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APPLICATION OF OZONE AIMING TO KEEP THE QUALITY OF STRAWBERRIES USING A LOW COST REACTOR[1]

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ABSTRACT - The strawberry is a fruit of high commercial value and has a rapid deterioration, and as the demand for healthy products, safe under the microbiological point of view and free from chemicals increases more and more, the method of application of ozone gas at a controlled atmosphere was proposed. The objective of this study was to verify the efficiency of ozone gas produced by a reactor, in order that small strawberries' producers may use it thus contributing to regional economies. Strawberries (*Fragaria ananassa*) Oso Grande variety, harvested in the region Minas Gerais were divided two groups: the first receive treatment with ozone and the second not. In the first group ozone was applied for 20 minutes from a reactor of Corona. The fruits were stored at 4 ° C for periods of 5, 10 and 15 days. The quality of the data was reported for the levels of total soluble solids (SS), titratable acidity (TA), pH, phenolic compounds (FC), ascorbic acid (AA), loss of fresh matter (LM %) and microbiological analysis (MA) in different storage times of ozonated and non ozonated fruits. The use of ozone gas was efficient for the strawberry postharvest. The levels of microorganisms are within acceptable limits and the physical and chemical properties were maintained.
Index terms : Strawberry, ozone and storage.

APLICAÇÃO DE OZONIO VISANDO MANTER A QUALIDADE DOS MORANGOS COM REATOR DE BAIXO CUSTO

RESUMO-O morango é uma fruta de alto valor comercial e tem uma rápida deterioração, como a demanda por produtos saudáveis, seguros sob o ponto de vista microbiológico e livre de produtos químicos aumenta cada vez mais, o método de aplicação do gás ozônio em uma atmosfera controlada foi proposto. O objetivo deste trabalho foi verificar a eficiência do gás ozônio produzido por um reator, a fim de que os pequenos produtores de morangos possam usá-lo, contribuindo, assim, para as economias regionais. Morangos (*Fragaria ananassa*) variedade Oso Grande, colhidas na região de Minas Gerais foram divididas dois grupos: o primeiro recebeu tratamento com ozônio e o segundo não. No primeiro grupo, o ozônio foi aplicado durante 20 minutos a partir de um reator de Corona. Os frutos foram armazenados a 4 ° C, por períodos de 5, 10 e 15 dias. A qualidade dos frutos foi relata a partir dos níveis de sólidos solúveis totais (SS), acidez titulável (AT), pH, compostos fenólicos (CF), ácido ascórbico (AA), perda de massa fresca (PM%) e análise microbiológica (AM), em diferentes tempos de armazenamento de frutos ozonizados e não ozonizados. O uso de gás ozônio foi eficiente para a pós-colheita de morango. Os níveis de microrganismos estão dentro dos limites aceitáveis e as propriedades físicas e químicas foram mantidas.
Termos para indexação: morango, ozônio e armazenamento.

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INDROTUCTION

The strawberry comes from a perennial herbaceous plant, common name strawberry, belonging to the genus *Fragaria* (Rosaceae), which includes about 20 different wild species (HANCOCK, 1999).

Among the nutritional benefits strawberry stands out due to high content of vitamin C and folic acid, great antioxidant power due to phenolic components and the ability to reduce the susceptibility to infections, its diuretic effect and its anti-inflammatory activity in rheumatism and gout (HANNUM, 2004; ROCHA et al., 2008).

The city of Datas, near Diamantina, located in upstate, is an example of this diversification (FERNANDES, 2008). With an average altitude of 1,270 meters and temperatures reaching 5 º C at night, Datas has new areas, subject to a lower incidence of pests and diseases (VIEIRA, 2009). The incentive of horticulture in the region has proven to be a viable alternative for families who worked previously only in mining, with low pay, uncertain and long-term. (FERNANDES, 2008).

The small fruits like strawberry, blackberry, raspberry, blueberry among other, have gained the attraction of consumers, in recent years, due to the high nutraceutical value. The farmers, due to the high value of the product on the market, have expanded gradually croplands boosting the local economy (CALVETE et al., 2008) .

This has led to an effort of supply chain in ways of preserving viable highly perishable products (MARTINS, 2009).

