

## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

### *Conservação de maçãs minimamente processadas através de revestimentos comestíveis de extrato de nabo e goma xantana*

Caroline Dellinghausen Borges<sup>1\*</sup>, Carla Rosane Barboza Mendonça<sup>1</sup>, Daiane Nogueira<sup>1</sup>, Ederson Schwenske Hartwig<sup>1</sup>, Josiane Kuhn Rutz<sup>2</sup>

<sup>1</sup> Universidade Federal de Pelotas (UFPel), Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Campus Universitário, Pelotas/RS - Brazil

<sup>2</sup> Universidade Federal de Pelotas (UFPel), Faculdade de Agronomia Eliseu Maciel (FAEM), Departamento de Ciência e Tecnologia Agroindustrial, Campus Universitário, Pelotas/RS - Brazil

#### \*Corresponding Author

Caroline Dellinghausen Borges, Universidade Federal de Pelotas (UFPel), Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Campus Universitário, Capão do Leão, prédio 4, Caixa Postal: 354, CEP: 96010-900, Pelotas/RS - Brazil, e-mail: caroldellin@hotmail.com

**Cite as:** Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum. *Braz. J. Food Technol.* v. 19, e2015038, 2016.

Received: June 03, 2015; Accepted: Feb. 05, 2016

#### Summary

The objective of this study was to evaluate the potential of turnip extract and xanthan gum in the conservation of minimally processed apples. The apples were washed, sanitized with sodium hypochlorite (200 ppm) for 15 minutes, peeled, and cut into eight pieces prior to being subjected to one of the following treatments in aqueous solution: A – water (control); B – turnip extract; C – turnip extract and CaCl<sub>2</sub>; D – xanthan gum, CaCl<sub>2</sub> and glycerol; E – turnip extract, xanthan gum, CaCl<sub>2</sub>, and glycerol. Subsequently, the freshly cut apples were dried under ventilation on nylon screens to ensure drying of the coatings, and then packed in polystyrene trays, covered with polyvinylchloride films and stored at 4 ± 1 °C for 13 days. The following parameters were evaluated: mass loss, firmness, colouration, pH value, soluble solids, and peroxidase/polyphenoloxidase activities. The edible coatings were found to be ineffective with respect to controlling mass loss, but the minimally processed apples coated with turnip extract maintained their initial levels of colouration, firmness and pH value. A considerable increase in peroxidase activity was registered for apples treated with turnip extract, suggesting that this effect may also be responsible for the reduction in browning. No advantage could be observed for the simultaneous presence of turnip extract and xanthan gum or calcium chloride. The turnip extract may represent an interesting alternative for applications to minimally processed apples, especially as it is a natural product, easily obtained, cost effective and contributes to the nutritional quality (e.g. as a source of calcium ions).

**Keywords:** *Minimally processed apple; Turnip; Enzymatic browning; Peroxidase; Edible coatings.*

#### Resumo

Objetivou-se, com este estudo, avaliar o extrato de nabo e a goma xantana na conservação de maçãs minimamente processadas. As maçãs foram lavadas, sanitizadas em solução de hipoclorito de sódio 200 ppm, por 15 minutos, descascadas e cortadas em oito pedaços, para após seguir com os distintos tratamentos em solução aquosa: A – Controle; B – extrato de nabo; C – extrato de nabo e CaCl<sub>2</sub>; D – xantana, CaCl<sub>2</sub> e glicerol; E – extrato de nabo; xantana, CaCl<sub>2</sub> e glicerol. Na sequência, as maçãs minimamente processadas foram dispostas sobre telas com incidência de ventilação, para a secagem do revestimento; em seguida, foram embaladas em bandejas de poliestireno revestidas de policloreto de vinila e armazenadas a 4 ± 1°C, durante 13 dias. Os seguintes parâmetros foram avaliados: perda de massa, firmeza, coloração, pH, sólidos solúveis e atividade das enzimas peroxidase e polifenoloxidase. Os revestimentos não foram eficientes no controle da perda de massa; entretanto, aqueles adicionados de extrato de nabo proporcionaram manutenção da coloração, da firmeza e do pH. Observou-se um aumento considerável na atividade da enzima peroxidase nas maçãs minimamente processadas tratadas com extrato de nabo, indicando que esse efeito seja o responsável pela redução do escurecimento. Não se observou vantagem na associação do extrato de nabo com goma xantana ou com cloreto de cálcio. O extrato de nabo pode representar uma alternativa interessante para aplicação em maçãs minimamente processadas, especialmente por ser um produto natural, pela sua facilidade de obtenção, pelo baixo custo e, ainda, por contribuir para a qualidade nutricional, pela riqueza em cálcio.

**Palavras-chave:** *Maçã minimamente processada; Nabo; Escurecimento enzimático; Peroxidase; Revestimentos comestíveis.*



## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

### 1 Introduction

Minimally processed apples (MPAs) are sold individually, together with other fruits, or as parts of cakes and salads. However, the inevitable cuts occurring during processing may lead to increased levels of enzymatic browning and a higher rate of water evaporation, which may compromise the appearance of the final product. Enzymatic browning is predominantly due to the oxidation reaction of phenolic compounds via polyphenoloxidase, causing the formation of *o*-quinone, which polymerizes to form the dark pigment melanin (LEE, 1999).

