

# Application of protein-phenolic based coating on tomatoes (*Lycopersicon esculentum*)

## Aplicação de coberturas proteicas e fenólicas em tomates (*Lycopersicon esculentum*)

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### Abstract

The aim of this study was to investigate the use of protein-phenolic based coating made from fermented rice bran on cherry tomatoes (*Lycopersicon esculentum*). Tests were performed with glycerol 3% (v/v), glycerol with protein-phenolic rice bran extract (5%), glycerol with protein-phenolic extract after 96 hours of fermentation (5%), and a control (without coating). The coated cherry tomatoes were kept at room temperature for 28 days. Mass loss, pH and acidity, total soluble solids, and carotenoids were determined every 96 hours. The coating made from the biomass extract reduced the carotenoid and acidity levels in the fruits studied by 17 and 21.1%, respectively, compared to the control. The coating proved an efficient barrier to water vapor with mass loss of 57% less than the control suggesting that it can be used as an alternative for vegetable tissue conservation.

**Keywords:** rice bran; fermentation process; conservation.

### Resumo

Este trabalho teve como objetivo estudar a utilização de películas, à base de compostos proteicos e fenólicos provenientes de farelo de arroz fermentado, em tomates (*Lycopersicon esculentum*). Foram realizados testes com: glicerol 3% (v/v); glicerol com extrato fenólico e proteico do farelo de arroz (5%); glicerol com extrato fenólico e proteico da biomassa gerada em 96 horas (5%), e um controle (sem a película). Os tomates revestidos foram mantidos à temperatura ambiente durante 28 dias, sendo determinados, a cada 96 horas, os seguintes aspectos: a perda de massa, o pH e a acidez, os sólidos solúveis totais e os carotenoides. A película elaborada com os extratos da biomassa reduziu os níveis de carotenoides e acidez dos frutos estudados em 17 e 21,1%, respectivamente, em relação ao controle. A película também foi eficiente como barreira ao vapor de água; assim, com perda de massa 57% inferior à do controle, sugere-se que esta poderá ser utilizada como alternativa para conservação desse tecido vegetal.

**Palavras-chave:** farelo de arroz; processo fermentativo; conservação.

## 1 Introduction

Tomato is climacteric fruit, and therefore it is a highly interesting system to study maturation and ripening processes because of the postharvest metabolic changes. Storage life is a function of several factors including transpiration, postharvest diseases, and quick ripening and senescence causing production loss (BOGGIO et al., 2000; CHEN et al., 2001).

Coating can act as barriers to moisture and oxygen during processing, handling, and storage (XU et al., 2007). The use of coating for protection purposes represents an economical advantage avoiding the need for climate controlled storage, which incurs operational costs and requires special equipment (FALCAO-RODRIGUES; MOLDAO-MARTINS; BEIRAO-COSTA, 2007). Biopolymers such as carbohydrates, lipids, and proteins can be used as coating with the purpose to modify the internal atmosphere of fruit and vegetables (CISNEROS-ZEVALLOS; KROCHTA, 2003). Polysaccharides have been

successfully used in coating formulation offering barrier against gases. However, due to their hydrophilic nature, they are not offer good barrier against humidity. Lipids offer excellent barrier against moisture, but they present problems related to oxidative stability. Protein-based coatings are known for being biodegradable and offering good barrier against gas. They also offer a mechanical protection, which increases the shelf life and minimizes food deterioration (CHO; PARK; RHEE, 2002). Proteins are made up of long carbonated chains, with 20 different monomers, which allows the necessary molecular interaction for coating generation (DANGARAN; TOMASULA; QI, 2009).

It was demonstrated that rice bran fermentation with the microorganism *Rhizopus oryzae* provides a significant increase in the protein content in the biomass, resulting in a continuous and cohesive matrix (OU et al., 2005; SILVEIRA; BADIALE-FURLONG, 2007) and in the antioxidant activity of

Received 27/8/2010

Accepted 21/5/2012 (005012)

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DOI: <http://dx.doi.org/10.1590/S0101-20612012005000081>

the phenolic compounds in the bran (OLIVEIRA et al., 2010). These properties may help protect against oxidative damage.

