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Stability of emulsion containing skipjack roe protein hydrolysate modified by oxidised tannic acid

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ABSTRACT

Stability of menhaden oil-in-water emulsion incorporated with skipjack roe protein hydrolysate (SRPH) at different levels (5 and 10%, w/v) was determined when oxidised tannic acid (OTA) at a level of 1% (w/v) was added before and after emulsification. During 14 days of storage, the addition of OTA yielded SRPH-emulsions with larger particle sizes (d_{43} and d_{32}), but less coalescence index (C_i) and flocculation factor (F_f), compared to those without OTA, regardless of OTA incorporation stage. Amongst all samples, emulsion stabilised by 10% SRPH showed the lower coalescence and flocculation, when 1% OTA was added after emulsification ($p < 0.05$). The stability of SRPH-emulsion added with OTA after emulsification as a function of OTA concentrations (0–2%, w/w) was assessed. When OTA concentrations increased, d_{43} and d_{32}, C_i and F_f decreased but ζ-potential value increased. Smaller droplets with less coalescence were obtained with increasing OTA concentrations. OTA slightly induced cross-linking of peptides, particularly those located at the interface. SRPH-emulsion containing OTA inhibited the formation of TBARS in a dose-dependent manner ($p < 0.05$). Therefore, SRPH along with OTA incorporation after emulsification could increase stability of emulsion and enhanced the oxidative stability.

1. Introduction

Marine oil is a rich source of polyunsaturated fatty acids (PUFAs), especially ω-3 and ω-6 fatty acids, which have been claimed for their health benefits (Ramakrishnan, Ferrando, Aceña-Muñoz, Lamo-Castellví, & Güell, 2013; Sahena et al., 2009). Nevertheless, the uses of marine oil in a processed food are limited, owing to its susceptibility to oxidation (Jacobsen, Let, Nielsen, & Meyer, 2008). For emulsion, the appropriate coating surrounding oil droplets in the system is another alternative means to lower oxidation (Dalgleish, 2006). Proteins are extensively used as emulsifiers in food products because they can decrease interfacial tension between oil and aqueous phase and form a continuous viscoelastic membrane-like film around oil droplets (Aewsiri et al., 2009; Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2013). Additionally, protein film surrounding oil droplets can play a role in prevention of lipid oxidation of oil droplets (Aewsiri et al., 2009). Some amino acids in proteins are able to scavenge free radical and chelate prooxidative metals (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2008).

Phenolic compounds from various plants have been reported to prevent lipid oxidation in fish oil-in-water emulsion, corresponding to their antioxidative properties such as radical scavenging, iron chelating, and reducing activities (Salminen, Heinonen, & Decker, 2010; Sekhon-Loodu, Warnakulasuriya, Vasantha Rupasinghe, & Shahidi, 2013). Nevertheless, the phenolic compounds in oxidised form may exert prooxidant in initiating free radical chain reaction (Michalak, 2006). Additionally, the oxidised phenolics can partially loss their reducing power or antioxidative activity (Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2010). However, they act as protein cross-linker (Aewsiri et al., 2013; Balange & Benjakul, 2009). The oxidised phenolic compounds are able to react with nucleophilic groups of several amino acids such as tryptophan, cysteine, methionine, histidine, tyrosine and proline, thereby inducing the cross-linking via those reactive groups (Kroll, Rawel, & Rohn, 2003). Aewsiri et al. (2009) reported that gelatin modified with oxidised tannic acid (OTA) via covalent interaction rendered the emulsion with high stability and could inhibit lipid oxidation of menhaden oil-in-water emulsion effectively throughout the storage of 12 days.

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Proteins from egg yolk including lipovitellin and phosvitin have been demonstrated as the potential emulsifiers (Daimer & Kulozik, 2009). Hydrophobicity of lipovitellin (lipoprotein) is involved in interfacial property, whereas repulsive force caused by phosphate moieties of phosvitin (phosphoprotein) favours the emulsion stability (Samaraweera, Zhang, Lee, & Ahn, 2011). Nevertheless, lipovitellin–phosvitin complex is less soluble and hydrolysis was implemented to increase solubility. Alcalase digestion was applied to skipjack roe in order to recover these proteins and augmented the solubility (Intarasirisawat, Benjakul, & Visessanguan, 2012). Skipjack roe protein hydrolysate having 5% degree of hydrolysis (DH) showed high emulsifying properties accompanied with antioxidative activity. Although high pressure homogenisation at 13.8 MPa could improve the stability of emulsion containing SRPH with 5% DH, the separated phases were also noticeable during extended storage (data not shown). To enhance stability of emulsion containing SRPH, the modification of protein films using phenolic compounds, particularly oxidised form, might increase emulsion stability via introducing the stronger film surrounding oil droplets. The incorporation stage of phenolic compounds into emulsion could also affect in emulsifying property of protein or hydrolysate. Furthermore, phenolic compounds with retained reducing capacity could function as antioxidant in the emulsion system. Those could contribute to enhanced stability. Therefore, the aim of this study was to investigate the characteristics and oxidative stability of menhaden oil-in-water emulsion stabilised by SRPH as influenced by OTA incorporation before and after emulsification.

