (Unochapecó) from November to June 2016. The tests were performed in six fold. And the procedures for the physicochemical analyses followed the methodologies proposed by authors [5].

2.1.4 Statistical analysis

The collected data was submitted to an analysis of variance with the F-test (Table 1), and the differences between means were compared through the Tukey test ($P \leq 0.05$). The computational application used was the SISVAR - system of analysis of variance for unbalanced [6].

III. RESULTS AND DISCUSSION

3.1 Weather Conditions

3.1.1 Air temperature

According to Figure 3, in the first period comprising of the months of October to December, the average temperatures were 22.99°C in the morning and 25.30°C in the afternoon. In the months of December and January, an average temperature of 26.65°C was obtained in the morning and 26.34°C in the afternoon, which were the highest recorded temperatures during the experiment. This phenomenon coincides with the occurrence of over 518.6 hrs of insolation [7], for the period. In the months of January and February, which were part of the third period, the findings for the morning and afternoon periods were 26.17 °C and 25.69°C, respectively. In the penultimate period, in the months of February and March, the average values were 24.08°C in the morning and 24.43°C in the afternoon. And in the fifth and last period, the average temperature values found in the months of March, April, May and June were 17.31°C in the morning and 17.98°C in the afternoon. It should be noted that this period had the lowest averages along the day, with the lowest recorded temperature (9.15 °C) (Figure 3). This correlates with the hours of sunshine according to the respective weather agency, which indicated that these months had the lowest sunshine rate of the experiment (155 hours), in line with the normal climatologic average of 159.8 hrs for this time of year [7]. This last period also registered a big difference between the minimum and maximum temperatures, reaching a variation of 14.6°C in four months. The protected environment can be cited as a conditioning factor, with the shaded area with dirt, the black screen over the roof and the irrigation made at irregular intervals and without frequency influencing the respective climatic data. That is, the black screen had a direct influence on the solar radiation conditions because of the imposed barrier and because the color tends to absorb and not reflect the photosynthetically active radiation (PAR).

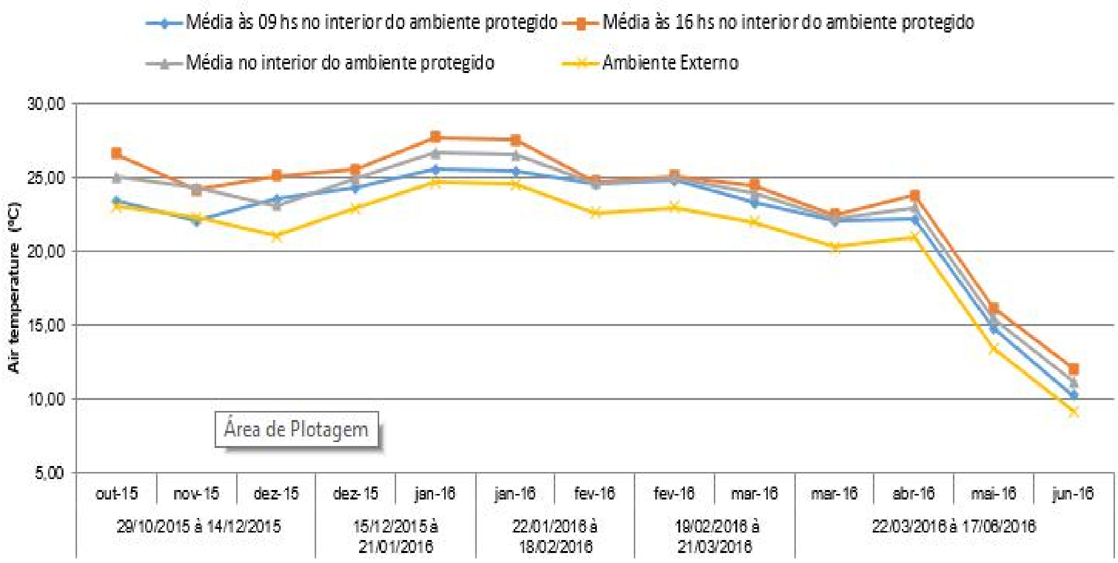

Fig.1: Air temperature recorded inside the protected environment and in the external environment (Chapecó, SC - 2015/2016 cycle)

