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Conservation of Sweet Grape tomatoes using albumin coatings containing phenolic compounds of *Spirulina* sp. LEB-18.

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ABSTRACT

The objective of this study was to evaluate the influence of protein globulin-based films and with addition of phenolic compounds extracted from *Spirulina* sp. LEB-18 in the conservation of tomatoes "Sweet Grape". The tomatoes were immersed in edible coatings, the first based on phenolic extracts derived from *Spirulina* sp. LEB-18 and ovalbumin (extracted from eggwhite), and the second based just on ovalbumin, beyond the control sample with only water immersion. Tests of pH, titratable acidity, color, soluble solids, mass loss, and visual analysis were taken every 96 h during the 20 day period. The results demonstrated that the coating made with phenolic compounds increased the shelf life of sweet grape tomatoes, maintaining physical chemical parameters such as pH, acidity, total soluble solids and loss of mass in 37%, 55%, 4.5% and 60%, respectively, lower than the control, conserving the color of the surface of the tomatoes for longer and inhibiting the fungal contamination.

KEYWORDS: *Solanum lycopersicum*; storage; coatings.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables in the world and the seventh species of most important crops, with the Asian continent as a leader of this market and in Brazil, according to the IBGE (2016), Goiás is the state with the largest production of tomatoes, with 21.4% of the total Brazilian production (BERGOUGNOUX, 2014).

Among the types of tomato recently launched in the market, stand out those of rounded forms (cherry) or grape type, reduced size, highlighting the intense red or yellow color for some hybrids, high firmness, resistance to disease and the nutritional value compared to other cultivars (JUNQUEIRA; PEETZ; ONODA, 2011).

Fruits and vegetables, when harvested, are still alive, with accelerated metabolism, since chemical changes are still in progress, using reserves and organic compounds rich in energy as sugars and starches, in order to maintain respiration and production of energy needed. Of all metabolic processes occurring in fruits and vegetables after harvest, respiration is the most important and can be affected by plant specific factors (internal) or by the environment (external), being the temperature the most influential factor (TERUEL, 2008).

Changes in tomatoes during the harvest process, until they reach the consumer, are mainly mechanical, physiological and pathological. Mechanical damages occur in handling of the product (harvest, selection, packaging, transport and display), and physiological and pathological damages occur mainly in the production phase, transport and display (FERREIRA; CORTEZ; HONÓRIO; TAVARES, 2006). These damages may cause disruption in the fruit tissue and make it an ideal substrate for the proliferation of microorganisms, molds and yeasts.

Therefore, techniques such as application of edible coatings, which have the function of coating fresh or minimally processed fruit and vegetables, have been used to preserve the quality, keeping moisture while reducing respiratation rate and slowing the degradation of organic acids, as well as to give shiny appearance and appealing to the product (CIPOLATTI; KUPSKI; ROCHA; OLIVEIRA; BUFFON; FURLONG, 2012).

The materials used for forming coatings may be composed by polysaccharides, proteins and lipids. Among these materials, the albumin protein has inherent properties making it an excellent component for the production of edible coatings, besides being soluble in water at pH 6.6 (DAMODARAN; FENNEMA; PARKIN, 2010; LOPES JUNIOR et al., 2010; REZVANI; SCHLEINING; SÜMEN; TAHERIAN, 2013). Studies with *Spirulina* sp. observed high levels of phenolic compounds that have antioxidant and antifungal activity, which confirms the efficacy against microorganisms even when applied in food (SOUZA et al., 2011; CHRIST-RIBEIRO, A.; BRETANHA; GIACOBBO; SOUZA; BADIALE-FURLONG, 2016; BRETANHA; CHRIST-RIBEIRO; GIACOBBO, G.; SOUZA; BADIALE-FURLONG, 2016).

Therefore, the aim of this study was to apply edible coatings prepared with ovalbumin and phenolic compounds from *Spirulina* LEB-18 on "Sweet Grape" tomatoes, to extend the shelf-life of the fruit.