2. Materials and methods

2.1. Chemicals/oil

Sodium azide (NaN_3) was purchased from Fluka Chemical (Buchs, Switerland). Acridine orange, Nile blue A, tannic acid, menhaden oil and sodium dodecyl sulphate (SDS) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Coomassie brilliant blue R-250 was obtained from Merck (Darmstadt, Germany). All reagents were of analytical grade.

2.2. Preparation of oxidised tannic acid and skipjack roe protein hydrolysate

Oxidised tannic acid (OTA) was prepared according to the method of Aewsiri et al. (2009). Tannic acid (2%, w/v) was dissolved in distilled water, followed by a pH adjustment to 9 using 1 M NaOH. Solution was then bubbled with high purity oxygen (99.5%) (Thai Industrial Gases PCL, Songkhla, Thailand) at 40 °C for 1 h for conversion of tannic acid to OTA with 70.15 ± 2% degree of conversion.

Skipjack roe protein hydrolysate (SRPH) was prepared as per the method of Intarasirisawat et al. (2012) to obtain a degree of hydrolysis (DH) of 5%. Briefly, the defatted roe was suspended in distilled water to obtain protein concentration of 20 mg/mL. The mixture was homogenised and pre-incubated at 50 °C for 20 min. The hydrolysis reaction was initiated by addition of Alcalase. The reaction was conducted at pH 8.0 and 50 °C for 1 h. The enzymatic reaction was terminated by heating at 85 °C for 15 min. DH of the resulting hydrolysate was measured as per the method of Benjakul and Morrissey (1997). The obtained skipjack roe protein hydrolysate (SRPH) with pH 6.98 was lyophylised using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark), placed in polyethylene bag and kept at −20 °C until use. SRPH was dissolved in distilled water and then adjusted to pH 9 using 1 M NaOH.

2.3. Preparation of emulsions

SRPH solutions with the concentrations of 5 and 10% (w/v) were emulsified with menhaden oil (oil volume fraction of 0.1) at a speed of 10,000 rpm for 2 min using a homogeniser (Model T25 basic, IKA LABORTECHNIK, Selangor, Malaysia). The coarse emulsions were then passed through a Microfluidics homogeniser (Model HC-5000, Microfluidizer, Newton, MA, USA) at the pressure of 13.8 MPa with fifteen passes. NaN_3 (0.02%, w/w) was added to the emulsions as an antimicrobial agent. Oil-in-water emulsions referred to as 'SRPH-emulsion' were stored at room temperature (26–28 °C). The samples were taken at day 1, 7 and 14 for analyses except for confocal laser scanning microscopy (CLSM), in which the samples were taken at day 1 and 14 of storage.

2.4. Effect of OTA incorporation on emulsion stability

OTA was added into emulsion containing SRPH (5 and 10%, w/v) to obtain the concentration of 1% (based on SRPH content). The addition of OTA was performed before and after emulsification. The resulting emulsions were termed 'pre-emulsified' and 'post-emulsified', respectively. To prepare pre-emulsified sample, SRPH solutions (pH 9.0) were added with OTA solution (pH 9.0) and the mixtures were continuously stirred using a magnetic stirrer (RO 10 power IKAMAG®, IKA LABORTECHNIK, Selangor, Malaysia) at 200 rpm at room temperature for 12 h. Thereafter, the mixtures were added with menhaden oil. Coarse and fine emulsions were prepared sequentially as described above.

For post-emulsified sample, coarse emulsion containing SRPH without OTA was subjected to high-pressure homogenisation with thirteen passes. Thereafter, OTA solution was added and the mixtures were homogenised for another two passes. The control for both processes was prepared in the same manner except OTA was excluded. The positive controls including emulsions containing Na-caseinate or Tween 20 at levels of 5 and 10% (w/v) were also prepared. The emulsion samples were kept at room temperature and taken for analyses. Emulsion yielding the low C_i and F_f was selected for further study.

2.5. Effect of OTA concentration on emulsion stability

Post-emulsified samples containing OTA at different concentrations (0, 0.1, 0.5, 1 and 2%, based on SRPH content) were prepared. The positive controls including emulsions containing Na-caseinate or Tween 20 at a level of 10% (w/v) in the absence of OTA were also prepared. Emulsions were stored at room temperature and taken for analyses.

2.6. Analyses

2.6.1. Particle size distribution

Particle size distribution of emulsions was determined using a liquid particle size analyser (LPSA) (Model LS 230, Beckman Coulter®, Fullerton, CA, USA) as per the method of Castellani, Belhomme, David-Briand, Guérin-Dubiard, and Anton (2006). Prior to analysis, an aliquot of emulsion (5 mL) was diluted with 1% (w/v) sodium dodecyl sulphate (SDS) solution (20 mL) in order to dissociate flocculated droplets. The surface-weighted mean particle diameter (d_{32}) and the volume-weighted mean particle diameter (d_{43}) of the emulsion droplets were measured.

2.6.2. Flocculation and coalescence

Emulsions were diluted with distilled water in the presence and absence of 1% (w/v) SDS. The flocculation factor (F_f) and coalescence index (Ci) were calculated using the following equations: