Food Research International 52 (2013) 64–74

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Potential applications of multiple emulsions in the development of healthy and functional foods

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ARTICLE INFO

Article history:
Received 18 December 2012
Accepted 21 February 2013

Keywords:
Multiple emulsions
Functional foods
Improving fat content
Encapsulation
Bioactive compounds
Sodium reduction

ABSTRACT

Recent advances in food and nutrition sciences have highlighted the possibility of modulating some specific physiological functions in the organism through food intake. The beneficial effects of functional foods derive from dietary active compounds, and therefore the design and development of these foods require strategies to control their presence. Thanks to their characteristics, multiple emulsions can be used to induce qualitative and quantitative changes in food composition. This review provides an overview of the specific developments and food applications of multiple emulsions ($W_1/O/W_2$) as a technological strategy to modulate the presence of active dietary compounds for the development of healthier foods including functional foods. It discusses food-grade $W_1/O/W_2$ emulsion applications to: a) improve fat content, both by reducing fat (calories) content and providing healthier fatty acid profiles; b) encapsulate (protect) bioactive compounds such as minerals, carotenoids, vitamins, microorganisms, lactoferrin, phenolic compounds, amino acids and oils; and c) reduce sodium content.

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1. Introduction

The role of dietary active compounds in human nutrition is one of the most important areas of concern and investigation in the field of nutritional science. The findings of research on this subject have

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0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.02.040

wide-ranging implications for consumers, health-care providers and nutrition educators as well as food producers, processors and distributors (FAO, 2007). New scientific evidence concerning the benefits and risks associated with particular aspects of dietary compounds is constantly emerging. The potential effects of nutrients and other components in the diet has led to the realization that it is possible to create food items with specific characteristics that are capable of influencing body function over and above meeting basic nutrition needs. Foods that have been satisfactorily shown to improve the state of health and wellbeing and/or to reduce the risk of disease, are denominated functional foods (Diplock et al., 1999); in fact they are currently an expanding market and one of the chief factors driving the development of new products. With consumers increasingly looking for healthy foods with added value, the most successful products are those which are able to claim an added health benefit.

Since the beneficial effects of functional foods derive from dietary active compounds (functional components), the design and development of these foods require strategies for defining/optimizing their presence, either by increasing the proportion of those that exhibit beneficial effects, or else limiting the content of others that have negative implications for health. Different strategies (technological or biotechnological) for production systems (raw materials from animal or vegetal sources), and for preparation, storage, and distribution or consumption processes, can be implemented to induce qualitative and quantitative changes in food composition and to optimize beneficial properties. With these strategies it is possible to introduce changes in the amounts and types of functional components with different potential implications for human health. Among the technological strategies used to design and develop functional foods are those based on changes in food transformation systems. The greatest versatility in the modification of a food's composition can be achieved thanks to the wide range of options for changing the ingredients used in their preparation, and consequently the presence of different bioactive compounds. With this strategy a number of approaches can be used to remove, reduce, increase, add and/or replace different components with physiological activity (Jiménez-Colmenero, 2007). The modification of the food formulation process also makes it possible to use traditional ingredients, and other ingredients specifically designed with certain attributes (nature or composition) that confer healthy properties. In this context the use of multiple (double) emulsions looks especially promising.

Multiple emulsions are multi-compartmentalized systems in which oil-in-water (O/W) and water-in-oil (W/O) coexist, where the globules of the dispersed phase themselves contain even smaller dispersed droplets (Garti, 1997). The most common forms are water-in-oil-in-water (W/O/W), but oil-in-water-in-oil (O/W/O) emulsions can also be used in specific applications. Water-in-oil-in-water emulsions consist of minute water particles (W_1) dispersed inside fat globules (O), which are dispersed in turn in a continuous aqueous phase (W_2) (Fig. 1). What we have, then, is a system ($W_1/O/W_2$) comprising three phases, two aqueous (one inner and another outer, generally with different compositions) and a lipid phase located between them and separated by two types of interphases which are stabilized by means of hydrophilic and lipophilic surfactants.

Potentially, W/O/W emulsions have some advantages over conventional O/W emulsions such as delivery systems for bioactive lipids and for encapsulation, protection and release of hydrophilic components (McClements, Decker, & Weiss, 2007). Because of their properties, among these is the ability to trap and protect various substances and control their release from inside one phase to another, these emulsions have been used as a means of micro-encapsulation in pharmacology (carriers for anti-cancer agents, hormones, steroids, etc.), cosmetics (easy application of creams with encapsulated compounds) and other industrial uses (Benichou, Aserin, & Garti, 2004; Kukizaki & Goto, 2007; Muschiolik, 2007). Double emulsions offer a means of preparing micro- and nano-capsulates (in solid or semi-solid form) containing hydrophilic and lipophilic compounds (Benichou et al., 2004). Multiple emulsions may offer some advantages for food applications, since it has been found to be a potentially useful strategy for producing low calorie and reduced fat products, masking flavors, preventing oxidation, and improving sensory characteristics of foods, or controlling the release of and protecting labile ingredients during eating and digestion (Benichou et al., 2004; Dickinson, 2011; McClements et al., 2007; Muschiolik, 2007). Since multiple emulsions offer the opportunity to enclose nutritional and bioactive compounds, and these emulsions could be used as food ingredients, they offer an interesting approach among the technological strategies used to optimize dietary active components in new food systems such as functional foods.

Numerous research articles have highlighted the potential for applying multiple emulsions in new food systems. However, most of the research has focused on the design, formation, structure and properties of the actual emulsion as affected by different variables associated with composition and preparation procedure, in an attempt to overcome the problems associated with the production of stable multiple emulsions. In the context of development of healthier foods (including functional foods), multiple emulsions have been reviewed as part of a wide variety of structured delivery systems mainly for the encapsulation of functional components (McClements, Decker, Park, & Weiss, 2009; McClements et al., 2007), with less attention to specific food applications. This article offers a detailed review of the specific possibilities and food applications of multiple emulsions as a technological strategy to incorporate dietary active compounds for the development of healthier foods, including functional foods. It discusses food-grade $W_1/O/W_2$ emulsion applications to improve fat content, to encapsulate bioactive compounds, and to reduce sodium content. This review does not deal with studies focusing on emulsification technology or the properties, stabilization and transport

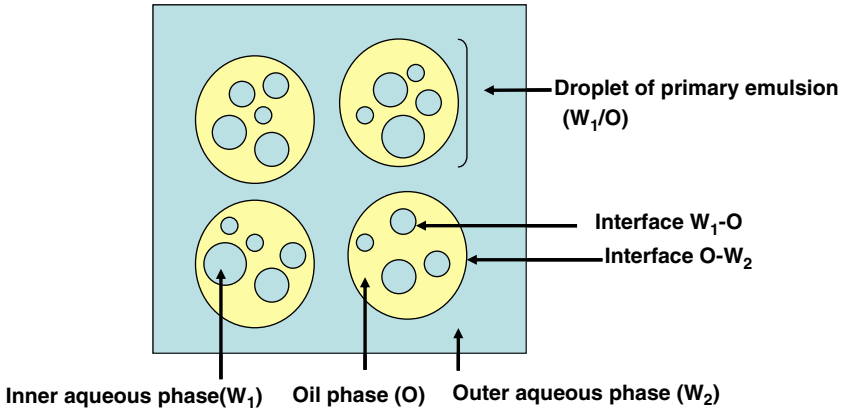

Fig. 1. Schematic representation of multiple emulsions ($W_1/O/W_2$).