Several vegetable extracts have already been evaluated to reduce enzymatic browning, not only due to an increased demand for natural instead of synthetic products, but also with regards to reduced production costs. Studies with this objective involving mushrooms (JANG et al., 2002), onions (LEE et al., 2007; ROLDÁN et al., 2008), oranges, lemons, apples, bananas, potatoes, eggplants, rice, wheat (OLIVEIRA et al., 2007), and pineapples (SUPAPVANICH et al., 2012) have been carried out.

The turnip (*Brassica rapa* L.) is considered to be a rich source of fibre, minerals, and calcium (GONDIM, 2010), and also contains high concentrations of vitamin C and antioxidants that may help to limit the formation of free radicals and deleterious oxidative reactions (SAEED et al., 2012). As compared to other vegetables, the turnip exhibits high peroxidase activity (FATIBELLO-FILHO; VIEIRA, 2002). In the presence of oxygen this enzyme catalyses the reduction of peroxides such as hydrogen peroxide, as well as the oxidation of a variety of other organic and inorganic compounds (KOBLOITZ, 2008; HAMID; KHALIL-UR-REHMAN, 2009). The activity of this enzyme in food is usually associated with browning and the development of off-flavours (KOBLOITZ, 2008). However, this effect seems to depend on the substrate, as well as on the presence or absence of oxygen and hydrogen peroxide. Some reports in the scientific literature have shown that peroxidase may discolour aromatic compounds, similar to the quinones formed by polyphenoloxidase (SELVAM et al., 2003; SILVA et al., 2012b). The results of these studies indicated that the same effect could potentially be induced in vegetables, thus minimizing browning. The turnip extract may be a natural option to reduce the browning caused by polyphenoloxidase in MPAs.

Edible coatings may be used as a vehicle for these extracts, since they immobilize the active compound in a polymer matrix, thus maintaining a high concentration of the active compound on the food surface (OUATTARA et al., 2001). One possible polymer for this purpose is xanthan gum, which is a polysaccharide synthesized by a phytopathogenic bacterium of the genus *Xanthomonas*. According to Freitas et al. (2013),

this gum can be used as an edible coating for MPAs and thus extend their shelf life. Therefore, the objective of this study was to evaluate the potential of turnip extract and xanthan gum in the conservation of MPAs.

### 2 Materials and methods

#### 2.1 Materials

The apples used (*Malus domestica* Borkh) of the cultivar Fuji, were grown in Vacaria/RS (Brazil), whereas the turnips used (*Brassica rapa* L.) purple top white globe were grown in Pelotas/RS (Brazil). The vegetables were selected according to size, colour and the absence of physiological defects. Only apples with soluble solids contents between 11 and 12 °Brix were chosen. The food grade xanthan gum used in this study was obtained from Shandong Fufeng Fermentation Co Ltd (China).

#### 2.2 Methods

The vegetables were washed, sanitized with sodium hypochlorite solution (200 ppm, 15 min), rinsed and peeled. The apples were manually cut in half with the aid of stainless steel knives and each half further cut into four pieces of approximately 2.0 cm × 6.0 cm in size. The turnip pieces were pulped using a food multiprocessor (Philips Walita) before being filtered. Distilled water was added to this filtrate in a 1:1 (v/v) ratio. All food processing was carried out at ambient temperature (15 °C) using chilled water.

The xanthan gum solution was prepared by slowly dissolving xanthan gum in distilled water at room temperature (r.t.; ca. 2 h), followed by heating to 60±1 °C for 20 min. This solution was stored at 4±1 °C for 24 h. Under vigorous stirring at r.t. (ca. 10 min), appropriate amounts of glycerol and/or CaCl<sub>2</sub> were added to this solution.

The following edible coatings were prepared in aqueous solution: **A** – water (control); **B** – turnip extract; **C** – turnip extract and CaCl<sub>2</sub> (1.0% w/v); **D** – xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v), and glycerol (1.0% v/v); **E** – turnip extract, xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v) and glycerol (1.0% v/v).

For the treatments **A-D**, the apple pieces were immersed in the respective solutions for 2 min, before being dried under ventilation on nylon screens for ca. 4 h. Treatment **E** was accomplished in two stages: initially, the MPAs were immersed in the turnip extract for 2 min and then dried, before being immersed in xanthan gum solution for 2 min, followed by drying again.

After treatment, the MPAs were packed in polyvinylchloride film-covered polystyrene trays (seven pieces per tray), and stored at 4±1 °C (80% relative humidity) for 13 days. The analyses were carried out on days 1, 3, 6, 9 and 13.

## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

The experimental design was completely randomized in a 5 × 5 factorial scheme with five treatments (A, B, C, D and E) and five evaluation periods (1, 3, 6, 9 and 13 days). The polyphenoloxidase and peroxidase activities were determined on days 1 and 13 of storage. Each treatment consisted of 63 pieces of apple, distributed on nine trays. Three trays per treatment were used for the evaluation of mass loss, one tray per treatment for the analyses at each evaluation period, while an additional tray served as a safety margin.

### 2.3 Evaluations

The mass loss was determined from the difference between the initial weight of the MPAs and that obtained at the end of the respective storage period (see Equation 1). Three trays each containing seven pieces of apple were used for the analysis of mass loss for each treatment, and the average of the results is expressed as a percentage of mass loss.

$$\text{Mass loss (\%)} = \left[ \frac{(m_{\text{initial}} - m_{\text{final}})}{m_{\text{initial}}} \right] \cdot 100 \quad (1)$$

The firmness of the apple pieces was determined using a texturometer (Stable Micro Systems TA.XTplus) with a P/2N probe. Four pieces of apple were used for the analysis of firmness, two measurements being made per piece, totalling eight repetitions per sample per analysis period. The results were expressed in Newton (N).

