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Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities.

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Abstract

Milk proteins are used to make emulsions, and maybe used to make nanoemulsions. Nanoemulsions are a nanotechnology with food applications, and possess superior physicochemical and sensorial properties compared to macro- and microemulsions. They are also able to deliver bioactive compounds when consumed. In this review, three aspects of food nanoemulsions will be examined: (1) the production and properties of food nanoemulsions, (2) emulsifiers/surfactant (ionic, non-ionic, phospholipid, polysaccharide, and protein) used in nanoemulsions production. The suitability of proteins and protein hydrolysates as nanoemulsifiers is discussed, with a particular focus on whey protein, (3) the potential of whey protein derived peptides as both emulsifiers and bioactive compounds in nanoemulsions delivery systems. Lastly, the potential delivery of bioactive peptides and other bioactive compounds within nanoemulsion systems is also discussed.

Keywords: Nanoemulsions; non-protein surfactants; whey protein and peptide emulsifiers; bioactive peptides; dual-functional peptides; nano-delivery systems
Highlights

- Nanoemulsions are a nanotechnology with food applications
- Whey protein peptides are potential emulsifiers in food nanoemulsions
- Bioactive peptides may act as functional and nutraceuticals agents
- Peptides serve a dual-functional role in foods as emulsifiers and bioactive compounds when consumed
- Nanoemulsions are delivery vehicles for bioactive peptides and compounds
1. Introduction

Milk proteins, and whey proteins in particular, are valued as important food ingredients because of their functional and nutritional properties (Christiansen et al., 2004; Sarkar et al., 2009), and have been extensively used as emulsifiers in foods (Chu et al., 2007; Dissanayake & Vasiljevic, 2009; Lee & McClements, 2010). Recent studies have proven the potential of whey protein ingredients as emulsifiers in nanoemulsions that have been tailored for food applications (Lee & McClements, 2010; Relkin et al., 2011; Shah et al., 2012). Nanoemulsions are a specific type of colloidal dispersion characterised by very small droplet sizes, usually covering the size range of 10–200 nm (Chu et al., 2007; Lee & McClements, 2010; Wulff-Pérez et al., 2009). Nanoemulsions enhance the solubility, transport, dispersibility, bioavailability and bioaccessibility of active food and drug components (e.g. carotenoids, α-tocopherol, antioxidants, polyunsaturated fatty acids, hydrophobic vitamins, flavour and aroma compounds), and can act as excellent encapsulation systems compared to conventional emulsions (Bilbao-Sáinz et al., 2010; Qian et al., 2012a; Wulff-Pérez et al., 2009).

The advantages of nanoemulsions over other emulsions are derived from the smaller droplet sizes which impart distinct physicochemical properties in nanoemulsions (e.g. bulk viscosity, optical transparency, and physical stability) compared to those of other emulsion systems (Cortés-Muñoz et al., 2009; Donsì et al., 2012; Kentish et al., 2008; Peng et al., 2010; Sonneville-Aubrun et al., 2004). Most studies conducted so far have concentrated on the use of the synthetic and low molecular weight surfactants (e.g. the tweens and spans) due to their excellent interfacial diffusivity, compared to large biopolymers such as proteins and polysaccharides (Donsì et al., 2012; Ghosh et al., 2013; Lee & McClements, 2010; Qian & McClements, 2011). However, concerns about the safety, toxicity and metabolism of these synthetic emulsifiers in the human body limit their application to food systems.
The majority of the studies using proteins have reported on the native protein but not its hydrolysates. While hydrolysates may possess enhanced interfacial diffusivity and emulsifying capacity, they have shown poor stabilising ability in conventional emulsion systems, preventing long term storage (Agboola et al., 1998a; Scherze & Muschiolik, 2001). Moreover, studies on emulsion have often focused on the crude hydrolysate which consists of heterogeneous mixtures of amino acids, and small, medium to large chain peptides (Scherze & Muschiolik, 2001). The prospect of stepwise fractionation to enrich sufficiently large peptides may generate peptides with adequate surface activities that are capable of stabilising these nanodroplets, compared to the microdroplets (Gauthier & Pouliot, 2003).

Aside from enhancing the emulsifying properties of proteins, the products of enzymatic hydrolysis may also possess bioactivities (e.g. antioxidant activity, antihypertensive activity, mineral carrier, immuno-stimulant, anti-thrombotic, and anti-gastric, opioid, antimicrobial, and anti-cancer activities) which can be beneficial for promoting good health (Adjonu et al., 2013; Gauthier & Pouliot, 2003; Korhonen, 2009; Korhonen & Pihlanto, 2006). These bioactivities are usually absent or latent in the native unhydrolysed protein, but can be released or enhanced upon hydrolysis (Adjonu et al., 2013). Nanodispersions may serve as efficient delivery vehicles for incorporating these bioactive peptides into food, subsequently increasing their utilisation by the body as functional and nutraceutical agents (Chu et al., 2007; Qian et al., 2012a; Relkin et al., 2008) thus, allowing them to more effectively express their bioactivities in vivo (Prego et al., 2006).

This review will focus on: (a) the formation of nanoemulsions; (b) emulsifier (non-protein and protein emulsifiers) effects on nanoemulsion properties; (c) the emulsifying property of whey protein hydrolysates and the potential for whey protein peptides as both nanoemulsifiers and bioactive compounds in foods (i.e. dual-functionality) and (d) the potential applications for nanoemulsions for bioactive peptide delivery. The terminologies
emulsifiers and surfactants are used interchangeably in the following discussion.

2. Nanoemulsions

2.1 Properties of nanoemulsions

Nanoemulsions are a technology that has food and pharmaceutical applications (Tarver, 2006), and their evolution has paralleled the development of efficient emulsification technologies (Cortés-Muñoz et al., 2009). An efficient emulsification process is able to form emulsions with small droplet sizes and narrow size distributions. These characteristics are, however, a function of the two opposing forces; droplet breakup and droplet-droplet coalescence (Jafari et al., 2006). These properties have been identified in several works (Donsi et al., 2012; Jafari et al., 2006; Qian & McClements, 2011) as being dependent upon several processes including:

- Homogenising mechanism
- Type, concentration and interfacial properties of surfactant/emulsifier
- Dispersed phase volume/mass fraction and viscosity
- Timescale of surfactant adsorption onto the surfaces of newly created droplet
- Frequency and timescale of inter droplet-droplet collision

Nanoemulsions, like microemulsions are transparent/translucent systems and as a result, they can be incorporated as components of food beverages and gels, nutraceuticals and pharmaceutical preparations without a loss of clarity (Figure 1) (Chu et al., 2007; Kentish et al., 2008; Wulff-Pérez et al., 2009). Increasing interest in nanoemulsions stems from the characteristic physicochemical properties that their small droplet sizes provide (Table 1). Their small droplet size allows for efficient delivery, accelerated release and rapid absorption of hydrophobic bioactive drug and food agents such as vitamin E, omega 3 fatty acids, flavonoids and various phyto-polyphenolic compounds (Balcão et al., 2013; Lee &
2.2 Formation of nanoemulsion