Table 1
Examples of $W_1/O/W_2$ emulsions prepared for characterization and encapsulation of bioactive compounds.

Main purpose	Content of inner aqueous solution (W_1)	Lipid oil (O)	Lipophilic emulsifier (% in oil phase)	Hydrophilic emulsifier (% in external aqueous solution, W_2)	Reference
Formation of multiple emulsions	PTSA	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (1–10%)	Decaglycerol monolaurate	Shima et al., 2004
Encapsulation of *Lactobacillus acidophilus*	MRS broth containing the washed bacteria	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (10%)	Decaglycerol monolaurate (3%)	Shima et al., 2006
Encapsulation of *Lactobacillus acidophilus*	MRS broth containing the washed bacteria	Octanoic acid triacylglycerol	Hexaglyceryl condensed ricinoleate (10%)	Decaglycerol monolaurate (3%)	Shima et al., 2009
Encapsulation of vitamin B_{12}	Vitamin B_{12} (1%), and NaCl (0.22%)	Tripalmitin	Tetraglycerin condensed ricinoloeic acid ester (10%)	Decaglycerin condensed stearic acid ester (1%)	Kukizaki & Goto, 2007
Encapsulation of aroma compounds	Aromatic compound (0.025%) and glycerin	Rapeseed oil	Span™ 80 (3%)	WPC, SC, skim milk power, soy protein, modified starch, maltodextrin, and arabic gum the $W_1/O/W_2$ were spray dried	Brückner, Bade, & Kunz, 2007
Encapsulation of carotenoids	Gellan gum, Panodan SDK and saponified oleoresin	Commercial blend of sunflower–canola–cartamo oils with oleoresin	PGPR (6.4%)	Gum arabic (17%), mesquite gum (66%), and maltodextrin (17%) the W/O/W were spray dried	Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, & Vernon-Carter, 2004
Formation of multiple emulsions	NaCl (0.1 M), gelatin (5%), and Poly R-478 (0.5%)	Sunflower oil	PGPR (4%)	WPI	Scherze, Knöfel, & Muschiolik, 2005
Encapsulation of L-tryptophan	NaCl (0.1 M), gelatin (5%), and L-tryptophan (0.05 M)	Semicrystalline oils (different oils with high melting points)	PGPR (2–8%)	WPI (1%). the $W_1/O/W_2$ were embedded in alginate gels	Weiss et al., 2005
Encapsulation of lactoferrin	Lactoferrin with sodium lactate, and sodium bicarbonate	22% butter fat, 78% corn oil	PGPR (0.1%)	WPI (30%) + 0.02% xanthan gum the $W_1/O/W_2$ were freeze-dried	Al-Nabulsi, Han, Liu, Rodriguez-Vieira, & Holley, 2006
Formation of multiple emulsions	Sodium caseinate (0.5%), and Poly R-478	Soybean oil	PGPR (0.5–8%)	SC (0.03–2%), phosphate buffer (0.1 M),	Su et al., 2006
Formation of multiple emulsions	0 and 15% WPI, without and with heating	Corn oil	PGPR (8%)	Tween 20 (0.5%)	Surh, Vladisavljevic, Mun, & McClements, 2007
Encapsulation of vitamin B_{12}	0.1 M NaCl + 5% gelatin + 1.0% vitamin B_{12}	MCT	PGPR (2, 8%)	0.7% SC or 2.1% SC–dextran conjugates	Fechner et al., 2007
Formation of multiple emulsions	SC (0.5%) or gum arabic, and Poly R-478	Soybean oil	PGPR (0.5–2%)	SC (0.5%) or gum arabic (0–15%)	Su, Flanagan, & Singh, 2008
Encapsulation of vitamin B_{12}	5% gelatin (hydrated) + NaCl 0.6% + vitamin B_{12} 0.03%	MCT	PGPR (2%)	SC (1% protein)	O'Reagan & Mulvihill, 2009
Encapsulation of magnesium	0.1 M Mg Cl_2	Olive oil, rapeseed oil, and MCT	PGPR (5%)	0.3 M lactose + 12% SC	Bonnet et al., 2009

