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ORIGINAL ARTICLE

Fabrication of Cassava Starch/*Mentha piperita* Essential Oil Biodegradable Film with Enhanced Antibacterial Properties

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KEYWORDS

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ABSTRACT: In this study, an edible film is prepared using *Mentha piperita* essential oil (MPEO) plasticized by sorbitol and glycerol in cassava starch matrix by the solution intercalation process. The effects of MPEO addition on the color, and antimicrobial activity (*Staphylococcus aureus* and *Escherichia coli*), as well as the barrier properties of active films were investigated. MPEO was incorporated into cassava starch biodegradable films at different level (1-3%, w/w). A low level of MPEO can obviously increased the water vapor permeability (WVP) of cassava starch/MPEO films. When the MPEO contents varied from 0-3% w/w, the lightness (L^*value) decreased from 95.63 to 89.17 while the a* value increased from 0.15 to 0.82. The cassava film showed antimicrobial properties against *S. aureus* and *E.coli* by using agar diffusion method. These findings showed that *Mentha piperita* essential oil has a good potential to be added to cassava to make antimicrobial coating or film for food and non-food packaging.

INTRODUCTION

In novel science, plastics have become one of the most widely used substances in different technologies. Plastic applications are important components in automobiles, home appliances, computer equipments, medical uses and packages [1]. In the UK, approximately 4.7 million tons of plastic is produced by the plastic industry every year. Synthetic plastics are not compatible with environment due to their non-renewable properties [2]. Biopolymers (natural polymers) are components that can be fabricated from different living organisms such as starch, cellulose, sugar, or other synthetic materials [3]. In the past decade, biopolymers were utilized to have limited applicability in food and packaging industry. Compared to the usual laminates, films, labels, and wraps based on fossil fuel resources, natural polymers seem to be a fine component from an environmental perspective [4]. Currently, various European countries such as Greece, Italy, and Finland are encouraging their universities and research centers to utilize these biopolymers for the development of biopackaging in food and non-food industries, e.g. several companies such as NatureWorks LLC and Imperial Chemical Industry (ICI) are focusing on the study of these bio-based polymeric materials. Packaging substances represent different features in barrier against water vapor, oxygen and carbon dioxide, the development and

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fabricated of modified atmosphere package (MAP) in section headspace, extended shelf life, and loss of food additives such as flavor, antioxidants, color, and antimicrobial agents [5]. The biofilm and edible coating material efficiency is mainly attributed to its capability against environmental damage, while protection food quality [6].

Biodegradable films based on polysaccharide are fabricated from pectin starch, cellulose, carrageenan, and alginates. Polysaccharide films show fine permeability properties against gases, resulting in extended shelf life of the end product [7] . Packaging based on starch like other natural polymers, when compared to synthetic plastic, starch films show several disadvantages, such as poor mechanical properties and their hydrophilic nature. When biopackagings are combined with plasticizer and other material such as essential oil, antioxidant compounds and nano-additive the resulting in antioxidant and nanobiocomposites packaging, it represents significant improvements in transparency of films and mechanical properties [8].

The result of different works showed that the addition of lipids [9], nanoparticles [10 - 13] and essential oil [14] could improve the properties of films.

Mentha piperita L., (Peppermint) belonging to the Lamiaceae family is commercially cultivated in the temperate regions particularly in Europe, North Africa, and North America, currently cultivated all over the world. In the past decade, Plants of *Mentha piperita* L., have been grown in Iceland and by ancient Egyptians [15]. Peppermint is a rich resource of pharmacologically active compounds such as menthyl acetate (3–10%), menthone (20–31%), menthol (29–48%), menthofuran (6.8%), polymerized polyphenols (19%) flavonoids, caffeic acid (12%), carotenes, betaine, tocopherols, tannins, and choline [16, 17]. Moreover, peppermint oil and its ingredients are commercially used in cosmetics, pharmaceutical and food industries [15].

Current research studied the antibacterial activities, barrier properties, and color of cassava film incorporated with various amounts of MPE.

The results demonstrate its potential as an active coating or novel formulation in biodegradable films for packaging.

MATERIALS AND METHODS

Materials

Cassava starch powder was obtained from SIM Company (Malaysia), and sorbitol and glycerol were of analytical grade from Liang Traco (Malaysia). *Mentha piperita* essential oil was purchased from Giah Essence Phitopharm Company (Iran).

Film preparation

Cassava starch and cassava starch-based essential oil films were fabricated using a solution intercalation method. Approximately 4g of cassava starch [18, 19] was dispersed in 100 mL of distilled water while stirring vigorously at 87°C for 45 min and then cooled to 37°C. Appropriate concentrations of *M. piperita* essential oil were added to the dispersion, to reach a final concentration of 1%, 2%, 3% wt, based on native starch, and added to starch dispersion. As the starch biofilms were brittle, 1.6g of liquid glycerol and sorbitol (1:3) [20] was added to the native starch suspension. The dispersions were poured onto a casting dish (16×16 cm) and then allowed to dry for 72h at room temperature. The cassava films were peeled from the casting dish and conditioned at 24°C and 55% RH. The pure biofilms based on cassava were similarly manufactured but without addition of essential oil.