The colouration was evaluated using a Minolta CR 400 colorimeter. The values obtained for  $a^*$ ,  $b^*$  and  $L^*$  were used to calculate the browning index (BI) according to Palou (1999) (see Equation 2):

$$\text{BI} = \frac{[100(X - 0.31)]}{0.172} \quad (2)$$

$$\text{Where } X = \frac{(a^* + 1.75L^*)}{(5.64L^* + a^* - 3.02b^*)}$$

Four pieces of apple were used for the colour analysis, with eight readings per analysis period, i.e. two readings per piece.

In order to establish the pH value, three samples (10 g each) were triturated with distilled water (100 mL), before reading using a pH meter (Digimed MD-20). The analysis was carried out according to the AOAC method (CUNNIFF, 1995). The soluble solids contents were determined in the liquid extract after triturating the sample. An Abbe-type bench refractometer (Analytikjena) with automatic temperature control was used for this purpose, and the results expressed in °Brix. Both evaluations were carried out in triplicate.

For each evaluation period, the enzymes were extracted from three pieces of apple that were frozen

(−18 °C, 10 days) in low-density polyethylene plastic bags. One gram samples were homogenized with 10 mL of 0.05 M phosphate buffer (pH 7, plus 1% polyvinylpyrrolidone) and filtered immediately. The homogenate was centrifuged (15 min, 7000 g, 3 °C) (MATSUNO; URITANI, 1972) and the resulting supernatant, known as the enzymatic extract, used to determine the polyphenoloxidase and peroxidase activities.

The polyphenoloxidase activity was determined in 1 mL aliquots of the enzyme extract, to which 3.6 mL of a 0.05 M phosphate buffer (pH 6) containing 0.1 mL of 0.1 M pyrocatechol were added. The solutions obtained were incubated (30 min, 30 °C), cooled in an ice bath, and the spectrophotometer reading (Belgium) recorded immediately at a wavelength of  $\lambda = 395$  nm. The polyphenoloxidase activity was expressed in units of enzymatic activity capable of inducing an absorbance change of 0.001 at 395 nm per gram of fresh pulp per minute (UAE.g<sup>-1</sup>.min<sup>-1</sup>) (CAMPOS; SILVEIRA, 2003).

The peroxidase activity was determined in 3 mL aliquots of the enzyme extract, to which a solution containing 5 mL of 0.02 M phosphate citrate buffer (pH 5), 0.5 mL of 30% hydrogen peroxide and 0.5 mL of guaiacol was added. The solution was incubated (5 min, 30 °C), cooled in an ice bath, and the spectrophotometer reading recorded immediately at a wavelength of  $\lambda = 470$  nm. The peroxidase enzyme activity was expressed in units of enzymatic activity capable of induce an absorbance change of 0.001 at 470 nm per gram of fresh pulp per minute (UAE.g<sup>-1</sup>.min<sup>-1</sup>) (MATSUNO; URITANI, 1972).

The results obtained were subjected to a variance analysis, and the mean values were compared between the treatments using the t test and Tukey's test (threshold level for significance: 5%), using the *Statistix 10* programme (STATISTIX, 2014). Polynomial regression analyses were carried out to describe the variables according to the storage periods.

## 3 Results and discussion

For all treatments, mass loss of the MPAs increased significantly during storage, even though no significant difference could be observed between the treatments for each analysis period (Table 1).

Xanthan gum has previously been used as an edible coating for minimally processed vegetables, especially in order to reduce mass loss (CORTEZ-VEGA et al., 2013; FREITAS et al., 2013; PIZATO et al., 2013). However, in these studies 0.5% of xanthan gum was used in comparison to 0.25% in the present study. This lower concentration was chosen in accordance with the results of preliminary studies, which showed that higher concentrations of xanthan gum could increase enzymatic browning in the presence of turnip extract, possibly by

## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

**Table 1.** Mass loss, browning index, firmness, pH values and soluble solids contents of the MPAs with added turnip extract and xanthan gum stored at 4±1 °C for 13 days.

