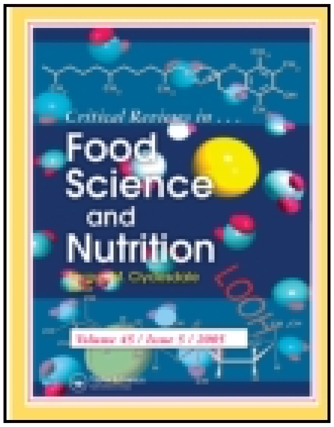

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Fruit Wastes Fermentation for Phenolic Antioxidants Production and Their Application in Manufacture of Edible Coatings and Films

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Fruit Wastes Fermentation for Phenolic Antioxidants Production and Their Application in Manufacture of Edible Coatings and Films

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Agro-industrial by-products are important sources of potent bioactive phenolic compounds. These compounds are of extreme relevance for food and pharmacological industries due to their great variety of biological activities. Fermentation represents an environmentally clean technology for production and extraction of these bioactive compounds, providing high quality and high activity extracts, which can be incorporated in foods using coatings/films wax-based in order to avoid alterations in their quality. In this document is presented an overview about importance and benefits of solid-state fermentation, pointing out this bioprocess as an alternative technology for use agro-industrial by-products as substrates to produce valuable secondary metabolites and their applications as food quality conservatives.

Keywords Fruit wastes, fermentation, phenolic antioxidants, edible coating, edible films

INTRODUCTION

Bioactive compounds are extra-nutritional constituents that typically occur in several plants and foods. They have numerous defense functions in plants, against several environmental factors such as light, temperature, humidity, and internal factors, including nutrients, hormones, etc. (Kähkönen et al., 2001). In addition, they play an important role in protection of plant tissue, because these compounds help to ensure healthy propagation of vegetable species. Large quantity of secondary metabolites occurs in seeds and peals of many vegetables and fruits (Vattem and Shetty, 2003). Those functions may be associated with relatively high contents of phenolic compounds in these foods. These compounds have been implicated in lower incidence of some human degenerative diseases including cancer (Lansky and Newmana, 2007), and with decreasing the risk factors to cardiovascular diseases (Kris-Etherton et al., 2002; Pérez-Jiménez et al., 2008). Moreover, phenolic compounds have been associated with antimutagenic effects (Negi et al., 2003) and good antioxidant activity (Vattem and Shetty, 2002; Randhir and Shetty, 2007; Lee et al., 2008; Sultana et al., 2008). Traditionally, some chemical processes have been employed to obtain bioactive phenolic compounds (BPC) from different vegetable sources such as *Jatropha dioica* stems, *Flourensia cernua* leaves (Aguilera-Carbo et al., 2008), pomegranate, apple, banana, citrus, and grape peels (Negro et al., 2003; Li et al., 2006; Sultana et al., 2008). However, due to non-specificity of these reactions, chemical procedures have large problems for BPC recovery, thus generating low yields; also, these processes have high environmental impact and in some cases they represent hazards to human health. In recent years, biotechnological hydrolysis for bioactive compounds production has attracted more attention. Solid-State Fermentation (SSF) represents a potential

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bioprocess for production/extraction of high stable and quality microbial products such as phenolic compounds for pharmaceutical and food applications. SSF has the advantage to utilize agro-industrial residues as support and nutrient source, besides providing a novel alternative to give a value-added to these non-utilized residues (Pandey, 2003).