Nanoemulsion droplets are only kinetically stable, in that the free energy of the separated oil and aqueous phases is always lower than that of the formed emulsion and therefore, nanoemulsions do not form spontaneously (McClements & Rao, 2011). To break large emulsion droplets into nanodroplets, a large external force (homogenisation pressure, Pa; energy applied per volume of liquid) must be applied during homogenisation in order to overcome the Laplace pressure \( p \) (Pa; difference in pressure between the convex and concave sides of a curved interface) and to break up the interface between the oil and water phases (Equation 1) (Cortés-Muñoz et al., 2009; Walstra, 1993). The Laplace pressure characterises the interfacial force that acts on droplets to keep them from being disrupted (McClements, 2005).

\[
p = \frac{2\gamma}{r} \quad \text{Eqn.1}
\]

Where \( r \) (m) is the principal radius of curvature of the droplets (assuming droplets are spherical) and \( \gamma \) (Nm\(^{-1}\)) is the interfacial tension between the two phases.

The high energy emulsification method requires the use of a high pressure valve homogeniser (HPVH), a microfluidiser, or an ultrasonicator (Jafari et al., 2006; Kentish et al., 2008; Lee & McClements, 2010; Mao et al., 2010). Droplet disruption within the homogeniser is a complex process arising from a combination of flow dynamics (laminar, turbulent and cavitational) which determines the nature of the disruptive forces (shear/viscous, elongational, inertial and cavitational) acting on droplets (McClements, 2005; Walstra, 1993). A more in-depth discussion on flow conditions within the homogeniser, disruptive forces and energy considerations can be obtained from (McClements, 2005),...
The choice of homogenisation technique and homogeniser is vital as it defines the type of flow conditions to which droplets are subjected in order to cause droplet breakup (McClements, 2005) and determines the smallest droplet size that can be produced during emulsification (Donsì et al., 2012; Mao et al., 2010). A comparison of nanoemulsification techniques applicable to food nanoemulsion production is given in Table 2. Other methods for producing nanoemulsions have been reported, such as: the membrane emulsification and electrical coaxial liquid jets (Acosta, 2009), the aqueous extraction-ultrafiltration method (Nikiforidis et al., 2011) and the typical low energy emulsification approaches, such as the spontaneous emulsification and solvent displacement techniques (Prego et al., 2006), and the phase inversion or persuasive technique (Bilbao-Sáinz et al., 2010; Wulff-Pérez et al., 2009). However, these methods have found little application to the food industry.

3. Emulsifiers/surfactants and co-surfactant systems in nanoemulsions

Nanoemulsion droplets exhibit an appreciable interfacial tension (Mao et al., 2010) and hence the choice of emulsifier/surfactant/co-surfactant systems is paramount for the efficient design of nanoemulsion systems (McClements & Rao, 2011). Surfactants influence the efficiency of droplet breakup by reducing the interfacial tension between the oil and water phases, adsorb onto the surfaces of newly created droplets and stabilise them through electrostatic repulsive and steric forces (Ghosh et al., 2013; Mao et al., 2010). In addition, the nature and properties of the emulsifier can affect the interfacial and bulk rheology of the nanoemulsion droplets, which is vital to the design of nanoemulsion systems tailored for a specific application. Surfactant properties also define the physicochemical, sensorial and functionality of the nanoemulsion produced.
3.1 Non-protein surfactants/co-surfactants systems

A diverse range of non-protein surfactants exist for formulating nanoemulsions and colloidal systems in general. Most studies on nanoemulsions formation have utilised low molecular weight synthetic emulsifiers/surfactant/co-surfactant systems (Table 3). They possess better interfacial diffusive properties compared to large biopolymers, such as proteins and polysaccharides. However, concerns about their safety, toxicity and metabolism may limit their application in food systems.

3.2 Whey protein and peptide emulsifiers

Dairy and plant proteins have been extensively used as emulsifiers in foods as they adsorb to the oil droplet interface, forming a strong and cohesive protective film that helps prevent droplet aggregation (Lee & McClements, 2010). They are also effective as emulsifiers in nanoemulsions (Table 4). Whey proteins (α-lactalbumin, β-lactoglobulin, bovine serum albumin, lactoferrins, and immunoglobulins) constitute about 20% of the total protein in milk (~80% caseins) and have a high nutritional value owing to their high essential amino acid content (Custódio et al., 2009). They are valued as important emulsifiers in food due to their amphiphilic properties (possessing both hydrophobic and hydrophilic residues) (Foegeding et al., 2002).

Whey proteins possess globular/rigid structures with buried hydrophobic residues which tend to negatively affect their functionality (Gauthier & Pouliot, 2003). Controlled enzymatic hydrolysis of whey proteins produces peptides that are smaller, possess fewer secondary and tertiary structures, and have a partially exposed hydrophobic core (Christiansen et al., 2004; Gauthier & Pouliot, 2003; Tirok et al., 2001). These characteristics account for their higher rate of diffusion to the oil/water interface and their ability to cover a larger area of the interface than the intact protein (Davis et al., 2005; O’Regan & Mulvihill, 2010). Their amphiphilic nature allows them to adsorb onto the surfaces of oil droplets (Tirok et al., 2001),
and stabilise the newly created emulsion droplets against destabilisation (van der Ven et al., 2001).

The performance of peptides derived from whey and other proteins is well known in conventional emulsions (Christiansen et al., 2004; Gauthier & Pouliot, 2003; Scherze & Muschiolik, 2001; Tirok et al., 2001), and is also seen in nanoemulsions. The utilisation of peptides from food proteins as nanoemulsifying agents is limited, however, the potential of whey protein isolate (WPI) hydrolysates as emulsifiers in nanoemulsions has been reported (Chu et al., 2007). β-carotene nanoemulsions formed by two WPI hydrolysates (with degree of hydrolysis (DH) 8.1% and 18.1%) had smaller droplet sizes (110.3 and 30.4 nm, respectively) than whey protein concentrate (WPC) and soy protein isolate (SPI) stabilised nanoemulsion (145.3 and 196.3 nm, respectively). Zeta potential measurement showed the WPI hydrolysates had droplet surface charge comparable to unhydrolysed SC and SPI but significantly higher than unhydrolysed WPI and WPC. With the increasing knowledge of the creation of nanoemulsions in the food industry, the potential of hydrolysed protein peptides as emulsifier ingredients in nanoemulsions warrants further investigation.