MATERIAL AND METHODS

As raw material was used the cepa *Spirulina* sp. LEB-18, courtesy of Biochemical Engineering Laboratory (FURG). *Spirulina* production was carried out in pilot plant located in Mangueira Lagoon, Santa Vitória do Palmar, RS, Brazil. In addition to this, we used the protein albumin, obtained from eggwhites coming from candy factories in the city of Pelotas, RS, Brazil (ovalbumin).

The tomatoes were purchased in the local market (Rio Grande, RS, Brazil). They were washed and stored at room temperature (25°C) with the coatings during the experiment. The "Sweet Grape" tomatoes were used weighing on average 12.2 ± 0.6 g and used, for each method applied, a sample n = 5.

EXTRACTION OF PHENOLIC COMPOUNDS AND OVALBUMIN

Phenolic compounds of biomass *Spirulina* sp. LEB-18 were extracted with methanol at a ratio of 1:5 (w/v) at 25 °C for 60 min, under orbital shaking (Tecnal TE-141) at 160 rpm. After this period and a break of 15 min, the solvent was added again (10 mL) and stirred for another 60 min. The methanolic solutions were taken to rotaevaporator (Quimis ®, Q-344B2), and the final residue was dissolved in distilled water. The extract was clarified (5 mL of 0,1M BaOH and 5 mL of 5% $ZnSO_4$) and allowed to stand for 20 min, centrifuged at 3220 g for 15 min, filtered and swelled with distilled water in a volumetric flask of 25 mL. The free phenol content was determined by the Folin-Ciocalteu method, in which the concentration was determined by spectrophotometer (Varian Cary 100) at a 750 nm using a standard curve of gallic acid (2.5 to 22.5 µg mL^{-1}) (SOUZA; PRIETTO; RIBEIRO; SOUZA; BADIALE-FURLONG, 2011).

The extraction of the ovalbumin was performed according to the methodology Sgarbieri (1996) where the eggwhite was diluted with distilled water in the ratio of 2:1 (v / v) under pH 6.6. Shortly after, material was centrifuged (Cientec 5000R) at 15,000 g for 20 min and the ovalbumin was precipitated and collected to be measured by the method of Lowry, Rosebrough, Lewis Farr and Randall (1951).

FORMULATION AND APPLICATION OF EDIBLE COATINGS

For the formulation and application of edible coating, the tomatoes were initially cleaned with water and afterwards immersed in solutions containing 1% calcium chloride ($CaCl_2$) for 5 min and randomly distributed into trays after application of the film corresponding formulation, being tomatoes without coating named as control. After dried, it was applied the coating on the fruit by immersion during 1 min in solutions corresponding to different coatings, leaving the natural drainage of excess solution. One of the coatings, we used the phenolic extract (20 mL) obtained from *Spirulina*, which was homogenised with 3 mL glycerol, 5 mL ovalbumin and enough distilled water to make the volume to 100 mL, based the methodology Cipolatti et al. (2012). The other coating was obtained with 3 mL glycerol, 5 mL ovalbumin and enough distilled water for completing volume 100 mL.

QUALITY EVALUATION OF FRUITS.

The evaluations of the quality of tomatoes were performed every 96 h during a 20-day period. The pH and titratable acidity tests were according to A.O.A.C. (2000); total soluble solids, determined by refractometer-Abbé (ATTO, 2WAJ); epidermal surface color using a colorimeter (Konica Minolta Chroma Meter CR-400/410) according to Oliveira, Martins, Santos, Gomes and Almeida (2012); mass loss according Brookfield, Murpay, Harker and Macrae (1997); and visual analysis comparing the applied tomatoes with the coatings and the control during the storage period. All determinations were performed in triplicate and the differences between treatments were estimated by ANOVA, with 5% significance level, according to Tukey, by the program Statistica 7.0.

RESULTS AND DISCUSSION

The phenolic extracts from *Spirulina* and the protein ovalbumin were quantified with values of 409.67 µg mL^{-1} and the content of 401.23 µg mL^{-1}, respectively.

Table 1 shows the pH and the titratable acidity, the soluble solids of tomatoes submitted to edible coatings and stored for a period of 20 days. The coating C1 showed less variation in pH and titratable acidity content during the 20 days of storage. Samples produced by coating C2 showed lower variations in pH values and content of titratable acidity than the control.