3.1.3 Relative humidity

The average recorded relative air humidity values inside the greenhouse are shown in Figure 4, revealing values in the morning and afternoon period of 71.90% and 70.23%, respectively. In the second period, the values were 71.57% in the morning period and 72.51% in the afternoon. In the following period, the percentages were 70.53% and 70.48% in the morning and afternoon, respectively. It should be noted that this period is considered the apex of the hottest season of the year, and the relative air humidity values were the lowest of the whole experiment. In the fourth period, the mornings had an average air humidity value of 71.05%, while in the afternoon it reached 71.77%. In the last period, the findings for the months of March to June were 76.70% in

https://dx.doi.org/10.22161/ijaers.5.7.34 ISSN: 2349-6495(P) | 2456-1908(O)

the morning and 74.62% in the afternoon. These months correspond with the fall and winter seasons and they had the highest air humidity percentages. A concomitant factor that should be taken into consideration is the rainfall of the four months, which amounted to 543 mm [7], and it should be noted that the highest level of rainfall of the experiment occurred in the respective period. The results of this work are in line with the study by [8], who pointed out that the optimal relative humidity for the cultivation of strawberries lies between 70 and 80%. As such, one could state that the humidity values presented lie inside the proper range for the development of the crop, preventing the emergence of diseases. The relative air humidity inside the greenhouse is directly related to the ambient temperature. At 16 PM, when the ambient temperature is highest, the relative air humidity had the lowest values in the four periods, while at 09 AM, when the ambient temperature is lowest because of the sharp drop in temperature during the night,the humidity values could reach 100% before sunrise. The relative air humidity inside the greenhouse proved to be higher, mainly in the fifth period, at the start of the fall and winter seasons. These results are associated with the fact that the water vapor values inside the greenhouse are extremely influenced by evapotranspiration, which increases the amount of water vapor in the air and, combined with the low permeability of the plastic film and lower rate of air renovation inside the greenhouse, leads to a greater accumulation of water vapor inside [9].

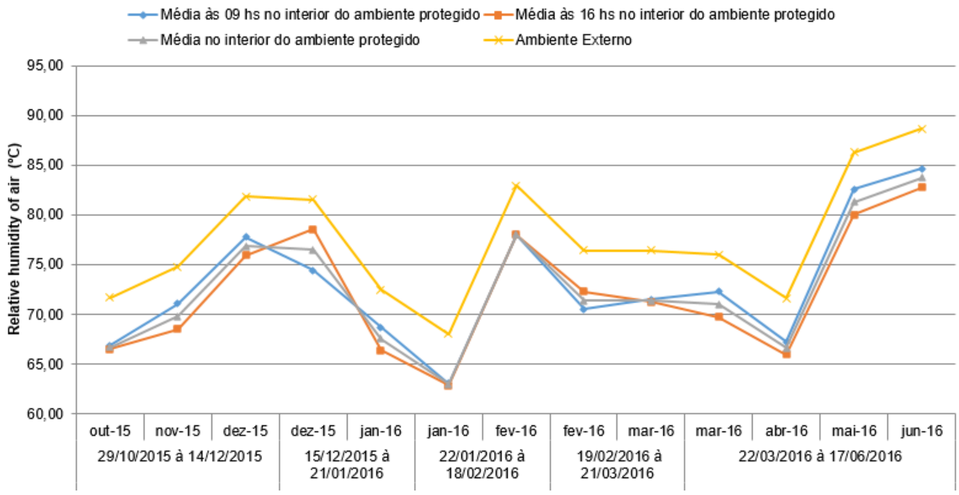

Fig.2: Relative humidity of air recorded inside the protected environment and in the external environment (Chapecó, SC - 2015/2016 cycle)

3.1.4 Light Intensity

Figure 5 reveals that the first period of the experiment had an average solar radiation in the morning period of 164.91 µmol.m-2.s-1 and 212.89 µmol.m-2.s-1 in the afternoon. During these months, the solar radiation rates were the most intense of the experiment, a fact that is associated with the total number of hours of sunshine, 421.2 hrs. As such, this was the period with the greatest insolation and lowest air humidity values. In the second period, the findings were 176.84 µmol.m-2.s-1 and 162.14 µmol m-2 s-1. In the next period, the morning averaged 151.23 µmol.m-2.s-1 of radiation and the afternoon 160.56 µmol.m-2.s-1. It should be noted that the lowest solar radiation rates were obtained during this period. This correlates with the rainfall levels, which reached 388.20mm according to the data of [7], totaling 27 days of rain in the two months that make up this period. In the second-to-last period, the findings were 164.56 µmol.m-2.s-1 and 162.14 µmol m-2 s-1. And in the fifth and last period, the morning had an average radiation value of 149.92 µmol.m-2.s-1 and the afternoon of 179.40 µmol.m-2.s-1. The solar radiation levels can also be associated with the presence of a the black shading screen, which was placed above the shade net in the greenhouse and therefore reduced the harmful effects of high solar radiation rates and temperatures on the plants.

