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# Edible candelilla wax coating with fermented extract of tarbush improves the shelf life and quality of apples

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ABSTRACT

Edible coating formulated with candelilla wax and fermented extract of tarbush was applied for immersion to evaluate its effects on the shelf life and quality of Golden delicious apples in marketing conditions stored at room temperature (27 ± 1 °C). Control treatments were apples without edible coating. Changes in appearance, weight loss, water activity and firmness were monitored during 8 weeks. A sensory evaluation of preference in taste and appearance was performed in the apples. Edible coatings were able to reduce significantly the change in appearance, weight loss, water activity and firmness ($p \leq 0.05$) in the apples. Results of the sensory evaluation demonstrated edible coating with fermented extract of tarbush did not alter appearance and taste of apples. According to the results, fermented extract of tarbush incorporated into the edible coating appear to be a promising preservation alternative and effective method improve the quality and shelf life of apples in marketing conditions.



## 1. Introduction

There is currently a high demand for healthier and more natural foods for human consumption. This has led to development of natural technologies for food conservation. In recent years, research has focusing on search of naturally occurring substances capable of acting as alternative antimicrobials and antioxidants in minimally processed fruits and vegetables (Saucedo-Pompa et al., 2009), in order to reduce the negative effects of maturation process, improving quality of foodstuffs, and impacting positively on food quality (Saucedo-Pompa et al., 2007).

A natural alternative to extend shelf life of these products during storage is the use of edible coatings, which is defined as a cover material that is applied to edible food to improve appearance, being an effective barrier to transmission of gases, solving problems of migration of moisture, oxygen, carbon dioxide and aromas (Cuq, Gontard, & Guilbert, 1995; Fernández-Álvarez, 2000), reducing maturation process, prolonging life and quality of a vegetable or fruit (Vernon, Pez, & Garc, 1999).

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Tarbush (*Flourensia cernua*) plant is widely distributed in the semiarid region of Northern Mexico and Southern United States of Amerca, which is currently only used empirically for infusions preparation to treat some diseases or ailments for the rural population in Mexico, particularly (Belmares, Garza, Rodríuez, Contreras-Esquivel, & Aguilar, 2009). Tarbush extracts or constituents have reported to possess antioxidant (Abou-Gazar, Bedir, Takamatsu, Ferreira, & Khan, 2004), anti-HIV (Gnabre, Ito, Ma, & Huang, 1996), antimicrobial, enzyme inhibitory, antitumor (MacRae & Towers, 1984), and anti-hyperglycemic (Luo et al., 1998) activities. Tarbush leaves contain substantial levels of polyphenolic compounds (Hyder, Fredrickson, Estell, Lucero, & Remmenga, 2005), which may have digestive properties (Belmares et al., 2009) and fungicide activity against phytopathogenic fungi such as *Rhizoctonia solani*, *Phytophthora infestans* (Gamboa-Alvarado, Hernández-Castillo, Guerrero-Rodríguez, Sánchez-Arizpe, & Lira-Saldivar, 2003), and *Colletotrichum* spp. (Tellez et al., 2001).

Polyphenols, particularly tannins are secondary metabolites present in different plants such as tarbush (Ventura et al., 2008) and act as a defense against animal and microbial attack (Belmares et al., 2009). It is important to note that the tannins are generally considered as inhibitors of microorganisms growth (Belmares et al., 2009).

Most phenolic compounds found in plants, as tarbush, are conjugated with sugars (mainly glucose) forming glycosides (De León et al., 2013). This combination reduces their ability to function, since the availability of the free hydroxyl groups in phenolic rings is diminished (Vattem & Shetty, 2003). That is why in this work was performed a tarbush fungal fermentation to remove the sugars and release the phenolic compounds catechin equivalents.

At the present time, the possibility of producing phenolic antioxidant present in tannins-rich bushes, such as tarbush mainly derivatives of biodegradation of the leaves, by fermentation processes, are a reality (Ventura et al., 2008). Plants can be used as substrate to produce powerful antioxidants by microbiological methods through fermentation (Belmares-Cerda, Reyes-Vega, Contreras-Esquivel, Rodríguez-Herrera, & Aguilar, 2003).