Treat.	Mass loss (%)					Polynomial regression
	1	3	6	9	13	
<b>A</b>	-	0.90±0.16 <sup>a</sup>	2.62±0.36 <sup>a</sup>	5.09±0.33 <sup>a</sup>	7.00±0.09 <sup>a</sup>	$y = -0.0002x^2 + 0.6096x - 0.7669$ R <sup>2</sup> = 0.9916
<b>B</b>	-	1.14±0.35 <sup>a</sup>	3.04±0.24 <sup>a</sup>	4.94±0.52 <sup>a</sup>	7.20±0.35 <sup>a</sup>	$y = -0.0023x^2 + 0.6379x - 0.6838$ R <sup>2</sup> = 0.9996
<b>C</b>	-	1.22±0.39 <sup>a</sup>	2.93±0.41 <sup>a</sup>	5.62±0.98 <sup>a</sup>	7.51±0.84 <sup>a</sup>	$y = -0.0047x^2 + 0.7112x - 0.8171$ R <sup>2</sup> = 0.9915
<b>D</b>	-	1.09±0.06 <sup>a</sup>	2.99±0.17 <sup>a</sup>	4.85±0.41 <sup>a</sup>	7.94±0.46 <sup>a</sup>	$y = 0.0112x^2 + 0.5029x - 0.5058$ R <sup>2</sup> = 0.9997
<b>E</b>	-	1.49±0.56 <sup>a</sup>	3.00±0.85 <sup>a</sup>	5.16±1.66 <sup>a</sup>	7.89±1.20 <sup>a</sup>	$y = 0.0053x^2 + 0.5748x - 0.4864$ R <sup>2</sup> = 0.998
Browning index						
<b>A</b>	33.73±4.09 <sup>a</sup>	33.35±3.44 <sup>a</sup>	35.66±1.63 <sup>a</sup>	34.94±3.00 <sup>ab</sup>	44.09±4.49 <sup>a</sup>	$y = 0.115x^2 - 0.8188x + 34.785$ R <sup>2</sup> = 0.9193
<b>B</b>	28.59±2.95 <sup>ab</sup>	26.25±1.89 <sup>b</sup>	28.58±1.79 <sup>b</sup>	29.57±2.71 <sup>bc</sup>	29.28±2.62 <sup>c</sup>	(ns) $y$ (average) = 28.45
<b>C</b>	24.22±4.37 <sup>b</sup>	28.61±3.62 <sup>ab</sup>	27.79±3.20 <sup>b</sup>	28.01±4.37 <sup>c</sup>	29.86±4.55 <sup>c</sup>	(ns) $y$ (average) = 27.69
<b>D</b>	31.40±3.25 <sup>a</sup>	32.69±1.42 <sup>a</sup>	37.25±3.46 <sup>a</sup>	36.32±3.65 <sup>a</sup>	37.36±2.66 <sup>b</sup>	$y = -0.0651x^2 + 1.4077x + 29.846$ R <sup>2</sup> = 0.8936
<b>E</b>	25.66±3.04 <sup>b</sup>	26.32±3.86 <sup>b</sup>	29.83±1.91 <sup>b</sup>	31.26±3.37 <sup>abc</sup>	32.78±3.07 <sup>bc</sup>	$y = -0.0284x^2 + 1.029x + 24.265$ R <sup>2</sup> = 0.9756
Firmness (N)						
<b>A</b>	3.15±0.44 <sup>a</sup>	2.98±0.48 <sup>ab</sup>	3.13±0.94 <sup>a</sup>	2.82±0.39 <sup>ab</sup>	2.50±0.42 <sup>c</sup>	$y = -0.0051x^2 + 0.0209x + 3.0824$ R <sup>2</sup> = 0.8971
<b>B</b>	2.66±0.51 <sup>a</sup>	2.59±0.29 <sup>b</sup>	2.95±0.77 <sup>a</sup>	2.82±0.51 <sup>ab</sup>	2.71±0.39 <sup>bc</sup>	(ns) $y$ (average) = 2.74
<b>C</b>	2.83±0.31 <sup>a</sup>	3.56±0.76 <sup>a</sup>	3.06±0.35 <sup>a</sup>	2.61±0.46 <sup>b</sup>	2.70±0.18 <sup>c</sup>	(ns) $y$ (average) = 2.95
<b>D</b>	3.07±0.67 <sup>a</sup>	3.37±0.50 <sup>ab</sup>	3.27±0.39 <sup>a</sup>	3.39±0.59 <sup>a</sup>	3.39±0.38 <sup>a</sup>	(ns) $y$ (average) = 3.32
<b>E</b>	2.87±0.28 <sup>a</sup>	2.98±0.39 <sup>ab</sup>	3.27±0.36 <sup>a</sup>	3.40±0.54 <sup>a</sup>	3.21±0.32 <sup>ab</sup>	$y = -0.0115x^2 + 0.2022x + 2.5858$ R <sup>2</sup> = 0.8019
pH						
<b>A</b>	3.74±0.08 <sup>ab</sup>	3.63±0.08 <sup>ab</sup>	3.67±0.05 <sup>a</sup>	3.77±0.11 <sup>ab</sup>	3.98±0.05 <sup>a</sup>	$y = 0.0047x^2 - 0.0425x + 3.7542$ R <sup>2</sup> = 0.9651
<b>B</b>	3.83±0.03 <sup>a</sup>	3.50±0.10 <sup>b</sup>	3.47±0.05 <sup>b</sup>	3.86±0.05 <sup>a</sup>	3.84±0.10 <sup>a</sup>	(ns) $y$ (average) = 3.70
<b>C</b>	3.64±0.03 <sup>bc</sup>	3.61±0.06 <sup>ab</sup>	3.59±0.09 <sup>ab</sup>	3.43±0.08 <sup>d</sup>	3.79±0.19 <sup>a</sup>	(ns) $y$ (average) = 3.61
<b>D</b>	3.59±0.04 <sup>c</sup>	3.82±0.02 <sup>a</sup>	3.73±0.07 <sup>a</sup>	3.62±0.03 <sup>bc</sup>	3.99±0.09 <sup>a</sup>	(ns) $y$ (average) = 3.74
<b>E</b>	3.61±0.04 <sup>bc</sup>	3.72±0.13 <sup>ab</sup>	3.68±0.03 <sup>a</sup>	3.51±0.04 <sup>cd</sup>	3.69±0.09 <sup>a</sup>	(ns) $y$ (average) = 3.64
Soluble solids (°Brix)						
<b>A</b>	11.80±0.10 <sup>ab</sup>	12.06±0.15 <sup>b</sup>	12.80±0.70 <sup>b</sup>	12.73±0.60 <sup>a</sup>	13.00±0.60 <sup>b</sup>	$y = -0.04x^2 + 0.7426x + 11.308$ R <sup>2</sup> = 0.8587
<b>B</b>	11.60±0.10 <sup>b</sup>	13.83±0.67 <sup>a</sup>	14.33±0.20 <sup>a</sup>	14.33±0.10 <sup>a</sup>	14.36±0.40 <sup>a</sup>	$y = -0.0096x^2 + 0.233x + 11.553$ R <sup>2</sup> = 0.9335
<b>C</b>	11.60±0.10 <sup>b</sup>	12.80±0.26 <sup>ab</sup>	12.80±0.10 <sup>b</sup>	13.10±0.70 <sup>a</sup>	13.06±0.55 <sup>b</sup>	$y = -0.0164x^2 + 0.431x + 11.454$ R <sup>2</sup> = 0.9682
<b>D</b>	11.80±0.10 <sup>ab</sup>	12.80±0.78 <sup>ab</sup>	13.20±0.20 <sup>ab</sup>	14.16±0.80 <sup>a</sup>	14.26±0.29 <sup>a</sup>	$y = -0.0182x^2 + 0.352x + 11.491$ R <sup>2</sup> = 0.8299
<b>E</b>	12.00±0.10 <sup>a</sup>	12.53±0.06 <sup>b</sup>	13.80±0.70 <sup>ab</sup>	14.43±1.00 <sup>a</sup>	13.90±0.10 <sup>ab</sup>	$y = -0.0311x^2 + 0.613x + 11.244$ R <sup>2</sup> = 0.9656

