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Encapsulation of flavonoid in multiple emulsion using spinning disc reactor technology

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ABSTRACT

Rutin (quercetin-3-rutinoside) and anthocyanin flavonoids have numerous biological activities which are beneficial to human health such as antioxidant and anti-inflammatory effects. In order to aid delivery of their health benefits, an attempt has been made to encapsulate rutin and Hibiscus anthocyanins in multiple emulsions using a spinning disc reactor (SDR) as a novel processing aid. The encapsulation of flavonoids may prolong their shelf-life and increase their bioavailability for absorption by the body (Munin & Edwards-Lévy, 2011).

The advantage of using SDR technology in the second stage of emulsification is that it does not break the droplets of the primary emulsion. The time-dependent stability of the multiple emulsions was investigated using particle size, microscopy, visual assessment and stability index measurements. At 2 wt.% emulsifier, Brij 78 was found to be capable of producing uniform droplets of the final W/O/W emulsion in the size range of 13–15 μm. The results show that the SDR technology can be used as an alternative process for making stable W/O/W multiple emulsions with a fairly narrow droplet size distribution.

Rutin and anthocyanins were successfully encapsulated within the internal aqueous phase of W/O/W multiple emulsions, giving an encapsulation efficiency of >80%. In the presence of flavonoids, a reduction in the average particle size has also been observed, possibly due to its surface active properties. Confocal laser microscopy confirmed the successful formation of SDR-processed multiple emulsions.

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1. Introduction

Multiple emulsions have a number of potential benefits over the conventional oil-in-water (O/W) emulsions for certain applications such as reducing fat content (Gaonkar, 1994; Lobato-Calleros, Rodriguez, Sandoval-Castilla, Vernon-Carter, & Alvarez-Ramirez, 2006) or encapsulation of the functional food components (Benichou, Aserin, & Garti, 2004) and active molecules (Kanouni, Rosano, & Naouli, 2002; Laugel, Chaminade, Baillet, Seiller, & Ferrier, 1996; Tokimitsu, Kobayashi, Uzu, & Arisawa, 1990) in the inner aqueous phase. Thus, multiple emulsions have potential as micro carriers of hydrophilic or lipophilic ingredients entrapped in their internal droplets which are subsequently released. Encapsulation within the inner emulsion can allow the masking of odour or taste and protection against oxidation by light or enzymatic degradation, to prolong shelf-life. Controlled release of the active ingredients can be produced by dilution, shear, or other agitation (Kanouni et al., 2002; Muschiolik, 2007).

Generally, multiple emulsions are prepared by a two stage emulsification process: firstly, a simple W/O emulsion is made using a low HLB (hydrophilic-lipophilic balance) emulsifier under intense homogenization conditions. In the second stage, the primary water-in-oil (W/O) emulsion is dispersed in an aqueous phase containing high HLB emulsifier under lower shear conditions, preventing rupture of the internal droplets as far as possible, to produce a W/O/W multiple emulsion (Pal, 2008).

The loss of the internal phase due to the excessive shear stress during the production of the secondary emulsion is a major problem and much research has been carried out to try to overcome this difficulty (Liu, Ma, Meng, & Su, 2005). The release rate of the internal droplets is directly proportional to the applied shear stress and only moderate shear can be applied in order to produce multiple emulsions that retain a significantly high percentage of the internal phase (Van der Graaf, Schroen, & Boom, 2005).

Hence it is desirable to use low shear device to prevent expulsion of the internal droplets to the external continuous phase in order to produce highly stable multiple emulsions (Pal, 2008). However, low-shear conditions cannot be used with most conventional emulsification equipment without yielding droplets that

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are unacceptably large or have an unacceptably wide droplet distribution, which eventually leads to unstable products (Van der Graaf et al., 2005).

In the recent years, there has been growing interest in the role of flavonoids in maintaining human health. Flavonoids have become a regular part of the human diet (Havsteen, 1983; Pierpoint, 1986) and are of importance as antiscorbutic (anti-scurvy) agents added to food (Roger, 1988).

Rutin (quercetin-3-rutinoside) is one of the primary flavonoids in a number of plants (Kim et al., 2005) such as buckwheat. It has numerous biological activities which are beneficial to human health such as antioxidant effect (Gao, Xu, & Chen, 2003; Kozlov, Ostrachovitch & Afanas, 1994), protective effect against hepatotoxicity (Janbaz, Saeed, & Gilani, 2002), and anti-inflammatory effect (Cruz et al., 1998; Guardia, Rotelli, Juarez, & Pelzer, 2001). Reynolds (1996), pp. 1679–1680 suggested that rutin can be used to improve capillary function by reducing abnormal leakage and it has been administered to reduce capillary impairment and venous insufficiency of the lower limb. However, the solubility of rutin and many other flavonoids in water (or oil) is low (Luo et al., 2011; Luo et al., 2012).

The only group of flavonoids that has reasonable solubility in water is the anthocyanins. Anthocyanins have a high potential for use as natural colourants due to their attractive orange, red, purple, and blue colours. However, they can be quite unstable chemically (Fennema, 2008) depending on the flavonoid concentration, pH, temperature, light intensity, the presence of metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products and sulphur dioxide, among others (Cevallos, Bolyvar, & Cisneros-Zevallos, 2004). The colour stability is generally more stable at low pH, e.g., pH 2 (Selim, Khalil, Abdel-Bary & Azein, 2008).