**3.3 Tailoring peptide functionality**

The emulsifying properties of peptides depend upon their characteristics (e.g. chain length/ molecular size, conformation, hydrophilicity and hydrophobicity) (Doucet et al., 2003; Gauthier & Pouliot, 2003). Protein hydrolysis to produce peptides with desirable functionalities is usually performed with enzymes due to their highly specific mode of action, ease of control, use of milder conditions, and tendency not to cause amino acid damage compared to chemical, thermal and microbial hydrolysis (Cheison et al., 2007). Careful enzyme selection means hydrolysates can be produced which are tailored specifically for food applications.
3.3.1 Enzyme type

Tryptic peptides of heat pre-treated WPI had higher emulsifying activity and emulsion stability than chymotrypsin, Alcalase and Neutrase peptides (Mutilangi et al., 1996). Trypsin hydrolysate contained large amphiphilic peptides that favoured emulsion formation and stability (Mutilangi et al., 1996). Trypsin hydrolysates of WPC were also found to have higher interfacial adsorption, emulsifying capacity and better storage stability compared to chymotrypsin WPC peptides (Gauthier et al., 1993; Turgeon et al., 1992; Turgeon et al., 1996). Different enzymes produced peptides with different numbers of hydrophobic regions because they cut in different places. The adsorption of peptide onto droplet interfaces depends on hydrophobic properties of the peptides, especially their surface hydrophobicity (Lam & Nickerson, 2013; Singh & Dalgleish, 1998; Tirok et al., 2001; Turgeon et al., 1992; Turgeon et al., 1996). Adequate surface hydrophobicity of whey protein hydrolysates is required to form strong and cohesive films around droplets, and allow hydrolysates to function as good emulsifiers (Lam & Nickerson, 2013). The surface hydrophobicity of whey protein hydrolysates may be reduced or increased depending on the specificity of the enzyme used, pre-hydrolysis heat treatment, as well as on the extent of hydrolysis (Adjonu et al., 2013; Mutilangi et al., 1996).

3.3.2 Degree of hydrolysis

The capacity of whey protein peptides to form and stabilise emulsion droplets is influenced by their DH (the percent of peptide bonds cleaved during hydrolysis). Increased DH increases peptide solubility and emulsifying ability (Lieske & Konrad, 1996), but if the DH becomes too high, it ultimately results in reduced emulsion formation and stability, and foam stability (Agboola et al., 1998; Lam & Nickerson, 2013; Scherze & Muschiolik, 2001; Singh & Dalgleish, 1998; Tirok et al., 2001). For example, the emulsifying capacity of WPC hydrolysates increased to a maximum capacity at 3% DH, with lower emulsifying capacity at
lower and higher DH (Lieske & Konrad, 1996). In more extensively hydrolysed whey protein products, the 10% DH showed better emulsion characteristics (e.g. smaller droplet sizes) followed by the 20% DH and then the 27% DH (Scherze & Muschiolik, 2001). Commercial whey protein hydrolysates with DH between 10 and 20% possessed better emulsifying capacity than hydrolysates with DH >20% (Singh & Dalgleish, 1998). As DH was increased from 10 to 45%, droplet size increased, and coarse emulsions exhibiting bimodal size distributions were formed. The decreased emulsifying properties when proteins are extensively hydrolysed can be attributed to a greater proportion of the peptides remaining in the continuous phase rather than adhering to the oil–water interface (Lam & Nickerson, 2013; Miñones Conde, & Rodríguez Patino, 2007), and increased peptide-peptide and protein-peptide interactions at the expense of peptide-oil interactions (Creusot et al., 2006). Thus, for good emulsifying properties, whey proteins must undergo limited hydrolysis in order to partially unfold their secondary and tertiary structures while minimising the degradation of their primary structure (Foegeding et al., 2002).

### 3.3.3 Peptide size

Emulsions formed by hydrolysate with increased small peptide content tended to show larger droplet sizes, multimodal size distributions, increased creaming and coalescence, with extensive oiling-off compared to the unhydrolysed protein (Agboola et al., 1998a; Singh & Dalgleish, 1998; Sinha et al., 2007; Tirok et al., 2001; van der Ven et al., 2001). A minimum peptide size of >2 kDa is required for good emulsify properties, as the size of the peptides dictates the steric stabilisation of emulsion droplets (Gauthier & Pouliot, 2003; van der Ven et al., 2001). Also, larger size peptides are more likely to have both hydrophobic and hydrophilic residues the same molecule. During emulsion formation, the hydrophobic side chains of proteins interact with the oil droplets and the hydrophilic residues will favour the aqueous phase and stabilise the droplets through steric effects (Dalgleish, 1997; Lam & Nickerson,
Although small peptides may be sufficiently surface active to form small droplets, their small size may be insufficient to prevent droplet aggregation post-emulsification, as a result of a loss of their steric stabilising property (van der Ven et al., 2001). In addition, larger peptides are capable of modifying the internal structure of an emulsion by forming interlocking networks and, hence, limit the tendency to creaming, flocculation and coalescence (Singh & Dalgleish, 1998; Tirok et al., 2001).

### 3.4 Stability of whey protein hydrolysate stabilised emulsions

Once whey protein hydrolysate emulsions are formed, they are subjected to various forms of instability, including creaming, sedimentation, coalescence, flocculation, aggregation and oiling-off (Agboola et al., 1998a; van der Ven et al., 2001). Rapid droplet destabilisation resulted from the formation of weak interfacial films around droplets that promoted the formation of larger emulsion droplets that creamed and coalesced faster (Agboola et al., 1998a; van Aken, 2003). Small droplets are generally stable to gravitational separation (creaming and sedimentation) as a result of their constant Brownian motion (Kentish et al., 2008).

Co-surfactants/emulsifiers, stabilisers and texture-modifiers are often required to improve the stability of whey protein hydrolysate emulsions (Table 3) (McClements & Rao, 2011; Tirok et al., 2001). Co-surfactants such as lecithins and monoglycerides may displace small peptides from the droplet interface or act in a synergistic role to compensate for the poor emulsion stability of protein hydrolysates (O’Regan & Mulvihill, 2009; Tirok et al., 2001). For example, phospholipids compete with for available interfaces and may result in less peptides adsorbing at the droplet interface as a result of the better interfacial properties of lecithin compared to the peptides making up the hydrolysate. Effectively, smaller size droplets may form due to the formation of a thinner adsorbed layer around droplets.
(McSweeney et al., 2008; Van der Meeren et al., 2005), making them highly stable to
creaming and sedimentation (Kentish et al., 2008). In addition, peptide-lecithin interactions
may lead to an increase in the overall charge and hydration at the oil droplet surface, resulting
in strong interfacial film favouring droplet stability (Agboola et al., 1998b; McSweeney et al.,
2008; Van der Meeren et al., 2005).