Currently, one food application of these phenolic compounds focusing on their use in edible coatings and films capable to increase shelf life of some food, because consumers demands less use of chemicals on minimally processed fruits and vegetables (Saucedo-Pompa et al., 2007; Saucedo-Pompa et al., 2009; Ochoa et al., 2011). Candelilla, carnauba, and bee waxes as moisture barrier (Bosquez-Molina et al., 2003; Saucedo-Pompa et al., 2007; Saucedo-Pompa et al., 2009; Ochoa et al., 2011), mesquite gum, pectin, chitosan, carboxymethyl cellulose as structural constituent (Bosquez-Molina et al., 2003; Ribeiro et al., 2007; Ponce et al., 2008), and ellagic acid and natural plant extracts as bioactive ingredients (Saucedo-Pompa et al., 2007; Ponce et al., 2008; Saucedo-Pompa et al., 2009; Ochoa et al., 2011) have been employed as main components of edible coatings and films. This document describes the biotechnological advantages of fermentation of fruit residues for production of potent phytochemicals with high antioxidant activities derived from fungal hydrolysis of polyphenols, particularly of tannins. This type of fermentation has been done using mainly SSF and its main feature is the possibility of generation of natural compounds with high commercial value. It emphasizes on technical aspects needed to optimize SSF conditions and their effect in filamentous fungi physiology. Also, it includes a detailed description of protocols used for recovery of antioxidants and applications in formulation of new active edible films. In addition, it is described the impact of antioxidants in conservation of whole and cut fruits. The use of natural waxes as main component of film and as carrier of antioxidants produced by fermentation is a relevant aspect of this contribution.

Solid-State Fermentation: General Aspects

SSF is referred as the bioprocess during which microbial growth and product formation occur on surface of solid materials near absence of free water; however, substrate must possess enough moisture to support growth and metabolism of microorganisms (Pandey, 2003). There are several important aspects, which should be considered to develop a bioprocess in SSF for production of any bioactive compound. These aspects include selection of a suitable microorganism, support/substrate that will be employed, optimization of fermentation parameters, and selection of purification steps of desired product (Pandey, 1992). Due to low moisture content of SSF process, the microorganisms most commonly used in this kind of technique are fungi and yeasts; however, bacteria cultures can also be used and manipulated in this bioprocess (Cho et al., 2008). SSF offers several advantages over submerged fermentation, such as higher productivity per reactor volume, lower costs, less space requirements, simpler equipment, and easier downstream processing (Pandey, 1992; Ustok et al., 2007). Another very important advantage is that, it permits the use of agricultural and agro-industrial byproducts as substrates, which might be converted into products with commercial value-added as organic acids, proteins, alcohol, and enzymes (Mamma et al., 2008). In the last 20 years, SSF has been utilized for enrichment or bioconversion of several ligno-cellulosic materials into bioactive phenolic compounds.

Bioactive Phenolic Compounds Production

Bioprocessing of several foods and plant materials using SSF and generally recognized as safe (GRAS) fungi such as *Aspergillus awamori*, *Aspergillus oryzae*, *Aspergillus sojae*, *Rhizopus azygosporus*, and *Rhizopus sp*. No. 2, provides strategies to improve nutraceutical properties and produce bioactive phenolic ingredients (Vattem and Shetty, 2002). An interesting application of SSF is production of foods enriched with antioxidant compounds. Lee et al. (2008), explored SSF of black beans, which are rich in polyphenolic compounds that have been demonstrated to scavenge free radicals. In general, their experimentation evidenced that 1-1-diphenyl-2-picyl-hydoxyl ($DPPH^{\cdot}$) free radical-scavenging activity, Fe^{2+} iron-chelating ability, as well as reducing power of black bean were higher in black bean koji than those in unfermented black bean due to an increment of phenolic through fermentation with a filamentous fungi commonly employed for preparation of fermented foods. Nevertheless, authors proved that black bean antioxidant activity enhancement depends on the starter microorganism. A significant increase of total methanol-extractable phenolic compounds and anthocyanins content in black beans was also observed after SSF. Therefore, enhancement of black beans antioxidant activity after SSF could be linked to phenolic compounds and anthocyanins content increase, since these compounds present antioxidant properties. Starzynska-Janiszewska et al. (2008) demonstrated that SSF with *Rhizopus oligosporus* (a GRAS fungi) caused a significant increase in the antiradical properties of fermented cooked seeds of grass pea, obtained high radical-scavenging activity values (with $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assay) strongly related with levels of phenolic compounds, which might improve functionality of fermented food for diabetes and ulcer management.