Mean values followed by the same lower case letter in the same column do not differ by Tukey's test ( $p < 0.05$ ). A minimum determination coefficient of 0.80 was used. Treatments: **A** – control (water); **B** – turnip extract; **C** – turnip extract and CaCl<sub>2</sub> (1.0% w/v); **D** – xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v) and glycerol (1.0% v/v); **E** – turnip extract, xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v) and glycerol (1.0% v/v).

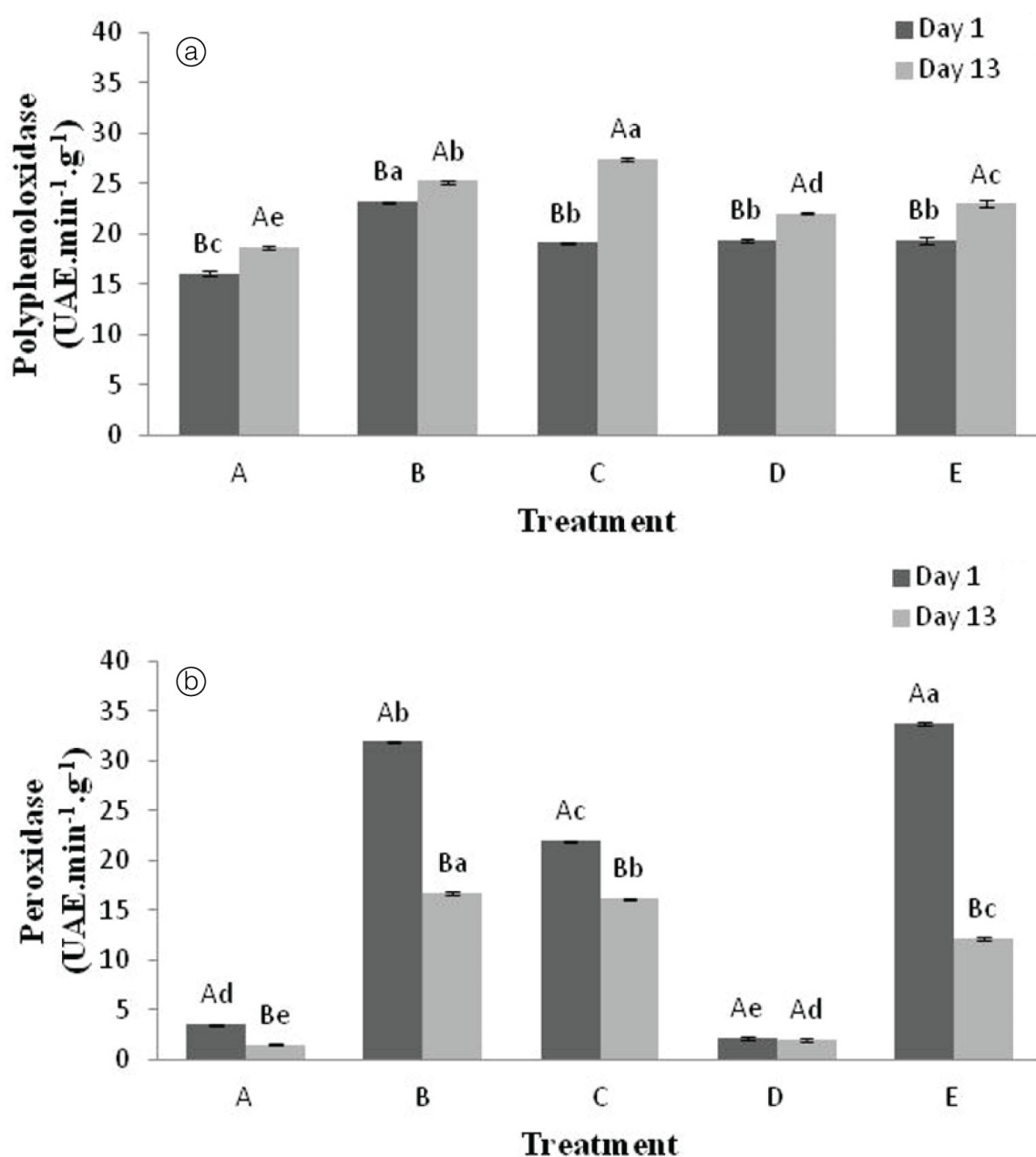
## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

providing lower permeability for oxygen. The influence of oxygen will be discussed in the following section.

