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Characterisation of fish oil emulsions stabilised by sodium caseinate

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Abstract

Fish oil emulsions varying in sodium caseinate concentration (25% w/w oil and 0.1–1.0% w/w protein, giving oil-to-protein ratios of 250–25) were investigated in terms of their creaming stability, rheological properties, the mobility of oil droplets and the oil/protein interaction at the interface. The presence of excessive protein in an emulsion (i.e., at 1% w/w) caused the aggregation of oil droplets through depletion flocculation, resulting in low creaming stability and high low-shear viscosity. At a lower protein concentration (0.1% w/w), when protein was limited, the emulsion droplets were stabilised by bridging flocculation and showed good stability to creaming. Shear-thinning behaviour was observed for both flocculated emulsions. A reduction in the low-shear viscosity and a Newtonian flow was obtained for the emulsion containing an intermediate concentration of protein (0.25% w/w). At this concentration, there was relatively little excess unadsorbed protein in the continuous phase; thus the emulsion was most stable to creaming. NMR was used to characterise these emulsion systems without dilution. Shorter T_2 values (by low-field ^{1}H NMR), for the emulsions containing both high (1% w/w) and low (0.1% w/w) amounts of protein, indicated increased restricted mobility of oils, caused by depletion or bridging flocculation. The line broadening in oil signals in the high-field NMR spectra (^{1}H, ^{13}C) indicated increased interaction between oil molecules and proteins at the interface with increasing protein concentration in emulsions. In addition, ^{31}P NMR spectra, which reflect the mobility of the casein component only, showed increased line broadening, with reduction in protein content due to the relatively higher proportion of the protein being adsorbed to the interface of the oil droplets, compared to that in the continuous phase (i.e., as the oil-to-protein ratio was increased). The T_2 values of resonances of the individual groups on oil molecules, obtained using high-field ^{1}H NMR, reflected their different environments within the oil droplet.

Keywords: Emulsion; Particle size; Creaming; Flocculation; NMR

1. Introduction

Many traditional food products, such as milk, yoghurt, mayonnaise, cake batters, ice creams, whipped toppings and dairy creamers, are emulsion-based. The physicochemical properties of emulsions play an important role in food systems as they directly contribute to texture, sensory and nutritional properties of foods (Dalgleish, 2006; McClements, 2005). More recently, emulsion systems are being utilised for delivery of lipophilic health-active compounds, such as long chain polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), where they provide convenient and practical means for delivering these nutrients in human diets (Augustin & Sanguansri, 2003; Keogh et al., 2001; Kolanowski, Swiderski, & Berger, 1999).

Proteins, particularly dairy proteins, are often used as emulsifiers in foods. The amphiphilic nature of proteins

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means that they have the ability to adsorb to an interface, lower surface tension and provide a viscoelastic layer which prevents coalescence of the droplets (Dickinson, 1999, 2006). However, the ability of various milk proteins and their fractions to form and stabilise emulsions differs, depending on their structure, conformation and state of aggregation (Agboola & Dalgleish, 1995; Dickinson, Golding, & Povey, 1997; Euston & Hirst, 2000). Sodium caseinate is a well-used ingredient because of its good solubility and emulsifying properties and its stability during heating (Dickinson, 1999). It is known that the amount of protein required to stabilise oil-in-water emulsions depends, not only on the structure of the protein at the interface, and the average diameters of the emulsion droplets, but also on the types of oils, i.e., whether they are hydrocarbons such as *n*-tetradecane or triacylglycerides, such as soya oil (Dickinson et al., 1997; Fang & Dalgleish, 1993). A critical concentration (c^*) of protein in the aqueous phase is often needed to provide the full coverage of emulsion droplets. However, it has been reported that the presence of excessive caseinate in an emulsion containing a high concentration of protein ($c > c^*$) can induce depletion flocculation of emulsion droplets, resulting in creaming due to the strong tendency of the casein submicelles to form small protein particles (Dickinson et al., 1997). On the other hand, when the protein is limited in an emulsion containing a low concentration of caseinate ($c < c^*$), the oil interface may not be fully covered by the available protein, thus the adsorbed caseins are shared between two or more oil droplets to form bridging flocculation (Dickinson, Flint, & Hunt, 1989). Therefore, it is important to obtain optimum emulsion stability with an intermediate concentration of caseinate ($c = c^*$), which gives full coverage of the oil–water interface without any significant excess of protein remaining unadsorbed in the emulsion, (Dickinson, 1999).