The distribution and marketing of the fruit to long distances are restricted due to their perishability and senescence as well as the occurrence of postharvest diseases that cause quantitative and qualitative losses. The strawberry preserves its good quality for up to two days when stored without refrigeration, and this short life is related in reducing its aromatic properties, flavor and characteristic luster, which is caused by deterioration or early decay (CUNHA, 2011).

Among the most sanitizers used in the industry food, the best known are the chlorinated compounds. However, the reduction of microbial efficiency coupled to the potential toxicity of the chlorination byproducts, is making this less and less attractive process (SILVA et al., 2011).

The application of ozone gas, an effective disinfection method, is gaining momentum in food processing due to its high power and sanitizing and rapid degradation, leaving no residues in treated food. These intrinsic properties allow the intake of ozonated without risks to health foods (CHIATTONE, 2008).

The objective of this study was to verify the efficiency of ozone gas in the application of strawberries using microbiological quality and chemical-physical characteristics in order that it may be used on small producers contributing to regional economies.

MATERIALS AND METHODS

Harvesting, washing and separation of fruits

Strawberries (*Fragaria ananassa* Duch) Oso Grande variety were collected (November 26th, 2012), approximately two pounds of strawberries in MapFruti farm located in the city of Datas geographical location: latitude -18 ° and -43 ° longitude - Minas Gerais.

Once collected, the strawberries were selected according to the degree of maturation (about 70% red color) and no injuries and later transported to the Laboratory of Biomass Technology of Cerrado (LTBC) and the Research Laboratory of Environmental Chemistry and Electrochemistry, Department of Chemistry Faculty of Exact Sciences UFVJM, Diamantina, Minas Gerais.

In LTBC, fruits were washed with tap water, separated by size and exemption from injury to treatment application and then were divided into two groups, with and without ozone, containing 100 fruits each.

Application of O_3 by the corona process

The group of strawberries that did not receive treatment was called control group. The second group was placed in a polystyrene box sealed and ozonated for 20 minutes on flow rate 1 g / h by the corona process by Ozonator IPABRAS .

Storage

The strawberries in the two groups after receiving the treatments were packed in polystyrene trays with dimensions (0.50 cm thickness and dimensions of 11.50 x 19.0 x 4.0 cm), each containing eight fruits, being covered with plastic film of polyvinyl chloride (PVC) and identified with the letter O (ozonated), NO (not ozonated), inside a cold room at 4 ° C properly sanitized. The fruits were stored for 15 days, being numbered 0, 5, 10, 15 (corresponding to the days of storage) .

Chemical and Microbiological Analyses.

The chemical and microbiological analyzes were performed during storage of 0, 5, 10, 15 days.

Soluble solids and pH

The SS were determined using a portable refractometer from three drops of sample, expressing the results in ° Brix, numerical scale of refractive index of a solution. The pH was determined by direct potentiometer and measured after immersing the sample in a glass electrode.

Titratable acidity

Initially an extract prepared by diluting 2 g of sample in 30 mL of water. Determined the concentration of titratable acids by titration of the filtrate with 10 mL 0.1 M $NaOH_{-1}$ standard and 3 drops of phenolphthalein indicator , and the results expressed as percentage citric acid per 100 g of sample (g/100g) (IAL, 2005).

Loss of fresh weight

The loss of fresh weight was evaluated considering the difference between the initial and final weight of the trays, according to equation (GUEDES, 2012) : LM (%) = P0 - Pt / P0

Where: LM (%) = Loss of fresh weight in% (w / w) , P0 = Weight of the fruit at the initial time (g) , Pt = Weight of the fruit at the end of time t (g) .

Phenolic compounds

The determination of total phenolics was performed adjusting the Folin – Ciocalteau (SINGLETON, 1965). Initially the extract was prepared with 3.0 g of the homogenized sample and added 150 mL of 80% methanol and stirred for 4 hours on a shaker.

For the determination of total phenolic 400 µL of the extract was added to 400 µL of Folin , waited for 5 minutes and adjusted to a volume of 10 mL flask and then 4000 µL solution of sodium carbonate 7%.

It made the absorbance reading at 750 nm using a quartz cuvette of 1 cm and optical spectrophotometer (UV mini - 1240, SHIMADZU). The results were obtained as gallic acid (GAE) / 100g .

