Antioxidant activity and physical properties of hydroxypropylmethylcellulose films enriched with essential oils

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Summary

In this study, the antioxidant activity and physical properties, such as water vapour permeability (*WVP*), oxygen permeability (*OP*), mechanical and optical properties of hydroxypropylmethylcellulose (HPMC) films containing essential oils of clove buds, sage and oregano were evaluated. Clove essential oil showed higher antioxidant activity in 2,2-diphenyl-1-picryl hydrazine (DPPH) radical-scavenging assay (IC_{50} = 2.71 $\mu g \cdot ml^{-1}$) and in a reduction power assay (IC_{50} = 53.54 $\mu g \cdot ml^{-1}$) but, in β-carotene-bleaching test, butylated hydroxytoluene (BHT) showed higher (IC_{50} = 62.86 $\mu g \cdot ml^{-1}$) antioxidant activity. The results showed that addition of essential oils into HPMC films led to a significant decrease in *WVP* (2.30–1.49 $ng \cdot Pa^{-1}m^{-1}s^{-1}$), *OP* (488.78–235.95 $ml \cdot \mu m \cdot kPa^{-1}m^{-2}d^{-1}$), colour differences (0–38.91), tensile strength (27.35–7.21 MPa), elongation at break (23.6–17.9%) and elastic modulus (537.54–202.12 MPa). Also, soybean oil without antioxidant was packed by HPMC films and some soybean oil samples after addition of the same essential oils and packed in glass bottles stored at accelerated conditions. The HPMC films combined with essential oils showed a moderate effect against soybean oil oxidation. In comparison between the films, HPMC film combined with clove essential oil was the most effective protectant against oxidation (43.3%).

Keywords

antioxidant packaging; edible film; essential oil; hydroxypropylmethylcellulose

Nowadays, consumers are more interested in using the natural-based ingredients and materials such as essential oils and edible films instead of synthetic antioxidants and non-biodegradable packaging materials [1]. The quality of most foods depends on the oxidation reactions of lipids. Essential oils as antioxidant agents are able to reduce lipid oxidation [2]. Essential oils of many herbs and spices have been found to have antioxidant activity, as essential oils of clove buds, sage and oregano are known as natural antioxidant agents [3, 4]. These antioxidant activities are largely dependent on the content and composition of phenolic compunds [2].

Edible films can be classified into three categories: hydrocolloids (polysaccharides and proteins), lipids (triglycerides and waxes) and composites films [5]. Edible films have unique properties such as biodegradability, and a potential to act as carriers of food additives (such as vitamins, colours, antioxidant and antimicrobial agents). Moreover, combination of edible films and essential oils can improve the storage quality of food products. For instance, chitosan coatings enriched with cinnamon oil improved the quality of refrigerated rainbow trout fish and led to a significant decrease ($p < 0.05$) in lipid oxidation [6]. Almond oil protected with hydroxypropylmethylcellulose (HPMC) film combined with ginger essential oil was shown to be very well preserved against lipid oxidation during 64 days of storage [7].

Cellulose derivatives have been shown, because of their excellent mechanical properties and film formation abilities, as very suitable materials to form edible films. HPMC, as a non-ionic hydrocolloid, showed excellent properties such as transparency, no taste, no odour, oil-resistance, high tensile strength (*TS*), as well as oxygen and aroma

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barrier properties. Based on this, HPMC is established as suitable for edible film formation purposes [8, 9]. However, HPMC films, due to their hydrophilic nature, have poor water vapour permeability (*WVP*). To overcome this defect, HPMC can be combined with hydrophobic materials such as essential oils, lipids, waxes, surfactants and resins [1, 10–12].

Despite many studies carried out on HPMC-based films, there is still only limited information available on essential oils incorporated into HPMC film for packaging applications. No information had been published on clove, sage and oregano essential oils HPMC edible films. Therefore, the objective of this work was to study:

- the antioxidant activity of clove, sage and oregano essential oils by using different methods (2,2-diphenyl-1-picryl hydrazine (DPPH) radical scavenging, β-carotene bleaching (*BCB*) and reducing power (*RP*));
- the effects of essential oils on HPMC film properties (*WVP*, *OP*, mechanical and optical properties);
- the effects of HPMC film combined with essential oils, as an active packaging, on the oxidation rate of soybean oil stored at accelerated conditions.

MATERIALS AND METHODS

Materials

Hydroxypropylmethylcellulose (HPMC; Sigma–Aldrich, Saint Louis, Missouri, USA), Tween 80 (Panreac Quimica, Barcelona, Spain) and 2,2-diphenyl-1-picryl hydrazine (DPPH) from Sigma Chemical (Sigma–Aldrich) were used. Methanol, acetic acid, sodium iodide, sodium thiosulphate, chloroform, butylated hydroxytoluene (BHT) and glycerol (purity > 97%) were obtained from Merck (Darmstadt, Germany). Soybean oil containing no antioxidant was purchased from Naz Vegetable Oil Refinery (Isfahan, Iran). Dried clove buds (*E. caryophyllata* Thunb.), were purchased from Isfahan Pakan Bazr (Isfahan, Iran), dried aerial parts of oregano (*Oringanum vulgare* L.) and sage (*Salvia officinalis* L.) were obtained from Shahrekord area (Chaharmahal and Bakhtiari Province, Iran).