Encapsulation of iron	WPI (15%), with 0.1% Fe	Corn oil	PGPR (8%)	Tween 20 (0.5%)	Choi et al., 2009
Encapsulation of vitamin C	Glucose (11.9%), NaCl (4.4%) and WPI. Sodium ascorbate (1–30%)	MCT R(+) limonene	PGPR (10%)	WPI/pectine (4/0.5%)	Lutz et al., 2009
Encapsulation of Ca	Calcium salts	Sunflower oil	PGPR (0.5, 1,0 2.0%)	Soybean milk containing 0.02% xanthan gum	Márquez & Wagner, 2010
Encapsulation of resveratrol	0.25% resveratrol in sol of 0.1 M NaCl, ethanol, and 2.5% WPI	Canola oil	PGPR (8%)	0.5% SC, and 0.1 M NaCl	Hemar et al., 2010
Formation of multiple emulsions	0.28 M NaCl	Sunflower oil	PGPR (4%)	Tween 20 + glucose	Pawlik, Cox, & Norton, 2010
Encapsulation of vitamin B_{12}	Hydrating gelatin (5%), NaCl (0.06%), and vitamin B_{12} (0.03%)	MCT	PGPR (2%)	SC or SC–maltodextrin conjugate	O'Reagan & Mulvihill, 2010
Formation of multiple emulsions	PTSA	Soybean oil	PGPR (4–8%)	WPI (2–6%)	Mun et al., 2010
Encapsulation of Mg	$MgCl_2$, hemimagnesium salt, phosvitin, gluconate, and lactose	Olive oil	PGPR (5%)	SC (12%), and lactose (0.2 M)	Bonnet, Cansell, Placin, David-Brian, et al., 2010
Encapsulation of Mg	$MgCl_2$ (0.1 M), lactose (0.02 M–0.2 M), and SC (1.9%)	Olive oil, and MCT	PGPR (5%)	SC (1.9–12.0%), and lactose (lactose 0.20 or 0.3 M)	Bonnet, Cansell, Placin, Anton, et al., 2010
Encapsulation of magnesium	0.1 M Mg Cl_2	Olive oil, and MCT	PGPR (5%)	Lactose (0.3 M), and SC (3.1–12%)	Bonnet, Cansell, Placin, Monteil, et al., 2010
Formation of multiple emulsions	D-glucose (40%) and NaCl (8%)	Sunflower oil	PGPR (1–6%)	Gum arabic (20%), xanthan gum (0.025–1.0%), and D-glucose	Leal-Calderón, Homer, Goh, & Lundin, 2012
Formation of multiple emulsions	Gelatin (0–10%) and NaCl (0–8%)	Canola oil	PGPR (6%)	Polysorbate 80	Sapei, Naqvi, & Rousseau, 2012
Encapsulation of vitamin E and vitamin B_2	Vitamin B_2 (0.5 mg/mL)	Soy oil containing vitamin E (20%)	PGPR (8%)	WPI (10%), polysaccharides (0.2–1%), methoxyl peptin, -carrageenan	Li et al., 2012
Encapsulation of anthocyanin extract	Anthocyanin extract, pectin and $CaCl_2$	Rapeseed oil	PGPR (2.5%)	Bile extract, whey protein isolate or protein–polysaccharide-conjugate with peptin amide	Frank et al., 2012
Encapsulation of glucose and B_1	Glucose (2%) + glycerol B_1 (1%)	MCT	PGPR (8%) + monoglyceride oleate (2%)	WPI (5%)-polysaccharide complexes (xanthan gum 0.5–6%, guar gum and locust bean gum)	Benichou et al., 2007
Encapsulation of ferrous bisglycinate	Ferrous bisglycinate (30%)	Mineral oil (food grade)	5% of PGPR/Panodan SDK® (6:4)	WPC:polysaccharides (gum arabic or mesquite gum or low methoxyl peptin) 2:1	Jiménez-Alvarado et al., 2009
Encapsulation of *Lactobacillus rhamnosus*	Sweet whey containing 8.02 log cfu/mL	Canola oil	8% (1 part of Panodan SDK® + 4 parts of PGPR)	Sweet whey or concentrate of sweet whey dispersions	Pimentel-González et al., 2009
Encapsulation of ascorbic acid	Ascorbic acid (30%), gellan gum (0.5%), and Panodan SDK® (8%)	Chia oil	6% (1 part of Panodan SDK® + 4 parts of PGPR)	Mesquite gum, maltodextrin, and WPC	Carrillo-Navas et al., 2012

MCT: medium chain triglyceride oil; Panodan SDK: esters of monoglycerides and diglycerides of diacetyl tartaric acid; PGPR: polyglycerol polyricinoleate; Poly R-478: water soluble dye marker; PTSA: 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt, used a marker; SC: sodium caseinate; WPC: whey protein concentrate; and WPI: whey protein isolate.

phenomena in multiple emulsions. There are various other reviews that deal with those aspects (Benichou et al., 2004; Garti, 1997; McClements et al., 2007; Muschiolik, 2007; van der Graaf, Schroën, & Boom, 2005).

2. Preparation of multiple emulsions

There are several methods for preparation of multiple emulsions, but most entail a double emulsifying process, since that is the best procedure for achieving stable, well-defined systems with a reproducible particle size. There is a first stage in which a water-in-oil emulsion (W_1/O) is formed by homogenizing an aqueous phase (W_1) and a lipid phase (O) in the presence of a lipophilic emulsifying agent capable of producing a W_1–O interface. In the second stage the W_1/O emulsion is homogenized with a new aqueous phase (W_2) with the help of a hydrophilic emulsifying agent (O–W_2 interface), thus producing a double emulsion ($W_1/O/W_2$). The homogenizing conditions are more energetic in the case of the primary emulsion (W_1/O), whereas the second step is carried out with less shear in order to avoid rupturing the internal droplets (W_1/O) (Garti, 1997). If homogenization is too mild, the resulting system is highly polydispersed, but if it is too intense, the encapsulation is less efficient.

The type and concentration of the surfactant affects the characteristics of primary and multiple emulsions. Several lipophilic emulsifiers have been used to form W/O primary emulsions, but polyglycerol polyricinoleate (PGPR, E 476) is by far the most commonly used (Tables 1 and 2). Although PGPR is apparently the most effective hydrophobic emulsifier available, its use is strictly regulated for many food product applications; moreover, its presence is readily detected as an unpleasant off-taste when more than 5% is added; therefore, reducing the use of or partially substituting PGPR with other food grade materials is desirable, especially when preparing multiple emulsions for functional food applications (Muschiolik et al., 2006). Two options have been assayed for this purpose: the use of various macromolecular materials (proteins and/or polysaccharides) in the inner aqueous phase to partially replace PGPR (Dickinson, 2011; Su, Flanagan, Hemar, & Singh, 2006), or the replacement of PGPR by natural emulsifiers (e.g. lecithin) (Knoth, Scherze & Muschiolik, 2005).

Two-step preparation of $W_1/O/W_2$ emulsions involves dispersal of the primary emulsion in the outer aqueous phase (W_2) with a secondary hydrophilic emulsifying agent (Tables 1 and 2), the nature and concentration of which affect the properties of the $W_1/O/W_2$ emulsion. Various types of emulsifying food proteins have been used for this purpose, such as whey proteins, sodium caseinate, etc., and also some hydrocolloid emulsifiers such as gum arabic, xanthan gum, modified starch, etc. (Tables 1 and 2). Various soluble polysaccharides (pectin, alginate, gellan, etc.) have also been added in the outer aqueous phase to act as stabilizing agents due to their thickening/gelling properties (Dickinson, 2011) and overcome the instability and release problem in multiple emulsions. Protein–polysaccharide conjugates (prepared by controlled heating), such as caseinate–dextran (Fechner, Knoth, Scherze, & Muschiolik, 2007) or caseinate–maltodextrin (O'Reagan & Mulvihill, 2010) have been used as secondary hydrophilic emulsifying agents (Tables 1 and 2).