The coatings provided increased the polyphenoloxidase activity as compared to the control, and regardless of the treatment, an increase in polyphenoloxidase activity was observed during the storage period (Figure 1A). Furthermore, the peroxidase activity also increased significantly in the MPAs treated with turnip extracts (**B**, **C** and **E**) as compared to untreated samples (Figure 1B).

Turnips have been used for the extraction of peroxidase due to the high activity of this enzyme in this vegetable (MATTO; HUSAIN, 2009; HUSAIN, 2010; SILVA et al., 2012a; SILVA et al., 2012b). However, during storage, a trend for a reduction in peroxidase activity was observed. According to Valderrama et al. (2002) peroxidases are relatively unstable and susceptible to inactivation in the presence of hydrogen peroxide. This auto-oxidative inactivation seems to proceed via several catalytic pathways, including haem oxidation



**Figure 1.** Polyphenoloxidase (a) and peroxidase (b) activities in MPAs treated with turnip extract and xanthan gum on the 1<sup>st</sup> and the 13<sup>th</sup> days of storage at 4±1 °C. Mean values followed by the same capital letter between the 1<sup>st</sup> and the 13<sup>th</sup> day do not differ by the t test ( $p < 0.05$ ). Mean values followed by the same lower case letter on each evaluation day do not differ according to Tukey's test ( $p < 0.05$ ). Treatments: **A** - control (water); **B** - turnip extract; **C** - turnip extract and CaCl<sub>2</sub> (1.0% w/v); **D** - xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v) and glycerol (1.0% v/v); **E** - turnip extract, xanthan (0.25% w/v), CaCl<sub>2</sub> (1.0% w/v) and glycerol (1.0% v/v).

## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

and the destruction of essential amino acids. In general, although there were significant differences between the treatments regarding the activities of polyphenol oxidase and peroxidase, both at the beginning and end of storage, the main effect observed was that of high peroxidase activity in relation to colour maintenance. During storage, no significant increase in the browning index was observed for MPAs subjected to treatments **B** (turnip extract) and **C** (turnip extract and  $\text{CaCl}_2$ ) (Table 1). However, MPAs submitted to the other treatments exhibited significantly increased browning index values during storage. This included MPAs treated with **E** (turnip extract and xanthan gum), albeit to a lesser extent. According to Husain (2010), peroxidase can catalyse the degradation/transformation of aromatic dyes either by precipitation or ring-opening reactions of the aromatic rings. Silva et al. (2012b) demonstrated the effectiveness of peroxidase in the discoloration of anthraquinone dyes, which have a similar structure to the quinones generated in the polyphenoloxidase-induced oxidation of phenolic compounds.

Hiner et al. (2001) and Silva et al. (2012b) suggested that the peroxidase-catalysed degradation of aromatic compounds proceeded initially *via* a reaction with hydrogen peroxide at the active site, whereby hydrogen peroxide was reduced to water and compound I, which is a reactive intermediate with a higher oxidation state as compared to the original enzyme. Subsequently, compound I oxidized a substrate molecule ( $\text{AH}_2$ ), whereby an aromatic dye substrate and a radical compound II were generated. Finally, compound II was reduced by a second substrate molecule, causing the enzyme to return to its original form.

Therefore, the initial differences observed for the browning index could possibly be attributed to the influence of the treatment during the 4 h of drying (Table 1), most likely due to peroxidase activity. Accordingly, the addition of turnip extract, including treatment **E**, resulted in lower browning rates, both at the beginning and end of storage.

Treatment **E** possibly induced lower permeability to oxygen *via* the additional coverage with xanthan gum, thus causing partially inhibited peroxidase activity relative to treatments with only turnip extract (**B**) or turnip extract and calcium chloride (**C**). In agreement with the study of Krochta & Mulder-Johnston (1997), the edible coatings promoted the formation of semi-permeable oxygen barriers. Moreover, some of the present authors' preliminary data (data not shown) suggested that the turnip-induced inhibition of enzymatic browning was higher in polystyrene containers coated with polyvinylchloride as compared to polyethyleneterephthalate packaging, since the former exhibits higher oxygen permeability relative to the latter. Accordingly, polyvinylchloride-covered

polystyrene trays were chosen as the packaging material in the present study.

The present results also suggested that in the presence of higher oxygen concentrations (i.e. in the absence of xanthan gum), peroxidase could degrade the polyphenoloxidase-generated pigment. Thus, despite the increased polyphenoloxidase-induced production of quinones at higher oxygen concentrations, it appears that peroxidase is able to subsequently degrade them. However, at lower oxygen concentrations (i.e. in the presence of xanthan gum), polyphenoloxidase still seems to be active, and accordingly, the peroxidase appears to be inhibited.

As far as firmness was concerned, different values were observed for the MPAs as a function of time. For example, the control treatment (**A**) showed a significant loss of firmness during storage (Table 1), possibly mainly due to the activity of pectinolytic enzymes.