Extraction of essential oils

According to the method explained by the Ozcan and Arslan [13], 100 g of dried and ground aerial parts of sage, oregano and clove buds were placed separately in a 600 ml round-bottom flask and hydro-distilled for 3 h using an essential oil distillation apparatus (Clevenger-type). The essential oils were dried over anhydrous sodium sulphate and stored in dark bottles at 4 °C until use.

Determination of antioxidant properties of the essential oils in vitro

Radical-scavenging activity using DPPH

Radical-scavenging activity (*RSA*) was measured by the •DPPH test as described previously [4] with some modifications. Briefly, 3 ml of methanolic solutions of essential oils at different concentrations (100, 200, 400, 600, 800 and 1 000 $\mu g \cdot ml^{-1}$) and BHT (100 $\mu g \cdot ml^{-1}$, 200 $\mu g \cdot ml^{-1}$) as a positive control were added to 1 ml of a freshly prepared 90 $\mu mol \cdot l^{-1}$ DPPH solution. The mixtures were incubated in the dark for 60 min at room temperature and then their absorbance was recorded at 517 nm (M350 Double Beam UV-Visible Spectrophotometer, Comspec, Leeds, United Kingdom). The percentage of •DPPH radical inhibition was calculated according to the following equation:

$$RSA = \frac{(A_B - A_S)}{A_B} \times 100 \quad (1)$$

where A_B represents the absorbance of the blank and A_S is the absorbance of the samples containing antioxidant. Blank sample contained all reagents except the antioxidant.

β-Carotene/linoleic acid bleaching assay

The *BCB* assays were carried out according to the method described by Kulisic et al. [2] with some modifications. One milligram of β-carotene was dissolved in 10 ml of chloroform. Four millilitres of this solution were added into a boiling flask containing 400 mg of Tween 80 and 40 mg of linoleic acid. Chloroform was completely evaporated at 50 °C by a rotary evaporator. Then, 100 ml of oxygenated distilled water was added to the residue at vigorous shaking to form a clear yellowish emulsion. Five millilitres of this emulsion were added into test tubes containing 0.2 ml of methanolic solutions of essential oils at different concentrations (100, 200, 400, 600, 800 and 1 000 $\mu g \cdot ml^{-1}$) and BHT (100 $\mu g \cdot ml^{-1}$, 200 $\mu g \cdot ml^{-1}$) as positive control and mixed thoroughly. The test tubes were incubated in a water bath at 50 °C for 180 min together with a negative control (blank) and the absorbance was measured at 470 nm every 30 min. Antioxidant activity (*AA*) was calculated according to the following equation:

$$AA = \left[1 - \frac{(A_{S0} - A_{St})}{(A_{C0} - A_{Ct})}\right] \times 100 \quad (2)$$

where As_0 and As_t were the absorbance values of test samples for 0 min and 180 min, respectively. Ac_0 and Ac_t were the absorbance values of blank at 0 min and 180 min.

Reducing power

Reducing power (*RP*) of essential oils was determined according to Oyaizu [14] with small modifications. One milliliter of methanolic solution of essential oils at different concentrations (100, 200, 400, 600, 800 and 1000 $\mu g \cdot ml^{-1}$) and BHT (100 $\mu g \cdot ml^{-1}$, 200 $\mu g \cdot ml^{-1}$) as positive control were added to 2.5 ml phosphate buffer (0.2 $mol \cdot l^{-1}$, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min. Then, 2.5 ml trichloroacetic acid (10%) was added to the mixture and it was centrifuged at 1500 $\times g$ for 10 min. Subsequently, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%). The absorbance was measured at 700 nm after allowing the solution to stand for 30 min at room temperature. The results of antioxidant activity of essential oils were reported as IC_{50} (the sample concentration that provides 50% inhibition; lower IC_{50} value indicated better protective ability of the essential oils).

Film preparation

Film solutions were prepared according to Atarés et al. [7] with minor modifications. HPMC powder (10 g) was dispersed in 200 ml deionized water at 80 °C, and stirred overnight. An amount of 5 g of glycerol as plasticizer, and 0.5 g of Tween 80 as emulsifier, were added to film solutions under vigorous stirring for 30 min. Then, 2 g of clove, oregano and sage essential oils were added separately (the ratio of HPMC : glycerol : Tween 80 : essential oil was 1 : 0.5 : 0.05 : 0.2) and homogenization was carried out using a vacuum high-shear probe mixer (IKA-T25 digital ultra-Turrax; IKA, Janke & Kunkel, Staufen, Germany) at 230 Hz for 10 min. After mixing, the dispersions were degassed at room temperature in a vacuum oven (Heraeus, Hanau, Germany). From here onwards, the four formulations will be referred to as H (HPMC without antioxidant), HC (HPMC containing clove essential oil), HO (HPMC containing oregano essential oil) and HS (HPMC containing sage essential oil). After degassing, films were made by pouring 200 ml of dispersions in the lids of glass boxes (20 cm × 17.6 cm) and then incubated for 48 h in an oven (at approx. 25 °C) under 40% relative humidity (*RH*) to dry on a leveled surface. About 56.82 $g \cdot m^{-2}$ of essential oils was present in each film.