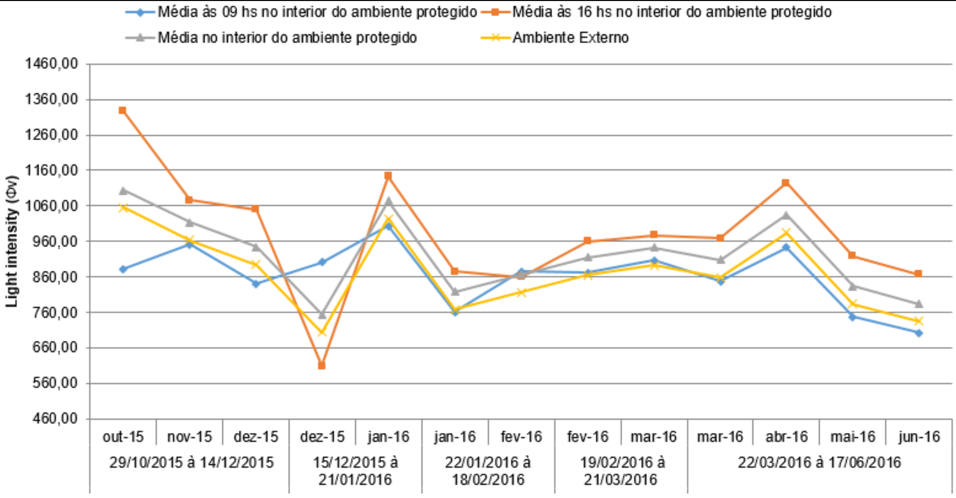

Fig.3: Light intensity recorded inside the protected environment and in the external environment (Chapecó, SC - 2015/2016 cycle)

3.1.1 Physico-Chemical Properties

3.2 Ph

The analysis of variance revealed no significant effect ($P < 0.05$) of the interaction between the concentration x period of application of the zinc oxide nanoparticles regarding the response variable pH (Table 2).

Aplication Period	Concentration		
	Recommended Dose (RD)	50% RD from Nano ZnO	100% RD from Nano ZnO
		----------(pH)----------	
1° Period (29/10/2015 à 14/12/2015)	3,66 aA	3,52 bB	3,55 abB
2° Period (15/12/2015 à 21/01/2016)	3,38 aD	3,35 aC	3,45 aBC
3° Period (22/01/2016 à 18/02/2016)	3,56 aAB	3,60 aB	3,53 aBC
4° Period (19/02/2016 à 21/03/2016)	3,50 abCB	3,55 aB	3,42 bC
5° Period (22/03/2016 à 17/06/2016)	3,65aA	3,76 aA	3,72 aA
CV (%)		2,50	

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test ($p\leq0.05$.
Table 2 - pH of strawberry pseudofruits submitted to applications of zinc oxide (ZnO) nanoparticles in a protected environment (Chapecó, SC - 2015/2016 harvest)

The pH values found were statistically equal ($P>0.05$) and showed no significant difference as a function of doses, period and their interaction, as can be seen in Table 2. The zinc oxide nano-structures don't influence the pH parameter because it is related to the fruit maturity, genotype, climatic condition, soil and fertilization parameters. The low pH can be associated with the growth speed of the plant, reiterating that zinc is a precursor of the auxin Indole-3-acetic acid (IAA), a growth hormone that promotes cell elongation and acts as a regulator of plant growth. As such, the increased speed of growth will influence the plant's degree of ripeness, making it have a limited availability to climatic factors, such as luminosity and temperature. This means pH does not accompany the ripening of the fruit, and consequently its rate goes down. The pH of strawberries ranges between 3.50 and 3.70 [10]. The findings in this experiment are in alignment with other studies. For the nano-zinc oxide concentrations of 50% and 100%, the mean of the five periods was 3.55, and for the nano-zinc oxide concentration of 100% it was 3.53. The results were similar to those found by the authors Françoso et al. (2008:11), who found mean values of 3.46. The results found here are close to those reported by Camargo (2008:12), which ranged between 3.50 and 3.77. Cantillano et al. (2003:13), on the other hand, found a pH of 3.23 for the cultivar Camino Real, 3.58 for the cultivar Ventana and 3.3 for the cultivar Aromas. Another study conducted in the city of Passo Fundo-RS also registered lower pH values, with Mendonça (2011:14), evaluating the quality of strawberry fruits in protected cultivation. For the cultivars Albion, Camarosa and Festival, he observed pH values equal to 3.23, 3.18 and 3.22, respectively. The results obtained in the present study corroborate those obtained by Pallamin (2007:15), who evaluated the cultivars Camarosa, Dover, Oso Grande and Sweet