Anthocyanins are also good natural antioxidants which may provide an array of health promoting benefits (Tsuda, Kato, & Osawa, 2000). Almajano, Carbo, Jimenez, and Gordon (2008) reported that W/O emulsions containing tea extracts have shown strong antioxidant activity against oil oxidation. However, anthocyanins have received less attention than other flavonoids; despite their widespread occurrence, possibly due to their instability. Multiple emulsions are a way of possibly protecting anthocyanins in foods.

Extracts of *Hibiscus sabdariffa* are known to contain a significantly high amount of anthocyanins and have been reported to decrease blood pressure (Haji Faraji & Haji Tarkhani, 1999; Onyenekwe, Ajani, Ameli & Garnamel, 1999) and have anti-tumour, immune-modulating and anti-leukaemic effects (Muller & Franz, 1992; Tseng et al., 2000). Wang et al. (2000) have reported protective effects against oxidative stress in rats.

In previous work (Akhtar & Dickinson, 2000) water-in-oil-in-water multiple emulsions were prepared via a two stage emulsification process using a jet homogeniser alone. The jet homogenisation produced multiple emulsions with a wide range of droplet sizes (0.5–16 μm), a highly poly dispersed system which had lower encapsulation efficiency (40–60%) due to high shear mixing. The aims of this study were to test the advantages of combining SDR technology with a jet homogenizer for producing multiple emulsions for effective encapsulation and protection of some of these flavonoids. The jet homogenizer is capable of reproducibly fine aqueous (or oil) droplets of a narrow size distribution, whilst the SDR can provide very controllable and low shear conditions for producing the secondary emulsion. The SDR equipment used for processing multiple emulsions is shown elsewhere (Akhtar, Blakemore, Clayton, & Knapper, 2009). The SDR is essentially a 20 cm diameter rotating disc heated up to 250 °C with a speed range of 200–3000 rpm. In the SDR, the emulsion phases are fed into the centre of the disc and the centrifugal force drives the emulsion phases towards the edge of the disc as a thin film. When the film breaks at the edge of the disc, it creates uniform emulsion droplets with a narrow droplet size distribution. The multiple emulsions formed were characterized and tested for their stability via particle size analysis, creaming, confocal microscopy and spectrophotometry.

2. Materials and methods

2.1. Materials

The low HLB lipophilic polymeric emulsifiers Arlacel P135 (polyethylene-30 dipolyhydroxystearate), HLB = 4–5, and Cithrol PG3PR (polyglycerol-3 polyricinoleate), HLB = 2–2, were purchased from ICI (Middlesbrough, England) and Croda (Hull, England), respectively. The high HLB hydrophilic emulsifiers, Brij 78 (polyoxyethylene (20) stearyl ether), HLB = 15.3, and Synperonic PE/F127, HLB = 16, were purchased from Croda Ltd (Hull, England). A pH 7 buffer was prepared from sodium dihydrogen orthophosphate dihydrate and di-sodium hydrogen orthophosphate, purchased from Fisher Chemicals (UK). Potassium chloride (>99%, reagentplus) was purchased from Sigma Aldrich and hydrochloric acid (37%, general purpose grade) was obtained from Riedel-de Haen, Germany.

Rutin trihydrate (Quercetin-3-rutinoside) (95%) was purchased from Sigma Aldrich (St Louis, MO, USA). Sunflower oil (refractive index 1.463) was purchased from a local supermarket (Morrison's, Leeds). *H. sabdariffa* (Rosella) plants were purchased from a local market in Nigeria and their species verified by the Agricultural Development Programme (ADP), Benin City, Nigeria. All solutions were prepared using double distilled water.

2.2. Preparation of rosella extract

Rosella extract was made by boiling 40 g of freshly ground dried calyx in 1560 g of water for 15 min. The solution was filtered though a 0.5 μm filter paper Whatman grade 1, then concentrated in a rotary evaporator (under vacuum) at 40 °C for 2 h. The concentrated extract was stored in a volumetric flask covered with aluminium foil and stored at 4 °C. The UV absorbance spectrum of Rosella was obtained by measuring the absorbance in the wavelength range of 250–550 nm using a spectrophotometer (CECIL CE3021, Tabot Scientific Ltd UK).

Fig. 1(a) shows a full spectrum of Rosella with maximum absorbance of 518.6 nm. Dilutions of the extract with pH 2 buffer were made in order to obtain a calibration curve, as shown in Fig. 1(b), so that the concentration of Rosella anthocyanins in the emulsions could be determined by measuring the absorbance of the serum layer. Absorbance measurements at 519 nm at each concentration were taken in triplicate.

2.3. Preparation of primary W/O emulsions

For encapsulating rutin, the aqueous phase was a pH 7 buffer prepared by combining 195 mL of 0.2 M NaH_2PO_4 with 305 mL of 0.2 M Na_2HPO_4. The oil phase was prepared by dissolving 4 wt% Arlacel P135 into sunflower oil with gentle stirring and heating at 50 °C. The water-in-oil emulsions (20 vol% water) were prepared at ambient temperature using a laboratory-scale jet homogenizer (Burgaud, Dickinson, & Nelson, 1990) working at the operational pressure of 300 bar For encapsulating the Rosella anthocyanin extract, a mixture of 50 mL of 0.2 M KCL plus 13 mL of 0.2 M HCl was used to make the aqueous phase of pH 2. Cithrol PG3PR emulsifier 1.6–4.5 wt% was dissolved in sunflower. The primary W/O emulsion (20 vol % aqueous phase) was prepared as above for the rutin system.

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