AAP Research Notes on Chemical Engineering

RESEARCH METHODS AND APPLICATIONS IN CHEMICAL AND BIOLOGICAL ENGINEERING

Ali Pourhashemi
Sankar Chandra Deka
A. K. Haghi
Editors

AAP APPLE ACADEMIC PRESS

CRC Press
Taylor & Francis Group

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Apple Academic Press Inc.
3333 Mistwell Crescent
Oakville, ON L6L 0A2
Canada

Apple Academic Press Inc.
1265 Goldenrod Circle NE
Palm Bay, Florida 32905
USA

Exclusive worldwide distribution by CRC Press, a member of Taylor & Francis Group

International Standard Book Number-13: 978-1-77188-768-7 (Hardcover)
International Standard Book Number-13: 978-0-42942-413-7 (eBook)

Library and Archives Canada Cataloguing in Publication

Title: Research methods and applications in chemical and biological engineering / edited by Ali Pourhashemi, Sankar Chandra Deka, A.K. Haghi.

Names: Pourhashemi, Ali, editor. | Deka, Sankar Chandra, editor. | Haghi, A. K., editor.

Series: AAP research notes on chemical engineering.

Description: Series statement: AAP research notes on chemical engineering | Includes bibliographical references and index.

Identifiers: Canadiana (print) 20190114924 | Canadiana (ebook) 20190114959 | ISBN 9781771887687 (hardcover) | ISBN 9780429424137 (ebook)

Subjects: LCSH: Chemical engineering—Research. | LCSH: Bioengineering—Research.

Classification: LCC TP165 .R43 2019 | DDC 660/.20721—dc23

Library of Congress Cataloging-in-Publication Data

Names: Pourhashemi, Ali, editor. | Deka, Sankar Chandra, 1965- editor. | Haghi, A. K., editor.

Title: Research methods and applications in chemical and biological engineering / editors, Ali Pourhashemi, Sankar Chandra Deka, A.K. Haghi.

Description: Toronto ; [Hackensack?] New Jersey : Apple Academic Press, 2020. | Includes bibliographical references and index. | Summary: "This research-oriented book consolidates and provides up-to-date experimental methods currently used in research for many branches of chemical and biological engineering. The book surveys essential ideas and research methodologies, concentrating on experiments as used in applications rather than on fine points of rigorous mathematics. Examples of important applications are reviewed in sufficient detail to provide the reader with a critical understanding of context and research methodology. Research Methods and Applications in Chemical and Biological Engineering presents a broad spectrum of chapters in the various branches of chemical and biological engineering, which demonstrate key developments in these rapidly changing fields. Chapters explore the design, development, operation, monitoring, control and optimization of chemical, physical and biological processes. Case studies are included in some chapters, building a real-world connection"-- Provided by publisher.

Identifiers: LCCN 2019020174 (print) | LCCN 2019980903 (ebook) | ISBN 9781771887687 (hardcover) | ISBN 9780429424137 (ebook)

Subjects: LCSH: Chemical engineering--Research. | Bioengineering--Research.

Classification: LCC TP165 .R47 2020 (print) | LCC TP165 (ebook) | DDC 660.6--dc23

LC record available at https://lccn.loc.gov/2019020174

LC ebook record available at https://lccn.loc.gov/2019980903

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PREFACE

Chemical and biological engineers create and develop processes to change raw materials into the products that society depends on, such as food, chemicals, pharmaceuticals, paper, plastics, and personal care products. Chemical and process engineers help to manage natural resources, protect the environment, control health and safety procedures, and recycle materials while developing and managing the processes, which make the products we use. Knowledge in the fields of chemistry, physics, biology, mathematics, and engineering sciences is necessary in order to apply processes developed on a laboratory scale and to adapt them for production on an industrial scale.

Biological and chemical engineering is a multidisciplinary research area in the cross field between biology, chemistry, physics, and engineering. It encompasses major cross-disciplinary areas such as disease and health, materials, environmental technologies, and food technology. Biological engineering is an interdisciplinary application of engineering principles to analyze biological systems and to expand the knowledge of chemical engineering into the biochemical realm.

Chemical engineering is an optimal combination of the molecular sciences (chemistry and biology), the physical sciences (physical chemistry and physics), the analytical sciences (math and computer programming), and engineering. Moreover, chemical engineering provides students with real-world experience through laboratory classes, hands-on operation of pilot-scale equipment, and research projects that result in clean energy production and medical applications.