The high tannin content of tarbush and the ability to be biologically degraded such compounds by solid state fermentation procedures, shows the potential value-added use of this plant material for recovery of fine chemicals such as monomeric antioxidants, such as gallic acid (Belmares et al., 2009).

The amount of aflatoxin present in the fermented plant material of tarbush (AF B1 409-612 ppt) as consequence of fungal growth was minimal considering the maximum permitted levels in food established in NOM-188-SSA1-2002 (300 mg/kg) and in the NOM-021-PSC-94 (20 ppb for AF B1), therefore these quantities fail to cause toxic effects in humans (Mercado-Martinez, 2006).

Waxes are effective in blocking the migration of moisture, being candelilla wax the most resistant one in comparation with carnauba wax and beeswax (Bósquez-Molina & Vernon-Carter, 2005). Candelilla *E. antisyphilitica* Zucc. is an endemic specie of the semiarid regions of the borderline between Mexico and USA; from this plant is obtained a wax, which is a substance generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (Saucedo-Pompa et al., 2009).

In Mexico, apple Golden delicious is one of the most important temperate fruits. The national production of Golden delicious apples is of 375,055 t per year and in the state of Coahuila produces approximately one tenth of national production (SIAP, 2012). The producer region is located in the Sierra of Arteaga mainly in locations like Carbonera, Los Lirios, El Tunal, Jamé, San Antonio of Alazanas and Huachichil (Zermeño-Gonzáles et al., 2008).

However, apple Golden delicious production is strongly affected by poor post-harvest management, presence of pests, particularly fungal pathogens and its short shelf life because it is a perishable climacteric type fruit, which means that even under refrigerated conditions, this fruit may be decomposed (Guerrero-Prieto et al., 2004).

The main objective of this work was to evaluate the effects of a candelilla wax based edible coating formulated with fermented extract of tarbush as a natural source of antioxidants on the shelf life and quality of apples Golden delicious in marketing conditions.

## 2. Materials and methods

### 2.1. *Raw materials*

Tarbush plant was provided by Fitokimica Mexicana S.A. de C.V. (Saltillo, México). Plant material was dehydrated in an oven at $60 \pm 1\,^{\circ}C$ for 48 h. Plant leaves were separated from stems, and pulverized in a miller. Powdered plant was stored in black plastic bags for later use. Refined candelilla wax was provided by Bioingenio Lifetech S.A. de C.V. (Saltillo, México). Arabic gum and jojoba oil were purchased in the local Market of Raw Materials in the Saltillo City, México).

Apples Golden delicious were purchased in the month of October (2011) from the central market in the City of Saltillo, Mexico. These fruits were produced in Arteaga Coahuila. The fruit selection criteria were: similar size, absence of skin damage, apparent absence of microorganisms, maturity and color. Fruits were sorted into completely randomized groups.

### 2.2. *Solid state fermentation (SSF) of tarbush leaves*

The strain of *Aspergillus niger* GH1 (DIA-UAdeC collection) was utilized for SSF of tarbush. Fungal spores were propagated in 250 ml Erlenmeyer flasks containing sterile potato dextrose agar and incubated at $30 \pm 1\,^{\circ}C$ for 5 days. SSF was carried out in tray reactors as follows: 50 g of powdered and dried plant were placed into each reactor. Then, moisture content was adjusted at 60 g/100 g with Czapek-Dox broth at an initial pH of 5.5 and inoculated with $2 \times 10^7$ *A. niger* spores per gram of dehydrated plant. Finally, reactors were incubated at $30 \pm 1\,^{\circ}C$ for 12 h. Samples were dehydrated in an oven at $60 \pm 1\,^{\circ}C$ for 24 h.

### 2.3. *Preparation of the aqueous extract*

About 50 g of fermented tarbush were homogenized in 500 ml of deionized water. This mixture was stirred vigorously for 60 s, then centrifuged for 20 min at $1500 \times g$, supernatant was filtered, using Whatman filter paper No. 1. The filtrate was stored in plastic containers at $4 \pm 1\,^{\circ}C$ and protected from light. The aqueous extract from fermented tarbush was lyophilized.