Characterization of the films

Thickness

Thickness of the films was determined using a digital micrometer (Electronic digital micrometer, DC-516, sensitivity 0.001 mm; Lutron, Philadelphia, Pennsylvania, USA).

Water vapour permeability

Water vapour permeability (*WVP*) was measured according to ASTM E96–80 Standard test method with some modifications [15, 16]. Circular cups with an average diameter of 2.5 cm and a depth of 8.5 cm were sealed by the test films. In each of the circular cups, 10 ml deionized water was poured to achieve 100% *RH*. The shiny side of the films was exposed to the inside circular cups with highest *RH*. Circular cups were placed into a desiccator containing silicagel to control the outside *RH* above the film (0% *RH*) and it was kept at 25 °C. This difference in *RH* corresponds to a driving force of 3179 Pa, expressed as water vapour partial pressure. The cups were weighed (every 6 h for a week) until a steady state was reached. *WVP* was calculated by the following equation:

$$WVP = \frac{S \times L}{\Delta P \times A \times 3\,600} \tag{3}$$

where *WVP* is expressed as grams per Pascal per metre and per second, *S* is slope determined on the basis of the weight loss vs time plot of the cups by linear regression (in grams per hour), *A* is the area of the film (in square metres), Δp is the vapour pressure differential across the film (in pascals) and *L* is the film thickness (in metres).

Oxygen permeability

Oxygen permeability (*OP*) was measured at 25 °C and 50% ± 5% *RH* after the samples had been equilibrated for a minimum period of 48 h. A volumetric permeability cell (Custom Scientific Instruments, Trenton, New Jersey, USA) was used to measure oxygen transmission rates through the films, according to the ASTM standard method D1434 [17]. *OP* was calculated from the following equation:

$$OP = \frac{Q \times L}{(P_1 - P_2) \times A} \tag{4}$$

where *Q* is gas flow through the films (in millilitres per day), *L* is thickness of the film (in millimetres), P_1 and P_2 are the differences between the oxygen partial pressures for two sides of the films (in kilopascals) and *A* is the film surface area exposed (in square metres).

Optical properties

Optical properties were determined on the shiny side of films pre-equilibrated at 50% ± 5% *RH* and 25 °C using colorimetric RGB system (Colour Analyzer Probe Meter, Model RGB-1002; Lutron). The value obtained with easyRGB software (Mathworks, Natick, Massachusetts, USA) was converted to *CIEL*a*b** scale, which is one of the most common methods for determining optical properties for films. The parameter L^* is the lightness and indicates the tendency to black or white, the parameter a^* is the value of variation from green to blue, and the parameter b^* is the variation from yellow to red [8]. Colour differences (ΔC) were calculated from the following equation:

$$\Delta C = \sqrt{(\Delta L)^2 - (\Delta a)^2 - (\Delta b)^2} \quad (5)$$

where

$$\Delta L = L_H - L_S \quad (6)$$

$$\Delta a = a_H - a_S \quad (7)$$

$$\Delta b = b_H - b_S \quad (8)$$

where the H index indicates the values observed for HPMC film without antioxidant as a reference and S index indicates the HPMC films containing essential oils (samples).

Mechanical behaviour

The film samples were cut at the size 25 mm × 100 mm and conditioned at 50% ± 5% *RH*, 25 °C for 48 h before the measurement. Mechanical properties of the film samples were measured using a Texture Analyzer TA-XT-plus (Stable Micro Systems, STM-20; Godalming, United Kingdom), with a 50 N load cell equipped with tensile grips (A/TG model) and films were stretched using a speed of 50 mm·min^{-1} [1]. Maximum tensile strength (*TS*), elongation at break (*EAB*) and elastic modulus (*EM*) were determined according to ASTM standard method D882 [18].

Packaging of soybean oil

Cylindrical packages of HPMC films (height 7.57 cm × diameter 3 cm) were made to pack and store the soybean oil samples without any type of antioxidant. The outer edges of the film were stuck together with clamp and the inner edges were covered by dispersion of the same film materials and exposed to air to get dried. Soybean oil was chosen because it is produced in a greater amount than any other oils in the world. Also, it is widely used for food purposes (cooking and salad oils, spreads and shortenings, mayonnaise and salad dressings. So, this is the product that might be packed by edible films in the future and thus it was selected as the product.

Preparation of soybean oil samples

In this study, two samples were prepared for storage of soybean oil and observation of the effect of HPMC films enriched with essential oils as an active packaging, as well as to study the direct effect of same essential oils on the rate of soybean oil oxidation.

