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Emulsion flocculation induced by saliva and mucin

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Abstract

Upon consumption of emulsions, mixing with saliva occurs. This article shows that whole saliva and a model mucin (pig gastric mucin, PGM) are able to induce extensive droplet flocculation. Saliva samples collected from several subjects at different times of the day always showed flocculation. However, there was a clear variation between samples from different individuals with respect to the structure of the flocs and reversibility of flocculation upon dilution.

Several aspects of PGM-induced flocculation, measured by microscopy, particle size analysis, demixing experiments and rheology pointed to depletion flocculation as the main mechanism of flocculation. However, although depletion may also be an important driving force in saliva-induced flocculation, the required mucin concentration seems to be considerably lower than for PGM. Therefore other interactions, such as bridging or specific binding, may be important as well.

The observed aggregation is expected to have implications for understanding sensory properties of emulsions. The viscosities of emulsions measured in vitro in the absence of saliva may deviate from the in vivo viscosities relevant for sensory perception, especially in case of liquid emulsions in which the droplets are not flocculated, such as milk.

Keywords: Emulsion; Saliva; Mucin; Aggregation; Depletion flocculation

1. Introduction

Food emulsions are exposed to a range of processing steps during consumption. They are mixed with saliva, heated or cooled to body temperature, air is introduced, the emulsions come into contact with oral surfaces and are exposed to complicated saliva flow profiles. Although, the physiology of the oral cavity has been clarified, little is known about the oral behavior of food emulsions. It is anticipated that saliva–emulsion interactions play an important role in understanding emulsion perception (Aken, Vingerhoeds, & de Hoog, in press).

Many factors, such as the flow rate, time of the day, type and size of the salivary gland, duration and type of the stimulus, diet, drugs, age, sex and blood type affect the amount and composition of secreted human saliva. Stimuli for increased salivation include the presence of food or irritating substances in the mouth, and thoughts of or the smell of food. Secretion of saliva ranges from 0.3 to 7 ml saliva per minute (Edgar, 1990) with about 0.5–1.5 l of saliva secreted per day (Humphrey & Williamson, 2001). Saliva has a pH of 6–7 and is composed of proteins, low molecular mass substances (mainly electrolytes) and more than 99% water (Humphrey & Williamson, 2001; van Nieuw Amerongen, Veerman, & Vissink, 2004; Zalewska, Zwierz, Zólkowski, & Gindzienski, 2000). The proteins in saliva (1–2 mg/ml) include enzymes, immunoglobulins, antibacterial proteins, proline-rich proteins (up to 45 wt% of total protein) and mucins (Humphrey & Williamson,

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2001). Mucins are highly glycosylated proteins with various functions in saliva. For example, they are responsible for the typical viscoelastic behavior of saliva, necessary for its retention in the mouth. The high molecular weight mucin (MUC5B) concentration in whole saliva is about 200 μg/ml (ranging from 30 to over 500 μg/ml, depending on the stimulus), i.e. 10–25 wt% of total salivary proteins (Fox, Bodner, Tabak, & Levine, 1985; van Nieuw Amerongen et al., 2004; Rayment, Liu, Offner, Oppenheim, & Troxler, 2000). The high degree of glycosylation keeps the protein chain in extended form, even under denaturating conditions (low pH, high pH, and presence of salts). The carbohydrate chains also protect the polypeptide chain from enzymatic degradation and play a crucial role in water binding.

Research reported in literature dealing with the interaction between emulsions and saliva has been directed towards, firstly, sensory analysis of emulsions in relation to saliva flow (Engelen, de Wijk, Prinz, & Bosman, 2003) and composition (Engelen et al., submitted for publication), secondly, physicochemical properties of emulsions in vitro (Barylko-Pikielna, Martin, & Mela, 1994; Kilcast & Clegg, 2002; Metcalf & Vickers, 2002; de Wijk, van Gemert, Terpstra, & Wilkinson, 2003) and, thirdly, towards flavor release from emulsions (de Roos, 2003) as measured in mouth models (Doyen, Carey, Linforth, Marin, & Taylor, 2001; van Ruth, King, & Giannouli, 2002; van Ruth & Roozen, 2000a,b) and in vivo via e.g. electronic noses (Miettinen, Tuorila, Piironen, Vehkalahti, & Hyvönen, 2002).

