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Full Length Research Paper

Chemical and structural characterization of Candelilla (*Euphorbia antisyphilitica* Zucc.)

R. Rojas-Molina[1], M. A. De León-Zapata[1], S. Saucedo-Pompa[1], M. A. Aguilar-Gonzalez[2] and C. N. Aguilar[1]*

[1]Department of Food Science and Technology, School of Chemistry. Universidad Autónoma de Coahuila, Unidad Saltillo 25 000. Saltillo, Coahuila, México.
[2]CINVESTAV, Center for Research and Advanced Studies, IPN Unit Ramos Arizpe, Coahuila, Mexico.

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This work describes the chemical and micro-structural characterization of candelilla (*Euphorbia antisyphilitica* Zucc) plant which produces a natural edible wax. Chemical composition analysis included total fat, protein, crude fiber, dry material, moisture, ashes, total of reducing sugars, hydrolysable and condensed polyphenols contents. Micro-structural characterization was made using Environmental scanning electron microscope (ESEM). Results demonstrated that contains a high level of lipids (15.9%), crude fiber (9%) and is a source of ellagic acid (2.2 µg/g). Candelilla wax is deposited in the external side of the stems surrounding fibers thus forming resistant structure. This is important for the development of novel methods for extraction, use and potential applications.

Key words: *Euphorbia antisyphilitica* Zucc, microscopy, condensed and total polyphenols, candelilla wax.

INTRODUCTION

The candelilla wax is a complex substance of plant origin, insoluble in water, but highly soluble in acetone, chloroform, benzene and other organic solvents. It is characterized by a high content of hydrocarbons (around 50%) and a relatively low quantity of volatile esters and is recognized by the Food and Drug Administration (FDA) as a substance Generally Recognized As Safe (GRAS) for application in the foods industry (Howe and Williams, 1990; Ochoa-Reyes et al., 2010).

Candelilla wax is traditionally extracted from *E. antisyphilitica* which is harvested in wild nature. Wax form a plant protection system to reduce excessive water loss. During wet season, wax deposits on the stem surface are minimal because there is no need for plant desiccation. The *Euphorbia* species that produce wax are native from Mexico, especially from the Northern states of this country (Coahuila and Chihuahua). To yield economical amounts of waxes, a desert-like climate is a required condition (Rojas-Molina et al., 2011)

Mexico has the potential to be the main producer of candelilla wax, however, the scarce knowledge about its chemical composition and plant anatomy is serious, limiting factor for more applications. Recently, our group has demonstrated that candelilla wax is an excellent biomaterial for elaboration of edible film that prolongs the physiochemcal and microbiological quality of cut-fresh and whole fruits (Saucedo-Pompa et al., 2007, 2009; Seydim and Sarikus, 2006). The main objective of this work was to determine the chemical composition and micro-structural characteristics of candelilla stems.

Recent studies in food packaging are focused on the use of biodegradable films, including materials made from plant sources (Seydim and Sarikus, 2006) as response to demands by consumers of less use of chemicals on minimally processed fruits and vegetables. Special attention has been centered to search for naturally occurring substances able to act as alternative antimicrobials and antioxidants (Saucedo-Pompa et al.,

*Corresponding author. E-mail: cristobal.aguilar@uadec.edu.mx. Tel: +52(844)416 9213. Fax: +52(844)439 0511.

2009). To develop the new kinds of edible films, it is necessary to consider the mechanical, physical and chemical factors involved in the storage of fruits. Besides, these films can confer nutritional and sensorial properties to the food if antioxidants, colorants or artificial flavors are added (Saucedo-Pompa et al., 2007). It is important to take into account the functional characteristics and the possible advantages in certain applications of new materials for food packaging.

MATERIALS AND METHODS

Vegetal material

Candelilla plants were collected in Coahuila State, Mexico (geographical latitudes: 26° 01' 07. 45" N and 101° 18' 51. 69" O). Samples were cut following the Official Mexican Norm-018-RECNAT-1999 (SAGARPA, 2008), which prohibits removing the root of the soil. Stems were deposited in polyethylene bags and transported to our laboratory.