Charlie, obtaining average pH values between 3.22 and 3.35.

3.3 Anthocyanin

The analysis of variance revealed a significant effect ($P < 0.05$) of the interaction between the concentration x period of application of the zinc oxide nanoparticles regarding the response variable Anthocyanin (Table 3).

Application Period	Concentration		
	Recommended Dose (DR)	50% RD from Nano ZnO	100% RD from Nano ZnO
	----------(mg/100g)----------		
1° Period (29/10/2015 à 14/12/2015)	23,29 aC	25,53 aD	24,32 aD
2° Period (15/12/2015 à 21/01/2016)	45,35 bB	54,49 aB	60,90 aA
3° Period (22/01/2016 à 18/02/2016)	62,08 aA	63,83 aA	49,16 bB
4° Period (19/02/2016 à 21/03/2016)	24,89 aC	28,82 aD	29,85 aD
5° Period (22/03/2016 à 17/06/2016)	29,95 bC	39,97 aC	39,87 aC
CV (%)		13,32	

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test (p≤0.05). Table 03 - Strawberry pseudofruitanthocyanins submitted to zinc oxide (ZnO) nanoparticle applications in protected environment (Chapecó, SC - 2015/2016 harvest)

Regarding the treatment with zinc oxide nanoparticles, the analysis of variance revealed a significant effect ($P < 0.05$) of the interaction between the concentration x period of application of the zinc oxide nanoparticles regarding the response variable. It should be noted, however, that the values obtained for period 3 were substantially above those found in other periods of the study, reiterating that, according to Clifford (2000:16), the levels can vary from 15 to 35 mg.100g-1 of fresh fruit, thus demonstrating values substantially above those found in the literature. The increased anthocyanin levels can be correlated with the plant's glucose concentrations, which is related to the factor that sugar plays in the central structure of anthocyanins, in addition to the fact that the zinc micronutrient directly influences the metabolism of carbohydrates. The atomic absorption spectroscopy technique reveals the incorporation of very small, but still higher quantities of zinc than present inside the seeds of the fruit. The results reveal that the zinc oxide nanoparticles are absorbed by the fibrous pericarp cells, forming nano-structured clusters on the surface, preferably anchored in the interfaces of the cells. Because of their nanometric dimensions, the nanoparticles form clusters that can serve as nano-nutrient reserves for the seed in the germination and following stages, and they are incorporated in small quantities by the seed. This is an indication that a large part of the zinc oxide nanoparticles is available on the surface and inside the pericarp of the strawberries [18]. In a study by the authors Weber et al. (2015:18), who compared the cultivars Camarosa, Camino Real and San Adreas, values of 55.92 and 56.34 mg/100 g of fresh fruit were obtained, respectively, with no significant difference between them. The cultivar San Andreas, on the other hand, differed significantly from the others, with 41.23 mg.100 g-1 of fresh fruit, similar results to those found in the present study, but in our case there was no statistically significant difference ($P>0.05$). The superior results of this study can also be correlated with Chaves (2014:19), who studied the same cultivar in the city of Passo Fundo/RS and obtained total anthocyanin values of 18.69 mg/100 g. This value is relatively low when compared to the results found in this study. In the same way, Maro et al. (2004:20) observed differences when comparing the total phenolic contents of the strawberry fruits of the cultivars Guarani, Dover and Sweet Charlie in the state of Minas Gerais. The cultivar Guarani had the highest anthocyanin content (19.5 mg 100 g-1), while Dover (14.3 mg 100 g-1) and Sweet Charlie (13.3 mg 100 g-1) had the lowest levels, with concentrations below those obtained in the study of Calvette et al. (2008:21) and the present study. Buendia et al. (2010:22) evaluated the chemical composition of phenolic compounds in 15 strawberry cultivars and demonstrated that total anthocyanins ranged from 20.2 to 47.4 mg100g-1 of fresh fruit, with a small variation in the concentration range. In the studies carried out by Castro et al. (2002:23) and by Pinto, Lajolo and Genovese (2008:24) with the cultivar Camarosa, the anthocyanin contents found were 48.2 and 43 mg.100g-1, respectively. This decrease in the levels may be related with the incidence of sunlight [26]. Strawberries are demanding when it comes to light, requiring high insolation during the growing period, an important factor in the photosynthesis process as well as in the definition of the chemical composition of the fruit. According to Klimov et al. (2008:26), anthocyaninsmay be influenced by temperature and radiation, so high temperatures and greater solar radiation are associated with higher concentrations of this compound. For this study, one could mention that the concentrations in the same period of peak of the anthocyanin rates (third period) coincided with extremely hot temperatures (Figure 3) and with quite significant insolation rates (378.6h) (Figure 5), but it should be reiterated that it wasn't the period with the highest solar radiation rates, according to the climate statistics. Folegatti et al. (1997:27) noted that the plastic cover changes the amount of overall radiation within protected environments, with mean values close to 63% of the total overall radiation outside. According to the aforementioned authors, these variations are strongly related to micro-climatic factors, the degree of ripeness, seasonality, and the cultivar variety.

