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Influence of *Ginkgo biloba* extracts and of their flavonoid glycosides fraction on the *in vitro* digestibility of emulsion systems

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ABSTRACT

The objective of this study was to investigate the influence of *Ginkgo biloba* extracts (GBE) and of their flavonoid glycosides fraction (FA) on the *in vitro* digestibility of emulsion systems. A soybean oil-in-water emulsion (10 wt% oil) was prepared using GBE or FA particles as emulsifiers. We showed that the droplet size distribution in the FA and GBE particle-stabilized emulsions was monomodal with mean droplet sizes of 0.8 and 1.0 μm, respectively. Moreover, after storage, the emulsions were relatively more stable against coalescence than Tween 20-stabilized emulsions. Our emulsions were also compared to conventional Tween 20 (0.1 wt%)-stabilized emulsions in terms of their lipid digestion kinetics by using an *in vitro* digestion model at pH 7.0 and pH 8.9. At both pH conditions, lipolysis in the FA- or GBE-stabilized emulsions was appreciably slower, and the total concentration of released fatty acids was much lower than in Tween 20-stabilized emulsions. The flavonoids adsorbed at the oil-water interface could barely be displaced by Tween 20, even at high concentrations (0.3 wt%), suggesting that the slower lipolysis observed in FA- or GBE-stabilized emulsions could be a result of the strong and irreversible adsorption of FA and GBE particles at the emulsion oil droplets. Furthermore, the bioaccessibility of flavonoids in FA-stabilized emulsions (39%) was considerably higher than in GBE-stabilized emulsions (11%).

1. Introduction

Triglycerides (TAG) are an important source of calories in a human diet (Ouellet et al., 2012). They are also responsible for tastes and flavours that are appreciated by so many people. However, excess storage of triglycerides in the human body may lead to obesity and cardiovascular diseases, which have emerged as major global health problems. According to the World Health Organization, the number of obese adults could reach 700 million by 2015 (Flegal, Carroll, Ogden, & Curtin, 2010). Therefore, understanding and controlling the digestibility of lipidic foods within the human gastrointestinal (GI) tract have become important challenges for researchers and food industries (Hu, Li, Decker, & McClements, 2010; McClements, Decker, & Park, 2008; Singh, Ye, & Horne, 2009; Vinarov et al., 2012).

About 70% of the TAG ingested are digested in the small intestine by pancreatic lipase (PL). It has also been reported (Golding & Wooster, 2010) that PL, co-lipase, bile salts, calcium, and even the food structure were important parameters influencing the digestibility of lipidic foods. Thus, there are several strategies preferred by researchers to control fat release within the gastrointestinal (GI) tract, including lipase inactivation, ingredient interactions, mass transport barrier, physical barrier, and reaction product accumulation (McClements & Li, 2010). Many of the emulsion-based delivery systems used to control lipid digestibility are based on at least one of these methods. For example, previous studies have reported that polysaccharides (Tsujita et al., 2007), green tea extracts (Juhel et al., 2000), chitin (Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013), chitosan (Mun, Decker, Park, Weiss, & McClements, 2006), and dietary fibres (Dunaif & Schneeman, 1981), which alter lipase inactivation and/or the emulsion structure, could effectively reduce the digestion rate. Moreover, emulsifiers (Chu et al., 2009; Mun, Decker, & McClements, 2007), solid particles (Tzoumaki et al., 2013), and multilayer emulsions (Mun et al., 2006) may also influence the rate and extent of lipid digestion. These studies indicated that controlling the oil-water interface could be an effective strategy for the management of lipid digestion. Recently, some flavonoids have been reported to stabilize oil-in-water emulsions through particle adsorption (i.e., Pickering emulsion) at the surface of oil droplets (Luo et al., 2011). These emulsions

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can be stable after a long period of storage. Interestingly, our previous study showed that an O/W emulsion containing stripped structured lipid as an oil phase with lecithin and *Ginkgo biloba* extract containing flavonoids glycosides displayed high emulsion stability. Therefore, this led us to investigate whether these kinds of solid particles would influence lipid digestibility.

Extracts from *G. biloba* (GBE) have been used in Europe for years for the treatment of symptoms associated with numerous cognitive disorders (Le Bars et al., 1997). GBE has also been reported to have strong free radical scavenging activity, antioxidant, and anti-atherosclerosis capacities (Chen et al., 2010; Kobus et al., 2009; Li, Liao, & Liao, 2004; Maltas, Vural, & Yildiz, 2011; Zahradníková, Schmidt, Sekretár, & Janáč, 2007). Researchers have reported that the positive effects of GBE may be due to the synergistic effects of flavonoids and triterpene lactones (Chen et al., 2010), which were accounted for 24% and 6% of GBE, respectively (Bastianetto et al., 2000). However, the poor liposolubility and water solubility of flavonoids resulted in low oral bioavailability, thereby limiting their applications (Chen et al., 2010). Pool, Mendoza, Xiao, and McClements (2013) reported that the bioaccessibility of quercetin was increased when quercetin was incorporated in nanoemulsions. Therefore, it is necessary to develop a new formulation in order to expand the application of GBE.

