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Preparation and characterization of improved gelatin films incorporating hemp and sage oils

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ABSTRACT

The aim of the study is to prepare and characterize gelatin films incorporating hemp and sage oil, to obtain an edible film which combines both food preservation and nutritional properties. Gelatin film-forming solutions were investigated in terms of wettability in relation to different foodstuff and their antimicrobial properties against *Escherichia coli*, *Staphylococcus aureus, Listeria innocua*, *Saccharomyces cerevisiae* and *Penicillium expansum*. The gelatin films were evaluated for their water vapor permeability, solubility in food simulants, moisture content, thickness and light barrier property. The steeper decrease in contact angles, surface tension and spreading coefficients was displayed by the sage oil-gelatin film solution and the mildest by the hemp oil-gelatin film solution. The most suitable foodstuffs to be coated in sage/hemp oil-gelatin films seem to be Golden Apple, pork meat and processed cheese, whereas the least suitable appear to be garlic, red bell pepper and cauliflower. Hemp oil has moderate antimicrobial action, while the sage oil displays a strong inhibition. Additive effect is expressed in case of all gelatin film solutions containing sage and hemp oils combinations. The sage oil-gelatin film displays the lowest water vapor permeability and moisture content, while the hemp oil-gelatin film results in the lowest thickness and highest light barrier property. Hemp oil-gelatin film is more soluble than the sage oil-gelatin film. Varying degrees in the investigated parameters can be noticed in cases of oils mixtures-gelatin films depending by the dominant oil.

1. Introduction

Maintaining the security along the entire food chain associated with the consumers' demands for fresh food which meets nutritional, sensorial and microbiological standards are the driving force of the studies done in the last decade in the food area. By extending the food shelf-life, the packaging contributes in protection of human health, as well as to food waste reduction. The new trend in food packaging research deals with design of edible films made from natural polymers whose recycling results in reducing the carbon foot-print associated to packaging production. Among the natural polymers, gelatin has attracted a great attention due to its versatility allowing its application in food industry, cosmetics, pharmacy and photography. Extracted from collagen in mammal, chicken or fish skins and bones the gelatin is made of triads of α-aminoacids with glycine at every third position and triads of hydroxyproline, proline and glycine (Fraga & Williams, 1985). Advantages and disadvantages of gelatin regarding the use in edible films have been shown. The suitable mechanical properties, low costs, efficient barrier properties against lipids, film forming capacity (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011), biodegradability and easy removal from the food surface strongly recommends development of gelatin use in edible films. Its high water binding capacity resulting in film swelling or dissolving during contact with aqueous foodstuff, as well as the poor moisture barrier properties (Gómez-Guillén et al., 2011) are the main impediments. By inclusion of bioactive compounds in the gelatin network the aforementioned impairments could be overcome and moreover, new protective and functional valences could be added.

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The inclusion of olive oil in gelatin matrix (Ma et al., 2012) gives it excellent light and moisture barrier properties whereas firmer gelatin film could be formed by casein addition (Pang, Deeth, Sopade, Sharma, & Bansal, 2014). By including the antibacterial compounds the properties of gelatin films are enhanced. Extensive studies reported inhibitory action of gelatin films against a wide range of food spoilage microorganisms following the addition of ethanol-propolis extract and/or essential oils, respectively (Bodini, Sobral, Favaro-Trindade, & Carvalho, 2013; Gomez-Estaca, Montero, Fernandez-Martin, Aleman, & Gomez-Guillen, 2009; Iturriaga, Olabarrieta, & Martínez de Marañón, 2012).

From a wide range of essential oils, sage oil (SO) has attracted a particular interest due to its strong inhibitory action against a broad spectrum of foodborn pathogens attributed to the presence of high concentration of monoterpenes (Sepahvand et al., 2014). The results of Kelen and Tepe (2008) indicate that *Escherichia coli* Hak 59, *Streptococcus pneumoniae* IK3, *Bacillus cereus* RK75, *Clostridium perfringens* Kukens-Turkey, *Candida albicans* A 117, *Candida krusei* ATCC 6258, *Acinetobacter lwoffii* ATCC 19002 and *Klebsiella pneumoniae* A 137 are extremely sensitive to the action of oils extracted from *Salvia aramiensis* and *Salvia aucheri* var. *aucheri*, respectively. The low values of minimum inhibitory concentration (MIC) reported by the Sepahvand et al. (2014) after testing the essential oil of the aerial parts of *Salvia sclareoides* against Gram-negative bacteria (15.6 μg/mL for both *Proteus vulgaris* ATTC 8427 and *K. pneumoniae* ATTC 500706) and Gram−positive bacteria (31.25 μg/mL for *Listeria monocytogenes* ATTC 1298 and 15.6 μg/mL for *Staphylococcus aureus* ATCC 25923) demonstrate its strong antibactericidal action. A broad antimicrobial spectrum against *S. epidermidis*, *B. cereus* and *B. subtilis* (Gram-positive bacteria) of whose MIC were 12.5 μg/mL and 25 μg/mL for both bacillus, respectively was also displayed by the *Salvia lanigera* essential oil (Tenore et al., 2011). Furthermore, the same oil was effective against some Gram-negative bacteria namely *E. coli*, *Salmonella typhi Ty2* and *Klebsiella pneumonia* (MIC = 50 μg/mL for all species), yeasts (*C. albicans*, 50 μg/mL) and molds (*Botrytis cinerea*, MIC = 50 μg/mL). The antioxidant activity of sage essential oil and extracts has been also demonstrated. Sepahvand et al. (2014) investigated the antioxidant capacity of oil from *S. sclareoides* using DPPH radical scavenging method and found that it is higher than BHT, a well known synthetic antioxidant. The results of Tepe, Daferera, Sokmen, Sokmen, and Polissiou (2005) place the antioxidant activity of methanol-water extract of *Salvia tomentosa* almost to the same level of BHT. Kivrak et al. (2009) reported that ethanol extract of *Salvia potentillifolia* (Turkey) has a DPPH radical scavenging activity almost at the same level of BHT while the methanol extracts of *Salvia runcinata*, *Salvia repens* and *Salvia stenophylla* (Africa) displays antioxidant activity in the range of 1/2 to 1/5 of C vitamin (Kamatou et al., 2005).