Stabilisers and texture modifiers, usually polysaccharides, also provide stability by
increasing the continuous phase viscosity as well as forming three dimensional networks
which trap and retard droplet movement, and limit droplet coalescence and creaming
(McClements & Rao, 2011). Increasing the concentration of polysaccharides (e.g. guar and
xantham gum) was found to reduce the collision frequency of droplets and the rate of
drainage from the droplet surface (Ye et al., 2004; Ye & Singh, 2006). In addition, emulsion
droplets were stable against creaming and coalescence as a result of improved droplet packing
that restricted the relative motion of emulsion droplets (Ye et al., 2004; Ye & Singh, 2006).
Protein-polysaccharide interactions can also modify the interfacial rheology, by forming
bulkier polymeric layers around emulsion droplets and provide enhanced stabilisation of
droplets through steric effects (Akhtar & Dickinson, 2007; O’Regan & Mulvihill, 2010).

3.5 Whey protein hydrolysate fractionation

Whey protein hydrolysates are a heterogeneous mixture of free amino acids, and short
to long chain peptides. Large concentrations of hydrophilic and short chain peptides can limit
their interfacial and steric-stabilising properties (Singh & Dalgleish, 1998; Tirok et al., 2001).
They can also promote adsorption and desorption mechanisms, resulting in an uneven, non-
continuous and weak mechanical stability of the adsorbed interfacial film, while increasing
the rate of re-coalescence and oiling-off (Tirok et al., 2001).

Peptide fractionation using membranes or chromatographic techniques have been used
to enrich large and surface active peptides from crude hydrolysates with enhanced food
functionalities (Gauthier & Pouliot, 2003; Gauthier et al., 1993; Korhonen & Pihlanto, 2006; Scherze & Muschiolik, 2001). A >10 kDa peptide fraction obtained after trypsin, chymotrypsin, Alcalase and Neutrase hydrolysis of WPC possessed higher emulsifying activity index and emulsion stability than the crude unfractionated hydrolysate (Mutilangi et al., 1996). Also, ultrafiltration of WPC hydrolysates produced a 1–30 kDa peptide fraction that possessed greater interfacial adsorption, emulsifying activity and stability than the unfractionated WPC hydrolysate (Gauthier & Pouliot, 2003; Gauthier et al., 1993; Turgeon et al., 1996). These fractions were composed of large amphiphilic peptides that favoured the formation of stronger interfacial films around emulsion droplets (Gauthier et al., 1993; Turgeon et al., 1996).

While no studies on the emulsifying properties of fractionated whey protein hydrolysates in nanoemulsions are known, studies using crude hydrolysates have predicted nanoemulsions with droplet sizes of 30–110 nm (Chu et al., 2007). The stability of such nanoemulsions has not been investigated, and little information exists on hydrolysate or peptide stabilised nanoemulsion. Moreover, elucidation of the factors that affect the nanoemulsifying potential of whey protein hydrolysates, such as DH, hydrolysate/peptide properties, lecithin and polysaccharide addition, and homogenisation conditions (e.g. pH, ion concentration) may provide a better understanding about the emulsifying properties of protein hydrolysates in food nanoemulsions.

4. **Bioactive peptides from whey protein**

Aside from modifications to their emulsifying properties and other functionalities, whey protein hydrolysates/peptides also possess bioactive properties (Hernández-Ledesma et al., 2011; Madureira et al., 2010). Bioactive peptides derived from enzymatically hydrolysed whey protein have the ability to promote good health in humans (Table 5). Many of these bioactive peptides have been isolated, purified, characterised and synthesised, and are
currently being marketed as specialty and functional ingredients (Gauthier & Pouliot, 2003; Gauthier et al., 2006; Korhonen & Pihlanto, 2006). These peptides have simple structures and are considered safe and healthy compounds which are easily absorbed by the human body (Li et al., 2004).

4.1 Whey peptides as dual-functional ingredients

Whey peptides perform a dual-functional role in foods as both emulsifiers (technological function) and bioactive compounds (biological function) important for promoting good health (Adjonu et al., 2013). However, studies on the emulsifying and biological functionalities of whey proteins, and proteins in general, have usually been conducted in isolation, although some studies have demonstrated these combined functionalities. Sinha et al., (2007) reported that a papain and a fungal protease treated WPC possessed high water solubility, increased foam overrun and had low emulsifying capacity. The hydrolysates also showed high ACE-inhibitory potencies and were suggested as potential ingredients for nutraceutical preparations. Gauthier & Pouliot, (2003) also demonstrated that some of the peptide sequences responsible for the emulsifying properties of whey proteins were also related to their ACE-inhibitory properties. However, the link between peptide bioactivity and their emulsifying properties was not demonstrated by these studies. Gauthier & Pouliot, (2003) and Sinha et al., (2007) only reported on the ACE-inhibitory peptides of these hydrolysates in food emulsion, but not their other bioactivities (Table 5). Table 6 summarises other studies reporting on the bioactivity and technological functions of peptides from other food proteins sources.

Whey protein bioactive peptides generally have a molecular weight of less than or equal to 10 kDa and sometimes greater (Pihlanto, 2006). These low molecular weight peptides show poor stabilising abilities in food emulsions (Agboola et. al 1998a, 1998b; van der Ven et al., 2001; Ye et al., 2004). Overcoming these problems may be possible by stepwise fractionation
using membrane and chromatographic techniques to generate different bioactive peptides from crude hydrolysates that are large and surface active and can stabilise emulsion droplets. These peptides may be effective as sole emulsifiers or in conjunction with other co-emulsifiers to form and stabilise nanoemulsion systems because of the smaller size of nanoemulsion droplets (Figure 2). The factors affecting the nanoemulsification abilities of these peptides (e.g. dispersion conditions, aqueous phase properties, oil mass fractions, surfactant/co-surfactant systems, and ion and salt concentrations) could be elucidated in order to better understand their interfacial properties in nanoemulsion systems.

In addition, the inclusion of bioactive peptides in nanoemulsions may extend their application in food and pharmaceutical industries because colloidal systems may serve as an excellent medium to incorporate these bioactive peptides into day-to-day foods. Active peptides that show dual-functionality could be isolated, characterised, sequenced and possibly synthesised, which has occurred for many other bioactive peptides. Also, whey protein peptides possess reduced allergenicity and are easy to digest, properties that are vital for formulating infant formulae and sports nutrition diets (Tirok et al., 2001). Inclusion of whey protein peptides in nanoemulsion could result in products with a modification to many of their macro-scale characteristics, such as texture, taste, sensory attributes, and shelf stability, leading to a variety of products with new functionalities (Silva et al., 2012).