To our knowledge, there is no published study on the use of phenolic and protein compounds obtained during solid state fermentation as coating for the preservation and extension of storage life of fresh fruit. The aim of this study was to investigate the use of protein-phenolic based coating made from fermented rice bran using glycerol as plastificant in order to extend the shelf life of tomatoes.

## 2 Materials and methods

### 2.1 Raw material

The rice bran used in this study was provided by IRGA (Instituto Rio-Grandense de Arroz); it was packed in 3 kg polypropylene bags and kept under refrigeration until use. The cherry tomatoes (*Lycopersicon esculentum*) were cultivated in the city of Feliz (Rio Grande do Sul State – RS).

### 2.2 Fermentation of the rice bran

Solid state fermentation (SSF) was carried out in tray bioreactors ( $29 \times 17 \times 5.5 \text{ cm}^3$ ). Rice bran substrate (100 g), with particle diameter of 0.5 mm (mesh 32 mm), was autoclaved in tray bioreactors forming a thin layer (2 cm). Next, it was homogenized with 45 mL of the nutrient solution ( $\text{KH}_2\text{PO}_4$  2 g.L<sup>-1</sup>,  $\text{MgSO}_4$  1 g.L<sup>-1</sup>,  $\text{NH}_2\text{CONH}_2$  1.8 g.L<sup>-1</sup> in HCl 0.4 N). The initial spore concentration of *Rhizopus oryzae* CCT 7560 was  $4.0 \times 10^6$  spores.g<sup>-1</sup> (BADIALE-FURLONG; CACCIAMANI; GARDA-BUFFON, 2007). Moisture was adjusted to 50% by the addition of sterile water, and the trays were covered with sterilized gauze to allow aeration. The incubation was carried out in a fermentation chamber (TECNAL TE – 403, Piracicaba, Brazil) at 30 °C, and one tray bioreactor was removed from the incubator every 24 hours for five days resulting in the biomasses of 0, 24, 48, 72, 96, and 120 hours, which were stored at -18 °C for later use (OLIVEIRA et al., 2010).

#### Protein extract

The method used to obtain the protein extract was adapted from Adebisi et al. (2008), which consisted of defatting fermented and unfermented rice bran with petroleum ether. Next, protein extraction was performed in alkaline environment (pH 9.5) using 10 g of defatted bran and 70 mL of NaOH 0.02 M adjusting the pH with NaOH 6 M. The mixture was stirred for 30 minutes at 160 rpm (MARCONI MA – 410, Piracicaba, Brazil), centrifuged (CIENITEC 5000R, Piracicaba, Brazil), and precipitated with acetone in a ratio of 1:3 (v/v). Protein was quantified by the method of Lowry et al. (1951).

#### Phenolic extract

The phenolic compounds were cold extracted with methanol 1:5 (w:v) using the method described by Souza et al. (2009). The antioxidant activity of these compounds was determined by the DPPH free radical sequestration method (SOUSA et al., 2007).

### 2.3 Formulation and application of the coating on cherry tomato (*Lycopersicon esculentum*)

The tomatoes were selected according to size and color (ruddy) (BRASIL, 1995), washed in water, and immersed in a sodium hypochlorite solution 100 mg.L<sup>-1</sup> for 5 minutes. The coating solutions were prepared dissolving glycerol and protein-phenolic extract from fermented and unfermented rice bran in distilled water. Three different treatments were evaluated: glycerol (3% v/v) (treatment 1); coating solution containing glycerol (3% v/v) and protein-phenolic (5%) rice bran extracts (treatment 2); coating solution containing glycerol (3% v/v) and protein-phenolic extracts (5%) from the biomass (treatment 3); and an uncoated control. The coating was applied by fruit immersion in the different treatments for 1 minute with further drainage of excess. Next, they were placed on trays and kept at room temperature (20-25 °C) for 28 days. The useful life of tomatoes coated with the different treatments was monitored every 96 hours.