3. Application of multiple emulsions in healthier food developments

Despite the above-mentioned possibilities for the use of multiple emulsions in foods, and the fact that several new food products based on double emulsions have been patented, such as salted creams (encapsulation of salt), aromatic mayonnaises, etc. (Garti, 1997), there are very few instances of actual application in new developments. This has been attributed largely to their thermodynamic instability, which means that multiple emulsions are highly susceptible to breakdown during storage or when exposed to environmental stresses commonly occurring in the food industry, such as mechanical forces, thermal processing, and chilling, or freezing (McClements et al., 2009). However, the properties of these delivery systems such as physical stability, must be considered at two different levels: the stability required as an intermediate food ingredient and the stability when incorporated into food matrix. As a food ingredient, the required stability varies according to the type of food and the processing conditions in each case; this often falls short of the stability required for other, different uses (e.g. pharmaceuticals or cosmetics) for which there are more references. When incorporated into food systems, the $W_1/O/W_2$ stability that is needed can vary with the type of food (solid, liquid, dairy products, meat products, etc.). In this regard, as well as meeting stability standards, this food ingredient should be compatible with the specific properties (flavor, texture, appearance, etc.) of the food matrix in which it is included. However, in most cases the $W_1/O/W_2$ emulsions that are formulated have not been incorporated in a food matrix, which means that their behavior in real foods is not known and hence neither is their impact on technological, sensory and microbiological properties of complex matrixes. At the same time, in order to study $W_1/O/W_2$ emulsion formation or improve their stability, non-food grade ingredients (such as emulsifiers, stabilizers, oil phase, etc., for non-food applications such as pharmaceutical or cosmetics) have been used in many cases, so that these emulsions are unsuitable for human consumption. All this undoubtedly rules out the application of many such results to real foods.

The location of bioactive compounds in multiple emulsions must be considered in order to understand their potential application as food ingredients in new edible systems. Bioactive components can potentially be located in a number of different molecular and physical environments within a $W_1/O/W_2$ emulsion (McClements et al., 2009). For instance, water-soluble compounds (minerals, vitamins, amino acids, peptides, fibers, etc.) can be included both in the inner aqueous phase (W_1), during formation of the primary W_1/O emulsion and in the outer phase (W_2), in the formation of the $W_1/O/W_2$. The hydrophilic bioactive ingredients in the inner phase (W_1) are trapped inside microcapsules with advantages (protection and controlled release of bioactive compounds and limitation of the effect of unwanted sensory attributes of these) which are useful for some food applications. Oil soluble components (n-3 polyunsaturated fatty acid-PUFA, conjugated linoleic acid-CLA, carotenoids, antioxidants, etc.) can be incorporated by dispersion in the oil phase. Moreover, the actual choice of lipid phase (for example oils rich in n-3 PUFAs or monounsaturated fatty acids-MUFAs) can serve as a strategy to favor the presence of functional ingredients. Additionally, surface-active functional components could be located at either the W_1–O interface or the O–W_2 interface (McClements et al., 2009).

There are three main reasons for considering $W_1/O/W_2$ emulsions for healthier food applications: to improve fat content, to encapsulate (protect) bioactive compounds and to reduce sodium content.

3.1. Improving the fat content of foods

Multiple emulsions can be used to modify qualitative and quantitative aspects of the lipid material in foods, improving their content through two main approaches: by reducing the fat content and by providing healthier fatty acid profiles. Encapsulation of oil in a double emulsion is considered in another section.

3.1.1. Multiple emulsions in fat (calorie) reduction of foods

In foods using oil-in-water emulsions, a $W_1/O/W_2$ emulsion can be used to reduce the fat content, since part of the lipid material is replaced by water particles dispersed inside it (Fig. 1). A double emulsion could be prepared with the same overall dispersed phase volume fraction and droplet size distribution as a conventional oil-in-water emulsion, but with reduced fat content. Consequently, it should be possible to produce reduced-fat products with similar physicochemical and sensory properties as full-fat products (Garti, 1997; McClements et

Table 2
Examples of utilization of $W_1/O/W_2$ multiple emulsions for food applications.

Main purpose	Content of inner aqueous solution (W_1)	Lipid oil (O)	Lipophilic emulsifier (% in oil phase)	Hydrophilic emulsifier (% in external aqueous solution, W_2)	Reference
Reduced-calorie food emulsion	NaCl (0.1 M)	Olive oil	Glycerol mono-dioleate (3%), and sucrose palmitate-stereate (5%)	Sucrose palmitate–stereate (3%)	De Cindio & Cacace, 1995
Reduced-fat white fresh cheese-like products	Gellan gum (0.1%), and Panodan SDK® (1.6%)	Canola oil	PGPR (6.4%)	Different biopolymers of gum arabic, carboxymethyl cellulose and low-methoxyl pectine	Lobato-Calleros et al., 2006
Reduced-fat white fresh cheese-like products	Gellan gum (0.1%), and Panodan SDK® (1.6%)	Canola oil	PGPR (6.4%)	Different biopolymers of gum arabic, carboxymethyl cellulose and low-methoxyl pectine.	Lobato-Calleros et al., 2008
Formulation of low-fat stirred yogurt	8% Panodan SDK® + 0.5% gellan gum	Canola oil	PGPR (8%)	Skim milk with amidated low-methoxy pectin (1%) or carboxymethyl cellulose (0.5%)	Lobato-Calleros et al., 2009
Whipped dairy cream with encapsulated Ca	Calcium salts	Sunflower oil	PGPR (0.5, 1.0 and 2.0%)	Soybean milk containing 0.02% xanthan gum	Márquez & Wagner, 2010
Improve fat content of meat systems	NaCl 0.6%	Olive oil	PGPR (6%)	Sodium caseinate (0.5%) or whey protein concentrate (6%)	Cofrades et al., in press

PGPR: polyglycerol polyricinoleate.

al., 2009). However, there has been very little use of multiple emulsions as fat replacers (food ingredients) in reformulation processes to produce low-fat (low-calorie) foods (Table 1). These studies have placed special emphasis on analyzing the influence of multiple emulsions on rheological and structural parameters of foods, with less attention to other aspects which are essential in order to gage the real possibilities of this strategy and its technological and sensory viability. In this connection, lactic analogs (cottage cheeses, yogurts) have been prepared by replacing milk fat with multiple emulsions (canola oil) and improving their nutritional quality by reducing the fat and saturated fatty acid contents (Lobato-Calleros, Recillas-Mota, Espinosa-Solares, Álvarez-Ramírez, & Vernon-Carter, 2009; Lobato-Calleros, Rodríguez, Sandoval-Castilla, Vernon-Carter, & Álvarez-Ramírez, 2006). Reduced-fat (range 15–26%) cheese-like products have been formulated with $W_1/O/W_2$ emulsion (stabilized with hydrocolloids), closely emulating some of the textural characteristics and showing similar preference scores to their full milk-fat counterparts (Lobato-Calleros et al., 2008). Márquez and Wagner (2010) reported that a double emulsion can work as a reduced-fat substitute for whipped dairy cream. The more promising way of reducing calorific content is with designer emulsion-based delivery systems (such as multiple emulsions) prepared using a non-digestible lipid phase (e.g. mineral oils) (McClments & Li, 2010). Non-digestible fat substitutes have been used in fat reduction strategies.