- After providing cylindrical packages made from HPMC films (with and without essential oils), 50 g soybean oil without antioxidant was infused into the packages and then a circular slice of the same film was put on the surface of soybean oil and stored at 40 °C for 60 days in an oven [7].
- Essential oils at different concentrations (200, 400, 600, 800 and 1 000 mg·l^{-1}) and BHT (100 mg·l^{-1}, 200 mg·l^{-1}) as a positive control were added directly to 50 g of soybean oil in dark bottles (without HPMC film) and mixed by a homogenizer (IKA-T25 digital ultra-Turrax) for 15 min at a speed of 80 Hz. A control sample was prepared under the same conditions but without any antioxidants. The samples were stored for 30 days at 60 °C in an oven [13].

Soybean oil oxidation throughout storage

To determine the effect of HPMC films containing essential oils on oxidation of soybean oil in comparison with same soybean oil added different concentrations of essential oils and BHT (as positive control) but packed in glass bottles similar to HPMC packs, peroxide value (*PV*) was measured by an AOCS method and was expressed as milliequivalents of O_2 per kilogram of oil [19]. Inhibition of oil oxidation during the storage period was expressed as oxidation inhibition ability (*OIA*):

$$OIA = 100 - \left[\frac{(PV_{S0} - PV_{St})}{(PV_{C0} - PV_{Ct})} \times 100\right] \quad (9)$$

where PV_{S0} and PV_{St} are PV of the samples at the beginning and the end of storage time, respectively. PV_{C0} and PV_{Ct} are PV of control at the beginning and the end of storage time, respectively.

Statistical analysis

Analysis of variance (ANOVA) and least significant difference (*LSD*) test using Statistical Analysis System (SAS 9) software (SAS Institute, Cary, North Carolina, USA) were used to calculate significant differences ($p < 0.05$). Each experiment was repeated three times and data

were reported as mean ± standard deviation. Five random positions were considered for determination of colour parameters and thickness of films. In antioxidant activity assays, IC_{50} values were obtained from in vitro analyses by regression from inhibition percentage vs concentration graphs.

RESULTS AND DISCUSSION

DPPH radical scavenging

The effect of essential oils on •DPPH radical scavenging is thought to be related to the hydrogen donation ability of essential oils to free radicals of •DPPH [2]. Tab. 1 shows IC_{50} values of clove, oregano and sage essential oils, as well as of BHT, as determined by DPPH method. The results indicated that all essential oils had significant ($p < 0.05$) scavenging abilities. Essential oil of clove had the strongest effects on •DPPH radical-scavenging activity, which were significantly ($p < 0.05$) higher than essential oils of oregano and sage. Viuda-Martos et al. [3] demonstrated that clove essential oils had a high phenolics content (898.89 mg of gallic acid equivalent per litre) and that the main compounds in clove essential oil were eugenol (85.5%) and β-caryophyllene (10.5%). Also, the phenolic compounds content could be used as an important indicator of the antioxidant capacity, and these compounds acted as antioxidants. The antioxidant activity decreased in the order: clove > BHT > oregano > sage. In numerous investigations, it was generally concluded that clove essential oil is a rich source of various types of antioxidants when compared to BHT, and clove essential oil shows varying degree of antioxidant activity in different model systems. Furthermore, for the assessment of the antioxidant activity of clove essential oil, different assays should be used. Viuda-Martos et al. [3] reported that •DPPH radical-scavenging of clove essential oil, ascorbic acid, BHT, thyme, oregano, sage and rosemary essential oils at 5 $g \cdot l^{-1}$ was 97.9%, 96.6%, 95.9%, 62.9%, 51.8%, 51.2% and 47.5%, respectively. Politeo et al. [20] studied the antioxidant activity of twelve herbal plant essential oils and observed that the clove essential oil (5 $g \cdot l^{-1}$) had highest (93%) •DPPH radical-scavenging activity, followed by basil (85%), laurel (68%), coriander (30%), nutmeg (24%), black pepper (14%), everlast (11%), marjoram (9%), mint (8%), cinnamon (6%), sage (5%) and fennel (2%).

β-Carotene bleaching

BCB method is usually used to estimate antioxidant abilities of the antioxidants to inhibit oxidation of lipids [2]. The IC_{50} values for BCB of clove, oregano and sage essential oils are shown in Tab. 1. The antioxidant activities decreased in the order: BHT > clove > oregano > sage. The results showed that IC_{50} value for essential oil of clove (75.64 $\mu g \cdot ml^{-1}$) was significantly ($p < 0.05$) lower than those of oregano (373.12 $\mu g \cdot ml^{-1}$) and sage (545.25 $\mu g \cdot ml^{-1}$). Kulisic et al. [2] demonstrated that the antioxidant ability of some standard compounds and oregano essential oil in β-carotene assay decreased in the order: BHT > α-tocopherol > oregano essential oil > ascorbic acid. Sahin et al. [21] showed that both methanolic extract and oregano essential oil were unable to effectively inhibit linoleic acid oxidation. Only 24% and 36% inhibitions were achieved at 2 $mg \cdot ml^{-1}$ concentrations, respectively, which were far lower than BHT (89%) at the same concentration.