### 2.4 Life cycle follow-up

The mass loss was determined by gravimetry, and it was expressed in g.100 g<sup>-1</sup> (%) of the initial mass of the fruit (ALI et al., 2010).

A digital pH meter was used for measuring the pH. The acidity was determined by neutralization with NaOH 0.1 N, and the results were expressed in citric acid percentage (ASSOCIATION..., 2000).

Soluble solids were determined (°Brix) in an Abbé refractometer according to (ASSOCIATION..., 2000) with diluted sample 1:5 (w:v). The total carotenoids were determined according to Carvalho et al. (2011) by extraction using acetone in the ratio of 1:8 (w:v) and stirring at 200 rpm in a shaker for 1 hour. Next, the extract was filtered in vacuum conditions by means of a Kitassato flask wrapped with aluminum foil to avoid photo-oxidation of pigments. The samples were washed three times with acetone for complete extraction of pigments. The content was transferred to a recipient containing 45 mL of petroleum ether. Next, distilled water was added to remove any remaining acetone solution. The pigment solution in petroleum ether was transferred to a volumetric flask and then diluted to 100 mL for further reading at the wavelength of 470 nm. The carotenoids content was expressed by Equation 1.

$$\mu\text{g.g}^{-1} = \frac{(A.V.10^6)}{M.100.A^{1\%}} \quad (1)$$

where: A is the solution absorbance at a wavelength of 470 nm; V is the final volume of the solution; A<sup>1%</sup> is the absorbance of a particular pigment in a specific solvent, and M is the sample mass taken for analysis. The absorptivity coefficient of the carotenoids extracts in petroleum ether is 3450.

### 2.5 Statistical analysis

All determinations were done in triplicate and the results were submitted to variance analysis (ANOVA) with significance level of 5% (p < 0.05) and Tukey Studentized Range test.

### 3 Results and discussion

#### 3.1 Selection of the extracts for coating application

The fermentation process increases nutrient availability in raw materials due to the changes resulting from the metabolic activity of microorganismos (WAINWRIGHT, 1995). In the present study, rice bran was fermented by the microorganism *Rhizopus oryzae* CCT 7560 resulting in an increase in the protein level from 155 (non-fermented bran) to 276, 348, 286, 332, 375, and 359 g.kg<sup>-1</sup> for 0, 24, 48, 72, 96 and 120 hours, respectively. There was an increase of 35.9% in the protein after 96 hours in relation to 0 hour. An increase of 400% was also observed in the phenolic compounds after 96 hours. According to Oliveira et al. (2010), this time interval resulted in an increase in the amount of protein and phenolic compounds with antioxidant capacity who found an increase of 49% in the protein and an increase of 366% in the phenolic compounds after fermentation by the same microorganism. The protein obtained by fermentation demonstrated an improvement in the gelling properties allowing an improvement in coating elasticity (DANGARAN; TOMASULA; QI, 2009). The increase in the antioxidant properties as a function of phenolic liberation makes the extract interesting to prevent oxidative process and favor the cross linking between protein chains. This represents a decrease in the permeability, which would prevent mass loss.

#### 3.2 Life cycle follow-up

Maintaining fruit mass indicates the integrity of the cell walls. It is related with the respiratory activity decrease, and it indicates the protective effect of coatings. Moisture is lost during transpiration when water is converted from liquid to gas. When produce is harvested, it loses its source of water, so recuperation from water loss is not possible. When water is lost, the turgor decreases and this water stress also causes metabolic alterations, changing enzyme activity, which may result in senescence acceleration (OLIVAS; BARBOSA-CÁNOVAS, 2009). Figure 1