3.4 Vitamin C

The analysis of variance revealed no significant effect ($P \leq 0.05$) of the interaction between the concentration x period of application of the zinc oxide nanoparticles regarding the response variable vitamin C (Table 4).

Application Period	Concentration		
	Recommended Dose (DR)	50% RD from Nano ZnO	100% RD from Nano ZnO
	----------(mg/100g)----------		
1° Period (29/10/2015 à 14/12/2015)	23,29 aC	25,53 aD	24,32 aD
2° Period (15/12/2015 à 21/01/2016)	45,35 bB	54,49 aB	60,90 aA
3° Period (22/01/2016 à 18/02/2016)	62,08 aA	63,83 aA	49,16 bB
4° Period (19/02/2016 à 21/03/2016)	24,89 aC	28,82 aD	29,85 aD
5° Period (22/03/2016 à 17/06/2016)	29,95 bC	39,97 aC	39,87 aC
CV (%)		13,32	

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test (p≤0.05).
Table 4 - Vitamin C of strawberry pseudofruits submitted to zinc oxide (ZnO) nanoparticle applications in a protected environment (Chapecó, SC - 2015/2016 harvest)

The vitamin C values found were statistically equal ($P>0.05$) and showed no significant difference as a function of doses, period and their interaction, as can be seen in Table 4. There is a peak in the vitamin C levels in the first period (Table 4), which corresponds to spring, with high temperatures, higher insolation rates and lower humidity. This corroborates the findings by the authors Lee and Kader (2000:28), adding that higher light intensities during the growth phase of plants consequently entail greater quantities of vitamin C in plant tissues.

It should be noted that the values found are far below those found by other authors. According to Domingues (2000:29), strawberries are a rich source of vitamin C, oscillating between 39 and 89mg/100g of fruit, with the average value being 60 mg.100g-1 of fruit.

Pinelli et al. (2011:38) evaluated the chemical characteristics of strawberries at different stages of maturation and found values for vitamin C of 23.16; 46.88 and 31.45 mg/100g of fresh pulp for the green, pink and mature Oso Grande cultivar, respectively. Campos et al. (2011:30), on the other hand, studied different post-harvest strawberry conservation techniques and observed values of 44.05 mg100g-1 of pulp in the fruits at harvest.

Webber (2015:18) found results of 69.31 and 42.29 mg100g for the cultivars Festival and San Adreas, respectively. In the following study, superior vitamin C values were found for the cultivars Oso Grande and Camarosa. For the cultivar Camarosa, on the other hand, Rocha et al. (2008:31) registered 73.14 mg.100 g-1. Lower vitamin C values were also found by Campos et al. (2011:30), who analyzed the post-harvest quality of strawberry fruits in Maringá-PR and obtained 44.05 mg100g-1 for the cultivar Camarosa at harvest.

The levels may vary depending on the ripeness, cultivar, season, conditions of cultivation, and storage conditionsand duration post-harvest, which may decisively influence the levels of this compound [32].