Other studies have also looked at the antioxidant activities of whey proteins and their peptides to inhibit lipid oxidation and rancidity in emulsions. Hu et al., (2003) studied the oxidative stability of salmon oil/water emulsion formed by whey protein emulsifiers (whey protein isolate, sweet whey, β-lactoglobulin and α-lactalbumin). All emulsions, especially those formed by β-lactoglobulin, showed greater oxidative stability with regard to the formation of hydroperoxide and headspace propanal, particularly at pH values below the isoelectric point of the proteins. Tong et al., (2000a, 2000b) and Peña-Ramos et al., (2004)
also reported the ability of whey protein fractions to inhibit the formation of thiobarbituric acid reactive substances (TBARS) in a salmon oil/water emulsion and a liposomal-oxidising system, respectively, and showed that larger molecular weight peptides (>3 kDa) were more effective as antioxidants than smaller molecular weight peptides (<3 kDa). The greater oxidative stability of emulsions occurred as a result of the ability of proteins to form cationic charges on the surface of emulsion droplets to repel transition metals, form protective films around droplets which hinders lipid hydroperoxide-transition interactions, chelate prooxidants and inactivate free radicals through sulphur containing amino acids and peptides (Hu et al., 2003; Peña-Ramos et al., 2004; Tong et al., 2000b). These studies, however, have looked at the antioxidant activities of whey peptides in food emulsions from a technological perspective rather than a biological perspective. It is possible the mechanisms of their antioxidant activities may differ from the technological and biological perspectives.

5. **Nanoemulsion delivery of bioactive peptides**

Bioactive peptides are ideal supplemental compounds to prevent or reduce oxidative damage to body organs, and the risk of hypertension and cardiovascular health diseases. Bioactive peptides have been identified in various food proteins such as caseins, soy, canola, and are finding great applications in the development of nutrition tailored functional foods (Phelan et al., 2009; Wang & De Mejia, 2005). However, such applications are limited due to the lack of appropriate delivery systems capable of protecting bioactive peptides from degradation (e.g. conformational changes and denaturation) during processing as well as during administration, because the structure and functionality of food proteins/peptide are positively correlated. In addition, the majority of bioactive peptides are not absorbed from the gastrointestinal tract into the blood, possibly due to poor delivery systems, although their effects have been proposed to be mediated directly in the gut through receptors on the intestinal walls (Korhonen & Pihlanto, 2006; Lee et al., 2007; Phelan et al., 2009).
A suitable delivery system should protect bioactive peptides from interactions with other food components, stabilise the peptides during processing and administration and should also enhance their absorption and transport across the intestinal mucosa to target sites (Balcão et al., 2013; Prego et al., 2006; Yang & McClements, 2013). Nano-delivery systems (e.g. nanoemulsion, nanoencapsulation, nanovesicles [liposomes]) (Figure 2) present a mechanism for the structural and functional stabilisation of bioactive proteins/peptides against denaturation by enzymatic digestion and a way to increase their biopharmaceutical and food applications (Balcão et al., 2013; Prego et al., 2006). In addition, their small droplet size may enhance the transport of bioactive peptides carried within nanodroplets as droplets may pass across the intestinal wall and facilitate their absorption, bioavailability and bioaccessibility (Martins et al., 2007; Watnasirichaikul et al., 2000).

The colloidal delivery of peptide drugs within pharmaceutical preparations is well known, whereas the delivery of bioactive peptides as part of food formulations has received little attention (Flanagan & Singh, 2006; Martins et al., 2007). Prego et al., (2006) demonstrated that when salmon calcitonin (a linear polypeptide hormone responsible for controlling blood calcium levels) was contained within nanoemulsion carriers, enhanced and prolonged intestinal absorption occurred.

Balcão et al., (2013) recently encapsulated lactoferrin (a whey protein fraction with bioactivities) within a water-oil-water nanoemulsion as potential antimicrobial formulation. Nanoencapsulated lactoferrin and lactoferrin in solution showed inhibitory effect against *Staphylococcus aureus*, *Listeria innocua*, *Bacillus cereus* and *Candida albicans*, but not Gram negative bacteria such as *Salmonella sp.*, *Escherichia coli* (*E. coli*) and *Pseudomonas fluorescens*. Nanoencapsulation of antimicrobial proteins could be extended to antimicrobial peptides derived from other food protein sources. Antimicrobial peptides from α-lactalbumin, β-lactoglobulin and caseins have been shown to be effective against Gram-positive and Gram-
negative bacteria (*E. coli, Helicobacter, Listeria, Salmonella* and *Staphylococcus, Listeria ivanovii*), yeasts and filamentous fungi (Hartmann & Meisel, 2007; Théolier et al., 2013).

Nanoencapsulation of antimicrobial peptides, coupled with the small droplet size of nanoemulsion droplets, may extend the applications food protein antimicrobial peptides across the food production chain. For example, they may be used for decontamination purposes and for extending the shelf life of food products.

Bioactive peptide products have inherent bitter tastes which tend to reduce their consumer acceptability (Komai et al., 2007; Pedrosa et al., 2006). Debittering techniques involving the removal of hydrophobic peptides by chromatography, absorption of bitter peptides on activated carbon or selective extraction with alcohols could result in a loss of bioactivity, as the majority of bioactive amino acids and peptides are hydrophobic in nature (Fitzgerald & O’Cuinn, 2006; Leksrisompong et al., 2012). Encapsulation of bioactive peptides within nanoemulsion delivery systems can be used to inhibit or reduce their bitter taste and off-flavours, as for other bitter and astringent compounds. Micro- and nanoencapsulation of polyphenolic compounds and other bitter compounds, such as chloroquine phosphate and trimebutine within polymeric coated multiple emulsions, reduced their off-flavours, astringency, bitterness, smell and increased their loading efficiency and bioavailability (Hashimoto et al., 2002; Munin & Edwards-Lévy, 2011; Sohi et al., 2004; Sun-Waterhouse & Wadhwa, 2013). Bitter tasting compounds dissolved in the internal phase of colloidal delivery systems can be shielded from interactions with taste sensors until target sites are reached (Hashimoto et al., 2002; Sohi et al., 2004; Sun-Waterhouse & Wadhwa, 2013). Such applications could be extended to encapsulate highly bioactive but bitter peptides within nanoemulsion system for fortification and supplementation purposes in foods.

Protein-coated nanoemulsions containing hydrophobic bioactive agents allow for the controlled release of active agents because proteins would have to undergo digestion before
release at target sites (He et al., 2011). Pea protein-coated antimicrobial nanoemulsions were noted to have longer bacteriostatic action against microorganisms, which was beneficial for products requiring long shelf stability, whereas sugar esters and other synthetic surfactant-coated nanoemulsions promoted quicker and faster antimicrobial effects (Donsì et al., 2012). Also, nanoemulsions formed with proteins, such as whey proteins, possess high biocompatibility with cells when applied as delivery systems, showing over 85% increase in cell viability compared to other synthetic emulsifiers such as Tween 80, Solutol HS 15, Poloxamer 188 and Cremophor (He et al., 2011).