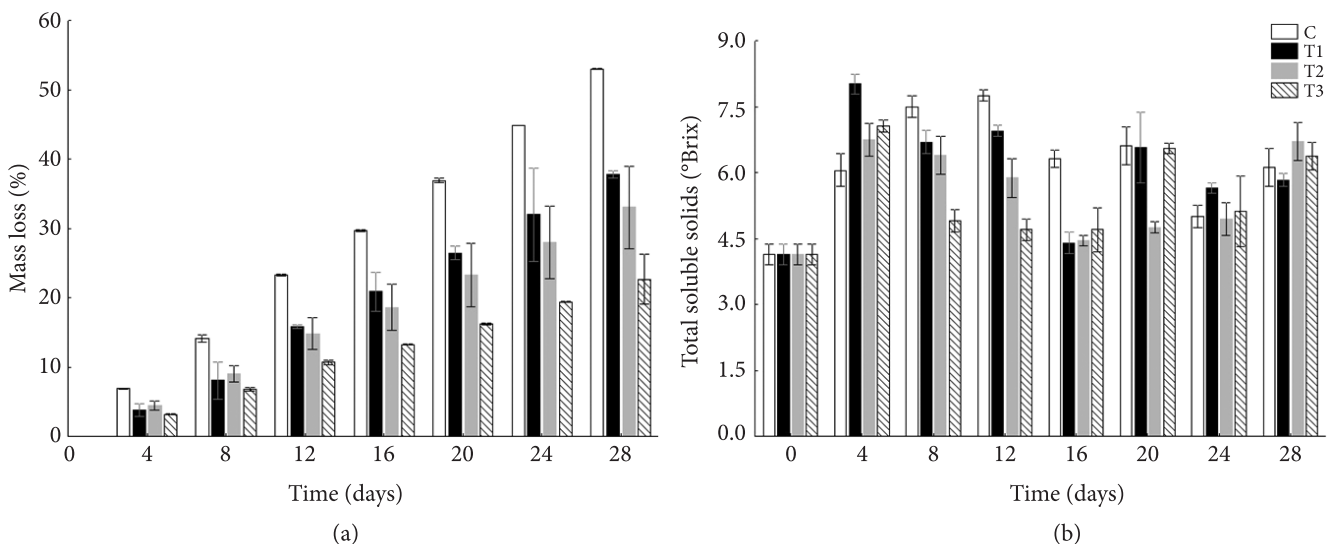
shows tomatoes mass loss (a) and total soluble solids when they were coated with the three treatments during 28 days.

The mass loss in the control group was the highest, 53% after 28 days of experiment, which can be related to the absence of the protective coating. The coating containing glycerol and extracts from the fermented bran (treatment 3) was more efficient as a barrier to mass loss, 43% lower than the control group on the last day of experiment. Fruit weight loss during senescence has been observed by other authors (YAMAN; BAYOINDIRLI, 2002; ALI et al., 2010), which results in the metabolic degradation of the cell wall decreasing the water retention capacity observed during senescence. In this case, the mass loss was decreased suggesting delayed senescence.

The weight loss reduction was probably due to the effects of the coating as a semi-permeable barrier against O<sub>2</sub>, CO<sub>2</sub>, moisture, and solute movement, thus reducing respiration, water loss, and oxidation reaction rates (BALDWIN et al., 1999) and this may influence, mainly, consumer's preference. This behavior can be due to the efficiency of the protein barrier created by the coating, which prevented the dissection of the fruits that tend to have the walls degraded and the water released causing tissue wilting.

It can be observed in Figure 1 that all treatments showed total soluble solids (TSS) increase. The increase in the TSS content during the experiment is due to the pectin degradation with the carbohydrates release. The results suggest that there was a delay in the degradation reactions, retarding fruit ripening. The treatments that reduced pectin degradation were those containing protein and phenolic compounds and smaller mass loss. García et al. (2010), investigating a chitosan coating on papaya, verified a similar behavior to that found in this study, an increase in TSS content for the coating with chitosan 1% in lactic acid, Tween 80, and oleic acid.

