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Structural, morphological and thermal behaviour characterisations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants

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ABSTRACT

Structural, morphological and thermal properties of fish skin gelatin films incorporated with basil and citronella essential oils at a ratio of 1:1 (w/w), as influenced by different surfactants (Tween-20, Tween-80 and soy lecithin at 25% based on essential oil), were characterised. Smoother surface and more homogeneous oil distribution was observed via SEM in films containing both basil and citronella essential oils when soy lecithin was used as surfactant. Essential oil containing gelatin films exhibited bi-layer morphology when Tween-80 was used as surfactant. FTIR results suggested the decreased protein–protein interaction in the matrix of gelatin film when essential oils were incorporated, depending on type of surfactants used. Films added with both essential oils had lower glass-transition and degradation temperatures than the control films, indicating a poorer protein–protein interaction in film network. Therefore, both essential oils and surfactants had the impact on molecular structure and thermal properties of resulting films.

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1. Introduction

Proteins are biopolymers capable of forming the film and their properties can be varied with many factors, e.g. level of plasticiser, amount and type of protein (Krochta, 1997). Gelatins from several sources have been widely used as film forming materials (Arvanitoyannis, 2002; Hoque, Benjakul, & Prodpran, 2011b; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2008; Tongnuanchan, Benjakul, & Prodpran, 2012). Gelatin film properties were determined by gelatin source and film making process (Limpisophon, Tanaka, & Osako, 2010). Gelatin films have excellent barrier properties against gas, volatile compounds and UV light (Hoque et al., 2011b; Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006). However, water vapour barrier property of gelatin films was poorer than that of other biopolymer films, due to the hydrophilic nature of gelatin and hydrophilic plasticiser required for film preparation (Jongjareonrak et al., 2006; Limpisophon et al., 2010; Tongnuanchan et al., 2012). Among plasticisers used, glycerol is a plasticiser of choice widely used to incorporate into gelatin film because it is well miscible with the gelatin molecule, providing excellent plasticising effect on gelatin film (Arvanitoyannis, 2002; Bergo & Sobral, 2007; Krochta, 1997). Moreover, glycerol is a major by-product, which generated by the production of biodiesel. Using glycerol as plasticiser for biodegradable film production is a potential way for increasing value of this low-grade by-product (Arrieta, Peltzer, Garrigós, & Jiménez, 2013; Ye, Xiu, Shahbazi, & Zhu, 2012).

The incorporation of non-polar or hydrophobic substances, such as oils, fats and fatty acids, are commonly used to improve the water vapour barrier property of hydrophilic biopolymer films (Limpisophon et al., 2010; Prodpran, Benjakul, & Artharn, 2007). Fat and lipids of different types have been successfully incorporated into protein-based films by different means including coating, lamination or multilayer and dispersion or emulsion to form composite films (Atarés, De Jesús, Talens, & Chiralt, 2010b; Bahram et al., 2013; Guerrero, Nur Hanani, Kerry, & de la Caba, 2011; Krochta, 2002).

Essential oils are natural volatile complex compounds formed as the secondary metabolites in plants (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Essential oils have been largely employed because of their antibacterial, antifungal, antiviral and antioxidant

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properties (Burt, 2004; Kordali et al., 2005). Therefore, the incorporation of essential oils into the films could provide antioxidant activity for resulting films. Atarés, De Jesús, et al. (2010b) studied the mechanical properties of soy protein isolate incorporated with cinnamon and ginger essential oil at different concentration (protein to oil mass ratios: 1:0.025, 1:0.050, 1:0.075 and 1:0.100). A slight decreasing trend of elastic modulus was observed as the oil content increased. Protein-based film incorporated with essential oil showed the increased water vapour barrier properties (Pires et al., 2011; Tongnuanchan et al., 2012) and possessed antioxidant and antimicrobial activities (Emiroğlu, YemişŸ, Coşkun, & Candoğan, 2010; Oussalah, Caillet, Salmieri, Saucier, & Lacroix, 2004; Seydim & Sarikus, 2006; Tongnuanchan et al., 2012). Seydim and Sarikus (2006) evaluated antimicrobial activity of whey protein isolate-based edible films incorporated with oregano essential oil and found that oregano essential oil added films exhibited larger inhibitory zone on *Staphylococcus aureus* with increasing levels of essential oil added. Recently, Tongnuanchan, Benjakul, and Prodpran (2013a) reported that gelatin film containing basil essential oil exhibited the highest DPPH radical- and ABTS radical-scavenging activities, followed by film containing citronella essential oil, while no differences in those activities were observed between films added with lemongrass and kaffir lime essential oils. Therefore, leaf essential oils such as basil and citronella essential oils can serve as hydrophobic substances with antioxidant activity and to be used to improve the property of biopolymer film as an active packaging. Furthermore, the amount of essential oils incorporated in the film directly affected properties and antioxidative activity of film. The lowest water vapour permeability and the highest radical-scavenging activity were observed in gelatin film when root essential oils were incorporated at a gelatin/essential oil ratio of 1:1 (w/w) (Tongnuanchan et al., 2013a). However, the disruption of protein–protein interaction in the film matrix arose from droplets of essential oils, thereby causing roughness or discontinuity of film matrix and decreasing strength of film (Atarés, Bonilla, & Chiralt, 2010a; Tongnuanchan et al., 2012). The increased content of essential oil droplets could enhance creaming and phase separation. Essential oils with low density, especially at high content were separated and localised at the upper surface of film (Tongnuanchan, Benjakul, & Prodpran, 2013b).