The study developed by Portela, Peil and Rombaldi (2012:33) evaluated the effect of the nutrient solution concentration on the characteristics of the phytochemical compounds found in strawberries. They found higher levels of vitamin C when there was an increase of the salt concentration in the nutrient solution. In the present study, there was no change in the salt concentration and the doses followed the recommendations of the fertilization and liming manual.

According to Smirnoff (1996:34), the incidence of solar light is a factor that stimulates the synthesis of L-ascorbic acid by plants. In the photosynthetic mechanism, L-ascorbic acid - the main active form of vitamin C - acts in the dissipation of excess light energy absorbed in the form of heat (when there is an excessive increase in luminosity) and also in the elimination of many reactive oxygen species [2].

In the study by Agar; Streif and Bangerth (1997:35), the authors noted that the activity of ascorbate oxidase, which promotes the oxidation of ascorbic acid to dehydroascorbic acid, can be seen as responsible for the loss of ascorbic acid. For the authors, the proposal of using treatments with nano-ZnO could reduce the diffusion of O2, decreasing the breathing rate, which should delay the oxidation of the ascorbic acid in the fruit. On the other hand, it has been reported that the loss of water can accelerate the loss of ascorbic acid due to increased oxidation [37]. This way, the treatments with nano-ZnO could probably affect the vitamin C levels in strawberries, changing the water content of the fruit.

Lee and Kader (2000:28) emphasize that losses in vitamin C may occur due to its sensitivity regarding low humidity conditions. As such, the findings in this study corroborate those of the aforementioned authors, since the periods with the lowest vitamin C levels - the fourth and fifth periods - had the lowest temperature and humidity averages, around 75.66% and 17.97°C, respectively.

According to Ferreira (2012:37), substantial losses of nutrients may occur with the storage of strawberries, especially of vitamin C, because of physiological and biochemical processes and because vitamin C is very sensitive and unstable, susceptible to degradation by light and heat.

In this work, the physicochemical analysis of vitamin C followed the methodology proposed by Tedesco (1995:5), who recommends the freezing of fruits in some analyses for the subsequent performance of the tests. The demands were collected daily in accordance with the periods, and the fruits were stored in freezers with temperatures below 0ºC for periods exceeding 20 days in most cases. It should also be noted that in some tests a minimum number of fruits was needed for the reading, which maintained them for an even longer time, until reaching the number of the sample. The low doses of vitamin C could therefore be associated with the possible losses in this process.

Pinelli (2005:38) raises very important considerations regarding the post-harvest storage of strawberries. In studies conducted by the author, he reports that the levels of vitamin C decrease when the fruits are stored at higher cooling temperatures. In his study regarding the amount of vitamin C in juices submitted to storage processes of 26 hrs,

3.6 Soluble Solids - Brix

The analysis of variance revealed a significant effect ($P \leq 0.05$) of the interaction between the concentration x period of application of the zinc oxide nanoparticles regarding the response variable Soluble Solids - Brix (Table 5).

Aplication Period	Concentração		
	Recommended Dose (RS)	50% RD from Nano ZnO	100% RD from Nano ZnO
		----------(°Brix)----------	
1° Period (29/10/2015 à 14/12/2015)	5,18 bAB	5,58 Bab	7,00 aA
2° Period (15/12/2015 à 21/01/2016)	6,00 bB	6,27 bA	7,18 aA
3° Period (22/01/2016 à 18/02/2016)	5,27 bAB	4,78 bB	7,35 aA
4° Period (19/02/2016 à 21/03/2016)	4,78 cC	5,77 bA	6,55 aA
5° Period (22/03/2016 à 17/06/2016)	7,25 aA	6,40 bA	7,25 aA
CV (%)		9,53	

Means followed by the same lowercase letter in the row and upper case in the column do not differ by Tukey test (p≤0.05).

Table 5 - ºBrix of strawberry pseudofruit submitted to applications of zinc oxide (ZnO) nanoparticles in protected environment (Chapecó, SC - 2015/2016 harvest)

In this study, the 100% concentration of nano zinc oxide differed significantly from the other doses, reaching a value of 7.35°Brix, indicating that the fruits with the addition of nano zinc oxide at a concentration of 100% had more sweetness in the fourth period than the nano zinc oxide doses at a concentration of 50% and also regarding the recommended dose of zinc in the fourth and other periods of the experiment (Table 5).