Vegetables and fruits are two of the most important sources of phenolic compounds in our diets, such as hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, catechins, and hydrolyzable or condensed tannins, among others (Kähkönen et al., 2001). In the food industry, after processing of fruits and vegetables, their solid wastes (seeds, peels, or whole pomace) contain several soluble sugars, fiber, and other hydrolysable materials that can be metabolized by a wide range of microorganisms (mainly filamentous fungi) into value-added products as bioactive compounds. In recent years, bioconversion of ligno-cellulosic materials using SSF bioprocess has received much attention for their practical applications in several agro-industrial processes, recent studies have had, as goal, the successful bioconversion of agro-industrial wastes such as cranberry pomace, pomegranate (seeds and husk), guava waste, among other materials into many BPC (Zheng and Shetty, 2000; Vattem and Shetty, 2002; Vattem and Shetty, 2003; Hernández et al., 2008; Robledo et al., 2008; Starzynska-Janiszewska et al., 2008). It has been observed that byproducts are very good source for several phenolic compounds such as catechin, epicatequin, quercetin, pelargonidin, punicalagin, punicalin and caffeic, chlorogenic, *p*-coumaric, quinic, gallic, and ellagic acids among others (Negi et al., 2003; Lansky and Newmana, 2007; Aguilar et al., 2008; Hernández et al., 2008; Robledo et al., 2008; Sultana et al., 2008). Clear examples of ligno-cellulosic materials that contain substantial amounts of phenolic compounds are pomegranate wastes. Hernández et al. (2008) evaluated the bioconversion of pomegranate husk as support and nutrient source in a SSF bioprocess for ellagic acid (EA) production using *Aspergillus niger* GH1, the authors reported the highest EA accumulation (12.3 mg per gram of substrate) at 96 h of culture, this amount was seven-fold higher than that obtained from unfermented pomegranate husk. These findings are consistent with those reported by Aguilar et al. (2008) whom demonstrated that pomegranate husk is a better source for EA production after fermentation than other vegetal materials such as creosote bush leaves. In 2009, Mexico produced more than 4680 tons of pomegranate fruit for the juice industry (SAGARPA, 2009), which represents a generation of almost 2507 tons of husks. According to Robledo et al. (2008), there is possibility to produce 8 kg of EA per ton of pomegranate husk. By this reason, there are possibilities to consider this agro-industrial residue as an alternative raw material for BPC. In addition, some authors have suggested the use of cranberry pomace for food grade phenolics production (Zheng and Shetty, 2000; Vattem and Shetty, 2002, 2003). In order to understand changes and mobilizations of simple phenolics and diphenyls and their antioxidant properties, Vattem and Shetty (2003) evidenced an increment in DPPH free radical-scavenging activity and β-carotene antioxidant protection factor, which were well correlated with the increase in phenolic compounds after cranberry pomace was processed by solid-state growth using *Lentinus edodes* (a food grade fungus) and two nitrogen sources, NH_4NO_3 and fish protein hydrolysate (FPH). Their investigation demonstrated that production of EA also may be improved for both aqueous and ethanolic extracts with yields that ranged from 116 μgg^{-1} of pomace for NH_4NO_3 and 107 μgg^{-1} of pomace for FPH treatment and 260 μgg^{-1} of pomace to 350 μgg^{-1} of pomace for the NH_4NO_3 and FPH nitrogen treatments, respectively. Therefore, the highest yields of EA were obtained from ethanolic extracts due to EA solubility.

SSF of ligno-cellulosic wastes for phenolic compounds production has also been reported using mixtures of other plant residues. Miszkiewicz and Jakubowski (2008) reported that antioxidant activity increased twofold after 12th day of fermentation with two mixtures, 9 g of Aronia pomace and 1 g of evening primrose, and 5 g of Aronia pomace and 5 g of evening primose waste, and this increase was related with an increment in phenolic compounds. Furthermore, bioconversion of soybean flour-supplemented with guava residue by *R. oligosporus* has evidenced that this kind of waste represents a novel strategy for enhancement of phenolic antioxidant content and a potential commercial value of guava wastes (Correia et al., 2004).