Unlike the multiple emulsions for use as food ingredients being referred to above, these systems could be formed directly on the food matrix. It is well known that comminuted meat products like frankfurters consist of oil droplets suspended in an aqueous phase of aggregated biopolymer molecules (conventional oil-in-water emulsion). Ritzoulis, Petridis, Derlikis, Fytianos, and Asteriou (2010) studied the use of water-in-oil emulsions (W_1/O) prepared with lard and diglycerides as a lipophilic emulsifier, to replace pure fat (lard) in model frankfurter sausage. During sausage manufacture the new previously-prepared oil droplets (W_1/O) were suspended in the aqueous phase (with soluble meat protein as a hydrophilic emulsifier) forming a $W_1/O/W_2$ emulsion, within meat matrix. Further heating (producing meat protein gelification) originated a gel/emulsion system. In these conditions, the substitution of fat globules by inverse water-in-fat globules was found to imitate a full-fat sausage adequately in mechanical tests.

3.1.2. Multiple emulsions for improving food fatty acid profiles

Because of their health implications, lipids are among the bioactive components (functional ingredients) that have received the most attention, particularly (in quantitative and qualitative terms) with respect to the development of healthier foods. Recommendations for optimal intake of total and unsaturated fatty acids have been proposed by a number of scientific authorities and nutritional organizations including the World Health Organization (WHO, 2003). In response to these considerations, numerous researchers are endeavoring to optimize the lipid contents and the fatty acid profiles of various foods in order to achieve a composition more in line with nutrient intake goals. For instance, oil (from plant and marine origin)-in-water emulsions have been used as animal fat replacers to produce healthier lipid content and lipid profile formulations in meat-based functional foods (Jiménez-Colmenero, 2007). In this regard, the choice of the most suitable lipid phase in multiple emulsions is a promising approach to modulating their presence in foods so as to obtain products more in line with health recommendations: i.e., with smaller proportions of saturated fatty acids (SFAs) and larger proportions of MUFAs or PUFAs (especially long chain n-3 PUFAs), better n-6/n-3 PUFA and PUFA/SFA ratios, and generally cholesterol-free.

Many of the studies on the formation and characterization of multiple emulsions have used mineral oils, hydrocarbon solvents, and to a lesser extent vegetable oils, as a lipid phase. Although some vegetable oils like canola, olive, sunflower, chia, etc., have been used to form $W_1/O/W_2$ emulsions (Table 1), their main function has been to act as a dispersed phase (Bonnet et al., 2009). However, lipid phases have also been considered for their potential health implications. Thus in addition to reducing fat content, the replacement of fats (for example animal fats) normally present in some foods (for example meat and dairy products) with multiple emulsions formulated with oils having healthier lipid profiles opens up new prospects for the development of potentially functional foods. But although a wide variety of oils of plant (olive, sunflower, linseed, chia, etc.) and marine (fish and algae) origin could be used in the preparation of multiple emulsions, only a fraction of them have been used (Table 1).

Olive oil is the most monounsaturated vegetable oil and has a high biological value, attributed to a high ratio of vitamin E to polyunsaturated fatty acids. Olive oil intake is associated with a wide range of health benefits, lessened risk of heart disease and breast cancer, and it has positive effects on colon cancer. Also, it has beneficial effects on postprandial lipid metabolism and thrombosis and inhibits low-density lipoprotein (LDL) oxidation (López-Miranda, Pérez-Martínez, & Pérez-Jiménez, 2006). Olive oil has been used in multiple emulsions as a lipid phase in the primary emulsion (Table 1) because of its advantages, both as a source of oleic acid (Bonnet, Cansell, Placin, David-Brian, et al., 2010) and for its technological properties (faster Mg release) in $W_1/O/W_2$ emulsions as compared with other oils (Bonnet et al., 2009). Cofrades, Antoniou, Solas, Herrero, and Jiménez-Colmenero (in press) reported that $W_1/O/W_2$ emulsions prepared with olive oil, PGPR as a lipophilic emulsifier and sodium caseinate and whey protein concentrate as hydrophilic emulsifiers showed good stability for longer than is usually required by the meat industry for use as a food ingredient in product reformulation.

When used as animal fat (pork back fat) replacers, they affect the physicochemical properties (water and fat binding properties, texture, and color) of gel/emulsion meat systems.

A double emulsion, using sunflower as a lipid phase, can work as a reduced-fat substitute for whipped dairy cream, with the benefit of being free of cholesterol and rich in unsaturated fatty acids (Márquez & Wagner, 2010). Chia (*Salvia hispanica* L.) contains the richest botanical source of α-linolenic acid known but does not contain any of the antinutritional compounds (total linamarin, linustatin, and neolinustatin) or vitamin B_6 antagonist factors present in other commercially-available sources of α-linolenic acid (Ayerza & Coates, 2005). Several studies have shown that n-3 PUFAs produce health benefits, establishing a cause–effect relationship between the dietary intake of α-linolenic acid and the reduction of blood cholesterol concentration (EFSA, 2009). Chia oil has been used as a lipid phase in the primary emulsion to improve the lipid profile of $W_1/O/W_2$ emulsions (Carrillo-Navas et al., 2012).

3.2. Encapsulation of functional components

$W_1/O/W_2$ emulsions have been used to encapsulate bioactive compounds in the inner aqueous phase (Table 1), although generally without further application of these new systems in food developments. Numerous advantages of encapsulation of hydrophilic bioactive compounds by means of $W_1/O/W_2$ emulsions have been reported (McClements et al., 2009), including: a) functional ingredients can be trapped inside the inner water droplets and released at a controlled rate or in response to specific environmental triggers e.g., in the mouth, and stomach, or small intestine; b) functional ingredients can be protected from chemical degradation by isolating them from other water-soluble ingredients that they might normally react with; c) water-soluble functional ingredients that have undesirable sensory qualities (e.g., bitter, astringent, or metallic flavors) can be trapped within the inner aqueous phase so that these undesirable sensory attributes are not perceived in the mouth during mastication.

The following is a description of the use of multiple emulsions for micro-encapsulation of functional components such as: minerals, vitamins, amino acids, polyphenolic compounds, probiotics etc. In some cases these encapsulated compounds have been used as a model species to test the release mechanism in these systems, for example Mg (Bonnet, Cansell, Placin, David-Brian, et al., 2010) and vitamin B_{12} (Fechner et al., 2007; O'Reagan & Mulvihill, 2009).