In contrast, treatments **B**, **C** and **D** maintained their initial levels of firmness, and treatment **E** showed a significantly increased level of firmness (Table 1). These results could be correlated with the substantial amounts of calcium already present in the turnip (UNICAMP, 2011), or, on the other hand, they could be due to the addition of calcium chloride, which is classified as a firming agent.

During storage, a significant increase in the pH value could only be observed for the control group, whereas the other treatments maintained the initial pH value (Table 1). A significant increase in soluble solids was observed during storage for all treatments (Table 1). However, this increase was probably related to the mass loss observed in all treatments, and the consequent increase in the sugar concentrations.

## 4 Conclusions

The experimental data obtained show that the turnip extract effectively reduced browning in the MPAs. This effect could be related to the peroxidase activity towards the phenolic/quinoidal compounds and the concentration of oxygen present in the sample. Apart from minimizing browning, the turnip extract facilitated the maintenance of physical and chemical characteristics such as firmness and the pH value. However, no advantage could be observed for the simultaneous presence of turnip extract and xanthan gum and/or calcium chloride. The coatings (xanthan and/or turnip extract) were not effective in controlling or preventing weight loss.

Moreover the study showed that turnip extract may represent an interesting alternative for applications in MPAs, especially as it is a natural product, easily obtained, cost effective and contributes to the nutritional quality (e.g. as a source of calcium ions).

**Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum**

Borges, C. D. et al.

**References**

- CAMPOS, A. D.; SILVEIRA, E. M. L. **Metodologia para determinação da peroxidase e da polifenol oxidase em plantas**. Pelotas: Embrapa Clima Temperado, 2003. 3 p. (Comunicado técnico, 87).
- CORTEZ-VEGA, W. R.; PIOTROWICZ, I. B. B.; PRENTICE-HERNÁNDEZ, C.; BORGES, C. D. Conservação de mamão minimamente processado com uso de revestimento comestível à base de goma xantana. **Semina: Ciências Agrárias**, Londrina, v. 34, n. 4, p. 1753-1764, 2013.
- CUNNIFF, P. (Ed.). **Official methods of analysis of the Association of Official Analytical Chemists**. 16th ed. Washington: AOAC, 1995. 1094 p.
- FATIBELLO-FILHO, O.; VIEIRA, I. C. Uso analítico de tecidos e de extratos brutos vegetais como fonte enzimática. **Química Nova**, São Paulo, v. 25, n. 3, p. 455-464, 2002.
- FREITAS, I. R.; CORTEZ-VEGA, W. R.; PIZATO, S.; PRENTICE-HERNÁNDEZ, C.; BORGES, C. D. Xanthan gum as a carrier of preservative agents and calcium chloride applied on fresh-cut apple. **Journal of Food Safety**, Malden, v. 33, n. 3, p. 229-238, 2013. <http://dx.doi.org/10.1111/jfs.12044>.
- GONDIM, A. **Catálogo brasileiro de hortaliças**. Brasília: Embrapa, 2010. 59 p.
- HAMID, M.; KHALIL-UR-REHMAN. Potential applications of peroxidases. **Food Chemistry**, Whiteknights, v. 115, n. 4, p. 1177-1186, 2009. <http://dx.doi.org/10.1016/j.foodchem.2009.02.035>.
- HUSAIN, Q. Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: a review. **Reviews in Environmental Science and Bio/technology**, Dordrecht, v. 9, n. 2, p. 117-140, 2010. <http://dx.doi.org/10.1007/s11157-009-9184-9>.
- HINER, A. N. P.; HERNÁNDEZ-RUIZ, J.; WILLIAMS, G. A.; ARNAO, M. B.; GARCÍA-CÁNOVAS, F.; ACOSTA, M. Catalase-like oxygen production by horseradish peroxidase must predominantly be an enzyme-catalyzed reaction. **Archives of Biochemistry and Biophysics**, New York, v. 392, n. 2, p. 295-302, 2001. <http://dx.doi.org/10.1006/abbi.2001.2460>. PMID:11488605.
- JANG, M. S.; SANADA, A.; USHIO, H.; TANAKA, M.; OHSHIMA, T. Inhibitory effects of 'enokitake' mushroom extracts on polyphenol oxidase and prevention of apple browning. **Lebensmittel-Wissenschaft und-Technologie**, Berlin, v. 35, n. 8, p. 697-702, 2002.
- KOBLITZ, M. G. B. **Bioquímica de alimentos-teoria e aplicações práticas**. Rio de Janeiro: Guanabara Koogan, 2008. 242 p.
- KROCHTA, J. M.; MULDER-JOHNSTON, C. Edible and biodegradable polymer films: challenges and opportunities. **Food Technology**, Chicago, v. 51, n. 2, p. 61-77, 1997.
- LEE, C. Y. Enzymatic browning reaction. In: F. J., FRANCIS (Ed.). **Encyclopedia of food science and technology**. New York: Wiley, 1999. p. 494-515.
- LEE, M. Y.; LEE, M. K.; PARK, I. Inhibitory effect of onion extract on polyphenol oxidase and enzymatic browning of taro (*Colocasia antiquorum* var. esculenta). **Food Chemistry Whiteknights**, v. 105, n. 2, p. 528-532, 2007. <http://dx.doi.org/10.1016/j.foodchem.2007.04.010>.
- MATSUNO, H.; URITANI, I. Physiological behavior of peroxidase isozymes in sweet potato root tissue injured by cutting or black root. **Plant Cell and Physiology**, Tokyo, v. 13, n. 6, p. 1091-1101, 1972.
- MATTO, M.; HUSAIN, Q. Decolorization of direct dyes by immobilized turnip peroxidase in batch and continuous processes. **Ecotoxicology and Environmental Safety**, New York, v. 72, n. 3, p. 965-971, 2009. <http://dx.doi.org/10.1016/j.ecoenv.2008.02.019>. PMID:18423852.
- OLIVEIRA, M. S.; DORS, G. C.; SOUZA-SOARES, L. A.; FURLONG, E. B. Atividade antioxidante e antifúngica de extratos vegetais. **Alimentos e Nutrição**, Araraquara, v. 18, n. 3, p. 267-275, 2007.
- OUATTARA, B.; SABATO, S. F.; LACROIX, M. Combined effect of antimicrobial coating and gamma irradiation on shelf life extension of pre-cooked shrimp (*Penaeus* spp.). **International Journal of Food Microbiology**, Netherlands, v. 68, n. 1-2, p. 1-9, 2001. [http://dx.doi.org/10.1016/S0168-1605\(01\)00436-6](http://dx.doi.org/10.1016/S0168-1605(01)00436-6). PMID:11545208.
- PALOU, E.; LÓPEZ-MALO, A.; BARBOSA-CÁNOVAS, G. V.; WELTI-CHANES, J.; SWANSON, B. G. Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. **Journal of Food Science**, Chicago, v. 64, n. 1, p. 42-45, 1999. <http://dx.doi.org/10.1111/j.1365-2621.1999.tb09857.x>.
- PIZATO, S.; CORTEZ-VEGA, W. R.; SOUZA, J. T. A.; PRENTICE-HERNÁNDEZ, C.; BORGES, C. D. Effects of different edible coatings in physical, chemical and microbiological characteristics of minimally processed peaches (*Prunus persica* (L.) Batsch). **Journal of Food Safety**, Malden, v. 33, n. 1, p. 30-39, 2013. <http://dx.doi.org/10.1111/jfs.12020>.
- ROLDÁN, E.; SÁNCHEZ-MORENO, C.; DE ANCOS, B.; CANO, M. P. Characterization of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties. **Food Chemistry**, Whiteknights, v. 108, n. 3, p. 907-916, 2008. <http://dx.doi.org/10.1016/j.foodchem.2007.11.058>. PMID:26065752.
- SAEED, M. K.; ANJUM, S.; AHMAD, I.; NISA, A.; ALI, S.; ZIA, A.; ALI, S. Nutritional facts and free radical scavenging activity of turnip (*Brassica rapa*) from Pakistan. **World Applied Sciences Journal**, Deira, v. 19, n. 3, p. 370-375, 2012.
- SELVAM, K.; SWAMINATHAN, K.; CHAE, K. S. Decolorization of azo dyes and a dye industry effluent by a white rot fungus