Enzymes Related with Phenolic Compounds Release

Several investigations have described the crucial role of some enzymes (esterases and glucocidases mainly) such as tannin acyl hydrolase, ellagitannin acyl hydrolase, α-amylase, laccase, and β-glycosidase during the release of phenolic compounds using SSF; phenolics are usually found in conjugated forms through hydroxyl groups with sugar and glycosides (Zheng and Shetty, 2000; Bhanja et al., 2008; Cho et al., 2008; Robledo et al., 2008; Starzynska-Janiszewska et al., 2008). Table 1 presents a list of microorganisms and phenolic-rich materials used to produce BPC on SSF systems and some of the enzymes related with their release.

β-glycosidase (β-D-glucoside glucohydrolase, E.C. 3.2.1.21) catalyzes the hydrolysis of glycosidic linkages in alkyl or aryl β-glucosides as well as glucosides containing only carbohydrate residues (Vattem and Shetty, 2003). It has been described as an enzyme capable of hydrolyze phenolic glycosides and releasing extractable free aglycones; therefore, it is related with phenolic compounds mobilization. It has been mentioned that fermented-crude extract of β-glycosidase

Table 1 Microorganisms and phenolic-rich materials used to produce BPC using SSF systems and enzymes related with phenolic compounds release

Plant material	Microorganism	Enzyme(s)	Reference
Cranberry pomace	*Lentinus edodes*	β-glycosidase	Zheng and Shetty, 2000
Cranberrypomace	*Rhizopus oligosporum*	β-glycosidase	Vattem and Shetty, 2002
Cranberry pomace	*Lentinus edodes*	β-glycosidase	Vattem and Shetty, 2003
Guava waste	*Rhizopus oligosporum*	β-glycosidase	Correia et al., 2004
Pomegranate seed and husk	*Aspergillus niger* GH1	Ellagin tannin hydrolase	Robledo et al., 2008
Aronia pomace	*Rhizopus oligosporum*	β-glycosidase and laccase	Miszkiewicz and Jakubowski, 2008
Soybean	*Bacillus pumilus* HY1	β-glycosidase	Cho et al., 2008
Rice	*Aspergillus oryzae* IFO- 30103	β-glycosidase and α-amilase	Bhanja et al., 2008
Pomegranate husk	*Aspergillus niger* GH1	–	Hernández et al., 2008

releases higher concentrations of phenolic compounds than a commercial enzyme. This was associated to the fact that crude β-glycosidase solution probably contains other enzymes such as esterases, which help to cleave inter-sugar linkages and release the corresponding glucosides, and then in liberation of phenolic aglycon moieties (Zheng and Shetty, 2000). Cho et al. (2008) reported an increment in phenolic aglycones (flavanols and gallic acid) after SSF of soybean with *Bacillus pumilus* HY1, while isoflavone glycosides, malonylglycosides and flavanol gallates were decreased; this phenomena was associated with bacterial β-glycosidase and esterase activities. Also, improvement of antioxidant potential of fermented rice has been associated with increment in phenolic compounds propitiated for β-glycosidase and α-amylase activities using a novel SSF bioreactor in which the title values of those enzymes were higher than a conventional SSF system (Correia et al., 2004). In addition, β-glycosidase activity has been related with an enriched of fermented cranberry pomace with EA (Zheng and Shetty, 2000). On the other hand, tanin acyl hydrolase has been reported as an enzyme able to release gallic acid, EA, or pyrogallol molecules from hydrolysable tannins (Mingshu et al., 2006). However, recently, Robledo et al. (2008) did not associate the EA release from ellagitannins of pomegranate husk with tannin acyl hydrolase activity, but they suggested the presence of an ellagitannin acyl hydrolase responsible for ellagitannins biodegradation. Laccase activity has also been related with phenolic concentration changes for enrichment of Aronia pomace and evening promise waste mixtures (Starzynska-Janiszewska et al., 2008).