3.2.1. Minerals

Calcium is an essential mineral for bone growth and dental health. Growing concern about bone health in people of all ages has prompted the food industry to respond by adding calcium to foods and beverages. Since the concentration of calcium is much lower in soybean than in cow's milk, calcium fortification of soybean milk has been considered to overcome this deficiency. However, this possibility is limited because the cation tends to bind to the soybean proteins and phospholipids, leading to the formation of aggregates and destabilization of the system. Multiple emulsions have been used as a means of isolating the Ca and avoiding those undesirable interactions (Table 1). In this connection Márquez and Wagner (2010) reported the preparation of $W_1/O/W_2$ emulsions with different calcium solutions in the inner aqueous phase and with soybean milk (along with xanthan gum as a hydrophilic emulsifier in the outer aqueous phase), polyglycerol polyricinoleate (PGPR) as a lipophilic emulsifier and sunflower oil as an oil phase. These emulsions have been reported to be suitable as a whipped dairy cream substitute with considerable calcium input.

Iron deficiency produces anemia, which is one of the principal public health problems, affecting a quarter of the world's population in both industrialized and developing countries. In this context ferrous bisglycinate has considerable potential as an iron food fortificant because its absorption in humans is not limited by the action of phytates or polyphenols. Ferrous bisglycinate supplementation is usually done by incorporating it directly in food systems as a solution, but there are two drawbacks: ferrous bisglycinate interacts with other food components, altering the taste of foods, which is detectable to consumers, and it is readily oxidized (Jiménez-Alvarado, Beristain, Medina-Torres, Román-Guerrero, & Vernon-Carter, 2009). In order to reduce or prevent these negative effects, ferrous bisglycinate has been entrapped in the inner phase of $W_1/O/W_2$ emulsions (Table 1), using mineral oil as a lipid phase, a mixture of lipophilic/hydrophilic emulsifiers (PGPR/Panodan SDK®) to form the primary emulsion, and stabilized with protein/polysaccharide complexes in the W_2 aqueous phase (Jiménez-Alvarado et al., 2009). Or again, since iron is one of the major pro-oxidants in foods, multiple emulsions have been used as an alternative strategy for physically isolating iron from an emulsified oxidatively unstable lipid substrate (Choi, Decker, & McClements, 2009). The iron (Fe^{3+}) was encapsulated within the inner aqueous phase of a primary emulsion prepared with corn oil as a lipid phase, and PGPR and Tween 20, as lipophilic and hydrophilic surfactants respectively (Table 1). Surprisingly, mixed fish oil and $W_1/O/W_2$ emulsions containing iron in the inner aqueous phase were less oxidatively stable than the mixed emulsions containing iron in the outer aqueous phase (Choi et al., 2009).

Magnesium plays a role as a physiological modulator affecting muscular contraction, cardiovascular function, and nerve impulse transmission. Due to a change in nutrition habits, the daily intake of magnesium is lower than the recommended value. Thus, magnesium supplementation of food could be an alternative to prevent magnesium deficiency and associated clinical disorders, for example hypertension, cardiovascular diseases, muscular weakness, and diarrhea (Bonnet, Cansell, Placin, Anton, & Leal-Calderón, 2010). However, magnesium addition in foods can induce chemical degradation and protein aggregation and generate an unpleasant taste. These drawbacks could be avoided or at least reduced by encapsulation of magnesium ions in multiple emulsions, which could be used in food applications (Bonnet, Cansell, Placin, David-Brian, et al., 2010; Bonnet et al., 2009). Magnesium has been encapsulated in the inner aqueous droplets of $W_1/O/W_2$ emulsions using triglyceride oils such as olive oil, rapeseed oil and medium chain triacylglycerols (lipid phase) and polymeric surface-active species such as sodium caseinate (SC)/peptin (hydrophilic emulsifier) and PGPR (lipophilic emulsifier). The use of chelating agents (phosvitin, SC) in the inner aqueous droplets has been reported to improve the encapsulated magnesium species in $W_1/O/W_2$ emulsions (Bonnet, Cansell, Placin, David-Brian, et al., 2010). On the other hand, when the emulsions were placed in the presence of pancreatic lipase, the triglycerides composing the oil phase were rapidly hydrolyzed by the enzyme, suggesting that magnesium could be available at the intestinal site (Bonnet et al., 2009).

3.2.2. Carotenoids

Carotenoids such as α-carotene, β-carotene, γ-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin, commonly found in vegetables and fruits, are being investigated as promising candidates for prevention of cancer, heart disease, and aging effects. Carotenoids have been suggested as candidate food components (in functional food development) for modulation of target functions related to defense against reactive oxidative species (Diplock et al., 1999). The incorporation of oil-soluble and water-soluble carotenoids in foods has a wider range of uses, although this presents some problems associated with instability (they are very sensitive to oxygen, light, and heat) and coloration of foods. Multiple emulsions can be used to overcome these limitations. Microcapsules containing both oil- and water-soluble carotenoids have been obtained by spray-drying of $W_1/O/W_2$ emulsions (Rodríguez-Huezo et al., 2004). Water-soluble carotenoids were included in the inner aqueous phase (with gellan gum and Panodan SDK®), while oil-soluble carotenoids were localized in the lipid phase (a commercial blend of sunflower–canola–cartamo oils) using PGPR as a

lipophilic emulsifier (Table 1). These authors reported that microcapsules obtained by spray-drying of $W_1/O/W_2$ emulsions with high solids contents showed the highest microencapsulation efficiency, and highest total carotenoids retention.

3.2.3. Vitamins

Vitamins can be incorporated in multiple emulsions in different ways depending on their solubility. While water-soluble (B-complex and C) vitamins have been encapsulated in $W_1/O/W_2$ emulsions, O/W/O emulsions can be more suitable in the case of lipid-soluble (A, D, E, and K) vitamins. Because of their known health implications, vitamin enrichment is a well-established strategy in the development of functional foods (Diplock et al., 1999).