Ascorbic Acid

The ascorbic acid content was determined by the colorimetric method of AOAC 2000 based on the reduction of 2,6- dichlorophenolindophenol -sodium (DFI), standardized with ascorbic acid. The solution was titrated with 2,6- dichlorophenolindophenol for the ascorbic acid solution until a pink hue. Similarly, titrated to a solution containing 5 g of sample and 50 ml of 1% oxalic acid according Strohecker, 1967. The results were expressed as ml of ascorbic acid per 100 g of sample.

MICROBIOLOGICAL ANALYSIS

Microbiological analysis began with the preparation and sterilization of culture media Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) .

Yeast and Molds

25 g strawberries samples

For counting yeasts and molds, originally withdrew the stems of strawberries and weighed 25 g strawberries samples, within a sterile bag. The samples of strawberries were macerated and added 225 mL of solution, 0.85% saline. Using the plating method on the surface were made dilutions of 10^{-1}, 10^{-2} and 10^{-3} (SILVA, 1997). Potato Dextrose Agar amid (PDA) acidified to pH 3.5 with the next solution tartaric acid at 10 % (w / v). Aliquots of 100 mL were plated on the agar surface, plating was carried out under aseptic conditions in triplicate and the plates were incubated at 25 ± 2 ° C for 5 days. The results were expressed as the number of units of colonies formed per gram of material, UFC g^{-1} (APHA, 2002).

Total mesophilic

To count mesophilic it was used the same method used in plating count of yeasts and molds, and amid Plate Count Agar (PCA). Aliquots of 100 mL were plated on the agar surface, plating was carried out in triplicate and the plates were incubated at 35 ± 2 °C for 2 days. The results were expressed as the number of units of colonies formed per gram of material (APHA, 2002).

Experimental design and statistical analysis

With the average values of the parameters studied for the different treatments over time in strawberries stored cold Multivariate analysis of variance, through the process of canonical variable (CV) Morrison, (1967) using the software SAS for Windows v.6.11 by PROG GLM procedure. With the largest eigenvalue canonical variable scores were obtained from the observation vector of each experimental unit of the variables studied, reducing it to a single value. These scores were subjected to analysis of variance with F - Wilks ' Lambda Test.

For comparison of individual chemical properties of fruits in treatment represented the data in tables and graphs .

RESULTS AND DISCUSSION

The values obtained from the observation vector of each experimental unit of the variables of the canonical variable (CV = 0.67480933 * Phenolic - 0.22684053 * Soluble Solids + 0.05237332 * 0.98484496 * AC Asc + pH + 0, 27037330 * AT with eigenvalue = 94%) revealed a significant difference between groups. The treatment versus time interaction was also significant at the 1% level of significance as shown in Table 1.

The results obtained in this study are shown in Figure 1, and we observed that treatment with ozone is the best to maintain the chemical characteristics of strawberries stored under refrigeration.

In their studies Chiattone (2008) shows that in addition to treating microbial agent perishable food with ozone improves quality and enhances the flavor of most foods, because it oxidizes pesticides and neutralizes ammonia and ethylene gases produced during the process of ripening and decay.

Strawberries treated only under refrigeration can be maintained without significant losses for up to five days when destined for fresh consumption and seven days when industrialized (SANTOS, 2007). Scalon (1996) found that the life of strawberries packed in plastic films of polyvinyl chloride (PVC) and stored at 4 ° C is 14 days. In this study strawberries, for both treatments kept at 4 ° C and packaged in PVC plastic was observed preservation of the physical and chemical characteristics throughout the study period of 15 days.

The variables that contributed positively in determining the canonical variable (CV) were pH, TA, ascorbic acid and phenolic compounds.

The acid pH is property of strawberries for industrial use, and the consumer market for raw fruits prefer low-acid fruits (CONTI, 2002).

The pH value expresses only the decoupled acid and has the power to buffer the solution, while the titratable acidity expressed as the amount of acid present.

It can be seen in Table 2 that the pH values increased to the 10th day, 0.10 to treatment without ozone and 0.15 with ozone, with the passage of storage time, there is a decrease in concentration of H + ions, becoming less strawberry acid. This increase is consistent with studies by Moraes et al. (2008), with minimally processed and kept under controlled atmosphere strawberries, in which there was a significant increase in pH in all treatments until the 7th day of storage.

According to the figures presented in Table 3, increased levels of acidity until the 15th day it was found, and the strawberries treated with ozone showed higher levels compared to the untreated, may mean that the treatment caused a decrease in respiratory rate of the fruits.