In addition, the actual mode of action of bioactive peptides when incorporated into foods are currently not well understood, possibly due to lack of methods available to determine these activities when they are included as components of foods, coupled with the complexity of most food matrices. Nanoemulsion may serve as a good substrate for use in assessing these bioactivities when peptides are added to foods. The factors that affect the solubility, loading efficiency, absorption and bioavailability, and control release of bioactive peptides could also be determined in nanoemulsion carrier systems. Furthermore, the pharmacokinetics, safety and biological fate of bioactive peptides could also be investigated because nano-delivery systems are model systems for control delivery studies (Li et al., 2012).

5.1 Delivery of other bioactive compounds

Ahmed et al., (2012) reported the high solubility of curcumin (a natural polyphenolic phytochemical extracted from turmeric spice) in nanoemulsions made with short, medium and long chain triacylglycerol oils (SCT, MCT and LCT, respectively). The average solubility was inversely proportional to the molecular weight of the carrier oil and increased as the average molecular weight of the oil decreased. SCT oils possess more polar groups per unit mass than MCT and LCT oils which may favour more dipole–dipole interactions with the
curcumin molecules and enhance solubilisation (Ahmed et al., 2012). Conversely, LCT (e.g. corn oil) and MCT (e.g. Miglyol® 812) oils enhanced the bioavailability and bioaccessibility of bioactive β-carotene and curcumin compared with SCT oils and flavour oils (e.g. orange oils) (Ahmed et al., 2012; Qian et al., 2012a). LCT and MCT oils contain long and medium chain fatty acids that are capable of forming mixed micelles possessing a large hydrophobic core to accommodate β-carotene and curcumin molecules (Qian et al., 2012a).

Wang et al., (2008) demonstrated the high anti-inflammatory activity of curcumin encapsulated within nanodroplets against 12-O-tetradecanoylphorbol-13-acetate-induced edema of mouse ear. Nanoemulsions containing 1% curcumin (nanoencapsulates) exhibited greater anti-inflammatory potencies (43% for 618.6 nm or 85% for 79.5 nm droplet sizes, respectively) than a 1% curcumin in 10% tween 20/water solution. The increased activity was attributed to the enhanced structure stability of nanoencapsulated curcumin against intestinal degradation, resulting in more efficient dispersibility and absorption. Also, encapsulation of resveratrol and curcumin within nanoemulsion delivery systems improved their water dispersibility, and their antioxidant properties were preserved as a result of protective effects from the nanoemulsion against degradation (Donsì et al., 2011).

The combined delivery of tocotrienols (one form of vitamin E) and simvastatin (a cholesterol lowering drug) by nanoemulsions was observed to increase their anticancer activity (Alayoubi et al., 2013). Nanoemulsion increased the solubility of tocotrienol (poor water solubility), and when they were encapsulated with simvastatin, enhanced their anticancer activity against human adenocarcenoma cells (Alayoubi et al., 2013). Nanoemulsions have also been made to encapsulate and deliver other hydrophobic bioactive food components in order to increase their food value (McClements & Rao, 2011). These include vitamin E (El Kinawy et al., 2012; Relkin et al., 2011; Yang & McClements, 2013), flavour oils (Ziani et al., 2012), β-carotene (Qian et al., 2012a, 2012b) and Coenzyme Q₁₀.
Antimicrobial nanoemulsions have also been demonstrated to be effective against various food-borne pathogens (Donsì et al., 2012; Sugumar et al., 2012). Nanoemulsions containing antimicrobial essential oils such as carvacrol, cinnamaldehyde, limonene, eucalyptus oils, were effective against *E. coli*, *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, *Bacillus cereus* and *Staphylococcus aureus* (Donsì et al., 2012). The antimicrobial effects of nanoemulsions depended on the concentration of the active agent loaded and the physicochemical properties of the surfactant/emulsifier used (Donsì et al., 2012). Also, sunflower nanoemulsions improved the shelf stability (microbiological, organoleptic properties and sensory qualities) of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20°C (Joe et al., 2012). The small droplet size of nanoemulsions allowed for efficient penetration through the cell walls of microorganism and exerted bactericidal effects against H$_2$S-producing and lactic acid bacteria (Joe et al., 2012).

6. **Summary**

The utilisation of whey proteins (WPI, WPC and β-lg) as nanoemulsifying agents has received considerable attention, with the majority of work concentrating on the use of the native protein rather than hydrolysates. This likely stems from hydrolysates apparently possessing poor stabilising ability in conventional emulsions. With the advent of nanoemulsion, the possibility of these peptides being capable of stabilising nanoemulsion droplets solely or in combination with other emulsifiers has not been addressed. In addition to their interfacial properties, whey peptides possess bioactive properties. Peptides stabilising emulsion droplets are consequently responsible for these bioactivities, thus, serving a dual-functional role in food systems. Studies that have been undertaken so far have addressed these two functionalities in isolation. A detailed study addressing the two themes of emulsifying and biological functionalities of hydrolysed whey proteins, and milk proteins in general, as a
single entity is needed in order to better understand their novel dual-functionality in foods.

With the increasing awareness of the link between diet and health, peptides possessing multiple functionalities are prospective additives for the fast growing functional food industry as nutraceutical and health promoting agents.

7. Acknowledgements

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8. References


functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry, 102*(4), 1317-1327.


of Food Science, 77(8), S282-S287.


Ziani, K., Chang, Y., McLandsborough, L., & McClements, D. J. (2011). Influence of
surfactant charge on antimicrobial efficacy of surfactant-stabilised thyme oil nanoemulsions. *Journal of Agricultural & Food Chemistry*, 59(11), 6247-6255.

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## Figure 1

### Type of Emulsions

<table>
<thead>
<tr>
<th>Type of Emulsions</th>
<th>Properties</th>
</tr>
</thead>
</table>
| **Nanoemulsion**  | - Droplet size: 10–200 nm  
- Appearance: Transparent/translucent to milky  
- High/low energy emulsification  
- Surfactant load: Medium (<1 to >10%)  
- Does not form spontaneously  
- Kinetically stable |
| **Microemulsion**  | - Droplet size: 4–200 nm  
- Appearance: Transparent  
- High/low energy emulsification  
- Surfactant load: Fairly high (10–30%)  
- Can form spontaneously  
- Thermodynamically stable |
| **Macroemulsion**  | - Droplet size: >1 μm  
- Appearance: Formula dependent  
- Conventional homogenisation  
- Surfactant load: Fairly low  
- Does not form spontaneously  
- Kinetically stable |
Figure 2

a

Non-protein surfactant

Bioactive peptide

Oil droplet

b

Protein/bioactive peptide emulsifier
Table 1: Nanoemulsion properties and applications