As mentioned above, fermentation bioprocess of vegetable and fruit wastes is a sustainable and environmentally friendly process to make appropriate use of food industry byproducts, plus getting a value of those wastes for bioactive ingredients production as phenolic compounds. For carrying out production of secondary metabolites and other products of fermentation, it is necessary to use GRAS microorganisms and specific conditions of fermentation parameters according to the specific residue to be used. Some important parameters that must be considered for production of phenolic compounds with antioxidant activity from agro-industry byproducts are: physical and chemical features of waste, which must possess a porous biodegradable or inert matrix. Water absorption and gas transfer capacity, low thermal conductivity, compressive strength, ability to scatter particles, and free contaminants and inhibitors of microbial growth must be considered too (Raimbault, 1998). These conditions are present in most of agro-industrial wastes employed in SSF. Other important parameters in fermentation of agro-industrial residues are temperature, moisture content, water activity, and pH. Temperature affects efficiency of microbial growth and metabolism, which generates around 15.9×10^6 JKg^{-1} of dry matter in response to the metabolic heat generated in SSF bioprocesses. However, the theoretical value for heat generation is in the order of 3.3×105 J/hKg on dry matter, which can cause overheating in different areas of the fermented material affecting microbial growth, enzyme activity, and hence phenolic compounds production (Robledo-Olivo et al., 2006).

Downstream Processing Phenolic Compounds

Downstream processing of phenolic compounds is the (ou one of the most important steps) major important step after waste fermentation, which involves its quantification and evaluation of its bioactive antioxidant activity. Diverse methodologies have been reported for extraction of phenolic compounds. In those methods, the authors used different ration of solvents. The techniques most widely referred are aqueous methanol (50%) and aqueous acetone (70%) with constant reflux at temperatures below 60°C, times ranging from 30 minutes to 12 hours, even refrigeration temperatures are reported for periods of 24–96 hours and in some cases they used the addition of HCl at low concentrations (Makkar, 2000; Belmares-Cerda et al., 2004; Koponnene et al., 2007). Besides, it is important to discuss about technologies for quantification of phenolic compounds, many of the reported methods are based on ability of these compounds to interact with other substrates and these methods are based on the fact that phenolic compounds are reducing agents. Concerning to quantification of total polyphenols, Folin-Ciocalteu method (Yu and Dahlgren, 2000) is the most commonly reported technique, where phosphotungstic and molybdic acids are reduced by oxidation of phenolic compounds, thus generating a mixture of oxides of tungsten and molybdate blue-absorbing at wavelength of 750 nm. For quantification of condensed tannins such as proanthocyanidins, HCl-butanol method is referred in literature; it is based on an oxidative depolymerization of condensed polyphenols, and formation of a chromofore in presence of iron (Wolf et al., 2008), which absorbs at a wavelength of 460 nm. Pink coloration is an indicative of flavon-4-ol type presence and orange coloring types 3-4 diol (Porter et al., 1986). Several fast methods have been developed for polyphenol quantification in ultraviolet light range, each polyphenolic group has a maximum absorbance at different wavelengths, for example, monomeric polyphenols have an absorbance range between 220–280 nm (Martínez-Valverde et al., 2000). Other techniques that involve the use of high performance liquid chromatography equipment (HPLC) for both separation and quantification of phenolic compounds have also been proposed. These methods use reverse phase column RP-C18 with octadecyl silane (Bianco et al., 1998; Amakura et al., 2000; Aguilera-Carbo et al., 2008), as well as polyacrylamide fillings 6 s has also been reported (El-Toumy and Rauwald, 2002) and for those proofs generally diode array detector are employed. The mobile phases more used are polar solvents such as methanol, acetonitrile mixed with acidificated water added to organic acids (formic, acetic, or phosphoric acids). Retention time depends on compound electronegativity and in some cases on its molecular weight; the mobile phase flow and solvent gradients are other important factors that must also be considered. Processing of samples for phenols extraction depends mainly on their origin, in the case of food or food wastes generated from them, different methodologies are described in the literature. Amakura et al. (2000) mentioned phenols extraction from samples of fruits and fruit juices using chloridric acid 0.1 $molL^{-1}$, the samples were washing up using Sep-Pak Plus tC18 and Bond Elut PSA cartridges and eluted with methanol, water and methanol-HCl 0.1 molL^{-1}, after that, the polyphenolic extract was evaporated to dryness to obtain gallic acid, chloragenic acid, cafeic acid, ferulic acid, and ferulic acid.