Vitamin C can protect tissues and cells against oxidative damage from free radicals and reactive oxygen (Diplock et al., 1999). Unfortunately, the stability of vitamin C is limited, affected as it is by factors such as high temperature storage, light, high pH values, and the presence of dissolved oxygen. Different procedures to protect vitamin C have been assayed for food application, including multiple emulsions (Carrillo-Navas et al., 2012; Lutz, Aserin, Wicker, & Garti, 2009; Su, 2008). Lutz et al. (2009) investigated the efficiency of sodium ascorbate encapsulation in multiple emulsions using PGPR (as a lipophilic emulsifier), two types of oils (medium chain triglycerides and R(+) limonene), and a blend of modified pectin with WPI. These authors reported the formation of stable multiple emulsions that were capable of releasing the entrapped molecules in a controlled pattern by manipulating the type of oil, the electrolytes and their concentrations, and using specific modified pectin to form a better interfacial film across the external interface. Su (2008) reported the stabilization of ascorbic acid in $W_1/O/W_2$ emulsions prepared using soybean oil, PGPR alone or in combination with SC as emulsifiers for primary water-in-oil emulsions and SC as the sole emulsifier for secondary emulsion. $W_1/O/W_2$ emulsions have been formulated incorporating ascorbic acid in the inner aqueous phase and chia oil (with PGPR) in the intermediate oil phase, and stabilizing the outer oil–water interface by associative adsorption of mesquite gum, maltodextrin and whey protein concentrate ternary blends (Carrillo-Navas et al., 2012).

The B vitamins work as co-factors in different enzyme systems in the body. Vitamin B_{12} has been suggested as a candidate food component (in functional food development) for modulation of target functions related to the cardiovascular system (Diplock et al., 1999). Multiple emulsions have been proposed as a strategy to encapsulate vitamin B_{12} (Fechner et al., 2007; O'Reagan & Mulvihill, 2009; O'Reagan & Mulvihill, 2010). A relatively low percentage encapsulation of vitamin B_{12} in double emulsion has been reported, since this compound is sufficiently small that it may rapidly diffuse from the inner aqueous phase across the intermediate oil phase into the outer aqueous phase (Fechner et al., 2007; O'Reagan & Mulvihill, 2010).

Food fortification has been considered as a long term strategy of choice in controlling thiamine deficiencies in populations with various health problems. Biopolymer adducts made of WPI–xanthan gum complexes have been used to stabilize double emulsions that contained entrapped vitamin B_1 in the inner aqueous phase (Benichou, Aserin, & Garti, 2007); the authors reported that the system could be used for protection and delivery of water-soluble nutraceuticals in foods and beverages.

Riboflavin (vitamin B_2) is an easily absorbed micronutrient with a key role in maintaining health in humans. Vitamin E acts as an antioxidant and protects the body's cells against damage (Diplock et al., 1999). Use of vitamin (B_2 or E)-fortified foods has been considered in attempts to increase vitamin intake. Lipid-soluble vitamin E and water-soluble vitamin B_2 have been encapsulated together in the same $W_1/O/W_2$ emulsion. Vitamin B_2 was encapsulated in the inner aqueous phase, while vitamin E was solubilized in the oil phase (soy oil combined with PGPR). Whey protein isolate (WPI)–polysaccharide complexes (hydrophilic emulsifiers) have the ability to retard vitamin B_2 release in simulated gastric conditions and undergo enteric release of vitamins in simulated intestinal conditions (Li et al., 2012). Stability and vitamin B_2 release from $W_1/O/W_2$ emulsions using kerosene oil, Span 80 and Tween 20, have been reported by Owusu, Zhu, and Dickinson (1992). The initial encapsulation yield was 92–98%, but the apparent yield stability decreased to 85–87% after heating. The authors conclude that processes of vitamin B_2 released from the multiple emulsions involve slow molecular diffusion through the intervening oil phase.

Vitamin A is essential for animal growth, the optical transduction system, immune system, and differentiation of epithelial tissue. Yoshida, Sekine, Matsuzaki, Yanaki, and Yamaguchi (1999) reported that an $O_1/W/O_2$ emulsion formulated with organophilic clay mineral is an effective carrier for stabilizing vitamin A, for cosmetic and pharmaceutical applications.

3.2.4. Microorganisms

Lactic acid bacteria have received increasing attention because of their beneficial effects for the health of their host (as probiotics), but their viability is affected by processing, storage, and the various digestive processes of their host such as an acidic stomach solution and bile acids. Microencapsulation is the best-known technique for providing a protective environment for microorganisms under adverse conditions (Heidebach, Först, & Kulozik, 2012). Several studies have shown successful microencapsulation and coating of bacteria using various encapsulating materials and methods. An alternative method for protecting probiotics is their inclusion in a $W_1/O/W_2$ emulsion. The double emulsion may serve as a suitable wrapper to encapsulate and protect probiotic bacteria during food processing, storage and passage through the human gastrointestinal tract. In this connection, protection of *Lactobacillus acidophilus* JCM 1132 against cytotoxic gastric juice (Shima, Morita, Yamashita, & Adachi, 2006) and bile acids (Shima, Matsuo, Yamashita, & Adachi, 2009) has been reported by incorporating the bacteria in the inner-aqueous phase of a $W_1/O/W_2$ emulsion. Pimentel-González, Campos-Montiel, Lobato-Calleros, and Pedroza-Islas (2009) established the viability of entrapment of *Lactobacillus rhamnosus* in a double emulsion using sweet whey as a hydrophilic emulsifier, concluding that the double emulsion protected *L. rhamnosus* against simulated gastrointestinal tract conditions.

3.2.5. Lactoferrin

Lactoferrin, the main iron-binding glycoprotein present in the milk of mammals, inhibits many bacteria, fungi, and parasites. It also has antioxidant, antiviral, anti-inflammatory, immune-modulating, and anticancer properties, and can promote the growth of probiotic bacteria like *Bifidobacterium*. Since different food components can interfere with some of these activities, multiple emulsions have been used to protect lactoferrin from contact with agents like divalent cations that interfere with its antimicrobial activity (Al-Nabulsi et al., 2006). Microcapsules were prepared as water-in-oil (W_1/O) emulsions using an aqueous phase containing lactoferrin emulsified with an oil mixture of butter fat plus corn oil and PGPR. The $W_1/O/W_2$ emulsions were produced using an outer aqueous phase consisting of a denatured whey protein isolate (WPI) solution. The inhibitory activity of the lactoferrin was evaluated against the meat spoilage bacterium *Carnobacterium viridans*. The results demonstrated that microencapsulation can enhance the antimicrobial activity of lactoferrin.