Chemical analysis

Analysis of fat, protein, crude fiber, ash and moisture contents were determined using the methodology reported by Association of Official Analytical Chemists (AOAC), (1980). Total and reducing sugar contents were evaluated with the Dubois et al. (1956) and Somogyi (1952) methods. Total polyphenols content evaluated with the Folin-Ciocalteu method (Makkar, 2003) and total condensed tannins content was evaluated with the HCl-Butanol method (Porter et al., 1986). Presence of phenolic antioxidants was also considered; catechin, gallic and ellagic acids were evaluated using a high-performance liquid chromatography (HPLC) method (Aguilar et al., 2004; Aguilera-Carbó et al., 2008). Each chemical analysis was conducted in triplicate.

Microscopic characterization

Fresh stem transversal sections of 1 mm of thickness were obtained with dissection equipment and fixed on the sample cell. Then, samples were metalized with a fine coating (60 nm) of gold. Structural observations were made using an Environmental Scanning Electron Microscope Philips XL30. Resolution at 30 kV was 3.5 nm and operation at low voltage (> 500 V). Interactions among the epicuticular wax layer, the intracuticular tissue and thickness of each internal tissue were measured by ESEM.

RESULTS AND DISCUSSION

A description of the chemical composition and micro-structural characteristics of the candelilla stems is reported with the aim of increasing the knowledge required to develop new strategies for the extraction of candelilla wax and design new food formulations based on this biomaterial. Results obtained from the chemical characterization are presented in Table 1. A reduced content of moisture was found in fresh candelilla stem samples. From the fat content, 30% corresponds to esters

Table 1. Proximal chemical composition of candelilla (*Euphorbia antisyphilitica* Zucc).

Component	Concentration (%)
Moisture	0.4±0.0006
Total solids	99.6±1.81
Lipids	15.9±1.119
Crude fibers	9.0±1.217
Proteins	2.3±0.0571
Ashes	10.9±0.315
Total sugars	0.27±0.043
Reducing sugars	0.16±0.021
Hydrolysable tannins	0.56±0.010
Ellagic acid	2.2±0.15 mg/g*
Gallic acid	0.6±0.03 mg/g*
Condensed tannins	0.16±0.013
Catechin	0.2±0.02 mg/g*

*Mean values and standard deviation of 3 replicates. Values reported as the milligrams of antioxidant per gram of plant (dry base).

esters of candelilla wax. At industrial level, a yield of 41.66 g of wax per kg of plant is considered as the best value (Ochoa-Reyes et al., 2010; Rojas-Molina et al., 2011). High levels of antioxidants (catechin) were also found. These results show the importance of the candelilla, as a source of this kind of bioactive compounds has not been mentioned earlier.

Candelilla plant is a potential source of catechin, gallic acid and ellagic acid. Similar values of protein, crude fiber, total and reducing sugar contents were also reported in literature (Martínez, 2002; Lopez-Guerra, 2006).

Figure 1 shows an image of surface of a young candelilla stem where wax was exposed after manual wringing of stems (Figure 1A), demonstrating that wax is easily recovered as thin layers. After removing these wax layers from the candelilla stem (Figure 1B), it was possible to see a green cuticular surface of epidermis. Also, this figure presents an image of the stem external surface (Figure 1C) using ESEM where it is possible to observe that wax is deposited as small granules, which grow protecting the steam as thick layers (Figure 1D). This explains the candelilla tolerance to cold and hot temperatures in the desert, its disease and insect resistance. Also, retention of low contents of water can be possible. Image of a stem transversal section shows different vegetal tissue layers: a cuticular surface of 491 nm, a polymeric matrix of 127 nm, a lignin layer of 261 µm, a phloem layer of 578 µm and xylem of 1.62 mm (Figure 1E). Cambium was also measured (190 µm). Figure 1F shows an image of the surface of the cereous layer composed by small follicles followed by an epidermis of spongy aspect. Lignin appears as a compact and fibrous layer due its function of hardness and strengthens of walls

