



## *In vitro* digestion of oil-in-water emulsions stabilized by whey protein nanofibrils



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### ABSTRACT

The effect of pH (3 and 7) and varied energy density of a high-pressure homogenization process on the stability of oil-in-water (O/W) emulsions stabilized by whey protein fibrils was evaluated. A dynamic digestion model comprising the simulation of stomach, duodenum, jejunum and ileum, has been used to evaluate O/W emulsions' behavior under gastrointestinal (GI) conditions. The emulsions did not separate phases during the storage period (7 days). The emulsions stabilized by whey protein fibrils were stable under simulated gastric conditions but were destabilized in the simulated intestinal conditions. In a similar way, the whey protein fibrils' dispersion showed a high resistance to proteolytic *in vitro* digestion by pepsin (gastric stage) but was more readily degraded by pancreatin (intestinal stage). This fact confirms the significant impact of the interfacial characteristics on emulsions' digestion. The percentage of free fatty acids (FFA) absorbed in the simulated intestinal conditions (jejunum and ileum) was much lower than the total percentage of FFA released due to the use of WPI fibrils as emulsifier. This work contributes to a better understanding about the behavior of O/W emulsions stabilized by whey protein fibrils within the GI tract; this knowledge is fundamental when considering the final application of this protein in food products.

### 1. Introduction

Oil-in-water (O/W) emulsions have significant potential for encapsulation of lipophilic bioactive compounds in food products. However, these systems are thermodynamically unstable which can lead to phase separation. Emulsions can be kinetically stabilized with addition of emulsifying agents (McClements, 2005) and by homogenization processes at high pressure, which enable the formation of small droplet sizes (Jafari, Assadpoor, He, & Bhandari, 2008). Whey proteins are widely used as emulsifying/stabilizing agents (Guzey & McClements, 2006; Walstra, 2003) due to their ability to form a thick protective layer onto the interface of oil droplets, increasing emulsion stability (Hu, McClements, & Decker, 2003; Pugnali, Dickinson, Ettelaie, Mackie, & Wilde, 2004). Amyloid-like fibrils from whey protein can be used as good gelling agents/thickeners and foam stabilisers because of their high length-to-width ratio (Loveday, Rao, Creamer, & Singh, 2009). Fibrils' potential as a new ingredient in food formulations is due to their robustness and rheological behavior in solution (Loveday, Su, Rao, Anema, & Singh, 2011). Changes in fibril

length affect directly functional properties such as foaming, emulsifying or stabilizing capacity (Kroes-Nijboer et al., 2012). The modification of protein fibril length can be promoted by: (i) altering the environmental conditions during or after fibril formation such as temperature *versus* heating time, ionic strength, pH and composition (Blijdenstein, Veerman, & Van der Linden, 2004; Kroes-Nijboer et al., 2012; Loveday et al., 2011; Mantovani, Fattori, Michelon, & Cunha, 2016; Serfert et al., 2014) or (ii) by applying mechanical treatments after the process of fibril formation using high shear or moderate shear (Oboroceanu, Wang, Magner, & Auty, 2014; Oboroceanu et al., 2011; Peng, Simon, Venema, & Van der Linden, 2016; Serfert et al., 2014). Previous studies have reported that mixing of emulsions stabilized by native whey proteins at low (Blijdenstein et al., 2004) or high fibril concentrations (Peng et al., 2016) can induce a depletion-flocculation process. However, the potential of fibril systems as a single emulsifier has not been fully investigated.

Understanding physiological events such as the digestion process of fibrils is of great importance for their application in food formulations. *In vitro* digestion of fibrils produced from  $\beta$ -lactoglobulin (Bateman,