The zinc oxide nanoparticles influenced the sugar levels of the pseudofruits because the micronutrient participates as a component in a large number of enzymes, including dehydrogenases, proteinases, proteases and phosphohydrolases, with the basic functions in the plant being related to the metabolism of carbohydrates, thus influencing the amount of sugar in the fruit.

Since the nanoparticles have nanometric dimensions, they can easily be absorbed by cellular membranes and be carried through the micro channels in the cellular structures of the plant. Under these conditions, the zinc oxide nanoparticles are carriers of the zinc nanonutrients to the cell regions that are still not serviced by traditional treatment methods, which explains the effectiveness of the nanoparticles in comparison to the zinc oxide particles as an effective and efficient absorption method of nano-nutrients.

The zinc oxide nanoparticles are absorbed in the fibrous cells in the pericarp of the seed and anchored in the form of clusters, preferably on the edges of the cells. As such, clusters are formed in the cavities of the cell interfaces and composed of zinc oxide nanostructures with smaller dimensions than the cavities. These characteristics favor the migration of the zinc oxide nanostructure to the inside of the pericarp of the strawberry seed, transforming the clusters into viable zinc reserves for the seed, with the availability of zinc oxide throughout its development stage.

The results show that the treatment process of strawberries with zinc oxide nanoparticles is efficient in increasing the °Brix concentrations of the fruit.

When compared with other studies, one can see that the results are similar to the °Brix levels of this study. In the study by Borsatti et al. (2009:39), who studied strawberries in the southwestern region of Paraná, the authors obtained soluble solid values for the cultivars Oso Grande, Festival, and Camarosa of 6.27; 7.08 and 6.76 °Brix, respectively.

The authors Resende et al. (2010:40) evaluated the soluble solids content of the fruits of four strawberry cultivars in Guarapuava-PR, and they found SS values for the cultivars Camarosa and Oso Grande equal to 4.43 and 4.80 °Brix, respectively.

Scolforo (2014:41) found values for the cultivars Camino Real and Sweet Charlie of 6.74°Brix and 7.31°Brix. Françoso et al. (2008:11), on the other hand, obtained °Brix values of 7.0 and 9.5 for the same varieties, while Cordenunsi et al. (2002:42) reached the values of 5.4 °Brix and 6.0 °Brix for the cultivars Dover and Capineiro.

The sugar content of the fruits of the cultivar Oso Grande were evaluated by Figueiredo et al. (2010:43) in the municipality of Lavras-MG, who obtained the average value of 4.4%. Superior sugar values were obtained for the cultivar Camarosa by Camargo et al. (2009:12), who evaluated the chemical characterization of fruits in different cultivation systems in the city of Guarapuava-PR and observed mean values of 5.65% and 5.92% of total soluble sugars in the organic and conventional cropping system, respectively. As can be seen, the findings are significantly below the values obtained in this study. Associating the sugar levels in the fruits with the climate parameters, Rios (2007:45) affirms that the quantity of sugars is directly related to the intensity of light and independent of the temperature and photoperiod.

In the period with the greatest Brix intensity (3rd period), the temperatures were considerably high, but within the recommended range for the cultivation of strawberries, which grow best in subtropical or temperate climates with mild temperatures between 15°C and 26°C, since excess heat and humidity leave the strawberry plants more susceptible to pests and diseases. In this case, the humidity in the period was considered low as a result of the temperatures reached. It should be noted that excess humidity can restrict the productive potential of plants. The insolation rates are also related to the temperature variable, the hours of solar radiation were significant, but it should be emphasized that it was not the period with the highest values. Longer days with higher average temperature favor the emergence of stolons and, consequently, new plants, while shorter days and lower temperatures favor blooming, especially of short-day varieties.

IV. CONCLUSION

Under the conditions in which the experiment was conducted, the resultsallow for the following conclusions:

- the treatment process of the strawberry crop with zinc oxide nanoparticles is efficient for the nutrients anthocyanins and soluble solids.
- the method for the analysis of vitamin C, which consisted in freezing the raw material, isprobably ineffective because it reduces the levels of ascorbic acid by freezing and crystallizing the sample.
- The climatic factors significantly influence the nutritional composition of anthocyanins and soluble solids.
- The 100% nanoparticle composition of the recommended dose proves to be more effective than zinc oxide in its natural form for the increase of the soluble solids values.

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