The pH and acidity as citric acid of the samples submitted to the different treatments (Table 1) showed no significant



**Figure 1.** Effect of different coatings: C (uncoated); T1 (glycerol); T2 (glycerol and rice bran extracts); T3 (glycerol and biomass extracts) on tomatoes mass loss (a) and total soluble solids (b).

**Table 1.** pH and acidity of tomatoes in the different treatments during storage.

Days	pH			
	C	T1	T2	T3
0	4.42 ± 0.01 <sup>aD</sup>	4.42 ± 0.01 <sup>aCD</sup>	4.42 ± 0.01 <sup>aB</sup>	4.42 ± 0.01 <sup>aE</sup>
4	4.46 ± 0.02 <sup>aC</sup>	4.40 ± 0.00 <sup>dcD</sup>	4.43 ± 0.01 <sup>caB</sup>	4.48 ± 0.01 <sup>baD</sup>
8	4.51 ± 0.01 <sup>aB</sup>	4.60 ± 0.05 <sup>aBC</sup>	4.43 ± 0.03 <sup>aB</sup>	4.50 ± 0.00 <sup>aC</sup>
12	4.50 ± 0.03 <sup>bb</sup>	4.50 ± 0.01 <sup>baB</sup>	4.48 ± 0.03 <sup>aA</sup>	4.60 ± 0.01 <sup>aA</sup>
16	4.41 ± 0.01 <sup>aD</sup>	4.33 ± 0.01 <sup>aE</sup>	4.36 ± 0.02 <sup>aC</sup>	4.40 ± 0.01 <sup>aF</sup>
20	4.32 ± 0.00 <sup>cabdE</sup>	4.48 ± 0.01 <sup>baB</sup>	4.20 ± 0.01 <sup>cdE</sup>	4.52 ± 0.01 <sup>aB</sup>
24	4.60 ± 0.20 <sup>bacda</sup>	4.60 ± 0.02 <sup>abcA</sup>	4.31 ± 0.02 <sup>caD</sup>	4.21 ± 0.00 <sup>dacG</sup>
28	4.43 ± 0.00 <sup>aCD</sup>	4.55 ± 0.02 <sup>aA</sup>	4.42 ± 0.02 <sup>aB</sup>	4.54 ± 0.01 <sup>aAB</sup>

Days	Acidity (% citric acid)			
	C	T1	T2	T3
0	0.80 ± 0.00 <sup>aE</sup>	0.80 ± 0.00 <sup>aE</sup>	0.80 ± 0.00 <sup>aC</sup>	0.80 ± 0.00 <sup>aD</sup>
4	1.20 ± 0.20 <sup>aC</sup>	1.25 ± 0.21 <sup>aA</sup>	1.25 ± 0.02 <sup>aB</sup>	1.18 ± 0.05 <sup>aBC</sup>
8	1.21 ± 0.02 <sup>acC</sup>	1.23 ± 0.03 <sup>aAB</sup>	1.21 ± 0.03 <sup>aB</sup>	1.02 ± 0.06 <sup>bcC</sup>
12	1.06 ± 0.02 <sup>bd</sup>	1.13 ± 0.00 <sup>aC</sup>	1.15 ± 0.02 <sup>aB</sup>	1.01 ± 0.02 <sup>bcC</sup>
16	1.20 ± 0.01 <sup>aCD</sup>	1.18 ± 0.01 <sup>aB</sup>	1.18 ± 0.00 <sup>aB</sup>	1.15 ± 0.02 <sup>aBC</sup>
20	1.35 ± 0.08 <sup>bb</sup>	1.08 ± 0.00 <sup>cd</sup>	1.53 ± 0.02 <sup>aA</sup>	1.14 ± 0.06 <sup>bcC</sup>
24	1.58 ± 0.06 <sup>aA</sup>	1.08 ± 0.02 <sup>bd</sup>	1.56 ± 0.06 <sup>aA</sup>	1.64 ± 0.10 <sup>aA</sup>
28	1.66 ± 0.06 <sup>aA</sup>	1.25 ± 0.02 <sup>ba</sup>	1.53 ± 0.11 <sup>abA</sup>	1.31 ± 0.09 <sup>bcB</sup>

Values are expressed as means ± SD. The values in each column with the same overwritten letter (lower case) are not significantly different ( $p < 0.05$ ). The values in each line with the same overwritten letter (uppercase) are not significantly different ( $p < 0.05$ ). C: uncoated; T1: glycerol; T2: glycerol and rice bran extracts; T3: glycerol and biomass extracts.