Chemical and biological engineering integrates chemistry and biology and uses this broad foundation along with engineering fundamentals to study the synthesis of new processes and products.

Chemical and biological engineering uses process engineering, transforms chemical and biological materials into high-value products and services, in a safe and cost-effective way. Chemical and biological engineers bring about the large-scale benefits of advances in chemistry, biotechnology, materials, and environmental sustainability to the real world.

Chemical or biological engineers could play a vital role in the creation and production of new medicines, nutritious foods, novel materials, better waste treatment methods, and a sustainable global future.

Research into bioprocess engineering is a major part of the wider chemical and biological engineering program and includes fermentation of functional foods, production of high-value pharmaceuticals, and bioconversion of waste into value-added chemicals.

This book is designed for exploring interdisciplinary research progress in life sciences, agriculture, food processing, and environment. It is also designed to accommodate students and researchers with broad interests within the field of chemical and biological engineering.

This research-oriented book is based on experimental methods and is aimed at the design, development, operation, monitoring, control, and optimization of chemical, physical, and biological processes.

This new research-oriented book provides innovative chapters covering the growth of educational, scientific, and industrial research activities among chemical and biological engineers and provides a medium for mutual communication between international academia and the industry. This book publishes significant research reporting new methodologies and important applications and latest coverage of physical chemistry and the development of new experimental methods. This collection presents to the reader a broad spectrum of chapters in the various branches of chemical and biological engineering, which demonstrate important developments in these rapidly changing fields. Case studies are included in some chapters, building a real-world connection. These case studies form a common thread throughout the book, motivating the reader and offering enhanced understanding.

CHAPTER 16

FUNCTIONALITY FEATURES OF CANDELILLA WAX IN EDIBLE NANOCOATINGS

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ABSTRACT

The use of lipids in the development of edible coatings is a viable alternative to conserve natural and fresh products, allowing the extension of the shelf life quality without causing any harm to the environment, compared to films prepared from synthetic plastics. By this reason, the most important lipid in this application is the wax, because it possesses the necessary properties to perform this function. Since its nature, the fruits tend to have a layer of wax that confers protection against pathogens, environmental, pests, etc. In this review, we report the importance of candelilla wax as a natural resource from the Mexican semi-arid region, as well as their uses in the development of edible coatings, because it is a biodegradable and edible material approved by the FDA. Its importance as an essential component of an edible coating made with this type of biomaterial is based in its action as a plasticizer. Also, we describe some reports of the

development of nutraceutical edible coatings formulated with the addition of natural phenolic antioxidants.

16.1 INTRODUCTION

The concern for the mass production of waste from packaging materials has meant a decisive shift in the vision toward the use of biodegradable materials, especially those coming from agricultural surpluses,[43] due to the growing demand by consumers of food made from natural products, has led to the innovation of new conservation technologies at agro-industrial level, which help prolong the shelf life of fresh fruit. However, the use of natural coatings cannot, nor intends to, replace the usage of traditional packaging materials, but it is necessary to take into account its functional characteristics and the possible advantages of behavior in certain applications.[2]

The technical challenges involved in producing food and preserving them with stable quality, indicate that the use of this type of coatings will be greater than what it currently is; however, despite the fact that the technical information available for the elaboration of edible covers is wide, it is not universal for all products, which implies a challenge for the development of specific coatings and films for each food.[35] In the particular case of fruits and vegetables for fresh consumption, the edible coatings provide an additional protective cover whose technological impact is equivalent to that of a modified atmosphere, which consists of a thin and continuous layer, made of materials that can be ingested, and provides a barrier to moisture, oxygen and solutes, this can completely cover the food or can be placed in the components of the product[32] and must ensure the stability of the food and prolong its useful life.

According to the conditions of storage of fruits and vegetables should be considered some factors whether mechanical or chemical involved in the design of films,[32] therefore represent a storage alternative for products that can be consumed in fresh.[35] The edible coatings that are being tested in post-harvest are mixed formulations of lipid compounds and hydrocolloids, whose main priority is the preservation and protection of products of plant origin, against microbial contamination generated during manipulation.[28] The use of candelilla wax has been reported as a basic component in the elaboration of edible coatings evaluating different factors involved in the conservation of fresh fruits. The candelilla wax is extracted from the wild plant *Euphorbia antisyphilitica* Zucc., it represents one of the main biopolymers that present biodegradable and edible properties, its structure is amorphous and its hardness is of an intermediate degree between that of the wax of carnauba and that of Bee.[33]