Another technique for phenols recovery was mentioned by Koponen et al. (2007), they used a three-step procedure with methanol-HCl and kept the sample at room temperature, followed by addition of methanol-HCl (2%) and vigorous mixing for 1 minute. The mixture was centrifuged at 3500 g for 10 minutes and supernatant was removed. After that, sediment was re-extracted with 50% methanol-HCl (1%), the supernatants were combined and made up to 50 mL with purified water. In this report, anthocyanins were quantified and the obtained compounds were: delphinidine, petunidin, peonidin, malvidin, and other hydrolysable tannins as elagitannins. Aguilera-Carbo et al. (2008) reported a method for extraction and analysis of ellagic acid, it is a simple method to recovery this powerful antioxidant phenolic compound. In their experimentation, the fermented waste was mixed with an immersion blender. Then, sample was placed in an ultrasonic bath at 40 kHz for 30 minutes. After that, an aliquot was taken and centrifuged, supernatant was decanted and precipitate was resuspended in methanol, filtered through 0.45 μm membranes and analyzed in HPLC system. This is a low-cost method; however, it must be evaluated on a pilot scale in first instance.

In 2005, Seeram et al. (2005) published an important ellagitannins recovery process from pomegranate husks waste, the methodology consisted in a percolation in water 1:5 (m/v), squeezed, blended, and filtered to yield a dark brown aqueous extract, polyphenols were separated in a XAD-16 resin column and eluted with methanol. Alcoholic-extractable polyphenols were dried in an evaporation system, in order to obtain the polyphenols rich powder. Efficiency of recovery was 77–85% of punicalagin and 80–95% of ellagic acid. Ellagitannins were purified onto Sephadex lipophilic LH-20 resin column previously reported by El-Toumy and Rauwald (2002) to separate pomegranate ellagitannins. This method was patented; however, it is expensive because cost of equipment and maintenance due to resin.

Edible Coatings and Films

Quality loss in fruit and vegetables often occurs between harvest and consumption periods (Wills et al., 1989), because of physical, chemical, enzymatic, and microbiological damages. Microbial contamination is the major concern for food industry, regulatory agencies, and consumers. By this reason, some technologies have been developed to protect fruits and vegetables from microorganisms attack. These microorganisms can be controlled by cooling, reducing water activity, acidification, atmosphere modification, thermal treatments, and covering foods with edible materials or applying chemical/natural antimicrobial compounds to prevent microbial contamination. Antimicrobial agents are chemical or natural compounds, which inhibit or retard microorganism growth; thus, avoiding alterations in product quality or safety. Antimicrobial agents are added to foods because microorganisms produce food poisoning (infectious agents and toxins producers) and food alterations. These food alterations may produce bad odors or flavors, texture problems, and color changes that affect human health (Davidson and Zivanovic, 2003). One of the main factors influencing survival and growth of microorganisms is medium acidity. Bacteria cannot grow at pH values below 3.9. Acidic foods with pH values below this limit are essentially protected against contamination of pathogenic bacteria. Some fruits such as oranges, pears, apples, and grapes are generally in this pH range because these fruits contain citric, malic, or tartaric acids. Sorbic acid is also present in fruits and it is extensively used to control microbial growth in foods. Acetic, lactic, and propionic acids are produced in fermented foods and they often play an important role in preventing growth of pathogenic bacteria (Beuchat, 2001).

In order to satisfy the growing consumer demands about less use of chemicals and minimally processed fruits and vegetables, more attention has been focused on natural antibacterial compounds production (Oya et al., 1996; Rojas-Graü et al., 2006). One option to produce and increase the potential of these agents is fermentation, since in this system can being obtained monomers, which in this way can increase their effectiveness. The antimicrobials include phenolic compounds from bark, stems, leaves, flowers, and organic acids present in fruits and phytoalexins produced by plants (Beuchat, 2001). Phenolic compounds are natural antioxidants derived from plant material extracts and they have become increasingly popular because as components of edible coatings and films, these products help to increasing shelf life of some foods. It has also been reported that phenolic compounds improve stability of lipid-containing foods and thereby preventing their sensorial and nutritional quality loss (Hemeda and Klein, 1990; Ozcan, 2003; Ponce et al., 2004; Sebranek, 2004).