<table>
<thead>
<tr>
<th>Property</th>
<th>Applications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravitational separation</td>
<td>The constant Brownian motion of the droplets makes them stable against gravitationally induced separation (creaming and sedimentation) and drainage in the manner observed for microscale and larger emulsion droplets.</td>
<td>Graves et al., (2005); Lee &amp; McClements, (2010); Peng et al., (2010)</td>
</tr>
<tr>
<td>Flocculation</td>
<td>Weak flocculation is prevented and this enables the droplets to remain dispersed with no separation.</td>
<td>Qian &amp; McClements, (2011); Graves et al., (2005)</td>
</tr>
<tr>
<td>Coalescence</td>
<td>1. The significant surfactant film thickness relative to droplet size prevents any thinning or disruption of the liquid film between the droplets.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. The small droplet sizes reduce the range of attractive forces acting between the droplets.</td>
<td></td>
</tr>
<tr>
<td>Large surface area</td>
<td>1. Improves the solubility, bioavailability and bioaccessibility of many functional ingredients such as carotenoids, phytosterols, and polyunsaturated fatty acids (PUFA).</td>
<td>Yuan et al., (2008); Qian et al., (2012a)</td>
</tr>
<tr>
<td></td>
<td>2. Enhances the bioavailability of peptides and proteins carried by nanocapsules due to enhanced surface interaction of nanocarriers with the absorptive epithelium and their protective effect for the associated peptide.</td>
<td>Prego et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>4. Nanodroplet delivery systems may increase the passive cellular absorption mechanisms and reduce the mass transfer resistances of antimicrobial essential oils (e.g. carvacrol, limonene and cinnamaldehyde, basil oil) against <em>Escherichia coli</em>, <em>Lactobacillus delbrueckii</em> and <em>Saccharomyces cerevisiae</em>.</td>
<td>Donsì et al., (2012); Ghosh et al., (2013)</td>
</tr>
<tr>
<td>Optical transparency / low turbidity</td>
<td>1. The dimensions of the oil droplets could be much smaller than the wavelength of light, making them transparent systems suitable for incorporation of active ingredients into many food beverages without loss of clarity.</td>
<td>Lee &amp; McClements, (2010); Qian &amp; McClements, (2011); Ghosh et al., (2013); Qian et al., (2012a); Kentish et al., (2008)</td>
</tr>
<tr>
<td></td>
<td>2. As optically transparent, they are associated with freshness, purity, simplicity, water-like and may lead to a large variety of products from water-like fluids to ringing gels.</td>
<td>Sonneville-Aubrun et al., (2004)</td>
</tr>
<tr>
<td>Fluidity</td>
<td>1. This may enhance spreading and interactions with taste sensory cells in the mouth.</td>
<td>Kentish et al., (2008)</td>
</tr>
<tr>
<td></td>
<td>2. At reasonable oil concentrations they are basically fluids and the absence of thickeners may give products easily absorbed by the skin culminating in a pleasant and aesthetic skin feel.</td>
<td>Sonneville-Aubrun et al., (2004)</td>
</tr>
</tbody>
</table>
Table 2: Comparison between different homogenisation techniques for nanoemulsion formation

<table>
<thead>
<tr>
<th>Property</th>
<th>Microfluidisation</th>
<th>HPVH</th>
<th>Ultrasonication</th>
<th>HEE/SD/E</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller droplet size</td>
<td>High</td>
<td>High at high disperse phase</td>
<td>High at higher</td>
<td>High with increasing organic solvent content</td>
<td>Jafari et al., (2006); Mao et al., (2010); Troncoso et al., (2012); Stang et al., (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>viscosity and geometry of the</td>
<td>oil concentrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>homogenisation nozzle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narrow size distribution</td>
<td>High</td>
<td>Moderate</td>
<td>Moderate</td>
<td>High with increasing organic solvent content</td>
<td>Jafari et al., (2006); Mao et al., (2010); Pinnamaneni et al., (2003); Troncoso et al., (2012); Lee &amp; McClements, (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Droplet stability</td>
<td>High</td>
<td>Moderate</td>
<td>High</td>
<td>Small droplets are stable to</td>
<td>Pinnamaneni et al., (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>gravitationally-induced separation</td>
<td></td>
</tr>
<tr>
<td>Ease of operation</td>
<td>Subject to</td>
<td>Easy to operate and clean</td>
<td>Straight forward</td>
<td>Large quantities of organic solvent required</td>
<td>Abismail et al., (1999); Freitas et al., (2006); Lee &amp; McClements, (2010)</td>
</tr>
<tr>
<td></td>
<td>equipment</td>
<td>operation and easy clean</td>
<td>operation and</td>
<td>which could be expensive and pose a threat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>contamination</td>
<td></td>
<td>easy to clean</td>
<td>to the environment</td>
<td></td>
</tr>
</tbody>
</table>