According to Table 1, initially the fruits presented pH and acidity on average of 4.4 and 1.3, during the storage days, the tomatoes coated C1 and C2 maintained the lower pH and acidity levels when compared to the control. These pH values close to neutrality are the most favorable to microbial growth, thus facilitating contamination. The tomatoes coated with C1 and C2 presented more acidic values, making it difficult to synthesize cellular components and the ability to divide the cells of the deteriorating microorganisms, making the flow of protons into and out of the cell uncontrolled due to changes in internal pH (Brown; Booth, 1991). According to Almeida (2014) the coating of tomatoes cv. Rasteiro content of potato starch films and essential oils of salvia and marjoram presented lower values of pH, providing greater stability at the end of the storage period of 288 h.

The coated tomatoes showed the tendency of an increase in the total soluble solids content by the end of the storage period. However, the control sample showed higher and significant additions for analyses of pH, titratable acidity and total soluble solids. As the maturation progresses, there is an increase in sugars content, due to the transformation of starch into simple sugars (Chitarra; Chitarra, 2005). It can be seen that the control sample showed more rapid maturation when compared to fruit subjected to the coatings tested in this study (Table 1).

These data are supported by García, Díaz, Martínez and Casariego (2010), which investigating a chitosan-based coating in papaya, verifing behavior similar to this study, i.e., an increase in soluble solids content in control samples as compared to samples coated with chitosan in 1% lactic acid. Wu, Lu and Wang (2016) studied the effects of application of oligosaccharides derived from japonica Laminaria-incorporated pullulan (in different concentrations) as coatings of cherry tomatoes and found that there was an increase in the shelf life of these fruits at room temperature.

Table 1. Results physicochemical of tomatoes submitted to the application of edible coatings

Parameters	Storage (days)	Treatments		
		C1	C2	Control
pH	1	4,42 ^{c,A}	4,40 ^{b,A}	4,39 ^{d,A}
	4	4,46 ^{c,A}	4,65 ^{a,b,B}	4,95 ^{c,B}
	8	4,52 ^{b,c,A}	4,39 ^{b,A}	5,24 ^{c,A}
	12	4,66 ^{b,B}	5,09 ^{a,b,B}	5,73 ^{b,A}
	16	4,86 ^{a,B}	5,21 ^{a,b,B}	5,78 ^{b,A}
	20	4,96 ^{a,C}	5,64 ^{a,B}	6,83 ^{a,A}
Acidity (%)	1	1,30 ^{b,A}	1,30 ^{c,A}	1,30 ^{c,A}
	4	1,28 ^{b,A}	1,38 ^{c,A}	1,58 ^{b,c,A}
	8	1,37 ^{b,A}	1,68 ^{c,A}	1,89 ^{b,c,A}
	12	1,55 ^{b,A}	1,86 ^{c,A}	2,05 ^{b,A}
	16	2,26 ^{a,B}	2,77 ^{b,B}	4,03 ^{a,A}
	20	2,78 ^{a,B}	3,85 ^{a,A}	4,31 ^{a,A}
Total soluble solid (° Brix)	1	7,68 ^{b,A}	7,68 ^{b,A}	7,68 ^{e,A}
	4	7,70 ^{b,A}	7,68 ^{b,A}	7,78 ^{d,A}
	8	7,69 ^{a,b,B}	7,63 ^{c,C}	7,89 ^{c,A}
	12	7,74 ^{a,b,C}	7,65 ^{c,B}	8,01 ^{b,A}
	16	7,77 ^{a,b,B}	7,73 ^{a,B}	8,14 ^{a,A}
	20	7,81 ^{a}	Cont.	Cont.

NOTE: Cont. = Microbial contamination; Equal and lowercase letters in the same column indicate that there is no significant difference during the storage by Tukey test for each determination (α <0.05). Equal and capital letters in the same line indicate that there is no significant difference between treatments by Tukey test for each determination (α <0.05). C1 = water, glycerol, ovalbumin and phenolic extract; C2 = distilled water, glycerol and ovalbumin; Control = distilled water.