The properties of both the interface and the continuous phase are important for understanding emulsion characteristics. Emulsions have been studied by numerous techniques, such as particle sizing, microscopy, rheology, and measurement of surface concentration, to characterise adsorbed protein at the interface and related physical properties of the emulsion (Agboola & Dalgleish, 1995; Dickinson et al., 1997; Euston & Hirst, 2000; Singh, Tamehana, Hemar, & Munro, 2003). All or most of these techniques involve some form of dilution. This dilution disrupts some structures that contribute to destabilisation, in particular, the structures of concentrated systems. The ability to study the stability of food emulsions in their undiluted forms may reveal subtle nuances about their stability.

The application of NMR spectroscopy for the analysis of liquid food systems has proved immensely valuable (Belton, Delgadillo, & Gil, 1998; Cornillon, 1998; Guillen & Ruiz, 2001). It may be used to measure the total fat content in fat-containing food products (Sorland, Larsen, Lundby, Rudi, & Guiheneuf, 2004; Veliyulin, van der Zwaag, Burk, & Erikson, 2005), coalescence of emulsions (Lee, McCarthy, & Dungan, 1998), the effect of fat crystals on the destabilisation of emulsions (Hodge & Rousseau, 2005), and the mobility of particles in fish oil-in-water emulsions (Shen, Udabage, Burgar, & Augustin, 2005). Over recent years, several NMR techniques have provided information on emulsion droplet measurement and the size distribution association in concentrated emulsions (Denkova et al., 2004; Kiokias, Reszka, & Bot, 2004; Lönnqvist, Khan, & Söderman, 1991). Both the dispersed and the continuous phase of emulsions can be characterised using NMR (Balinov, Mariette, & Söderman, 2004; Goudappel, van Duynhoven, & Mooren, 2001). Other researchers (Mine, 1997; ter Beek et al., 1996) have used ^{31}P NMR to investigate the adsorption behaviour of caseins and lipid–protein interaction at an oil-in-water interface. The advantage of using NMR is that it can be applied to concentrated emulsions without pre-treatment or dilution of the sample. Acquisitions of the measurement data are usually fast and do not require excessive sample volumes. Therefore, NMR measurements could provide new insights into the destabilisation mechanisms of emulsions due to the ability to study concentrated emulsions.

The aim of this work was to examine whether NMR could provide further information that would complement that obtained by other conventional techniques used for characterising emulsions. The examination of emulsion systems involved the use of low and high-field ^{1}H NMR and high-field ^{13}C NMR and ^{31}P NMR. ^{1}H and ^{13}C NMR were chosen to provide information about the oil components as it was expected that these spectra would be dominated by the oil molecules, which are more mobile than the colloidal protein components. ^{31}P NMR measurements enabled the determination of the protein distribution in the system, i.e., in bulk aqueous vs. the interface as only the phosphoseryl residues on caseins would be observed. In this study, fish oil-in-water emulsions, prepared using varying amounts of sodium caseinate (25% w/w oil, 0.1–1.0% w/w protein; oil-to-protein ratio of 250–25) were first characterised in terms of their stability to creaming and their rheological properties in relation to the emulsion particle size and structuring behaviour, by light microscopy. NMR techniques were then employed to study the mobility of oil and protein components at the interface in the emulsions with distinctive stabilisation characteristics.

2. Materials and methods

2.1. Materials

Sodium caseinate (Alanate 180) was obtained from New Zealand Milk Products Pty Ltd. (Rowville, VIC, Australia). It contained 92.6% protein, 4.2% moisture, 1.17% sodium and 0.02% calcium. Fish oil (HiDHA R 25N FOOD – steam deodorised) was supplied by Clover Corporation Ltd. (Sydney, NSW, Australia). Deuterium oxide