Similar results were found by Aguiar (2011), in the treatment of strawberries with immersion in ozonated water without soaking the fruits showed lower levels of citric acid over the course of days of storage.

The stability of vitamin C increases as the temperature decreases, reaching a maximum at temperatures below -18 ° C, which can observed at the table 4. However, losses were detected in frozen foods by oxidation, probably because, with very rapid thawing of the products, free themselves from oxidative enzymes within the cell that can react with vitamin C (ORDÓÑEZ, 2004).

In this study the values found for ascorbic acid showed a point of maximum on the 10th day after that day there was a reduction in ozonated fruits which showed less difference, thus getting higher levels of ascorbic acid at the15th day .

The data presented in Table 5 show that there was a variation of phenolic compounds in relation to the treatment and storage period. Comparing the initial and final times, it can be observed that both showed a decrease, where the group treated with ozone showed greater fall. Such compounds being directly involved in the ripening of fruits, that is, are related to the color, flavor and other characteristics, the results can be explained by the fruit harvest date, end of season, so the larger portion of fruit at the beginning the study was ripe.

Chitarra (2005) mentions that during fruit ripening, soluble phenolic condensation occurs which bind to other cellular components therefore making it insoluble. Thus, phenolics are detected at higher levels in green fruit.

According to Table 6 shows that when comparing the results of the first and last day there was a decrease and these similar result was found in the study of Toledo (2011) with strawberries receiving ozone gas and Aguiar (2011) with strawberries subjected to immersion in ozonated water. Yamashita et al., (2006) explains that this drop is due to the fruits using sugars as an energy source to maintain their metabolic activity.

Vieites et al., (2006) found in their studies with different concentrations of O_2 and CO_2 values of soluble solids in the sample decreased at the end of the experiment, data are in agreement with the results found with strawberries.

Evaluating Table 7 the maximum percentage of mass loss found for strawberries treated with

ozone stored at 4 ° C 15 days was 2,74%. In this study, the analysis of fresh matter loss has not be taken into consideration when the treatments compared by canonical variable because the analysis has no repetitions. Although higher than that found by Bender et al. (2010) who in his studies with strawberries stored at 4 ° C for 12 days got maximum fresh weight loss of 0,9%, this value is lower than that found for strawberries, treated with ozone and within maximum loss tolerable for strawberries is 6% mass according to GARCÍA, 1998; CALEGARO, (2002).

Table 8 shows the results of microbiological analyzes , BDA , strawberries submitted or not to ozonation treatment after 0, 5 , 10 and 15 days .

In Brazil, there is no specific legislation setting limits for molds and yeasts in fruits. However, Normative Instruction No. 01 of January 7th, 2000 of the Ministry of Agriculture, Livestock and Supply (MAPA) establishes the maximum limit of 5 x 10^3 CFU g^{-1} for counting yeasts and molds in fruit pulps " in nature " (SOUZA, 2011).

According to Table 8 can be noted that strawberries had since the beginning of the treatment lot of molds and yeasts, already had close to the limit, 5 x 10^3, the MAPA quantities, a pointer to this number can be the date of their harvest which occurred at the end of a crop.

When comparing the values obtained for the two treatments, strawberries treated with ozone showed lower values and within the allowed range at 0 and 10 days since the non ozonated on 5th day of treatment were already unfit for consumption, concluding that ozonation treatment maintained the quality of strawberries in relation to yeasts and molds up to 10 days .

As for yeasts and molds currently there is no legislation to limit quantity Bacteria in fruits "in natura" does not have a law, it can only make a comparison between the values obtained in both treatments, as can be seen in table 9 on the results of the microbiological analyzes, PCA, strawberries submitted or not to ozonation treatment after 0, 5, 10 and 15 days.

TABLE 1- Summary of multivariate analysis of variance.

	Variance Multivariate	Canonical variable
Cause of variation	F-Wilks' Lambda Teste	Medium square
Tretament (T)	33.52**	83.79**
Time (t)	12.00**	29.90**
T x t	33.52**	1.73**
Error	-	0.06
CV (%)	-	0.54

** Significant 1%.

TABLE 2 - pH values in *Fragaria ananassa* submitted or not to ozonation treatment at different storage times. The expressed values are an average of the results in triplicate.