To the best of our knowledge, the potential of flavonoid-stabilized emulsions to control lipid digestion and to increase flavonoid bioaccessibility has not yet been explored. Thus, the purpose of the present study was to use an *in vitro* digestion model to examine the influence of GBE and of the flavonoid glycosides fraction collected from GBE (FA) on lipolysis. In this study, the lipid digestibility of GBE or FA solid particle-stabilized emulsions was compared to that of conventional emulsions stabilized by Tween 20. We hypothesized that the properties of GBE or FA particles would allow the formation of a layer around the surface of the oil droplets. As a result, the newly formed physical barrier at the interface would prevent PL and bile salts from accessing the lipid droplets, thereby inhibiting lipolysis. For this reason, we carried out particle size characterization, microscopic observations, and surface coverage determination of flavonoids and competitive displacement of flavonoids by Tween 20 experiments. In summary, this study aimed at investigating whether GBE and FA particles could influence lipid digestibility and whether there was any correlation between the emulsion composition and inhibition of lipid hydrolysis. Furthermore, we investigated the bioaccessibility of flavonoids in GBE and FA after they were incorporated in O/W emulsion systems. Our results may have important implications for the control of lipid release in the GI tract and of the bioaccessibility of functional compounds in foods and pharmaceutical formulas.

2. Materials and methods

2.1. Materials

Soybean oil (SBO) (CJ Co., Seoul, Korea) was purchased from the local market (Daejeon, Korea). Pancreatin (P3293), type II lipase from porcine pancreas (L3126), bile salts (B8756), porcine bile extract (B8631) and bovine serum albumin (BSA, A7906) were obtained from Sigma Chemical Corp. (Poole, UK). Sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), monopotassium phosphate (KH_2PO_4), potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), hydrogen chloride (HCl), and sodium hydroxide solution (0.05 N, NaOH) were purchased from Daejung Chemicals & Metals Co., Ltd. (Korea). Urea was purchased from Junsei Chemical Cl., Ltd. (Japan).

2.2. Emulsion preparation

An aqueous phase containing 0.02% sodium azide as a preservative was prepared with a 20 mM bis–tris buffer solution (pH 7.0). FA powder, GBE powder or Tween 20 (100 mg) were dispersed in 90 g of aqueous phase using an ultrasonic processor for 1 min. Oil-in-water emulsions (100 g) were prepared using soybean oil (10 g) in the mixture (90 g of aqueous phase with 100 mg of FA, GBE or Tween 20). The mixture was kept in a warm water until pre-homogenization using a Silverson mixer (Model L4RT, Silverson Machines, UK) at a speed of 5000 rpm for 2 min. The resulting premix was then passed through a microfluidizer (M-110Y, Microfluidics, MA, USA) twice at 20 MPa.

2.3. Measurement of particle size

The volume-surface mean diameter d_{32} the emulsion droplets was determined using a laser diffraction instrument (Mastersizer S, Malvern Instrument, Worcestershire, UK) (Luo et al., 2012).

2.4. Microscopy

Approximately 10 μL of the emulsions were placed into laboratory-made well slides. A coverslip (0.17 mm thickness) was placed on top of each well, ensuring that there was no air gap (or bubbles) trapped between the sample and the coverslip. The samples were imaged with 100× oil-immersion objective lenses, at approximately 10–20 μm below the surface of the coverslip, in order to minimize hydrodynamic (and other) interactions with the coverslip.

2.5. In vitro simulated small intestine digestion

A potentiometric automatic titrator (AT-400E, Kyoto Electronics Manufacturing Co., Ltd., Japan) was used to measure the amount of free fatty acids (FFA) released from the emulsion samples. The titration pH was set at 7.0 and 8.9 based on the experimental conditions. Thirty-six millilitres of duodenal and bile juices (2:1, v/v) (Table 1) were mixed in a bottle in order to simulate small intestinal fluid (SSIF) according to Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005). For oil-SSIF type samples, 300 mg of soybean oil with or without 3 mg of extract powder (GBE or FA powder) were dispersed into 36 mL of SSIF in a beaker (100 mL), followed by ultrasonic processing (VC750, Sonics & Materials Inc., Newtown, CT, USA) for 1 min (~6200–6300 J) to prepare simulated digesta. After ultrasonication, the pH of simulated digesta was 7.6–8.4. The pH was then adjusted to 6.8 or 8.6 if necessary. For emulsion-SSIF type samples (Tween 20-, GBE-, or FA-based emulsions), 3 mL of 2-day-old emulsions were dispersed into 36 mL SSIF in a beaker (100 mL) in order to simulate digestion. Lipolysis experiments were carried out after addition of 1 mL SSIF containing 53 mg of pancreatin and 35 mg of type II lipase to 36 mL simulated digesta in a beaker and the mixtures were agitated at 150 rpm at 37 °C. The volume of used NaOH was recorded every minute in order to calculate the percentage of released fatty acids, the digestion rate (k, μmol s^{-1} m^{-2}), and the half digestion time ($t_{1/2}$, min) according to Li and McClements (Li & McClements, 2010).

$$\mathrm{FFA}(\%) = \frac{V_{\mathrm{NaOH}(t)} \times M_{\mathrm{NaOH}}}{W_{\mathrm{lipid}} \big/ M_{\mathrm{lipid}} \times 2} \times 100 \quad (1)$$

Where V_{NaOH} (in mL) is the volume of sodium hydroxide required to neutralize the FFA produced, M_{NaOH} is the molarity of the sodium hydroxide solution used (0.05 M), W_{lipid} is the total weight of oil (g)