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Design of the interface of edible nanoemulsions to modulate the bioaccessibility of neuroprotective antioxidants

M. Plaza-Oliver [a,b,1], J.Fernández Sainz de Baranda [a,b,1], V. Rodríguez Robledo [a,b], L. Castro-Vázquez [a,b], J. Gonzalez-Fuentes [a,b], P. Marcos [a,b], M.V. Lozano [a,b], M.J. Santander-Ortega [a,b,*], M.M. Arroyo-Jimenez [a,b,**]

[a] Cellular Neurobiology and Molecular Chemistry of the Central Nervous System Group, Faculty of Pharmacy, University of Castilla-La Mancha, Albacete, Spain
[b] Regional Centre of Biomedical Research (CRIB),University of Castilla-La Mancha, Albacete, Spain

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ABSTRACT

Most frequently the use of bioactive molecules for the supplementation of food and beverages is hampered by stability limitations or inadequate intestinal absorption. This work evaluates *in vitro* the role that the interface of the nanoemulsion has on the physicochemical properties, the stability behavior and the enzymatic degradation after oral intake. For that purpose three soybean oil (SB) formulations were studied. These formulations were based on the emulsifier lecithin but modified with two non-ionic surfactants Pluronic® F68 (PF68) or Pluronic® F127 (PF127) yielding (i) SB-NE (only lecithin on the interface), (ii) SB-NE PF68 (lecithin plus PF68) and 9 (iii) SB-NE PF127 (lecithin plus PF127). All the formulations tested were low polydispersed and showed a size of about 200 nm and ζ-potential of −50 mV. The *in vitro* colloidal stability assay showed that lecithin itself was able to promote that formulations reach unaltered to the small intestine and facilitate the absorption of the antioxidant payload on a tunable fashion there (with *in vitro* bioaccessibility values from around 40% up to a 70%). PF68 was able to sterically stabilize the formulation against the aggregation induced by the pH and electrolytes of the simulated gastrointestinal track; however, this surfactant was easily displaced by the lipases of the simulated intestinal milieu being unable to modulate the digestion pattern of the oil droplets in the small intestine. Finally, PF127 displayed a strong steric potential that dramatically reduced the interaction of the oil droplets with lipases *in vitro*, which will compromise the capacity of the formulation to improve the bioaccessibility of the loaded antioxidant.

1. Introduction

Currently, the food industry is trying to implement the valuable knowledge acquired in the pharmaceutical field for designing new nanostructures able to overcome biological barriers. These nanostructures would be used to encapsulate active therapeutic molecules for food and beverage products supplementation in order to increase and homogenise the bioavailability of the active molecule (Acosta, 2012; McClements and Xiao, 2012; Porter et al., 2007). In this case the concept would not be the treatment of a disease but the diet-based prevention of chronic diseases such as central nervous system pathological aging diseases or cardiovascular diseases among others (Acosta, 2012; Yang and McClements, 2013).

Nevertheless, the direct incorporation of bioactive molecules such as antioxidants to food or beverage matrices is mainly hampered by (i) the modification of the product organoleptic properties; (ii) the degradation of the labile bioactive due to oxidative processes, pH, temperature changes or during the manufacturing and storage of the food or beverage products; (iii) the premature degradation of the bioactive after oral intake; (iv) the limited intestinal absorption (Acosta, 2012; Fang and Bhandari, 2010). All these factors can result in the refusal of the product by the consumer or in low bioavailability of the bioactive after ingestion, clearly hampering its efficacy (Scalbert and Williamson, 2000). As introduced before, the need to overcome these limitations has brought the food and beverage industry to consider the use of nanotechnology. Nanoparticles for the encapsulation of food supplements would allow not only to

* Corresponding author at: University of Castilla-La Mancha, Medical Sciences, Albacete, Spain. Tel. :+ 967 59 92 00x2239.
** Corresponding author at: Tel. :+ 967 59 92 00x8249.
E-mail addresses: manuel.santander@uclm.es (M.J. Santander-Ortega), Mariamar.Arroyo@uclm.es (M.M. Arroyo-Jimenez).
[1] These authors have equally contributed to this work.

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protect the food/beverage product from the modification of its organoleptic properties by the bioactive molecules, but also to preserve the nutritional properties of those molecules against degradation processes during the manufacturing and storage phases, as well as to protect the cargo up to its release at the target site (Acosta, 2012; McClements and Xiao, 2012; Porter et al., 2007).

One of these nanoplatforms that potentially may improve this landscape are lipid nanostructures. These structures are used as food and beverage supplements for the encapsulation of lipophilic antioxidants to protect its cargo before releasing in the target site. In this sense, one key feature of the system is the maintenance of their nanometric entity up to its arrival to the small intestine (Acosta, 2012; Golding et al., 2011; Li et al., 2012; McClements and Xiao, 2012; Porter et al., 2007; Salvia-Trujillo et al., 2013). Size will affect the interaction of the nanostructure with the enzymes and others macromolecules present in the intestinal milieu. This interaction will promote the digestion of the nanostructure, and then, the enzyme triggered partition of the therapeutic antioxidant from the nanostructure matrix towards the mixed micelles and vesicles formed by bile salts, phospholipids and degradation products of the lipid nanostructure (Acosta, 2012; McClements and Xiao, 2012; Porter et al., 2007). Afterwards, these mixed micelles and vesicles with a therapeutic antioxidant enriched inner core will be absorbed by the enterocytes resulting in the release of the antioxidant to the systemic circulation in a reproducible and efficient fashion (Acosta, 2012; McClements and Xiao, 2012; Porter et al., 2007).