Natural Waxes: Carnauba, Bees, and Candelilla Wax

Natural waxes have been used as a moisture barrier of edible coatings and films. They are esters of fatty acids with alcohols of high molecular weight. The waxes are substances highly insoluble in aqueous media and at room temperature are strong and tough. On plants, waxes are covering stems that prevent water loss by evaporation; this property is given for the epicuticular wax (Jeffree, 1996; Post-Beittenmiller, 1996; Heredia, 2003). Cuticular waxes are a mixture of hydrophobic compounds that are composed predominantly of aliphatic lipids, such as long-chain fatty acids and their derivatives. Moreover, waxes may contain other compounds such as triterpenoids and phenylpropanoids (Nawrath, 2006). Waxes provide protection and structure due to their hydrophobic capacity; also, acting as a lubricant and waterproof skin, leather, and feathers in animals, and leaves and fruits in plants. A clear example is the formation of honeycombs of bees (QuiniNet.com, 2009). There are other plant responses to water limitation such as partial or

Table 2 Characteristics of natural waxes

Wax	Fusion point (°C)	Ash	Acid value	Saponification value	Ester value	Reference
Bees	65.1	1.8%	7–24	85–107	72–79%	Kohl et al., 2003
Carnauba	82.5–86	0.05–0.50%	2–10	78–88	80–85%	MULTICERAS, 2009
Candelilla	68.5–73	0.7%	12–22	43–65	39%	Kuznesof, 2005

total closure of specialized small holes found in the leaves, called stomata, which prevent plant dehydration (Del Carmen-Lallana et al., 2006). This phenomenon just not results in a decreased water loss through leaves, but also in a reduction of CO_2 intake, which affects directly photosynthetic process (Del Carmen-Lallana et al., 2006). Waxes have been used for long time and a variety of applications from food industry to their use in equipment propulsion spacecraft; this is the case of candelilla wax, which has been used as a fine lubricant (CONAFOR, 2002). Traditionally, it haas also been used to make candles, lighting, as wood protector, cloth and leather cover, as a preservative, and waterproofing among other applications. On cosmetics, waxes are used in forms of creams or ointments because of their anti-inflammatory and healing properties. Another application is as cosmetic depilatory, since they adhere to hair and it is easy to remove (Rojas-Molina, 2008). Waxes are also used as microencapsulation agents, especially for substances with odors and flavors (Tharanathan, 2003).

Natural waxes can be used during fruits and postharvest management and they may be considered as an alternative to maintain fruit quality and reduce losses during this stage (Tharanathan, 2003). However, according to Taek-Hwang et al. (2004) wax extracted from sorghum grain is a high-cost procedure due to the low yield obtained (around 0.2%). On the other hand, since the beginning of the last century, carnauba wax extraction has been one of the most important economic activities in countries such as Brazil (López-Araujo, 2008). This wax has been used in developing sails, drugs, patches, ointments, soaps, phonograph records, materials for polishing, grease lamps, and lubricants. Carnauba wax is obtained from palm *Copernicia cerífera* leaves, a Brazil endemic plant and for its properties of brightness, hardness, and high fusion point, it has also been used for chew-gum, lipsticks, paintings, etc. (Vandenburg and Wilder, 1970). Carnauba wax is a very important ingredient in preparation of emulsions, also known as edible coatings or films. Coatings based on carnauba wax have been applied on surface of vegetable products where they showed different rates of CO_2, O_2, and water vapor permeability, those phenomena were related with material properties, and coatings concentration and thickness. The right combination of these factors varies for each fruit, according its physiological characteristics (Amarante et al., 2001).

On the other hand, beeswax is a material composed for esters of alcohols with C_{24}–C_{33} and fatty acids with C_{18}–C_{36} and its fusion point range from 61°C to 65°C. It is liposoluble and soluble in organic solvents (Vit, 2005). Beeswax quality is determined by its purity and color, although it is noteworthy that all species of bees produce wax and each has chemical and physical properties slightly different, but wax with light color has better price for keeping less impurities (Kohl et al., 2003). Depending on production area, waxes have a great variation in their chemical composition. Table 2 shows some characteristics of natural waxes.