Candelilla wax is considered a GRAS substance by the FDA, so it has multiple applications in the food industry,[18] that is why it is used for the elaboration of edible roofs, providing one of the best barriers permeable to moisture and gases that are product of the metabolism of the fruit. Candelilla is a perennial plant that develops in semi-desert climates, almost devoid of leaves. The candelilla is one of the plants that grow in the wild with greater number of applications of use. It is reproduced by both aerial and underground stem shoots and seed. The collection of the candelilla plant for the production of natural wax has been one of the most important economic activities in five states of the Mexican Republic. It is currently being used in more than 20 different industries around the world, mainly in the United States, the European Union, and Japan. Its distinctive properties confer on it the category of raw material essential for the manufacture of cosmetics, inks, paints, adhesives, coatings, brighteners and polishes, electrical insulators, integrated circuits, chewing gums, fruit coatings for export purposes, thinners and hardeners of other waxes, candles against insects, among others (National Forestry Commission 2009).

At the pharmaceutical level, the candelilla is recognized in several therapeutic properties. Currently in Mexico, there are research projects aimed at technological improvement in the process of extraction and purification of timber and non-timber forest products with high commercial value, in particular, the Department of Food Research of the Faculty of Chemical Sciences of the Autonomous University of Coahuila have reported the elaboration of edible roofs with the addition of natural antioxidants produced by fungal fermentation in solid medium, they have presented positive results for the conservation of fresh fruits,[38] providing a barrier against pathogenic microorganisms that may possibly damage the fruit[39] and give the consumer a health benefit, thanks to the presence of antioxidants, which have anti-tumor, anticarcinogenic, and anti-cancer properties.[38]

16.2 NATURAL WAXES USED IN THE FORMULATION OF EDIBLE NANOCOATINGS

The use of waxes to cover the fruits by immersion, is one of the oldest methods, practiced for the first time in China,[26] since the early 12th century,[30] retarding perspiration in lemons and oranges, as they are more effective in blocking the migration of moisture, specifically during seasonal changes and continues to be used in other types of fruits,[26] being the candelilla one of the most resistant.[9]

Recently, De Leon-Zapata et al. (2018) demonstrated that emulsions of candelilla wax can be nano-structures used to prolong the shelf life of apples at industrial level. Natural waxes applied to fresh perishable products to reduce respiration are: beeswax, carnauba wax, candelilla wax, and rice bran wax,[9] also paraffin waxes are some of the waxes prepared and used in the elaboration of edible coatings, which are also used as microencapsulation agents, specifically for substances that provide fruit smells and flavors.[43] Edible waxes are significantly more resistant to moisture transport than most other lipid or non-lipid films,[9] in addition to preventing the softening caused by enzymatic hydrolysis of plant cells and membrane components during the cutting process,[44] however, it is important that wax covers in fresh or perishable fruits is not completely waterproof, which causes anaerobes favoring the physiological disorders that shorten the half-life.[45] Waxing is a conservation technique widely used by marketers, supermarkets and exporters in the world, whose method generates a barrier of protection between the product and the environment to prevent the fruit from breathing less or deteriorate faster, this wear is characterized by the loss of moisture or dehydration of horticultural products and is a deterioration factor so we must try to maintain an optimum quality of the product.[45]

16.3 CANDELILLA WAX (*Euphorbia antisyphilitica Zucc.*)

The candelilla wax is extracted from the wild plant *Euphorbia antisyphilitica* Zucc., which is formed by esters of long-chain fatty acids that create a protective surface in the plant.[38] It is insoluble in water, but highly soluble in acetone, chloroform, benzene, and other organic solvents.[33]

It presents a wide variety of applications, being currently used in more than 20 different industries around the world (Table 16.1).

Its distinctive properties confer on it the category of essential material for the manufacture of cosmetics, inks, adhesives, coatings, emulsions, polishes, and pharmaceutical products. In the cosmetics industry, for being a good plasticizer,[11] it is also used in the manufacture of chewing gum, in the smelting, molding industry, in manufacturing various products in the electronic and electrical industries. There are many other applications where it is currently used, including cardboard coatings, crayon manufacturing, paints, wax candles, lubricants, paper coatings, anticorrosives, waterproofing, and Fireworks.[11] Candelilla's wax is recognized by the Food and Drug Administration of the United States of America (FDA), as a natural safe-GRAS substance, generally recognized as safe-for application in the food industry, therefore it is widely used in various sectors of the branch.[18] Because candelilla wax is an edible wax, it is being used for the elaboration of natural coatings that can retard the ripening and ageing of fruits and vegetables, maintaining a controlled atmosphere on the exterior surface, which allows the protection of the product in the face of environmental, transport and storage conditions.[33]

TABLE 16.1 Commercial Importance of Mexican Candelilla Wax.