In the specific case of lipophilic therapeutic antioxidants, such as vitamin E, marketed food supplements products have shown low and very variable bioavailability results (Acosta, 2012; Eitenmiller and Lee, 2005; McClements and Xiao, 2012). Hence, the entrapment of the lipophilic molecule in lipid nanostructures, already incorporated into the food or beverage matrix during the manufacturing process, have improved the magnitude and reduced the variability of the antioxidant intestinal absorption after oral intake (Acosta, 2012; Gong et al., 2012; Hatanaka et al., 2010; Li et al., 2012; McClements and Xiao, 2012; Salvia-Trujillo et al., 2013; Scalbert and Williamson, 2000; Yang and McClements, 2013). Similar results were obtained with other hydrophobic antioxidants such as β-carotene and curcumin (Acosta, 2012; Anand et al., 2010; Salvia-Trujillo et al., 2013; Takahashi et al., 2009).

Under this scenario, the success of the establishment of nanocarriers loaded with specific bioactive molecules as food or beverage supplements depends on:

1. Our capacity to tune up the surface of the nanostructure to avoid the premature degradation/aggregation of the formulation and to selectively trigger the release of the cargo at the desired target (small intestine in our case). Among others factors, nanostructures are specially characterized by a high area/volume ratio (Israelachvili, 2010), this fact means that the design of the interface composition of the formulation is a key point to control not only the formulation process but also the colloidal behavior of the nanostructure once formulated (Israelachvili, 2010; Santander-Ortega et al., 2006, 2012). The interaction of the lipid nanostructures with the surrounding medium can be controlled by the superficial charge of the particles through a repulsion electrostatic potential of interaction, or by the incorporation of non-ionic polymers that will avoid the aggregation of the particles by the presence of electrolytes through a steric repulsion potential (Israelachvili, 2010). The proper coating of the lipid nanostructure with non-ionic polymers will also modulate the interaction of the particle with the macromolecules present in the physiological fluids, such as the lipases responsible of the system digestion in the small intestine (Santander-Ortega et al., 2006, 2009; Tobio et al., 2000).
2. Our capacity to design lipid nanostructured matrices able to properly accommodate the bioactive molecule (hydrophobic antioxidants in our case). It is already reported that the lipid matrix of nanostructures will affect the loading capacity of the formulation, the hydrodynamic mean size of the system and the bioavailability of the antioxidant (Li et al., 2012; Saberi et al., 2013; Salvia-Trujillo et al., 2013; Yang and McClements, 2013). In this line, compared to medium chain triglyceride oils (MCT), long chain triglyceride oils (LCT) have shown excellent properties to encapsulate and improve the bioavailability of α-tocopherol a lipophilic antioxidant with proved neuroprotective properties (Eitenmiller and Lee 2005; Yang and McClements, 2013).
3. Additionally, as food supplements, all the raw materials should be of food grade and the incorporation of the nanostructures to the food product should not significantly increase the final market price.

Bearing this in mind, the *in vitro* analysis of the effect of the interface composition of a lipid nanostructure was proposed as an improvement of the *in vivo* bioavailability of therapeutic antioxidants with already demonstrated neuroprotective properties. In order to achieve this aim three different oil nanoemulsions that share the same LCT oil matrix composed by soybean oil, which could help to improve the bioavailability of hydrophobic antioxidants were designed (Yang and McClements, 2013). The first oil nanoemulsion (SB-NE) has been formulated with an interface composed only by lecithin, a well-known emulsifier able to facilitate the formation of colloidally stable nanometric oil droplets thanks to an electrostatic repulsion potential (Israelachvili, 2010; Klang and Valenta, 2011; Santander-Ortega et al., 2010; van Nieuwenhuyzen and Szuhaj, 1998). It is important to highlight that this emulsifier can also modulate the interaction of pancreatic enzymes with the inner oil core of the nanoemulsion and then control the digestion of the oil droplets in the small intestine (Mun et al., 2007; Torcello-Gomez et al., 2011a; Wulff-Perez et al., 2010). The other two types of soybean nanoemulsions have been formulated using lecithin plus PF68 (SB-NE PF68) or PF127 (SB-NE PF127). These two non-ionic surfactants share the same poly(oxyethylene)–poly(oxypropylene)-poly(oxyethylene) (POE_a–POP_b–POE_a) architecture but differ on the POE/POP block length. These differences will have an effect on both, the adsorption of Pluronic® onto the O/W interface of the nanostructure and its capacity to modulate the interaction of the nanostructure with the surrounding medium (Santander-Ortega et al., 2009; Torcello-Gomez et al., 2013; Wulff-Perez et al., 2012). This effect was analyzed when the role of the interface composition of the formulation on the colloidal behavior of the systems after oral intake was evaluated, thus achieving the first statement. In order to address the second one, the encapsulation capacity of the nanoemulsions initially for three model hydrophobic antioxidants, *i.e.*, gentisic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid, with interesting neuroprotective properties was analyzed (Garrido et al., 2012; Joshi et al., 2006). Next, the concept bioaccessibility was introduced, which is the fraction of antioxidant in the mixed micelles and vesicles after the digestion of the lipid nanostructure in the small intestine (Li et al., 2012; McClements and Xiao, 2012; Mun et al., 2007; Saberi et al., 2013; Salvia-Trujillo et al., 2013; Yang and McClements, 2013)). Therefore, the capacity of the soybean nanoemulsion to improve the bioaccessibility of hydrophobic antioxidants with neuroprotective capacity as a function of the interface composition was studied.