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Effects of coconut (*Cocos nucifera* L.) protein hydrolysates obtained from enzymatic hydrolysis on the stability and rheological properties of oil-in-water emulsions

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ABSTRACT

The effect of coconut protein hydrolysates (CPHs), obtained from limited proteolytic hydrolysis of coconut protein concentrate (CPC), on the stability and the rheological properties of oil-in-water (O/W) emulsions was investigated in systems containing 0.2 g protein and 10 mL of virgin olive oil or sunflower oil in 90 mL aqueous phosphate buffer at pH 6.9. The CPC, from by–products of virgin coconut oil processing, was hydrolyzed with Alcalase at various enzyme to protein substrate ratios and hydrolyzation times to yield CPHs with a degree of hydrolysis in the range of 8.25–14.22%. The results showed that the stability and rheological properties of the emulsions depended on the reaction conditions and the degree of hydrolysis (DH). The CPHs with a DH of 8.25–10.17% contained proteins and peptides capable of forming stabilized emulsions, whereas less stable emulsions were obtained with CPHs with a DH of 11.04–14.22%. Additionally, all the O/W emulsions exhibited a shear-thinning behavior. Sunflower O/W emulsions stabilized by CPH were less viscous, showed less shear thinning and were more resistant to flocculation than the olive O/W emulsions. Thus, CPC can be converted into CPHs that had emulsifying activities by proteolytic hydrolysis at DH 8.25–10.17% using Alcalase.

1. Introduction

Emulsions are found in a large number of everyday foodstuffs, cosmetics and pharmacies. In the case of foodstuffs, they usually are in the form of oil–in–water (O/W) emulsions, such as deserts, yogurts, sauces and beverages. Emulsified oils also have important influences on the texture of emulsion food products like yoghurt and mayonnaise (Dickinson, 2012). However, these emulsions are actually thermodynamically unstable since they consist of at least two immiscible fluids that tend to destabilize through flocculation, coalescence and creaming (Damodaran, 2005; McClements, 1999; Wang, Wang, Li, Adhikari & Shi, 2011).

The most commonly used methods to improve the stability of O/W emulsions are to utilize emulsifying agents such as protein, which are surface active compounds that adsorb at the interface between the oil and water, facilitate droplet formation and stabilize

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the emulsion by lowering the surface tension (Dickinson, 1994). A thin layer of the protein also prevents droplet aggregation by generating repulsive forces and/or by forming a physical barrier between droplets (Bouyer, Mekhloufi, Rosilio, Grossiord & Agnely, 2012; Dickinson, 1994; Lam & Nickerson, 2013). In many cases the emulsifying agent used consists proteins that can act as an emulsifier, stabilizer and thickener (Dickinson, 2012; Hoffmann & Reger, 2014), because they reduce the movement of droplets by increasing viscosity or changing viscoelastic properties, such as the viscosity and flow behavior of a continuous phase (Bouyer et al., 2012; Amine, Dreher, Helgason & Tadros, 2014).

Soy protein has generally been applied in a wide range of food formulations due to its widespread availability, low cost and good functional properties (Molina, Papadopoulou & Ledward, 2001; Palazolo, Mitidieri & Wangner, 2003; Shao & Tang, 2014; Wang et al., 2011), and there have been numerous technical studies using soy proteins as emulsifiers (Dickinson & Matsumura, 1991). However, the emulsifying properties of soy protein are mainly utilized as processing aids in concentrated emulsions, such as comminuted meat emulsions, and their use as an emulsifying agent in dilute emulsion products is very limited (Keerati-u-rai & Corredig, 2011; Shao & Tang, 2014). Moreover, many people do not want to eat foods containing soy protein due to its beany flavor, allergies to soy, objections to using genetic modified organisms (which forms an increasing proportion of cultivated soy) and other dietary restrictions. For these reasons, the development of new proteins from alternative low cost sources, such as coconut oil by-products, is a new and interesting alternative.