Importing country	Imported candelilla wax (2016, USD)	Increase in purchase in the last 5 years (%)
Germany	11,000.00	23.3
China	9900.00	11.0
Italy	5600.00	23.6
France	5200.00	10.0
Spain	3800.00	27.5

Source: International Trade Center.

16.4 EFFECT OF CANDELILLA WAX AS PLASTICIZER ON THE FUNCTIONALITY OF AN EDIBLE COATING

Plasticization is a very important factor in the formulation, since they affect the mechanical properties[25] and physical of the coating (elasticity, flexibility, permeability, and wettability),[35] because they alter the mobility of the chain, the diffusion coefficients of gas or water and the structure of the films,[25] reducing the intermolecular forces between the polymer chains and increasing the free volume,[36] consequently, there is more space for water molecules to migrate, as well as hydrophilic plasticizers such as glycerol, are compatible with the polymeric material that forms the film and increase the absorption capacity of polar molecules such as water.[17] The increase in permeability with the plasticizer content may be related to the hydrophilicity of the plasticizer molecule,[5] because the permeability to the water vapor increases as the plasticizer content increases, however up to 30% glycerol content, that increase is relatively mild, later observed a more pronounced increase.[8]

Cellulose-based coatings, are very efficient barriers to the permeability of oxygen and their property of barrier to the water vapor,[29] these can be improved with the addition of lipids as plasticizers,[29] since they generally increase the permeability of the same.[36] The application of a lipid layer on the surface of fruits replaces the natural waxes of the cuticle, which may have been partially removed during washing.[9] The edible wax covers of candelilla have different functional properties, because when mixed with oils and polymers of high molecular weight as natural gums, they have an effect on the fruit to be coated, avoiding weight loss,[8] the use of candelilla wax on combined edible roofs has been amply evidenced by Bosquez-Molina et al., who demonstrated that the covers with this material and rubber mesquite create a modified atmosphere inside the fruit, to retard the process of maturation and senescence in a way similar to that of a controlled atmosphere that is much more expensive,[8] also avoids an increase in the production of ethylene and the hauling of additives that retard the discoloration and microbial growth (Gahouth 1991),[2] allow to control the respiration of the product, providing better permeability and texture, since it modifies the mechanical properties; Fulfilling the function of Plasticizer,[3] weakening the intermolecular forces between the polymer chains, increasing the flexibility of the coating.[8] The use of a hydrophilic or hydrophobic plasticizer will produce a coating with similar characteristics (Kester et al. 1986). Lipids such as candelilla wax are good plasticizers, so this natural wax product has shown its advantages over most synthetic waxes used in this industry,[11] they are compounds of low volatility and function as plasticizers, which are added to the coating,[28] considering themselves two forces, one among the forming molecules of the film, called cohesion and another in the coating and substrate, called adhesion,[24] in order to reduce the fragility, increasing the flexibility, hardness, and resistance to cutting, as they decrease the intramolecular forces of the polymer chains, thus producing a decrease in the strength of cohesion, tension and in the vitreous transition temperature.

Candelilla wax covers, among others, can be used as a support when adding preservatives or other additives on the surface of foodstuffs, mainly fresh fruits and vegetables to prolong periods of post-harvest storage, which consist of an emulsion made of waxes and oils in water, which are sprinkled in the fruits to improve their appearance, brightness, color, softness, control its maturity, and retard the loss of water.[15] The emulsion originated in the elaboration of an edible cover based on candelilla wax must present an adequate homogenization of the system and in this way guarantee the uniformity in the size and distribution of the particles of the dispersed phase,[8] as it will be reflected in the final barrier properties such as water vapor permeability and gases.[8] It is important to know the volumetric fraction of the dispersed phase of the wax emulsion of candelilla, as it influences much on the appearance.