Through the surface color data of the skin of "Sweet Grape" tomatoes subjected to different treatments and stored for 20 days (Table 2), it was observed a tendency to higher values of L until the 8th day for all samples, and then decreased until 16th day, reflecting an increase in the darkening of tomatoes to red, probably due to carotenoid synthesis (Cipolatti et al., 2012). At 20th day, tomatoes subjected to the C2 coating and the control showed microbial contamination. For this reason, the color reading on the surface of the fruit was not performed. However, before the 20th day, it was observed a definite trend of the influence of different coatings on this color parameter during storage of the samples.

All samples showed the highest value of a* to the 16th day of storage, except for the control (12th day). Also there was no definite trend of the influence of different coatings on this color parameter during storage of the samples.

Table 2. Color parameters of the surface of tomatoes subjected to different coatings during storage

Parameters	Storage (days)	Treatments		
		C1	C2	Control
L	1	37,89 ^{d,A}	37,89 ^{c,A}	37,89 ^{b,A}
	4	38,83 ^{b,A}	35,96 ^{d,B}	36,06 ^{e,B}
	8	39,79 ^{a,b,B}	42,02 ^{a,A}	38,59 ^{a,C}
	12	38,12 ^{c,B}	38,12 ^{b,B}	36,78 ^{c,A}
	16	34,83 ^{d,B}	33,85 ^{e,B}	36,56 ^{d,A}
	20	34,83 ^{d,A}	Cont.	Cont.
a*	1	10,20 ^{e,A}	10,20 ^{e,A}	10,20 ^{e,A}
	4	19,79 ^{b,B}	21,19 ^{b,A}	15,77 ^{d,C}
	8	18,23 ^{d,B}	18,14 ^{c,B}	19,52 ^{b,A}
	12	18,92 ^{c,B}	17,01 ^{c,B}	20,25 ^{a,A}
	16	22,64 ^{a,A}	21,3 ^{a,A}	17,65 ^{c,C}
	20	22,64 ^{a,A}	Cont.	Cont.
b*	1	19,80 ^{d,A}	19,80 ^{b,A}	19,80 ^{c,A}
	4	19,34 ^{c,B}	22,61 ^{a,A}	17,31 ^{e,C}
	8	22,32 ^{b,B}	19,31 ^{b,C}	21,81 ^{a,A}
	12	23,42 ^{a,A}	18,47 ^{c,B}	18,88 ^{d,B}
	16	18,98 ^{e,B}	17,67 ^{b,B}	20,65 ^{b,A}
	20	18,98 ^{e,A}	Cont.	Cont.
H (°)	1	60,01 ^{a,A}	60,01 ^{a,A}	60,01 ^{a,A}
	4	54,41 ^{b,A}	46,69 ^{c,B}	47,43 ^{d,B}
	8	51,06 ^{c,A}	46,72 ^{c,C}	48,71 ^{c,B}
	12	51,08 ^{c,A}	47,49 ^{b,B}	43,70 ^{e,C}
	16	40,04 ^{d,B}	39,77 ^{d,B}	49,44 ^{b,A}
	20	40,04 ^{d,A}	Cont.	Cont.

NOTE: Cont. = Microbial contamination; Equal and lowercase letters in the same column indicate that there is no significant difference during the storage by Tukey test for each determination (α <0.05); Equal and capital letters in the same line indicate that there is no significant difference between treatments by Tukey test for each determination (α <0.05). C1 = water, glycerol, ovalbumin and phenolic extract; C2 = distilled water, glycerol and ovalbumin; Control = distilled water.

The b* values indicate an increase followed by a decrease to the samples subjected to the coatings, whereas for the control there was no tendency to increase or decrease as a function of storage time. The samples submitted to C2 coating are differentiated of sample submitted to C1 coating for presenting an increase only until the 4th day of storage, followed by a gradual decrease in the subsequent period. This suggests that the C1 coatings can keep the fruits until the 12th day with the coloration closest to the yellow, since the parameter b* measures the yellow / blue coordinate indicating, thus, a greater stage of maturation. This reaction was not as effective when compared to the C2 coating, which at the 4th day of storage obtained the highest value of b* and the control at 8th day.