### 2.4. Formulation and application of the edible coatings

Edible coatings were prepared according the procedure reported by Saucedo-Pompa et al. (2007). For emulsification, arabic gum was homogenized using a homogenizer (International TM Mod. Ll-17 V) in distilled water at room temperature (27 ± 1 °C) and then heated to 80 ± 1 °C. The candelilla wax and jojoba oil were added and homogenized all components at 15,000 rpm for 15 min. Fermented extract of tarbush was added at 500 ppm in the edible coating, based on a previous work reported by De León et al. (2013).

Edible coatings were prepared in two formulations named as follows: AWCE, edible coating with fermented extract of tarbush and AWC, edible coating without fermented extract. Controls were apples without edible coating AWOC. The apples were washed and disinfected with a chlorine solution (500 ppm) for 5 min and dried in one room with room temperature (27 ± 1 °C) registered with a thermometer. The apples were dipped in the edible coating for 2 s and then dried using a fan at room temperature (27 ± 1 °C) registered with thermometer for 2 min.

### 2.5. Evaluation of fruit during the storage

The apples were stored in marketing conditions at room temperature (27 ± 1 °C). Experiment was evaluated with four replicates per sampling. In order to evaluate the shelf life of fruit and the functionality of edible coating, several parameter of quality were measured each week during a storage period of 8 weeks. These parameters were: loss of weight, with the help of an analytical balance Ohaus Model E-02130 (Parsippany, USA). Fruit appearance using a digital camera Panasonic DMC-LZ2. Loss of firmness using a universal penetrometer model Humboldt H-1200 (Chicago, USA) and water activity (aW) using an Aqualab Series 3 device (Decagon, USA) at room temperature (27 ± 1 °C).

### 2.6. Sensory evaluation

The sensory evaluation was performed after applied treatments AWC and AWCE with the finality of knowing if the applications of the coatings change the appearance (considering the homogeneity of the coating, the presence of cracks or spots) and taste of apples in comparison with control apples AWOC. Panel of evaluation was integrated by 104 untrained panelists (males 63, females 41, age 20–24). Panelists were asked to rank the samples according their preference in appearance and taste of the fruit (1 for the most preferred and 3 for the least preferred) in an answer sheet previously provided. Data were analyzed using a Kramer's multiple comparison tests as modified by Joanes (1985). It was used a significance level of 5% for statistical tests.

### 2.7. Experimental design and data analysis

Experiments were established under a complete randomized block design. Analysis of variance and the Tukey multiple range test for means comparison were used to analyze the data.

## 3. Results and discussion

In all treatments, fruit weight loss increased during the storage period (Fig. 1). Apples with AWCE resulted with lower weight loss (20 g/100 g) however there was not significantly difference ($p \leq 0.05$) with respect to apples with AWC (27 g/100 g) (Fig. 1). The highest loss of weight was found in control apples AWOC (37 g/100 g) from the 5th week (Fig. 1). The weight loss occurs because after the fruit harvest it has a natural tendency for weight loss, mainly by transpiration process (Krochta & De Mulder-Johnston, 1997). It has been shown that the use of lipids as coatings decreases loss of humidity (Kester & Fenema, 1986) in fruits as papayas (Guilbert, 1988), apples (Assis & Pessoa, 2004), mangoes (Hoa & Marie Noelle, 2008), green bell peppers (Beaulieu, Park, Ballew Mims, & Kuk, 2009) and lemons (Bisen, Pandey, & Patel, 2012).

The results of weight loss (Fig. 1) are related to the firmness and water activity of fruits because at the end of the storage period the apples coated with AWCE ($p \leq 0.05$) maintain the initial values of firmness (205–271 mm/10) (Fig. 2) and aW (0.98–0.97 at 27 ± 1 °C) (Fig. 3) compared to apples coated with AWC and control apples AWOC, because the weight loss during transpiration of apples by the lack of a protective barrier (Fig. 1). This is attributed to reduction of open area of the emulsified solids network, which restricts transport of water vapor from the inside (Baez et al., 2001) by closure of the stomata, reducing transpiration and microbial activity (Bisen et al., 2012).