16.5 FUNCTIONAL EDIBLE COATINGS BASED ON CANDELILLA WAX SUPPLEMENTED WITH PHENOLIC ANTIOXIDANTS

Additives are used to impart mechanical, nutritional, and organoleptic properties to edible roofs,[42] these may be of the plasticizer type (polyhydric alcohols, oils, and fatty acids), surfactant and emulsifier type (fats, oils, and polyethylene glycol) chemical preservatives (benzóico acid, sodium benzoate, sorbic acid, potassium sorbate, and propionic acid),[2] as well as antimicrobial agents, antioxidants, dyes, flavorings, and calcium as a firming agent of cell membranes, among others,[42] can be applied to control and modify surface conditions, reducing some of the degrading reactions.[13,19] The maintenance of microbial stability can be obtained using edible coatings with antimicrobial action and combined with refrigeration and controlled atmosphere. For fruits, waxes are usually used with addition of sorbic acid and sorbates as antifungal.[13,19]

The influence of a given additive will depend on its concentration, chemical structure, degree of dispersion in the film and degree of interaction with the polymer.[28] Some chemicals and natural products are used as antioxidants or as microbicides; ascorbic acid, citric, and lemon juice are used as antioxidants and salts of 5–acetyl–8–hydroxyquinoline or strong inorganic acids such as H_2SO_4 or H_3PO_4 are used as microbicides with very good results. The use of these substances does not prevent or retard the maturation so, other methods should be used together for this purpose, such as the application of gamma radiation that modifies the ripening time of the fruit according to its dose; however, some defects have been detected, such as the darkening of the pulp becoming coffee or by contrast discoloration. Good results have been reported when radiation is less than 7 J/kg.[20]

Zhang et al.[46] reported that the use of ascorbic acid, isoascorbic acid, and acetyl cysteine reduced darkening in litchi.[10] They used combinations of 4–hexylresorcinol, isoascorbic acid, $CaCl_2$, and acetyl cysteine to reduce changes in apple slices. Luo et al.[31] controlled darkening in apple slices using 4-hexylresorcinol mixed with ascorbic acid. Baldwin et al.[2] observed better protection with the addition of ascorbic acid in edible roofs. Ruiz-Cruz et al.[37] showed the positive effect of different antioxidants (independent and mixed) in inhibiting the darkening of cut fresh pineapple, because when antioxidants are used in combination with other technologies: treatments with heat, modified and controlled atmospheres, edible covers, gamma radiation, and electromagnetic pulses the darkening in fruits is inhibited.

Saucedo-Pompa et al.[38] reported for the first time the use of gallic acid, ellagic acid, and aloevera in the formulation of an edible cover based on candelilla wax, as anti-darkening additives of fruits, showing excellent results, even the controls with the cover without antioxidants were better compared with the fruits without edible cover.[38] The addition of ellagic acid and aloevera as natural antioxidants to the edible candelilla wax cover showed positive results when applied to fresh fruits, this due to the protective barrier that represents the cover of wax of candelilla as physical barrier of the coating, allowing greater control of gases, greater permeability and therefore the better control in the respiration of the fruit and in turn the addition of antioxidants, they intervene inhibiting microbial growth, as well as the possibility of providing a benefit to the health of the consumer, due to the anti-cancer and anti-tumor properties that present this type of antioxidants.[38] Saucedo-Pompa et al.[39] reported that when applying the edible cover based on candelilla wax with the addition of ellagic acid and aloevera to freshly cut avocados decreases the aqueous activity of the fruit, these results indicate that for freshly cut fruits reduction in weight loss is very important and the use of edible covers carrying natural antioxidants is an excellent tool to control weight loss[1,21,23] The apple slices with the edible covers with ellagic acid and aloevera kept to a greater extent the initial firmness, these covers had a protective effect on the firmness of freshly cut fruits. The texture of freshly cut fruits was improved with the application of edible candelilla wax covers, these results are similar to those reported by Ghaouth et al.,[22] who applied edible covers in tomato. Saucedo-Pompa et al.[39] reported antifungal properties of ellagic acid and aloe vera as part of the formulation of a candelilla wax-based edible cover which was applied in avocados, its functionality allowed to increase the resistance to the invasion of common phytopathogenic fungi and prolong its shelf life, improving the physical and chemical quality of the product. According to the results obtained by Saucedo-Pompa et al.,[39] the concentration of antioxidant influences the speed of water loss, as it is directly proportional to the increase in the concentration of antioxidant.