Previous research on the characterization of the proteins in coconut milk has been aimed at its potential as a value–added product, medical food (Remya, Chikku, Renjith, Arunima & Rajamohan, 2013), emulsifier systems (Karaman, Yilmaz, Dogan, Yetim & Kayacier, 2011; Tangsuphoom & Coupland, 2005; Thaiphanit & Anprung, 2016a) and as a protein nutrient (Ng, Mohammad, Ng & Jahim, 2015). Coconut protein has also been found to have potential in health promotion and disease prevention. It has a large proportion of arginine (Thaiphanit & Anprung, 2016a), a potent anti-diabetic activity (DebMandal & Mandal, 2011; Salil, Nevina & Rajamohan, 2011) and antioxidant activity (Thaiphanit & Anprung, 2013). Moreover, recent studies on the physicochemical and emulsion properties of coconut proteins have shown that they contain a large proportion of non-polar amino acids, 11S globulin (known as cocosin) and 7S globulin (Tangsuphoom & Coupland, 2008a; DebMandal & Mandal, 2011; Chambal, Bergenståhl, & Dejmek, 2012) similar to that of soy protein (Dickinson & Matsumura, 1991; Palazolo et al., 2003). Additionally, coconut protein is believed to govern the emulsion stability of coconut milk (Tangsuphoom & Coupland, 2008a, b). Coconut protein is surface active and it can be used to prepare O/W emulsions using high pressure valve homogenization (Onsaard, Vittayanont, Srigam & McClements, 2005; 2006). Accordingly, coconut protein has been shown to have the potential of acting as an emulsifier. However, it was not able to create emulsions as stable as those made with commercial protein isolates, such as whey protein isolate (Onsaard et al., 2006). A number of strategies have been suggested to improve the functional properties of proteins including chemical, thermal or enzymatic modification (Sinha, Radha, Prakash, & Kaul, 2007; Palazolo, Sobral & Wagner, 2011; Thaiphanit & Anprung, 2016a, b). However, enzymatic hydrolysis may be preferable to chemical treatments because of the milder process conditions and minimal formation of by–products (Yust, Pedroche, Millán-Linares, Alcaide-Hidalgo & Millán, 2010).

In most applications of hydrolases, it is desirable to achieve a complete degradation of the substrate, such as in a saccharification process in a starch industry or a clarification process in a beverage industry (Adler–Nissen, 1982). In contrast, a limited enzymatic hydrolysis has been widely applied to increase the functionality of protein by its modification (Adler–Nissen, 1982; Martinez, Baeza, Millan & Pilosof, 2005; Tsou, Lin, Chao & Chiang, 2012). If the hydrolysis is not limited, it will usually result in a hydrolysate with uninteresting functional and organoleptic properties (Adler–Nissen, 1982). A convenient measure of the course of the limited hydrolysis reaction is the degree of hydrolysis (DH), which is generally defined as the fraction of peptide bonds cleaved and it is often expressed as percentage, Eq. (1):

$$\%DH = (n/n_T) \times 100 \quad (1)$$

where n_T is the total number of moles of peptide bonds present in one mole of protein and n is the number of moles of peptide bonds cleaved per of mole of protein. When molar mass of a protein is not known or the protein sample is a mixture of various proteins, n and n_T are expressed as the number of peptide bonds per gram of protein (Damodaran, 2008).

In general, enzymatic protein modification is highly influenced by DH, enzyme specificity and the intrinsic characteristics of each substrate (Segura-Campos, Espinosa-García, Chel-Guerrero, & Betancur-Ancona, 2012; Soares de Castro, Bagagli, & Sato, 2015). Notably, an extensive DH can produce large amounts of free amino acids and short–chain peptides that decrease the emulsifying properties of proteins. In contrast, a limited DH exposes hydrophobic and hydrophilic residues, enhances the amphiphilic characteristics of proteins and improves emulsification (Soares de Castro et al., 2015). However, there is no information regarding the effects of intact coconut protein concentrate (CPC) and the derived coconut protein hydrolysates (CPHs) obtained from the enzymatic hydrolysis on the stability and rheological properties of O/W emulsions. Since the global coconut oil production has been increasing over the past decade, especially for virgin coconut oil, the production of waste by–products, such as coconut skim milk and solid substances, is increasing. Therefore, these “by–products” were used as the raw material in this study. Moreover, consumer awareness about dietary fat is increasing, and vegetable oils can be used as an alternative to solid fats to produce O/W emulsions with a healthier fatty acid profile that are rich in antioxidants (Protonotariou, Evageliou, Yanniotis & Mandala, 2013). At this point, emulsions formulated with “olive oil” or “sunflower oil” instead of saturated fat can include a claim of higher amounts of monounsaturated and polyunsaturated fatty acids.

The objective of this study was to investigate the effect of CPHs with a limited DH obtained from the enzymatic hydrolysis of CPC on the stability and rheological properties of virgin olive oil and sunflower O/W emulsions. The study will provide valuable information for the predicting of the physical stability of these kinds of emulsions, and the benefit of using CPHs as a potential emulsifier in the food industry.

2. Materials and methods

2.1. Materials

Fresh coconut wet processing by–products were supplied by the 100 Phan Ma-phrau Thai group at Prachuapkhirikhan Province of Thailand, and then stored at −20 °C until used. Alcalase 2.4 L (FG; EC 3.4.21.6) from *B. licheniformis* was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Scientific Equipment Co. Ltd., Roongsarp-Chemical (Partnerships), CTI & Science Co. Ltd. and Gibthai Co. Ltd., Bangkok, Thailand. Commercial virgin olive oil and sunflower oil were both purchased from a local supermarket.