Characterization of cassava starch/M. piperita essential oil films

Color evaluation

Color evaluation was conducted with a Minolta (Model CM-3500D; Osaka, Japan) on edible films using the CIELAB color indicators, L*(lightness/darkness), a*(redness/greenness), and b*(yellowness/blueness). A zero-transmittance calibration plate CM-A100 was used to calibrate the colorimeter and air was employed as full transmittance.

Antimicrobial effects

Two typical food pathogens such as Gram-positive bacteria, *S. aureus* ATCC 25923 and Gram-negative bacteria, *E. coli* ATCC 10536 were used to assay the antimicrobial activity of biodegradable films using an agar diffusion method [21]. Biodegradable Films were cut into disc pieces and placed in plates containing Mueller Hinton agar (Merck, Germany) with 1 cc of inoculums about 10^5–10^6 CFU/cc of S. *aureus* (gr positive) and *E. coli* (gr negative). Antibacterial effect of the treatment and control edible film were measured by computing inhibition zone against the microorganisms on *S. aureus* (gr+) and *E. coli* (gr-) on Mueller Hinton agar.

Water vapor permeability (WVP)

The WVP of the pure and films containing MPEO was studied according to the modified method [22, 23] of ASTM standard E96-05. Film pieces were mounted on glass cups filled with deionized water up to 1.5 cm underneath the film. The glass weighing cups were placed in a desiccator with silica gel. The glass cups were weighed every 120 min for a period of 14 h. The measured WVP of the films was determined using the following equation

$$WVP = WVTR \times L / \Delta P \times A$$

Where: WVTR is the water vapor transmission rate (g), L is the average film thickness (m), ΔP is the partial water vapor pressure variance (Pa) through two sides of the film, and A is the area of the cup (m^2). Three replicates of each film were tested.

Statistical analysis

The determination of color factors, antimicrobial properties, and WVP were evaluated with individually fabricated biofilms in triplicate. One-way ANOVA was used to compare the data. The significance of test mean was measured ($p< 0.05$) with the Tukey's post hoc test by SPSS software.

RESULTS AND DISCUSSION

Appearance of active films

Cassava starch film samples prepared without and with MPEO easily separated from the casting dish. As essential oil amount increased, active film samples become less transparent but with a smooth surface without pores.

Film color

The Colorimetric parameters changes of the active film sample incorporated with MPEO are presented in Table 1. The L* parameter varied from 89.17 to 95.63. Incorporation of MPEO markedly affected biofilm lightness, as seen in the reduced L* parameter at increased concentrations of essential oil. A statistical significant difference ($p < 0.05$) in L* value was markedly presented after 2% of MPEO incorporation.

Decreased lightness occurred in alginate film treated with different amounts of garlic oil [24].

The results of this research, results represented that an increase in essential oil level leads to increased *a** value. Furthermore, *a** parameter was observed to increase from 0.15 to 0.82. The color tended to redness, as showed by the increase of *a** parameter. The *b** value varied from 1.21 to 3.09. As shown in Table 1, incorporation of MPEO affected the *b** parameter of the cassava starch films. The *b** parameter tended to increase as less levels of MPEO were added.

Ghasemlou, et al. [25] reported that corn starch containing *M. pulegium* oil exhibited increased *b** value and decreased *a** value when compared to control sample. They pointed out that this was due to the increase in diffuse reflectance provoked by light scattering in the lipid droplet (*M. pulegium* oil) lowers both the film's lightness and the light scattering intensity. These results indicated that addition of the MPEO changed the color of biofilm samples from white to yellowish.

These findings is consistent with the results of another study [26] where alginate film sample became more gloomy with the addition of oregano essential oils.

Table 1 Colorimetric parameters of cassava/MPE films.

MPE Concentration (%)	L^*	a^*	b^*
0.0	95.63 ± 0.31a	0.15 ± 0.03d	1.21 ± 0.11c
1	95.3 ± 0.28a	0.26 ± 0.03c	1.29 ± 0.1c
2	93.87 ± 0.34b	0.46 ± 0.00b	2.02 ± 0.44b
3	89.17 ± 0.22c	0.82 ± 0.08a	3.09 ± 0.22a

Reported values are mean ± standard deviation. Different letters in colorimetric parameters showed significant difference at $P<0.05$

Antibacterial activity

The findings of the antimicrobial test of active film containing MPEO against two selected bacteria are shown in Fig. 1. Incorporation of MPE at 1 w/w% started to show a clear inhibition zone represented by the absence of *S. aureus* and *E. coli* growth around the film discs. Results revealed that antibacterial properties of edible film samples increased by higher essential oil content. Moreover, *S. aureus* was indicated to be more resistant to MPEO-incorporated film sample as compared to *E. coli*. Results suggested that the ingredient of MPEO could act as antibacterial compound in the cassava starch film sample and inhibiting target microorganisms.