The results were evident when observed the appearance of the fruits after storage period (8 weeks). Because edible coatings (AWC and AWCE) affect the appearance in a minor level compared to control apples (Fig. 4). However apples coated with AWCE showed better appearance compared to apples coated with AWC (Fig. 4).

The use of fermented extract of tarbush as one part of edible coating of candelilla wax decreases in greater proportion the weight loss, maintained the firmness and aW in fruits

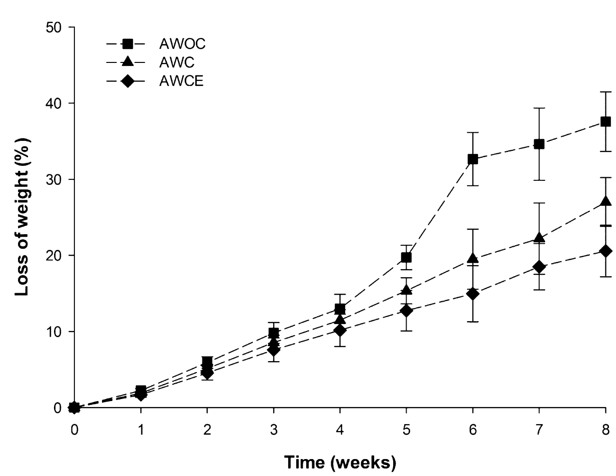



**Fig. 1 – Effect of edible coating on the loss of weight of apples with AWOC (■), AWC (▲) and AWCE (◆).**

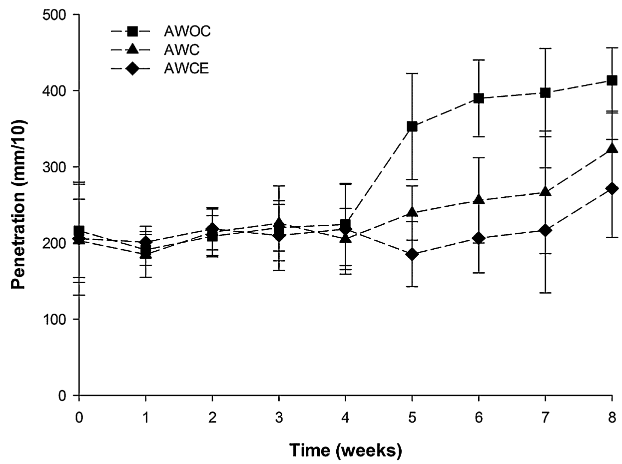



**Fig. 2 – Effect of edible coating on firmness of apples with AWOC (■), AWC (▲) and AWCE (◆).**

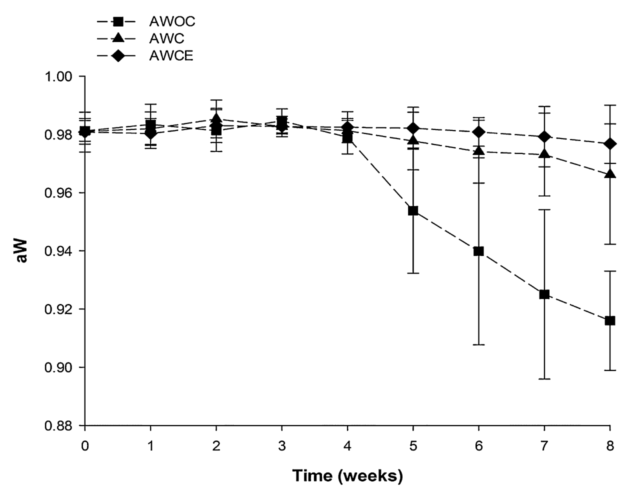



**Fig. 3 – aW of apples with AWOC (■), AWC (▲) and AWCE (◆).**

that an edible coating of candelilla wax alone. De León et al. (2013) reported that the fermented extract of tarbush used in this work contains from 0.3 to 0.35 mg of phenolic compounds catechin equivalents per gram of plant, in addition to have antioxidant activity (60–65%) and antifungal against *Fusarium oxysporum* and *Penicillium expansum*. It is possible that the phenolic compounds released during fungal fermentation of tarbush interact by hydrogen bonds with each of the chemical structures of the components of edible coating, mainly polysaccharide chains and proteins contained in gum arabic (Pasquel, 2001) because to the ability of the hydroxyl groups of the phenolic compounds of tarbush to form complexes with polysaccharides and proteins (Aguilar et al., 2007) and thereby create a more closed structure than that formed on the edible coating without extract of tarbush.