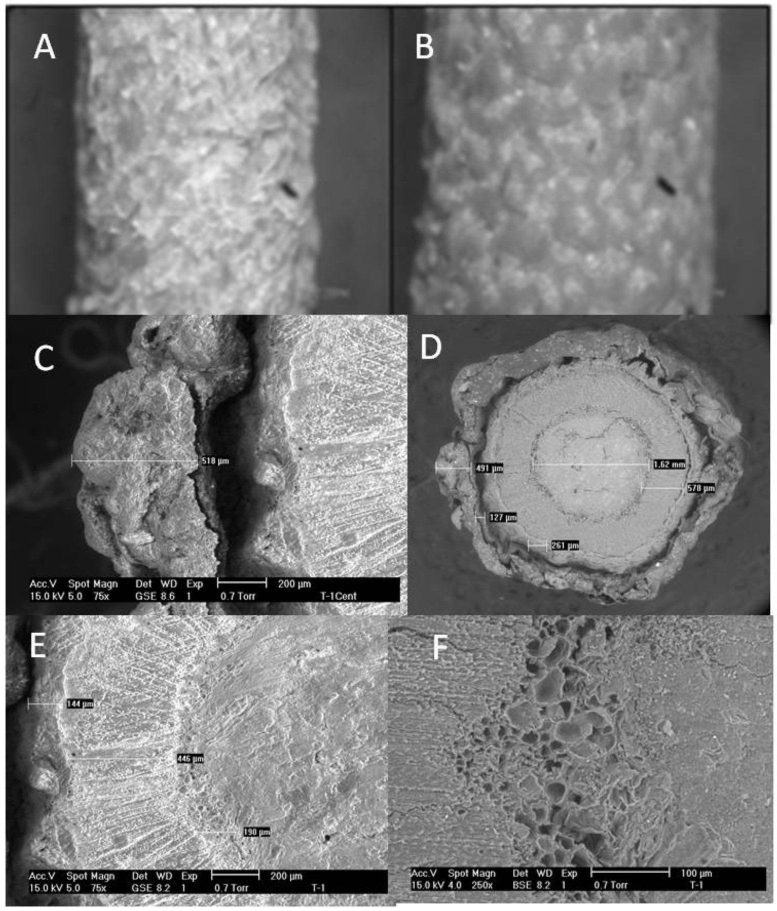

Figure 1. Images of candelilla stems (A) with wax and (b) after manual compression (without wax). Images C and D show the candelilla wax around the fibers of stem and the different tissues. Images E and F show an amplification of the phloem and xylem tissues.

of steam.

Candelilla wax has been successfully used for formulation of edible films, and their efficiency for prolonging the shelf life quality and improving the protection barrier properties of different fruits has been evaluated in whole (Saucedo-Pompa et al., 2009) and fresh-cut avocados (Saucedo-Pompa et al., 2007), guava fruits (Tomás et al., 2005), Valencia oranges (Hagenmaier, 2000), Persian limes (Bosquez-Molina et al., 2003), apples (Bai et al., 2003; Alleyne and Hagenmaier, 2000), bananas (Siade and Pedraza, 1977), grapefruits (Lakshminarayana et al., 1974) and limes (Paredes-López et al., 1974). Also, it was possible to produce edible films from their protein films, plasticized with candelilla wax and their sensorial (Kim and Ustunol, 2001), and thermal properties were evaluated (Kim and Ustunol, 2001a).

Candelilla wax edible films exhibit water vapor and oxygen permeability properties similar to carnauba wax and are not dependent of temperature; that of beeswax (Greener-Donhowe and Fennema, 1993) may be due to its chemical composition, which consists of about 57% hydrocarbons and 29% wax esters, with the remainder consisting mainly of fatty alcohols and fatty acids (Scora et al., 1995; Findley and Brown, 1953). Also, Candelilla wax possesses a relatively small amount of volatile esters (6%) relative to other natural waxes (Tolluch, 1973). This wax has been generally-recognized-as-safe (GRAS) status, with no limitations on use levels other than current good manufacturing practice, for surface finishes, chewing gums and hard candies (Bennet, 1975; ACT, 1984).

However, information of candelilla wax extraction is scarce but it is known that this wax is obtained through an ancient, deficient and contaminant process which uses high amounts of concentrated sulfuric acid (Ochoa-Reyes et al., 2010). This study revels that it is possible to adapt several modern technologies that can be applied to remove the candelilla wax, substituting the traditional process which implies the use of excessive amounts of concentrated sulfuric acid.

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