Reducing power

Tab. 1 shows the IC_{50} values for *RP* assay of essential oils of clove, oregano and sage as well as BHT, which reflect the potential of reducing Fe^{3+} to Fe^{2+}. *RP* decreased in the order: clove > BHT > oregano > sage. Interestingly, again in this method, clove essential oil had a higher power than other essential oils and BHT ($p < 0.05$).

Tab. 1. Antioxidant activity of different essential oils and BHT in model systems in vitro.

	IC_{50} [$\mu g \cdot ml^{-1}$]		
Sample	DPPH method	BCB method	*RP* method
Clove essential oil	2.71 ± 0.05 d	75.64 ± 3.88 c	53.54 ± 1.03 d
Oregano essential oil	834.30 ± 5.32 b	373.12 ± 2.45 b	888.86 ± 1.7 b
Sage essential oil	907.74 ± 5.26 a	545.25 ± 5.83 a	1362.42 ± 1.42 a
BHT	12.89 ± 1.72 c	62.86 ± 1.62 d	61.56 ± 0.87 c

IC_{50} – the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for RP.
DPPH method – •DPPH radical-scavenging method, BCB method – β-carotene-bleaching method, *RP* method – reducing power method, BHT – butylated hydroxytoluene.
Means followed by different letters in the same column are significantly different (LSD test, $p < 0.05$).

Tab. 2. Thickness, water vapour permeability, oxygen permeability, elongation at break, tensile strength and elastic modulus of the films.

Film	H	HC	HO	HS
Thickness [μm]	135.44 ± 2.44^{a}	130.04 ± 2.14^{b}	115.18 ± 3.16^{d}	122.62 ± 1.46^{c}
WVP [ng·Pa^{-1}m^{-1}s^{-1}]	2.30 ± 3.02^{a}	1.77 ± 6.09^{b}	1.61 ± 4.72^{c}	1.49 ± 8.79^{d}
OP [ml·μm·kPa^{-1}·m^{-2}·d^{-1}]	488.78 ± 20.46^{a}	393.03 ± 14.48^{b}	235.95 ± 28.63^{d}	287.42 ± 24.16^{c}
Elongation at break [%]	23.6 ± 3.8^{a}	17.9 ± 2.4^{b}	19.8 ± 2.3^{b}	20.8 ± 2.1^{ab}
Tensile strength [MPa]	27.35 ± 4.57^{a}	12.34 ± 3.35^{b}	7.21 ± 2.64^{c}	9.25 ± 3.38^{bc}
Elastic modulus [MPa]	537.54 ± 86.41^{a}	478.96 ± 67.48^{a}	202.12 ± 52.15^{b}	255.56 ± 74.21^{b}

Means followed by different letters in the same row are significantly different (LSD test, $p < 0.05$).
H – hydroxypropylmethylcellulose (HPMC) without antioxidant, HC – HPMC containing clove essential oil, HO – HPMC containing oregano essential oil, HS – HPMC containing sage essential oil, *WVP* – water vapour permeability, *OP* – oxygen permeability.

Viuda-Martos et al. [3] reported that clove essential oil showed the highest ferric reducing capacity in terms of Trolox equivalents, followed by oregano, sage and rosemary essential oils. The strong activity of clove essential oil can be due to the presence of eugenol, which is known to have antioxidant activity. Politeo et al. [20] demonstrated that the clove essential oil had the highest reducing power (88 mmol·l^{-1}), which was followed by basil (7 mmol·l^{-1}), laurel (2 mmol·l^{-1}), black pepper (1 mmol·l^{-1}) essential oils. Essential oils of sage, coriander, nutmeg, everlast, marjoram, mint, cinnamon and fennel showed the weakest capacity (< 1 mmol·l^{-1}). Gulcin et al. [22] showed that the reducing power of clove essential oil and standard antioxidants (at 15–45 μg·ml^{-1}) were in the following order: clove essential oil > BHA ≈ BHT > α-tocopherol > trolox.

Characterization of HPMC films

Several edible films of HPMC (H, HC, HO and HS) were prepared. Data on average thickness of the films, measured to determine the properties of the films, are shown in Tab. 2. Thickness of the composite films is highly dependent on the concentration of dry matter, film preparation method and composition and type of additives (e.g. essential oil, glycerol and Tween 80) in the edible films. Likewise, addition of glycerol as plasticizer could reduce the intermolecular forces and increase the flexibility of HPMC chains, *WVP* and thickness [8, 23].