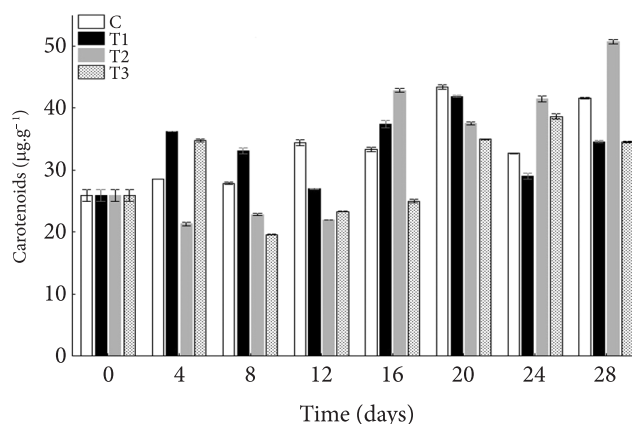
difference ( $p < 0.05$ ). Davila-Avina et al. (2011), studying tomatoes coated with mineral oil, found that the initial pH of the fruit coated with mineral oil was 4.2, similar to that found in this study, 4.4. After the 24<sup>th</sup> day, treatment 3 was lower comparing with the others showing that the application of coatings modified the internal atmosphere and the endogenous CO<sub>2</sub> and O<sub>2</sub> concentration of the fruit retarding ripening.

Acidity is directly related to the organic acids concentration in the fruits, and it depends on several intrinsic and extrinsic factors such as: cultivars, fertilization, soil management, irrigation, and some physiological factors (leaf area, organic biosynthesis, etc.) (CHIEN; SHEU; YANG, 2007). During the 28 days of storage, a tendency toward increasing acidity was observed.

The variations in the total carotenoids contents of the tomatoes during the 28 days of storage are shown in Figure 2. (GARCÍA et al., 2009). In this study, this aspect can be observed since, during the tomatoes storage, there was an increase in the carotenoids content. The increase was of 60.5% in the control and 33.2% in treatment 3 at end of the storage period indicating that the use of coating also provides a delay in the chlorophyll degradation.

Ilahy et al. (2011) compared the lycopene content present in tomatoes cultivar 'Rio Grande' at different maturation stages and found 1.6 µg.g<sup>-1</sup> in green, 8.2 µg.g<sup>-1</sup> in green-orange, 26.7 µg.g<sup>-1</sup> in orange-red and 97.0 µg.g<sup>-1</sup> in red-ripe tomatoes, which confirms the increase in the pigment with the maturation degree evolution.

The reactions during fruit maturation and senescence are independent; however, in order to improve shelf life, the rate of



**Figure 2.** Effect of different coatings: C (uncoated); T1 (glycerol); T2 (glycerol and rice bran extracts); T3 (glycerol and biomass extracts) on tomatoes carotenoids content.

chemical reaction should be decreased. The coating obtained in treatment 3 proved able to retard the tomatoes senescence by decreasing the main metabolic reactions.

#### 4 Conclusion

The use of protein-phenolic based coating made from the biomass fermented by *Rhizopus oryzae* (CCT 7560) combined with glycerol on tomatoes (*Lycopersicon esculentum*) decreased maturation reactions indicated by the decrease in the mass loss rate, pH, and acidity and increase in total carotenoids in relation to the control (without coating). Therefore, the use of coating provides an extended conservation period and also adds value to the product.



## Acknowledgements

The authors are grateful to the IRGA Institute (Instituto Rio Grandense do Arroz) for supplying rice bran.

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