The antimicrobial activity of κ-carrageenan film containing *M. pulegium* oil [27] has been reported against bacteria pathogens, such as *S. aureus* and *E. coli*. Moreover, the inhibitory effect of thymol and carvacrol has been found in S. *aureus* on the surface of cheese [28].

Ghasemlou, et al. [25] reported that the antimicrobial activity of corn starch films containing *M. pulegium* oil and *Z. multiflora Boiss* oil are attributed to their main compounds such as phenolic monoterpenes.

In another study, Mahboubi and Haghi [29] showed the presence and role of piperitenone, terpineol, pulegone and piperitone as the main antimicrobial constituents of *M. pulegium* oil.

Some components found in *M. pulegium*, such as phenolic monoterpenes [25] piperitenone, terpineol, pulegone, and piperitone [29] are known to have inhibitory effect against microorganisms.

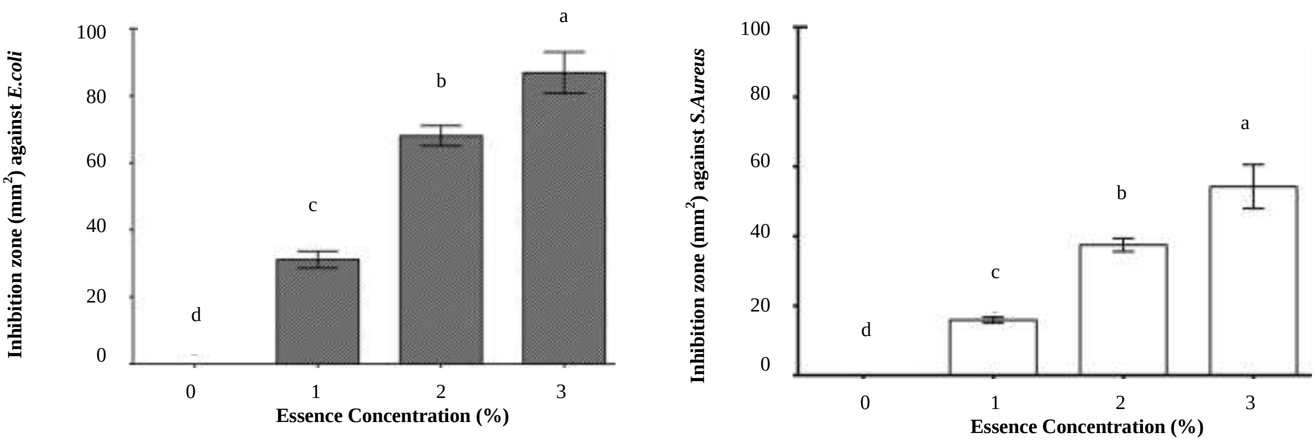

Figure 1. Effects of MPE concentrations on antimicrobial activity of cassava films. Antibacterial activities of different amounts of MPE incorporated in cassava based films against (a) *E.coli* (b) *S.aureus.*

Water vapor permeability

Table 2 shows the WVP of biodegradable film with the incorporation of various amounts of MPEO. As expected, the neat film had the lowest WVP amongst the films and the WVP of the biodegradable films gradually increased with the addition of MPEO. It was related to the addition of MPEO to natural polymer and regular structure of the biopolymer chains destruction. WVP of the biodegradable sample films ranged from 2.9 to $5.9\times10^{11} gm^{-1}s^{-1}Pa^{-1}$, and there was a statistically significant difference among the active films at amounts of 1 and 2 w/w%.

Addition of garlic oil into alginate film increased moisture content at different concentration in the biodegradable film [24]. Studies recently stated that the incorporation of thyme and clove essential oils to chitosan film resulted in WVP increase [30].

The increased in WVP of edible films containing garlic oil is attributed to the hydrophobic characteristics and extended intermolecular interactions [24].

Increases in WVP of film containing *Z. multiflora Boiss* oil were previously reported for carboxymethyl cellulose film. It was related to addition of *Z. multiflora Boiss* oil microdroplets to CMC film which reduced the film cohesion [31].

Table 2. WVP of cassava/MPE films

MPE Concentration (%)	**WVP×10^{11} [gm^{-1}s^{-1}Pa^{-1}]**
0.0	2.9±0.04d
1	3.6±0.25c
2.0	3.6±0.15b
3	5.8±0.01a

Reported values are mean ± standard deviation. Different letters in WVP showed significant difference at P<0.05

CONCLUSIONS

The characterization of films based on cassava was affected by the incorporation of MPEO. The results indicated that cassava starch/MPEO film had antimicrobial activity on the two bacteria used in this study. *M. piperita* essential oil significantly decreased L* value, while increasing a* and b* values of the biofilms. The barrier properties of the control film samples were significantly reduced by addition of MPEO.

Results indicated that cassava film containing MPEO has a good potential to be applied as an active film that controls Microbial population.

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Conflict of interests

The authors declare that they have no conflict of interest

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