Tretament	Time (days)			
	0	5	10	15
Without ozone	3.49	3.54	3.59	3.59
With ozone	3.51	3.51	3.66	3.61

TABLE 3 - Values of acidity expressed in ml % for *Fragaria ananassa* submitted or not to ozonation treatment at different storage times. The expressed values are an average of the results in triplicate.

Tretament	Time (days)			
	0	5	10	15
Without ozone	139.12	154.05	163.81	171.16
With ozone	150.35	162.60	166.40	181.60

TABLE 4 - Values of ascorbic acid expressed in ml 100g-1 sample of *Fragaria ananassa* submitted or not to ozonation treatment at different storage times. The expressed values are an average of the results in triplicate.

Tretament	Time (days)			
	0	5	10	15
Without ozone	5.74	9.15	10.08	6.82
With ozone	5.89	8.99	9.15	8.06

TABLE 5 - Values of Phenolic Compounds *Fragaria ananassa* in ml $100g^{-1}$ sample of strawberries submitted or not to ozonation treatment at different storage times. The expressed values are an average of the results in triplicate.

Tretament	Time (days)			
	0	5	10	15
Without ozone	0.57	0.61	0.53	0.48
With ozone	0.56	0.58	0.48	0.39

TABLE 6 - Values of soluble solids (º Brix) expressed in ml/100g sample of *Fragaria ananassa* submitted or not to ozonation treatment at different storage times. The expressed values are an average of the results in triplicate.

Tretament	Time (days)			
	0	5	10	15
Without ozone	6.67	6.80	6.37	5.77
With ozone	7.00	6.27	6.43	5.53

TABLE 7 - Values of loss mass expressed as a percentage of loss of fresh weight of *strawberries* submitted or not to ozonation treatment evaluated at the end and start time.

Tretament	Replication	
	1	2
Without ozone	2.82	2.38
With ozone	2.74	2.17

TABLE 8 - Analysis of molds and yeasts, obtained sample dilution 10^{-1} (UFC / g) in *Fragaria ananassa* submitted or not to ozonation treatment at different storage.

Tretament	Time (days)			
	0	5	10	15
Without ozone	7.5×10^3	1.3×10^4	3.0×10^4	3.0×10^4
With ozone	4.87×10^3	9.6×10^3	3.7×10^3	1.05×10^4

TABLE 9 - Analysis of mesophiles obtained sample dilution of 10^{-1} (UFC / g) in strawberries submitted or not to ozonation treatment at different storage times.

Tretament	Time (days)			
	0	5	10	15
Without ozone	NS	1.03×10^3	1.57×10^4	1.57×10^4
With ozone	NS	0.77×10^3	8.13×10^3	8.33×10^3

NS = Not significant.

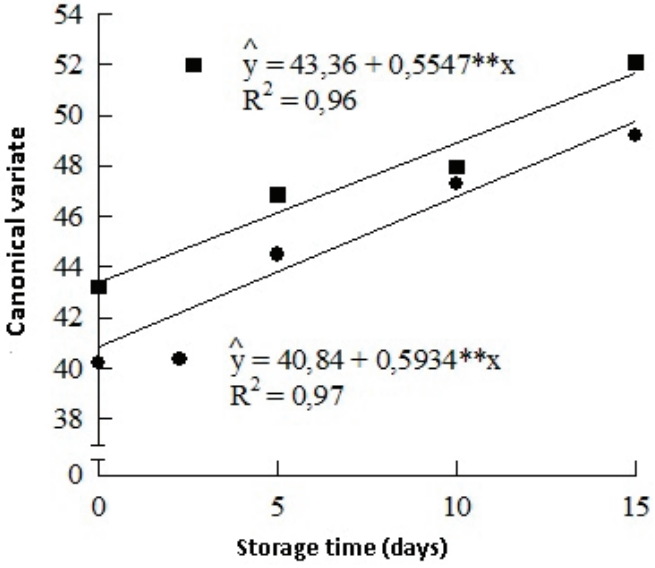

FIGURE 1- Score from the multivariate analysis as a function of storage time (days) with no treatment (●) and with (■) ozone (** significant at the 1% level by t test) canonical variable (CV). The canonical variable is in the y-axis of Figure 1.

CONCLUSION

From the discussion of individual results as well as analysis with the canonical variable related to various physic an chemical factors proposing a single efficiency curve for the period evaluated, it is concluded that the application of ozone gas to maintain the physic-chemical and microbiological characteristics is efficient for storage of fruits for up to 10 days.

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