The values of H (°) or hue angle showed a decrease between the first and the fourth day for all coatings during storage. However, the sample submitted to the C1 coating presented a smaller reduction in relation to the other coatings until the 12th day. The "h" parameter defines the basic coloring and represents the hue of the tomatoes. The larger the color angle (h) obtained means that the color of the fruit is closer to yellow and the lower the angle, the color approaches red. Therefore, the C1 coating presented the highest values of H ° indicating a higher preservation of the maturation state and the color of the fruit, unlike the C2 coating and the control that during the storage acquired the faster red color, and, consequently, the maturation and deterioration (Borguini; Silva, 2005). The results of this manuscript are supported by Das, Dutta and Mahanta (2013), who found that the values of L (Brightness and clarity) for the tomatoes coated with rice starch-based films containing coconut oil, as well as leaf extract of tea and control, declined during the initial maturation in storage up to 8 days. The b values (contrary to the values of L) showed an increase until 8th, followed of decline until the 20th day. The authors reported that the color change of fruits corresponds to a decrease in chlorophyll content and an increase in carotenoid synthesis, which reflects processing the chloroplasts to chromoplasts. Likewise Das, Dutta and Mahanta (2013) observed that tomatoes stored at room temperature showed greater changes in the values of a* and H (°) until the 12th day of storage, corroborating with results of this study. Therefore, with the data set results (color) showed that with coatings of phenolic extracts apllied in tomatoes showed a protective action to preserve the color of tomatoes "sweet grape."

Figure 1 presents mass loss during storage of tomatoes subjected to different treatments. The control sample stands out greater mass losses in almost all periods analyzed reaching in 16th and 20th storage days losses of 35 and 86%, respectively. The C1 treatment presented mass loss, except on the first day, lower than the other samples in all periods of storage, reaching the highest losses of 23% and 32% for the 16th and 20th storage days, respectively.

The reduction of the loss was probably due to the coating effect as a semipermeable barrier to O_2, to CO_2, to moisture and to the movement of solutes, thus reducing the breathing, loss of water and oxidation reactions (Cipolatti et al., 2012). This behavior may be due to the barrier efficiency as a function of lipophilicity attributed to the composition of coatings, which prevent water loss from the fruit that tend to have degraded walls and the released water, causing tissue shriveling. According to Oliveira, Leite, Aroucha and Ferreira (2011), the coating of tomatoes with gelatin biofilm influenced the loss of mass and ripening process of fruits. The hygroscopic character of the utilized biofilm caused a greater weight loss the fruits during the storage period, and the concentration used 20% gelatin prevented the normal ripening of the coated tomatoes.

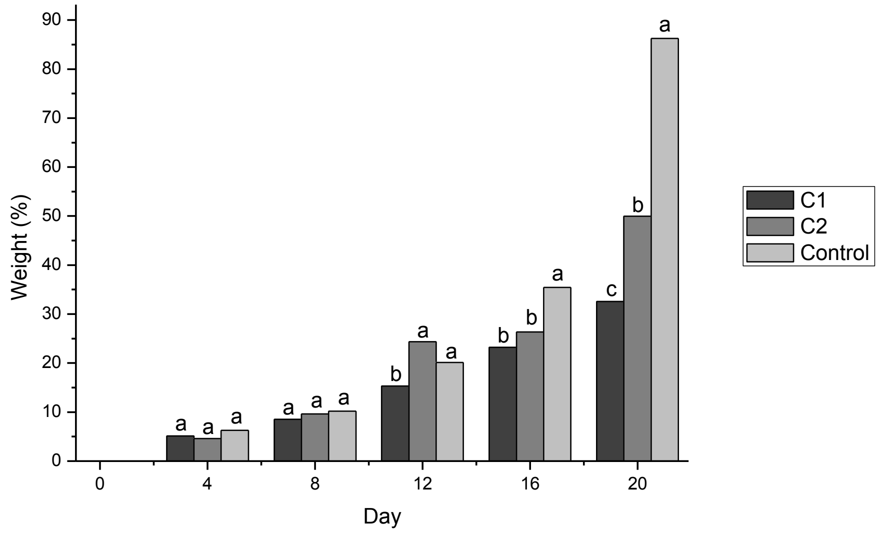

Figure 1 - Mass loss on tomatoes subjected to different edible coatings during storage. C1 - destilled water, glycerol, ovalbumin and phenolic extract; C2 - destilled water, glycerol and ovalbumin; Control - destilled water.