We previously reported a strong aggregating effect of saliva on both protein- and surfactant-stabilized emulsions (van Aken, Vingerhoeds, & de Hoog, in press). In addition, explorative experiments were done to gain insight in oral behavior of commercial emulsions (van Aken et al., in press). Dairy emulsions (1.5 and 3% fat homogenized pasteurized milk and 40% whipping cream) were taken in the mouth for 1 min and subsequently spat out. Droplet aggregation was observed in the spat-out mixtures. Moreover, the spat out mixtures were clearly 'slimy' and contained loose droplet aggregates, which were often elongated in structure. The sliminess of the spat out saliva–emulsion mixture appeared to increase upon increasing fat content.

The aim of this paper is to further investigate the observed aggregation phenomena and to elucidate the physicochemical effects of saliva on protein-stabilized food emulsions, with emphasis on the role of high molecular weight mucins. The high molecular weight salivary component mucin is in this study modeled by pig gastric mucin (PGM), which consists of both MUC5AC and MUC6 (Nordman et al., 2002). Especially MUC5AC has large similarities to the high molecular weight human salivary mucins (MUC5B) (van Klinken, Einerhand, Büller, & Dekker, 1998; Offner, Nunes, Keates, Afdhal, & Troxler, 1998). PGM induces rapid depletion flocculation of the studied emulsions. This aggregation phenomenon will affect the texture and rheological properties of emulsions. We anticipate that it will also influence the sensory perception of food emulsions.

2. Materials and methods

2.1. Materials

β-Lactoglobulin and β-casein were purified from bovine milk (de Jongh, Gröneveld, & de Groot, 2001; Swaisgood, 1982) and had protein contents of 92.5 and 92.6 wt%, respectively. Whey protein isolate (Bipro, Davisco International) contained 71% β-lactoglobulin, 12% α-lactalbumin, 5% bovine serum albumin, 5% immunoglobulins, 2% salt, 1% lactose and 4% moisture. Sodium caseinate (DMV International, Veghel) contained 90 wt% protein, 5.0 wt% moisture and 0.08 wt% calcium. Porcine gastric mucin (PGM) was obtained from either ICN Biomedicals (lot number 90877 with 71.4% purity; Ohio, USA) or Sigma (Type 3; cat number M1778; batch number 013K7029). NaCl (p.a.) and Tween 20 were obtained from Merck (Shuchardt, Germany) and thiomersal (97%) was purchased from BHD, Poole, UK. Sunflower oil (Reddy, Vandemoortele, the Netherlands) was purchased from a local retailer.

Saliva was collected in the morning from healthy subjects (2 male; 4 female; age 28–43 years) by drooling into collecting tubes and was kept on melting ice for maximally 6 h. The samples were in most cases vortexed (vortexing did not affect the results), the pH was determined and cellular debris was removed by centrifugation (11,000*g*, 10 min). Dr E.C.I. Veerman (Vrije Universiteit, Amsterdam) kindly provided parotis saliva of two healthy male subjects (41 and 51 years old) that was collected as described earlier (Lashley, 1916; Veerman, van den Keybus, Vissink, & van Nieuw Amerongen, 1996), and was stored for maximally 3 weeks at −20 °C before use. Freezing of Parotis saliva induces only minor changes in the overall protein composition (Francis, Hector, & Proctor, 2000).

2.2. Mucin dispersion and characterization

PGM (2 wt%; from ICN) was dispersed in 0.1 M NaCl solution (except when stated otherwise) with 0.02 wt% thiomersal during 2 days at ambient temperature (25 °C). The pH was brought to 6.7 with 0.5 M NaOH.

The molecular weight and radius of gyration of PGM were determined, after centrifugation to remove undissolved material, by gel permeation chromatography at two different pH values. At pH 6.7, the M_W of PGM was 2.2 million Da and the weight-average radius of gyration (R_G) was 49 nm. Lowering the pH to 3.0 hardly affected the R_G (53 nm). Plotting the logarithm of the radius against the logarithm of the molar mass resulted in a slope of 0.47 or 0.51 at pH 3.0 and 6.7 respectively, indicating a flexible