## Conservation of minimally processed apples using edible coatings made of turnip extract and xanthan gum

Borges, C. D. et al.

*Thelephora* sp. **Bioresource Technology**, Barking, v. 88, n. 2, p. 115-119, 2003. [http://dx.doi.org/10.1016/S0960-8524\(02\)00280-8](http://dx.doi.org/10.1016/S0960-8524(02)00280-8). PMID:12576004.

SILVA, M. C.; CORRÊA, A. D.; AMORIM, M. T. S. P.; PARPOT, P.; TORRES, J. A.; CHAGAS, P. M. B. Decolorization of the phthalocyanine dye reactive blue 21 by turnip peroxidase and assessment of its oxidation products. **Journal of Molecular Catalysis. B, Enzymatic**, Amsterdam, v. 77, p. 9-14, 2012a.

SILVA, M. C.; CORRÊA, A. D.; TORRES, J. A.; AMORIM, M. T. S. P. Descoloração de corantes industriais e efluentes têxteis simulados por peroxidase de nabo (*Brassica campestris*). **Química Nova**, São Paulo, v. 35, n. 5, p. 889-895, 2012b.

STATISTIX. **Statistix 10: data analysis software for researchers**. Tallahassee: Statistix, 2014. Available at: <<http://www.statistix.com/free-trial/>>. Accessed on: 1 oct. 2014.

SUPAPVANICH, S.; PRATHAAN, P.; TEPSON, R. Browning inhibition in fresh-cut rose apple fruit cv. Taaptimjaan using konjac glucomannan coating incorporated with pineapple fruit extract. **Postharvest Biology and Technology**, Amsterdam, v. 73, p. 46-49, 2012. <http://dx.doi.org/10.1016/j.postharvbio.2012.05.013>.

UNIVERSIDADE ESTADUAL DE CAMPINAS – UNICAMP. **Tabela Brasileira de Composição de Alimentos – TACO**. 4th ed. Campinas: UNICAMP; NEPA, 2011. 161 p.

VALDERRAMA, B.; AYALA, M.; VÁZQUEZ-DUHALT, R. Suicide inactivation of peroxidases and the challenge of engineering more robust enzymes. **Chemistry & Biology**, London, v. 9, n. 5, p. 555-565, 2002. [http://dx.doi.org/10.1016/S1074-5521\(02\)00149-7](http://dx.doi.org/10.1016/S1074-5521(02)00149-7). PMID:12031662.