Water vapour permeability

The efficiency of the film with or without essential oils as a water barrier was estimated by determination of *WVP*. Data on *WVP* of the films at 40% *RH* and 25 °C are presented in Tab. 2. The results indicated the highest and lowest *WVP* values for H and HS films, respectively. In general, addition of essential oils to films led to improvement of *WVP* and a significant decrease was observed ($p < 0.05$). This behaviour was expected, as an increase in the contents of hydrophobic compounds, such as essential oils, usually leads to improvement of water barrier properties of films [24]. However, results of a previous study showed that addition of essential oils into bioactive films resulted in an increase in *WVP*, which was ascribed to discontinuities in the polymer network caused by lipid droplets [25]. Therefore, influence of essential oils on *WVP* of some of edible films can be different. HC showed the highest *WVP* among films incorporated with essential oils (HO and HS). This could be due to low solubility of clove essential oil in water and to a lower content of hydrophobic compounds in comparison to sage and oregano essential oils. On the other hand, *WVP* values were higher than reported by other authors for HPMC films [7, 24]. These differences are linked to the use of high amounts of glycerol as a plasticizer to increase *WVP*. Glycerol is an effective plasticizer that becomes less dense with a larger free volume in the matrix of films. It has a high capacity to interact with water and facilitates its high mobility, solubilization and permeability through whole films [23]. Atarés et al. [7] studied *WVP* of HPMC films with incorporated ascorbic acid, citric acid and ginger essential oil, at 20 °C and 53–75% *RH*, and reported that *WVP* was significantly reduced in particular by ginger essential oil. Also, they showed that *WVP* was greatly dependent on the environment conditions like *RH* gradient and temperature. Sánchez-González et al. [24] reported that, with an increase in contents of tea tree, bergamot and lemon essential oils, *WVP* was significantly reduced.

Oxygen permeability

Tab. 2 shows *OP* values of HPMC-based films

Tab. 3. Colour parameters of the films.

Film	*L**	*a**	*b**	Δ*C*
H	95.47 ± 0.2[a]	0.45 ± 0.06[a]	–1.3 ± 0.19[c]	0
HC	93.39 ± 0.23[b]	0.43 ± 0.07[a]	–0.35 ± 0.04[b]	5.24 ± 1.59[c]
HO	89.34 ± 0.1[d]	0.20 ± 0.08[b]	–0.15 ± 0.09[a]	38.91 ± 2.15[a]
HS	90.3 ± 0.22[c]	0.21 ± 0.04[b]	–0.20 ± 0.04[ab]	27.98 ± 1.43[b]

Δ*C* – colour differences, H – hydroxypropylmethylcellulose (HPMC) without antioxidant, HC – HPMC containing clove essential oil, HO – HPMC containing oregano essential oil, HS – HPMC containing sage essential oil.
Means followed by different letters in the same column are significantly different (LSD test, $p < 0.05$).

with and without essential oils. The results indicated that essential oils had a significant effect on reduction of *OP* in the HPMC films ($p < 0.05$), H showing the highest *OP*. The rate of *OP* decreased for the films containing oregano, sage and clove essential oils, respectively. Generally, *OP* was increased by addition of plasticizers [23, 26] but, in this study, blending essential oils with glycerol led to a decreased *OP*, which was even lower than the control film. This could be explained by filling the pores in films by smaller molecules formed by hydrolysis [27]. De Moura et al. [28] reported that free hydroxyl groups of natural red colour, via hydrogen bonding with HPMC matrix, make more stable and compact structure of the film matrix. According to some previous studies, in which essential oils were added to the films, *OP* of the films decreased and this might have been related to the lipophilic nature of essential oils [29, 30].

Optical properties

The colour parameters of the films are presented in Tab. 3. The results demonstrate that the film formulation had a significant effect ($p < 0.05$). In this respect, addition of essential oils showed more significant effect on optical properties of the films than other ingredients. In general, significant differences between those parameters (*L**, *a** and *b**) were observed to be associated with the nature of essential oils, and were significantly ($p < 0.05$) lower in films containing essential oils. The composite films were more opaque than control HPMC films. HC showed the highest lightness in comparison to HO and HS. This may be due to the whiter colour of clove essential oil. Colour difference (Δ*C*) was calculated to summarize the changes in *L**, *a** and *b** values. HC showed the lowest Δ*C*, as compared with H and HO, which had the highest Δ*C*. This was due to a lower decrease in *L** and *a** values, and a lower increase in *b** values for HC. These results are similar to those obtained by Sánchez-González et al. [24], who also observed that the addition of essential oils to the HPMC and chitosan matrix led to a significant decrease in gloss properties and transparency of the composite films.

Mechanical behaviour

Tab. 2 presents data on *EAB, TS* and *EM* of HPMC films with different essential oils equilibrated at 25 °C and 40% *RH*. In general, addition of essential oils to the films led to a significant decrease ($p < 0.05$) in the mechanical properties in most of films, which can be attributed to the presence of structural discontinuities. Between the films incorporated with essential oils, HO induced a greater decrease in *TS* and *EM* values of the films. As shown in Tab. 2, the highest *EAB*, *EM* and *TS* were observed in films with no additives (H film). They were significantly ($p < 0.05$) reduced when essential oils were added to the films, although HS and HC had no significant difference ($p > 0.05$) for *EAB* and *EM*, or showed little variations. Sánchez- González et al. [1] reported that the addition of tea tree essential oil caused a significant decrease in *TS* and *EM* of HPMC films, although had no significant effect on *EAB*. Hosseini et al. [25] reported that the addition of oregano essential oil caused a significant decrease in *TS* and *EM* of fish gelatin–chitosan composite films, although no significant effect was observed regarding *EAB*. Atarés et al. [7] declared that this phenomenon could be due to weakening of the polymer chain aggregation associated to the oil presence and the liquid state of essential oils at room temperature, as well as plasticity of essential oil droplets due to stretching of the structure. This coincides with the results reported by other authors for addition of essential oils to HPMC matrix [24, 29]. However, it was observed that the nature of essential oils had a slight effect on mechanical behaviour of HPMC films [31].