**Table 1 – Preference results performed by the means obtained from each treatment of Golden delicious apples.**

| Treatment | Taste | | | Appearance | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total |
| AWOC | 2.0[a] | 2.0[a] | 2.0[a] | 2.1[a] | 2.5[a] | 2.3[a] |
| AWC | 2.1[a] | 2.0[a] | 2.1[a] | 2.0[a] | 1.8[b] | 1.8[b] |
| AWCE | 1.7[a] | 1.9[a] | 1.8[a] | 1.8[a] | 1.6[b] | 1.7[b] |

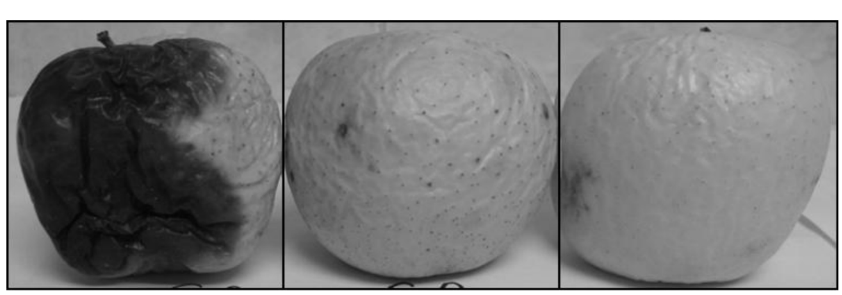
[a,b]Values with different letters differ significantly ($p \leq 0.05$) in the same column.

Ochoa-Reyes et al. (2013) reported that an edible coating of candelilla wax with extract of tarbush prevents further weight loss, improve firmness and the appearance of green bell peppers that edible coating alone. Similar results have been reported on the application of edible coatings based waxes with other natural active compounds as ellagic acid in apples, bananas and avocados (Saucedo-Pompa et al., 2007, 2009; Ochoa et al., 2011), extract of propolis in papayas (Barrera-Bello, Gil-Loaiza, García-Pajón, Durango-Restrepo, & Gil-González, 2012) and lemon essential oil in apples (Wan-Shin, Hye-Yeon, Nak-Bum, Ji-Hyun, & Kyung-Bin, 2014).

Results of sensorial evaluation demonstrated that in the male preference it was found that there was no significant difference ($p \leq 0.05$) in the appearance of apples coated with AWC, AWCE and control apples AWOC (Table 1). On the other hand, the female preference showed significant differences ($p \leq 0.05$) in the appearance of control apples AWOC and apples coated with AWCE and AWC (Table 1). There were no significant differences ($p \leq 0.05$) between untrained panelists (males and females) in the results of the preference of fruit taste. It was found that the presence of edible coating with fermented extract of tarbush AWCE did not alter the appearance and taste of apples.

## 4. Conclusions

Application of candelilla wax-based edible coating with fermented extract of tarbush as natural antioxidants source



a b c

**Fig. 4 – Appearance changes in treatments AWOC (a), AWC (b) and AWCE (c) after 8 weeks of storage.**

resulted in a positive effect reducing the weight loss and maintain the water activity and the firmness improving significantly the quality and shelf life of apples Golden delicious for 8 weeks in marketing conditions. This edible coating did not alter appearance and taste of apples in the sensory evaluation. Even the application of edible coating AWC improves the shelf life and quality of apples. This work is an initial effort for the use of phenolic compounds derived from tannin biodegradation during the fungal solid-state fermentation of tarbush leaves as an alternative to increase the value and use of these plants widely distributed in semi-arid regions of Mexico.

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