At the beginning of the new millennium, a new era in the area of food and nutrition sciences has become increasingly intense: the area of food–medicine interaction increasingly recognized as the “functional foods” that accepts the role of food components, as essential nutrients for the maintenance of life and health and as non-nutritional compounds but that contribute to prevent or retard the chronic diseases of the ripe age. Initially regarded as a passing curiosity, the idea of food formulation based on health benefits that its non-nutritional components could provide to the consumer, it has become an area of great interest today for large food companies,[6] so the addition of natural antioxidant additives from the group of phenols such as ellagic acid and aloevera to an edible cover, they represent a viable alternative for the development of nutraceutical roofs and enter in the field of functional foods.[37] In addition to offering a novel and comfortable presentation for the consumption of antioxidants by consumers and the VES improve the quality of shelf life in fruits[37] and avoid losses, by the attack of microorganisms, this due to the antifungal and antibacterial activity of the antioxidant additives.[38]

16.6 PHENOLIC COMPOUNDS AS NATURAL ANTIOXIDANTS AND THEIR FUNCTIONAL ACTIVITY IN THE EDIBLE COATING

Phenols are also antioxidants and as such trap free radicals, preventing them from joining and damaging the molecules of deoxyribonucleic acid (DNA), a critical step in initiating carcinogenic processes, they also prevent lipid peroxidation, which, being free radicals can cause structural damage to normal cells, interfering with the transport of molecules through these membranes affecting cell growth and proliferation.[41] These phytonutrients, include a large group of compounds that have been subject to extensive research as preventive agents of diseases, which protect plants against oxidative damage and carry the same function in the human organism, whose main characteristic is its ability to block the action of specific enzymes that cause inflammation, but also modify the metabolic steps of prostaglandins and thus protect the agglomeration of platelets, according to data obtained from experimental studies, it seems that there are some possible mechanisms for the action of phenols, inhibiting the activation of carcinogens and therefore block the initiation of the process of carcinogenesis.[27] Phenolic compounds come from barks, stems, leaves, flowers, organic acids present in fruits, and phytoalexins produced in plants,[7] such as caffeic, chlorogenic, p-coumaric, hydroxicinnamic, cinnamic, ferulic, and quinic acids that are present in plants which are used as spices, these acids present antimicrobial activity so they can retard the rot of fruits and vegetables, in fact it has been shown that tannins and tannic acid also present antimicrobial activity.[7] Antimicrobial additives can inactivate essential enzymes, reacting with the cell membrane or altering the function of the genetic material, it has been observed that the pH and temperature affect the antimicrobial activity of these compounds.[14]

The antioxidant compounds prevent the negative effects of free radicals on tissues and fats, reducing the risk of cardiac disturbances by avoiding the oxidation and cytotoxicity of LDL in vitro, decreasing the aterogenicidty.[4,40] Vitamins C, E, and β-carotene that prevent the oxidation of the LDL fraction of cholesterol, reduce the risk of coronary alterations, as well as possessing anti-cancer properties, whose protection measure consists of increasing the ingestion of fruits and vegetables, as well as foods containing antioxidant nutrients to protect from oxidation to LDL mentioned and thus avoid its oxidative modification and atherogenic formation.[40]

Biomaterials such as candelilla wax have had a significant role in the coating of food, as it is a natural and biodegradable material, so it does not harm the environment besides being a natural safe-GRAS, generally recognized as safe-recognized by the FDA, for its application in the food industry and can be used as an alternative in the elaboration of natural edible covers, reducing the use of synthetic polymers as derivatives of petroleum, which, when discarded, present a slow degradation in the environment compared with biomaterials, representing serious pollution problems. The edible wax covers of candelilla, allow controlling the respiration of the product, providing better permeability and texture, since it modifies the mechanical properties; fulfilling the function of plasticizer. In addition, additives such as nutrients, dyes, antioxidants, antimicrobials can be added, which, as additives, make the edible cover a functional food.

At present, the realization of edible roofs is of lesser proportion compared with the elaboration of synthetic roofs, but the main advantage of edible covers made with biomaterial such as candelilla wax, are easy to produce and quickly biodegradable, so it is a natural technique of conservation of fresh fruits.

It is important to consider the elaboration and commercialization of edible candelilla wax covers with the addition of antioxidants such as ellagic acid and aloe vera for the conservation of fresh fruit products in post-harvest, since it will allow to extend its shelf life avoiding microbial contamination, due to the antioxidant potential of ellagic acid and aloe vera, in addition to providing a benefit to the health of the consumer thanks to the anti-tumor characteristics, anticarcinogenic, and anti-cancer agents that present these antioxidants of natural origin.

ACKNOWLEDGMENTS

This work is part of the project "design of a high-performance process in the extraction of high quality candelilla wax and formulation of products of final use from the prepared wax," financed by the project CONAFOR-CONACYT-S0002-2008-C01-91633.

KEYWORDS

- **coatings**
- **wax**
- **candelilla**
- **biodegradable**
- **edible**

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ISBN: 978-1-77188-768-7
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