3.2.6. Phenolic compounds

Resveratrol is a naturally occurring polyphenolic compound which has attracted much attention over recent years due to interest in its health benefits, including its antioxidant capacity, cardioprotection, anticancer activity, anti-inflammatory and other effects. However, resveratrol has limited solubility in water, is sensitive to oxidation and undergoes a light-induced conversion from the trans to the cis

isomer. Thus, it is important to develop encapsulation strategies to protect and target delivery of resveratrol. In this connection, the potential of multiple emulsions to encapsulate resveratrol for food applications has been reported by Hemar, Cheng, Oliver, Sanguansri, and Agustin (2010). Due to the poor solubility of resveratrol, different inner aqueous phases containing select components were used to enhance the retention of resveratrol. The primary emulsions were made with added canola oil containing PGPR and re-emulsified in water containing sodium caseinate and NaCl. Less than 10% of the total encapsulated resveratrol was released to the external continuous aqueous phase, demonstrating the potential of multiple emulsions to encapsulate resveratrol for food applications. Maisuthisakul and Gordon (2012) developed a system for emulsion stabilization based on formulations containing gum arabic, maltodextrin and alginate as coating materials in $W_1/O/W_2$ emulsions containing phenolic mango seed kernel extract. This extract is a good source of phenolic antioxidants with metal chelating and tyrosinase inhibiting activities.

Anthocyanins belong to the most important group of hydrophilic plant pigments and have strong antioxidant, anticarcinogenic and immune modulating effects. Outside their natural environment, these molecules are extremely unstable. Encapsulating them in multiple emulsions is one possible way to stabilize them for use in bioactivity studies or functional foods (Frank et al., 2012). Anthocyanin-rich bilberry extract has been encapsulated in an inner aqueous phase containing a gelling agent like peptin, PGPR as a lipophilic emulsifier, and various hydrophilic emulsifiers (bile extract, whey protein isolate and protein–polysaccharide-conjugate). Frank et al. (2012) reported that anthocyanins can be stabilized in the inner phase of double emulsions and released under gastrointestinal conditions. The release rate of free fatty acids during incubation is independent of the emulsifier used. The dominant release mechanism for entrapped matter is coalescence of the inner W_1-droplets with the surrounding W_2-phase.

3.2.7. Amino acids

L-tryptophan is an essential amino acid and its source is dietary only. It has been suggested as a candidate food component for modulation of target functions related to behavior and mental performance. Stability and L-tryptophan release from model $W_1/O/W_2$ emulsions based on kerosene oil, Span 80 and Tween 20 have been reported by Owusu et al. (1992). The release of encapsulated L-tryptophan from polysaccharide gels (alginate gel containing maltodextrin) with embedded multiple emulsions was studied by Weiss, Scherze, and Muschiolik (2005). L-tryptophan was enclosed within the inner aqueous phase of the $W_1/O/W_2$ emulsion prepared with semicrystalline oil phases (different concentrations and types of high melting point) and lipophilic surfactant PGPR in the oil phase. Differences in release of L-tryptophan could be ascribed to the composition (melting point) of the lipid phase, while the temperature reduction had a relatively high impact on L-tryptophan release.

3.2.8. Oils

In addition to the strategies described in Section 3.1, multiple emulsions have been used to improve fat content in food by encapsulating healthier oils. In this connection, orange oil has been encapsulated in the inner phase of an $O_1/W/O_2$ emulsion (O_1 orange oil, W water and O_2 rapeseed oil). In order to make this system suitable for use in dry mixes, it was secondarily coated with wall materials of lactose and caseinate using a spray drying technique (Edris & Bergnstahl, 2001). A delivery system with fish oil as the core of polymeric wheat gluten has been developed by microencapsulation of an $O_1/W/O_2$ emulsion and subsequent heat-polymerization. This encapsulation method was effective for protecting fish oil from oxidation (Liao, Luo, Zhao, & Wang, 2012).

3.3. Multiple emulsions in sodium reduction of foods

There is strong evidence that salt (sodium) consumption is currently the major factor increasing blood pressure and thereby cardiovascular disease. Since the reduction in population salt intake worldwide would produce a major improvement in public health (WHO, 2003), the ongoing challenge for food manufacturers is thus to generate products with reduced sodium levels while maintaining their sensory acceptability to the consumer. $W_1/O/W_2$ emulsions could be used as a strategy to reduce salt (sodium) in foods. Multiple emulsions can be designed to influence taste perception (for example, acid, salty, and bitter tastes) by modifying the extent to which the aqueous phase interacts with oral surfaces (Dickinson, 2011). These systems can be used in the food industry, where an outer aqueous phase is more acceptable in terms of palatability (Garti, 1997). Since only the salt contained in the outer aqueous phase of multiple emulsions will be perceived, the salt contained in the inner aqueous phase would not contribute to saltiness perception (Koliandris, 2010). So, if salt is only present in the outer aqueous phase of the $W_1/O/W_2$ emulsion, the perception of saltiness in the product could be greater than is justified by the actual salt content. Given such conditions, salt reductions of up to 80% have been postulated (Norton & Norton, 2010). Further research is needed to address this sodium reduction strategy.

4. Future research

Emerging interest in functional foods requires the technology to produce healthier foods. It is clear that multiple emulsions have great potential as delivery systems for functional food components, but there are still a number of important issues that need to be addressed before this technology can be successfully employed in the food industry. Most of the studies related to multiple emulsions preparation are not suitable for use in food applications because they are not easily scaled up, they are not cost effective or they may require the use of non-food grade ingredients making these double emulsions unsuitable for human consumption (O'Reagan & Mulvihill, 2010). When multiple emulsions are designed for food application – and more specifically in the development of healthier foods, as well as aspects relating to the presence of bioactive compounds – consideration must also be given to the nature and concentration of the components used in their formation, including the oil phase, the type of emulsifiers, or the inner and outer aqueous phases.

$W_1/O/W_2$ emulsions are prepared for use mainly as intermediate products (food ingredients), which can be used in the technological strategies habitually pursued to optimize the presence of bioactive compounds in foods. For this purpose, any properties required have to be established on two levels. As food ingredients, the required characteristics will depend on the management strategies used by the industry for the type of food (beverages, meat-based foods, dairy products, etc.) and processing conditions (heating, freezing, etc.). In any case the storage times and conditions will generally be very different from those employed in other non-comparable industrial uses (e.g. pharmaceuticals or cosmetics), for which there are more references. Also, once incorporated in a food matrix the multiple emulsions have to have compatible structures that help lend the product the desired characteristics, without presenting unwanted features in terms of technological and sensory viability.

Additionally, most researches have focused on the design, formation, structure and properties of $W_1/O/W_2$ emulsions themselves as affected by different variables in order to achieve specific properties (high stability and encapsulation efficiency), without considering their potential food applications. As a result, it is not known how they will behave in a food matrix or hence what impact they will have on the technological, sensory and microbiological properties of complex matrixes of real foods. A new step is therefore necessary to

determine the extent to which the food-grade $W_1/O/W_2$ emulsion technology that has been developed in recent years can be successfully used in food products, especially in healthier approaches.

Acknowledgments

This work was supported by projects AGL2011-29644-C02-01, Consolider-Ingenio 2010: CARNISENUSA (CSD2007-00016) and Plan Nacional de I+D+i.

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