Figure 2 shows the visual analysis of tomatos subjected to different treatments during storage through photographs taken during the period of 20 days. It is observed that the C1 coating showed an increase in shelf life, preserving against the microorganisms and the damage to the surface of the tomato peel.

According to Figure 2, it can be seen that the C1 samples showed no signs of fungal contamination or morphological changes of wrinkling and shriveling fruit, remaining unchanged until the end of the experiment, in great condition and presentation. Tomatoes coated with ovalbumin showed greater stability than the control samples having damage from the 16th day. The control showed only minor damage from the 12th day of storage, being the 20th day with complete degradation of the fruit.

The hydrophilic materials exhibit good solubility in aqueous media, thus favoring a better dispersion of the solute and a more homogeneous formation of the coating. In the case of this study, with the chemical structure of the compounds employed, allowed complete solubilization and formation of a kind of gel for both coatings. Phenolic compounds and Albumin are materials with structures in which there is a predominance of amino groups or hydroxyl and characterized by polar covalent bonds that have carbon chain sites partially positively charged, and the other, negatively, favoring the accumulation and rearrangement of the molecules polar, and particularly water, around these sites. Therefore, hydrophilic coatings are suitable for fruit with bright aspects for having affinity for water, preserve the aspect hydrated, keeping longer the shiny surface and increase the shelf life (Assis; Britto, 2014).

With the application of the phenolic compound in the coating (C1), became shelf life of tomato greater, since their action is related to their chemical structures, wherein the gallic acid is majority, followed of caffeic acid in the matrix of *Spirulina* sp. LEB-18. These compounds are able to penetrate the cell wall and inducing physical change and / or chemical or affect metabolic pathway, can also harm to obtain the energy required to maintain cell viability, possibly due to the lower availability of substrate and hence makes energy Lowest to produce cell wall components (Pagnussat; Del Ponte; Garda-Buffon; Badiale-Furlong, 2014).

Figure 2 - Photographs of tomatoes subjected to different treatments and stored for a period of 20 days. (A) 1st day; (B) 4th day; (C) 8th day; (D) 12th day; (E) 16th day; (F) 20th day. C = control with destilled water; C2 = treatment with ovalbumin, glycerol and destilled water; C1 = treatment with ovalbumin, glycerol, destilled water and phenol extract.

CONCLUSION

The samples submitted to edible ovalbumin coating containing phenolic extracts derived from *Spirulina* sp. LEB-18 (C1) has been shown to be promising for the conservation of "sweet grape" tomatoes, preserving physicochemical characteristics, inhibiting fungal contamination and, consequently, increasing fruit shelf life compared to other treatments.

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Conservação de tomates Sweet Grape utilizando revestimentos de albumina contendo compostos fenólicos (*Spirulina* sp. LEB-18).

ABSTRACT

O objetivo deste trabalho foi avaliar a influência de películas à base de proteína globulina e de películas com adição de compostos fenólicos extraídos da *Spirulina* sp. LEB-18 na conservação de tomates tipo "Sweet Grape". Para isto, os tomates foram imersos por um min em películas comestíveis, uma à base de extratos fenólicos extraídos da *Spirulina* sp. LEB-18 e globulina (extraída de clara de ovo), e outra à base de globulina, além da amostra controle apenas com imersão em água. Foram realizadas a cada 96 h durante o período de 20 dias, análises de pH, acidez total titulável, de cor, de sólidos solúveis totais, de perda de massa e análise visual. Os resultados demonstraram que a película elaborada com compostos fenólicos aumentou a vida útil de tomates tipo "sweet grape" mantendo parâmetros físico químicos como pH, acidez, sólidos solúveis totais e perda de massa em 37%, 55%, 4,5% e 60% menores que o controle, conservando a cor da superfície dos tomates por mais tempo e inibindo a contaminação fúngica.

PALAVRAS-CHAVE: *Solanum lycopersicum*; armazenamento; revestimentos.

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