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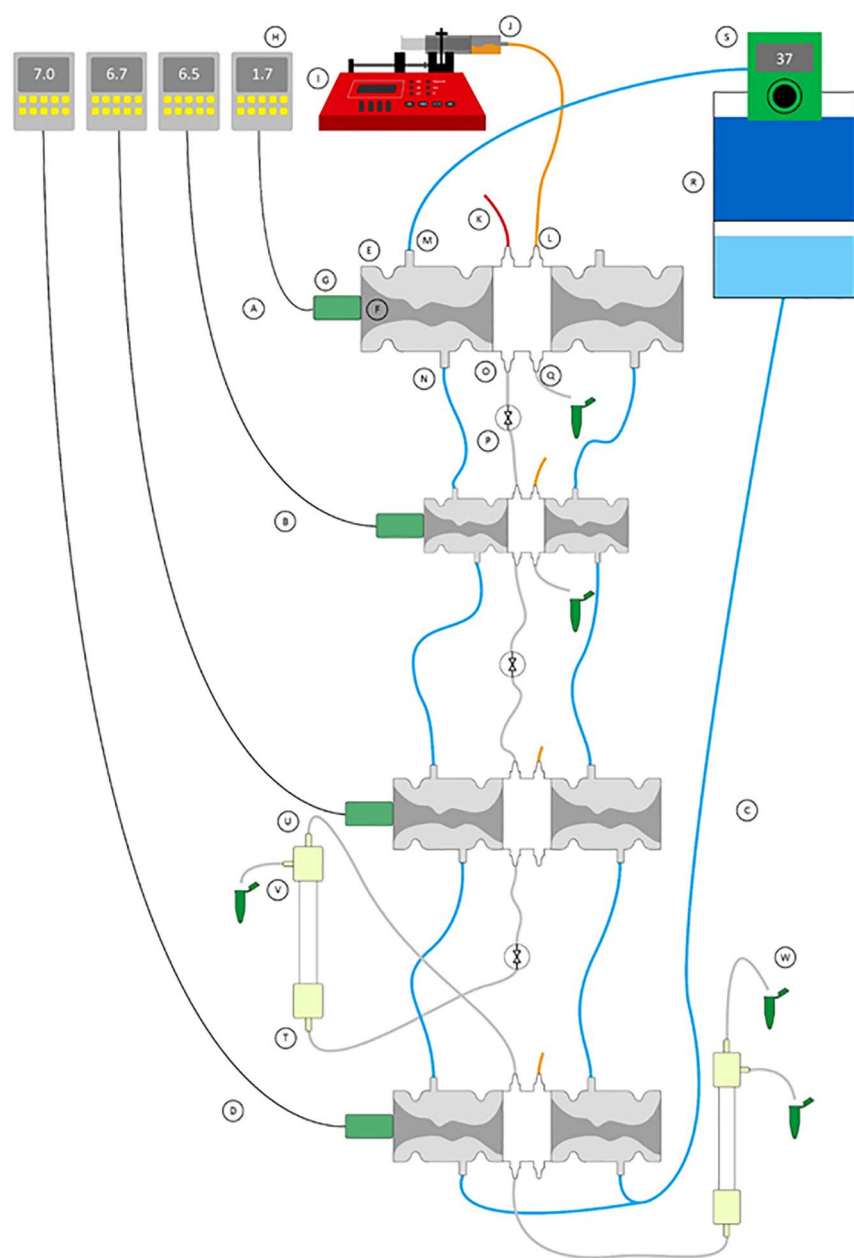


Fig. 1. Representative schematic of the *in vitro* GI system where A) stomach compartment; B) duodenum compartment; C) jejunum compartment; D) ileum compartment; E) glass reactor; F) silicon bags; G) pH electrode; H) pH monitor; I) syringe pump; J) digestion secretion syringe; K) sample inlet; L) digestion secretion inlet; M) water at 37 °C inlet; N) water outlet; O) digestion products outlet; P) valve; Q) digestion sample; R) water bath at 37 °C; S) water heater; T) hollow fiber membrane inlet; U) hollow fiber membrane outlet; V) jejunum/ileum filtrate and W) ileum delivery sample.

Ye, & Singh, 2010) or whey protein isolate (Lassé et al., 2016) has been reported. However, the digestibility of these nanostructures at oil-water interface should be also evaluated if they are applied as emulsifiers since the digestion response of emulsions depends not only on the nature of food matrix but also to their interfacial properties (Pafumi et al., 2002). The bioavailability of lipid components when emulsions are applied as delivery systems is another relevant point that must be taken into consideration. Lipolysis begins in the stomach at low acidic pH (around 2), which is ideal for gastric lipase activity. In this step, mainly free fatty acids and diacylglycerols are produced from the ingested triacylglycerol hydrolysis. The lipid hydrolysis occurring in the stomach facilitates the subsequent lipid hydrolysis in the duodenum by pancreatic lipase since it facilitates fat emulsification and, consequently, lipase activity (Pafumi et al., 2002). Thus, the lipid digestion process consists in an interfacial reaction since its occurrence depends on lipase adsorption onto the oil droplet surface (Torcello-Gomez, Maldonado-Valderrama, Martin-Rodriguez, & McClements, 2011). Considering these aspects, studying the response of the interfacial composition of emulsions subjected to the gastrointestinal tract

conditions is important for a better control of lipid bioavailability (McClements, Decker, & Park, 2007; Mun, Decker, & McClements, 2007) and to define the final application of the encapsulation system.

This work aimed at studying the influence of pH (3 and 7) and energy density of the high-pressure homogenization process on the stability of O/W emulsions stabilized by whey protein fibrils. A dynamic digestion model comprising the simulation of stomach, duodenum, jejunum and ileum, has subsequently been used to evaluate the behavior of O/W emulsions under gastrointestinal conditions. Whey protein fibrils dispersed in aqueous solution were also subjected to simulated gastrointestinal conditions to understand the influence of fibrils on emulsions' digestibility.

## 2. Materials and methods

### 2.1. Materials

Soybean oil (Soya, Bunge Alimentos S.A., Gaspar, Brazil) was purchased in the local market. Whey protein isolate (WPI) (protein content

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