Candelilla wax is a complex substance of vegetable 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 it is recognized by the FDA as a GRAS substance for application in foods (Saucedo-Pompa et al., 2007). The scientific name of candelilla plant is *Euphorbia antisyphilitica*; its common name was derived from the form of its large, straight wax stems that look like candles. This plant normally grows in desert environments, mainly in calcium-rich soils and is native of Mexican and USA's Southwestern arid zones (Saucedo-Pompa et al., 2007).

In recent years, food packaging research has focused on biodegradable films, including coatings and films made from vegetal sources. The most critical factor in sensory quality and shelf life of food products is moisture loss. Some polysaccharides used for making films present a good capacity for formation of those films, with efficient mechanical and barrier properties of O_2; however, for their hydrophilic properties, they not present adequate water vapor permeability (Kuznesof, 2005). It has been reported that incorporation of lipid materials into the films can improve barrier property, due to generation of new chemical bonds (Kester and Fennema, 1985). For their food grade properties, carnauba, bees, and candelilla waxes can be used for application in the food industry (MULTICERAS, 2009) as an important component of coatings and films. Cáceros et al. (2003) suggested that use of natural waxes such as carnauba, bees, and candelilla waxes keeps better quality of mandarins than a conventional wax (polyethylene wax). Furthermore, a mixture of candelilla wax and beeswax has been employed as a moisture barrier on Persian limes (Bosquez-Molina et al., 2003). In this study, these coatings were characterized by having poor water vapor permeability performance; however, if candelilla wax is mixed with mesquite gum and mineral oil, it could reduce the natural decay rate of Persian limes.

Wax-based coatings also can serve as a carrier for antimicrobial and/or antioxidant compounds, in order to maintain high concentrations of preservatives on the food surface. Polymeric and monomeric phenolic compounds as tannins and caffeic, ferulic, and *p*-cumaric acids have been related with growth inhibition of some microorganisms such as *Clostridium botulinum*, *Bacillus cereus*, *Escherichia coli*, *Lysteria monocytogenes*, *Staphylococcus aureus* and *Vibrio parahaemolyticus,* *Aspergillus flavus*, *A. parasiticus*, *A. versicolor*, *A. ochraceus,* and *Penicillium urticae* (Beuchat, 2001). Puupponen-Pimiä et al. (2001) demonstrated that myricetin (a kind of flavonoid), derived from the flora of human gastrointestinal tract, inhibited the growth of lactic acid bacteria. Edible films based on candelilla wax and ellagic acid have been used to reduce successfully the negative effects caused by *Collecotrichum gloeosporoides* on "Hass" avocado and therefore its appearance and quality were significantly improved (Saucedo-Pompa et al., 2009). In addition, antioxidants are used in combination with process technologies such as heat treatments, modified and controlled atmospheres, edible coatings and films, gamma radiations and electric pulses, in order to avoid the browning of fruits.

Saucedo-Pompa et al. (2007) evaluated the addition of gallic and ellagic acids on candelilla wax-based films with the purpose of assay the effect of addition of natural antioxidants on shelf life quality of fresh-cut fruits (avocado, banana, and apple). They reported that formulations with those bioactive compounds reduce color changes. These findings are consistent with those reported by Ponce et al. (2008), who evidenced the great advantage of using chitosan coatings enriched with high-antioxidant activity oleoresins on prevention of browning reactions, which typically result in quality loss in fruits and vegetables.

FINAL COMMENTS

Based on what is mentioned above, SSF is an interesting and promising bioprocess for production and extraction of phenolic compounds, which have important applications in foods. In this paper, the most recent findings in the field of phenolic compounds production using agro-industrial byproducts are presented. Also, it includes the use of natural waxes as main component of edible coatings and films and it described how they act as carrier of antioxidants to improve quality of some food products. Although the use of edible coatings and films cannot replace the use of traditional packaging materials, it is necessary to take in account their functional characteristics and the possible advantages in certain applications.

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