Therefore, the preparation of emulsion films generally requires proper surfactant in order to provoke the stable state of two-phase emulsion system with homogeneity of oil droplet distribution. Different surfactants incorporated would contribute to film structure or morphology (oil droplet distribution) and film properties differently. Prodpran et al. (2007) prepared fish muscle protein-based film containing palm oil, in which Tween-20 was used as surfactant. Peng and Li (2014) also used Tween-20 to prepared chitosan-based film incorporated with lemon, thyme and cinnamon essential oils. Tween-80 has been reported to prepared soluble soybean polysaccharide film added with essential oils (Salarbashi et al., 2013), whey protein concentrate film containing cinnamon essential oil (Bahram et al., 2013), cassava starch blend film (Brandelero, Yamashita, & Grossmann, 2010). Tanaka, Ishizaki, Suzuki, and Takai (2001) prepared fish protein–lipid emulsion films using lecithin as surfactant. Furthermore, Limpisophon et al. (2010) reported the use of sucrose stearate as surfactant for preparation of gelatin film incorporated with stearic and oleic acids. More recently, the physico-chemical properties of gelatin/essential oil films as affected by different surfactants used were reported (Tongnuanchan et al., 2012). However, little information regarding the effect of different surfactants on fish gelatin–essential oil emulsion film, particularly on its microstructures, molecular characteristics and thermal properties has been reported. Thus, the present study was undertaken to investigate morphological, molecular and thermal properties of emulsion films based on fish gelatin incorporated with basil and citronella essential oils in the presence of different surfactants used.

2. Materials and method

2.1. Chemicals

Glycerol and soy lecithin (HLB = 4.0) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tween-20 (HLB = 16.7) and Tween-80 (HLB = 15.0) were obtained from Merck (Darmstadt, Germany). All chemicals are of analytical grade.

2.2. Fish gelatin and essential oils

Fish gelatin produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). Essential oils from the leaves of basil (*Ocimum basilicum*) and citronella (*Cymbopogon nardus*) were purchased from *Botanicessence* (Bangkok, Thailand).

2.3. Preparation of film from fish gelatin incorporated with different essential oils and surfactants

Gelatin powder was mixed with distilled water to obtain the protein concentration of 3.5% (w/v). The mixture was heated at 70 °C for 30 min. Glycerol at 30% (w/w) of protein content was used as a plasticiser. Prior to the addition into solution, essential oils were mixed with various surfactants (Tween-20, Tween-80, soy lecithin) at 25% (w/w, based on essential oil). Subsequently, the prepared essential oils were added into the solution at gelatin/essential oil ratio of 1:1 (w/w). The suspension was homogenised at 22,000 rpm for 3 min using a homogeniser (IKA Labortechnik homogeniser, Selangor, Malaysia). The dissolved air in the suspension was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

To prepare the films, the suspension (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air-blown for 12 h at room condition (27 ± 2 °C and 75 ± 10% relative humidity (RH)). The films were further dried at 25 °C and 50 ± 5% RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subjected to analyses. Control films were prepared from solution containing gelatin and glycerol without essential oils and surfactants.

2.4. Characterisation of fish gelatin–essential oils emulsion film

Prior to analyses, films were conditioned in desiccators containing dried silica gel for 2 weeks and 1 week in desiccators containing P_2O_5 at room temperature (25–30 °C) to obtain the most dehydrated films.

2.4.1. Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were visualised using a scanning electron microscope (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section, samples were fractured under liquid nitrogen prior to visualisation. Then, the samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, West Chester, PA, USA) in order to make the sample conductive. The photographs were taken at an acceleration voltage of 15 kV.

2.4.2. Differential scanning calorimetry

Thermal properties of films were determined using a differential scanning calorimeter (PerkinElmer, Model DSC-7, Norwalk, CT,

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