Protective ability against soybean oil oxidation

OIA values of HPMC films, determined by *PV*, are presented in Fig. 1, in comparison with the same soybean oil with different concentrations of essential oils and BHT, packed in glass bottles si-

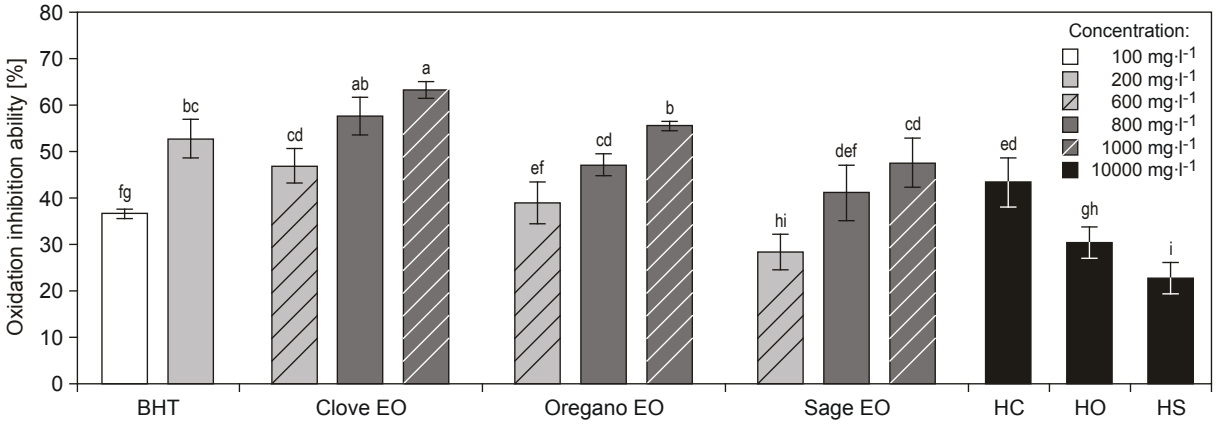

Fig. 1. Oxidation inhibition ability of HPMC films enriched with clove, oregano and sage essential oils, and at direct addition of these essential oils in soybean oil.

Columns marked by different letters are significantly different (LSD test, $p < 0.05$).
BHT – butylated hydroxytoluene, EO – essential oil, HC – hydroxypropylmethylcellulose (HPMC) containing clove essential oil, HO – HPMC containing oregano essential oil, HS – HPMC containing sage essential oil.

milar to HPMC packs. Because of the low effect of 200 mg·l^{-1} and 400 mg·l^{-1} concentrations of essential oils on *OIA*, those data are not shown in Fig. 1. *PV* in all of the samples progressively increased during the storage period. It was 0.56 meq·kg^{-1} and 85 meq·kg^{-1} for the control (soybean oil without antioxidant) in the beginning and end of the storage period, respectively. In order to compare the antioxidant behaviour of the composite films (containing essential oils) and directed adding of essential oils in soybean oil at different concentrations, a value of 85 meq·kg^{-1} (for reference samples) was chosen as the base of comparison of the different conditions in respect to oxidation patterns during the incubation period.

As shown in Fig. 1, *OIA* of the samples of soybean oil containing essential oils packed in glass bottles and composite films was below 63.3% (this value was determined for clove essential oil at 1000 mg·l^{-1} direct addition to soybean oil). However, *OIA* values of the soybean oil packed in composite films were significantly ($p < 0.05$) lower than those for samples packed in glass bottles with high concentrations of essential oils. No significant difference was observed ($p > 0.05$) comparing the rate of oxidation in the samples containing BHT at 100 mg·l^{-1} concentration, packed in glass bottles, with samples packed in HO film, but HC film showed a higher *OIA*. However, samples packed in HO and HS films showed a larger extent of oxidation reactions than the samples containing BHT at 200 mg·l^{-1} in glass bottles. The lowest extent of oxidation was determined for the samples containing 800 mg·l^{-1} and 1000 mg·l^{-1} clove essential oil packed in glass bottles. Tab. 4 shows the *PV*

Tab. 4. Peroxide values of soybean oil samples packed with or without HPMC films during storage.