The benefic impact on human health of hemp oil (HO) is world wide recognized. Due to a well-balanced proportion of essential fatty acids which could not be synthesized by the human body, namely linoleic acid (C18:2; n-6) and α-linolenic acid (C18:3; n-3) in the ratio of between 2:1 and 3:1 (Teh & Birch, 2013), the various benefic properties are associated to hemp seeds oil. Rezapour-Firouzi et al. (2014) reported an improvement of the extended disability status score and activity of liver enzymes in multiple sclerosis patients who received the co-supplemented hemp seed oil and evening primrose oil (9/1 ratio). Linolenic acid in hemp oil exerts anti-inflammatory, antihypertension, anti-vasoconstrictive, anti-cancer and anti-thrombotic actions, reduces the low-density lipoprotein (LDL), increases metabolic rates and fat burning (Erasmus, 1999) and together with other polyunsaturated fatty acids also presented in hemp oil are able to heal wounds and increase immunity (Harbige, Layward, Morris-Downes, Dumonde, & Amor, 2000). Moreover, a recent study demonstrated the antimicrobial properties of hemp oil. The work of Nissen et al. (2010) screened the inhibitory activity of oils extracted from three legal hemp varieties (Carmagnola, Fibranova and Futura) against seven microorganisms belonging to Gram-positive bacteria, seven microorganisms belonging to Gram-negative bacteria and 8 yeasts. The results show that the hemp oils can significantly inhibit the microbial growth at different levels depending on the variety and sowing time. Among all studied varieties, the oil coming from Futura variety inhibited all Gram (+) and Gram (−) bacteria.

Due to their abundance in biologically active compounds, sage and hemp oils are promising natural alternatives that may extend the shelf-life, microbiological safety and nutritional values of food. Taking into account the benefits of each oil their inclusion into gelatin film could result an edible film with high added-value providing both food protection and nutritional properties. Although many papers in the field of edible films were published, according to the best of our knowledge there are only a few concerning the influence of oils mixtures and none related to inclusion of hemp oil. In this line, the aim of the paper is to discuss some properties of gelatin films incorporated with sage and hemp oils, pure or in mixtures, in terms of food wettability, water vapor permeability, solubility, thickness, antimicrobial and optical properties.

2. Materials and methods

2.1. Bioactive compounds

Cold pressed hemp seed (*Cannabis sativa* L.) oil (boiling point of 185 °C) and sage (*Salvia officinalis*) oil (boiling point of 210 °C), purchased from Canah International SRL-Romania and Elemental Company-Romania, respectively were incorporated alone or as mixtures in the gelatin film. The chemical compositions of oils are provided in their technical specifications (Elemental-Salvie ulei esential pur, 2014; Organic-Hempseed-Oil, 2014).

2.2. Formulations and preparation of the film-forming solutions

Previous experiments developed by varying the ratios of ingredients resulted in selection of five recipes that lead to films with desirable physical and chemical properties (brittleness, wettability, film integrity, mechanical properties, light transparency, antioxidant activity, water vapor permeability, antimicrobial efficiency). Gelatin film free of oil was used as control. Film-forming solutions were prepared by dissolving 4 g of bovine hide gelatin of type B (Bloom 150, Sigma−Aldrich Chemical Co., USA) per 100 mL of distilled water at 80 °C and stirring (1200 rpm) for 30 min. A volume of 0.6 mL glycerol (99% purity, Sigma−Aldrich Chemical Co., USA) was added as a plasticizer at 45 °C followed by stirring for another 15 min. The oils, previously mixed for 5 min at 1200 rpm in soy lecithin (analytical grade) as surfactant (HLB = 4.0, Sigma−Aldrich Chemical Co., USA) were added when the film-forming solution has reached the temperature of 35 °C in the proportions indicated in Table 1, followed by 10 min of stirring at 1200 rpm.

2.3. Characterization of the film-forming solutions

2.3.1. Contact angle (θ), liquid vapor interfacial force (γ_{LV}), surface tension (γ_{SL}), critical surface tension (γ_C) and wettability measurements

The measurements were performed on the outer surface of vegetal and animal foodstuffs (Table 2), purchased from a local supermarket (Baia Mare − Romania). Some of them were selected on the basis of their potential to be film-coated due to their high perishability (e.g. meat, cheese, apple, tomato). Others, whose