Film or EO	EO concentration [mg·l^{-1}]	Peroxide value [meq·kg^{-1}]
H film	0	85.89 ± 4.41^{a}
HC film	10000	48.67 ± 3.05^{gfh}
HO film	10000	59.78 ± 4.02^{e}
HS film	10000	66.33 ± 1.60^{d}
BHT	100	53.24 ± 2.76^{f}
	200	39.83 ± 1.98^{gih}
Clove EO	200	60.28 ± 3.56^{jk}
	400	52.23 ± 2.75^{f}
	600	44.66 ± 2.06^{gih}
	800	35.72 ± 2.26^{jk}
	1000	31.08 ± 2.00^{k}
Oregano EO	200	79.01 ± 1.02^{b}
	400	72.33 ± 2.52^{c}
	600	51.34 ± 4.34^{f}
	800	44.49 ± 2.41^{ih}
	1000	37.55 ± 2.28^{j}
Sage EO	200	80.80 ± 2.89^{b}
	400	76.67 ± 2.08^{cb}
	600	60.13 ± 4.27^{e}
	800	49.52 ± 4.77^{gf}
	1000	44.17 ± 2.77^{ih}

Peroxide value is expressed as milliequivalents of O_2 per kilogram of oil (values represent mean ± standard deviation). Means followed by different letters in the same column are significantly different (LSD test, $p < 0.05$).
EO – essential oil, H – hydroxypropylmethylcellulose (HPMC) without antioxidant, HC – HPMC containing clove essential oil, HO – HPMC containing oregano essential oil, HS – HPMC containing sage essential oil, BHT – butylated hydroxytoluene.

of soybean oil samples packed with different film formulations, and soybean oil samples containing different concentrations of essential oils that were not packed with HPMC film, at the end of storage. After 30 days, the samples with highest concentrations of the essential oil showed the highest antioxidant activity (Tab. 4). During the storage time, the oxidation rate of the samples containing 800 $mg \cdot l^{-1}$ and 600 $mg \cdot l^{-1}$ clove essential oils were comparable with a concentration of 200 $mg \cdot l^{-1}$ of BHT, and the samples containing 400 $mg \cdot l^{-1}$ clove essential oil were comparable to BHT at 100 $mg \cdot l^{-1}$ (not significantly different, $p > 0.05$). Ozcan and Arslan [13] evaluated the effect of some essential oils on the stability of hazelnut and poppy oils and reported that cinnamon oil was the most effective in relation to lipid oxidation of crude oils, followed by clove and rosemary essential oils. Clove essential oil is more effective than essential oils of oregano and sage, which is mainly due to a higher eugenol concentration in clove essential oil [13]. It was been reported that *OIA* of sage essential oil mainly depends on the contained phenolic compounds, such as β-thujone, 1,8-cineole and camphor [32], whereas in oregano essential oil, it is related to high contents of phenolic monoterpenes, such as thymol and carvacrol [2]. Viuda-Martos et al. [3] found that clove essential oils had higher phenolic concentration than both oregano and sage essential oils. In the present study, *OIA* of oregano and sage essential oils that were directly added to the soybean oil were better in comparison to BHT at the end of storage. Tab. 4 shows that the protection provided by HC was more effective (48.67 $meq \cdot kg^{-1}$) than those provided by other films, but its effect was weaker, at the end of storage, than if the same essential oils were added directly to the soybean oil at high concentrations (800 $mg \cdot l^{-1}$ and 1000 $mg \cdot l^{-1}$). HS in comparison with the two other films (HC and HO) showed the lowest activity (66.33 $meq \cdot kg^{-1}$) but, comparing to the soybean oils containing oregano and sage essential oils, it had an acceptable effect, even at lower concentrations (200 $mg \cdot l^{-1}$ and 400 $mg \cdot l^{-1}$). HC demonstrated a higher *OIA* (44.1%) than the two other films (HO and HS) (Fig. 1). This might be due to the facilitated oxygen transport in the matrix and the weakened oxygen barrier properties of the films [7]. Also, the highest concentration and strongest phenolic compounds in clove essential oil resulted in high *OIA* in HC film. Phoopuritham et al. [33] reported that *PV* of soybean oil packaged in cellulose-based pouches containing some essential oils increased linearly with the storage time, while clove and butylated hydroxyanisole showed a similar behaviour ($p > 0.05$) and an acceptable activity after eight weeks of storage. Finally, previous studies showed that, in comparison to some essential oils, *OIA* of clove essential oil during food heating processes had a better response in higher-temperature than in lower-temperature conditions [34].

CONCLUSION

The results obtained by different methods of evaluating the antioxidant activity showed that clove, oregano and sage essential oils may be considered as good sources of natural antioxidants. The results obtained by *PV* determination during the storage period showed that essential oils from clove, oregano and sage can stabilize soybean oil. Adding essential oil to HPMC films effectively protected soybean oil against oxidation. The protective effect of films was dependent on both the presence of active antioxidant compounds in essential oils and the structure of the films. On the other hand, *OP* and *WVP* were reduced by adding the essential oils. Films containing clove essential oils showed the highest *OIA*, *OP* and *WVP*, whereas this high activity was exclusively related to the chemical structure of the phenolic compounds as an active antioxidant agents in clove essential oil. Also, inclusion of essential oils in HPMC led to a significant decrease in the *EAB*, *TS*, *EM*, lightness and transparency of the films. Likewise, the type of the essential oil played a more important role in films properties. Nevertheless, more research is still needed to investigate the effect of bioactive films with incorporated essential oils on microbial growth, flavour retention, methods of film formation and disintegration in time, to improve film properties and the potential applications.

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