HPVH: High pressure valve homogenisation, HEE/SD/E: High energy emulsification/solvent displacement/evaporation
<table>
<thead>
<tr>
<th>Emulsifier type</th>
<th>Homogenisation method</th>
<th>Oil phase concentration (%)</th>
<th>Emulsifier concentration (%)</th>
<th>Oil phase</th>
<th>Droplet diameter (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decaglycerol monolaurate (DML, ML750)</td>
<td>Microfluidisation / HPVH</td>
<td>0.03, 1</td>
<td>1, 10</td>
<td>β-carotene in sunflower oil</td>
<td>115–279</td>
<td>Mao et al., (2009); Mao et al., (2010)</td>
</tr>
<tr>
<td>Polyoxyethylene sorbitan monolaurate (Tween 20)</td>
<td>Microfluidisation / HPVH</td>
<td>0.03, 1</td>
<td>1, 10</td>
<td>β-carotene in sunflower oil</td>
<td>117–280</td>
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</tr>
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<td></td>
<td>Microfluidisation</td>
<td>4</td>
<td>1.5</td>
<td>β-carotene in corn oil, Miglyol 812 and orange oil</td>
<td>140–170</td>
<td>Qian et al., (2012a)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation</td>
<td>5</td>
<td>1–10</td>
<td>Corn oil</td>
<td>113–143</td>
<td>Qian &amp; McClements, (2011)</td>
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<tr>
<td></td>
<td>Microfluidisation / solvent evaporation</td>
<td>0.3</td>
<td>0.5</td>
<td>β-carotene in hexane</td>
<td>40–260</td>
<td>Tan &amp; Nakajima, (2005)</td>
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<td>Microfluidisation</td>
<td>10</td>
<td>1</td>
<td>Thyme oil / Miglyol 812 oil</td>
<td>160–176</td>
<td>Chang et al., (2012)</td>
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<td>Microfluidisation</td>
<td>10</td>
<td>1</td>
<td>Thyme oil / corn oil</td>
<td>170–196</td>
<td></td>
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<tr>
<td>Polyoxyethylene sorbitan monopalmitate (Tween 40)</td>
<td>Sonication</td>
<td>15</td>
<td>5.6</td>
<td>Flaxseed oil</td>
<td>135</td>
<td>Kentish et al., (2008)</td>
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<td>Microfluidisation</td>
<td>5</td>
<td>0.5</td>
<td>Thyme oil / corn oil</td>
<td>164–196</td>
<td>Ziani et al., (2011)</td>
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<tr>
<td>HPVH</td>
<td>20/4/1</td>
<td>1</td>
<td>PCL-liquid / Lipoid S-75 / α-tocopherol</td>
<td>170</td>
<td>Hoeller et al., (2009)</td>
<td></td>
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<tr>
<td>Emulsifier type</td>
<td>Homogenisation method</td>
<td>Oil phase concentration (%)</td>
<td>Emulsifier concentration (%)</td>
<td>Oil phase</td>
<td>Droplet diameter (nm)</td>
<td>Reference</td>
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<tr>
<td>Sucrose palmitate</td>
<td>Ultra high pressure homogenisation</td>
<td>8/2, 10</td>
<td>1</td>
<td>d-limonene, trans-cinnamaldehyde, carvacrol in sunflower oil</td>
<td>130–168</td>
<td>Donsì et al., (2012)</td>
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<tr>
<td>Sucrose laureate</td>
<td>HPVH</td>
<td>20/4/1</td>
<td>1</td>
<td>PCL-liquid / Lipoid S-75 /α-tocopherol</td>
<td>161</td>
<td>Hoeller et al., (2009)</td>
</tr>
<tr>
<td><strong>Ionic</strong></td>
<td></td>
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<tr>
<td>Pluronic F 68</td>
<td>Ultrasonication</td>
<td>25</td>
<td>1–2.5</td>
<td>Olive</td>
<td>379</td>
<td>Wulff-Pérez et al., (2009)</td>
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<td></td>
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<td></td>
<td>Sesame</td>
<td>368</td>
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<td></td>
<td></td>
<td></td>
<td>Soybean</td>
<td>380</td>
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<tr>
<td>Sodium dodecyl sulphate</td>
<td>Microfluidisation</td>
<td></td>
<td></td>
<td>Silicone oil</td>
<td>150</td>
<td>Graves et al., (2005)</td>
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<td></td>
<td>Microfluidisation</td>
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<td></td>
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<td></td>
<td>Microfluidisation</td>
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<tr>
<td></td>
<td>5</td>
<td>1–10</td>
<td>Corn oil/octadecane</td>
<td>92–131</td>
<td>Qian &amp; McClements, (2011)</td>
<td></td>
</tr>
<tr>
<td><strong>Zwitterionic</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Phospholipids e.g. Lecithins</td>
<td>HPVH</td>
<td>10</td>
<td>1–5</td>
<td>*Neobee 1053</td>
<td>120</td>
<td>Donsì et al., (2011)</td>
</tr>
<tr>
<td></td>
<td>HPVH</td>
<td>20</td>
<td>1.5</td>
<td>*Neobee 1095</td>
<td>110</td>
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<td>Polysaccharide</td>
<td></td>
<td></td>
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<tr>
<td>Low-methoxyl pectin,</td>
<td>Ultra-Turrax</td>
<td>20</td>
<td>0.5–3</td>
<td>Itraconazole in chloroform</td>
<td>200–900</td>
<td>Burapapad et al., (2010)</td>
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<tr>
<td>Emulsifier type</td>
<td>Homogenisation method</td>
<td>Oil phase concentration (%)</td>
<td>Emulsifier concentration (%)</td>
<td>Oil phase</td>
<td>Droplet diameter (nm)</td>
<td>Reference</td>
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</tr>
<tr>
<td>Amidated low-methoxyl pectin, High-methoxyl pectin</td>
<td></td>
<td></td>
<td></td>
<td>Itraconazole in Miglyol® 812</td>
<td>&gt;2000</td>
<td></td>
</tr>
<tr>
<td>Succinylated waxy maize starch/Octenyl succinate starch (Purity Gum 2000/OSA/Hi-Cap)</td>
<td>HPVH</td>
<td>10</td>
<td>15</td>
<td>Neobee 1053</td>
<td>140</td>
<td>Donsì et al., (2011)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation / HPVH</td>
<td>1</td>
<td>10</td>
<td>Neobee 1095</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microfluidisation/ Sonication</td>
<td>5, 10, 15</td>
<td>30/10 (40)</td>
<td>Peppermint oil/MCT oil</td>
<td>184-228</td>
<td>Liang et al., (2012)</td>
</tr>
<tr>
<td>Maltodextrin/H-Cap</td>
<td></td>
<td></td>
<td></td>
<td>Fish oil</td>
<td>174-274</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d-Limonene</td>
<td>518-919</td>
<td></td>
</tr>
</tbody>
</table>

* Neobee 1053 is a low melting temperature lipid and Neobee 1095 is a high temperature melting lipid (Donsì et al., 2011).
### Table 4: An overview of protein stabilised nanoemulsions

<table>
<thead>
<tr>
<th>Emulsifier type</th>
<th>Homogenisation method</th>
<th>Oil phase concentration (%)</th>
<th>Emulsifier concentration (%)</th>
<th>Oil phase</th>
<th>Droplet diameter (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein isolate (WPI)</td>
<td>HPVH</td>
<td>15, 30, 45</td>
<td>4.3</td>
<td>Pea nut oil</td>
<td>146–236</td>
<td>Cortés-Muñoz et al., (2009)</td>
</tr>
<tr>
<td></td>
<td>High energy emulsification / solvent evaporation</td>
<td>10</td>
<td>1</td>
<td>Corn oil</td>
<td>75–121</td>
<td>Lee &amp; McClements, (2010)</td>
</tr>
<tr>
<td></td>
<td>HPVH</td>
<td>0.03, 1</td>
<td>1, 10</td>
<td>β-carotene in sunflower oil</td>
<td>160–373</td>
<td>Mao et al., (2009; Mao et al., (2010)</td>
</tr>
<tr>
<td></td>
<td>HPVH</td>
<td>20</td>
<td>4.5</td>
<td>α-tocopherol in palm oil</td>
<td>200–500</td>
<td>Shah et al., (2012)</td>
</tr>
<tr>
<td>Whey protein concentrate (WPC)</td>
<td>Microfluidisation</td>
<td>0.1</td>
<td>1</td>
<td>β-carotene in hexane</td>
<td>145</td>
<td>Chu et al., (2007)</td>
</tr>
<tr>
<td>β-lactoglobulin (β-lg)</td>
<td>HPVH</td>
<td>20</td>
<td>1</td>
<td>Soy oil</td>
<td>350</td>
<td>Sarkar et al., (2009)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation</td>
<td>5</td>
<td>1–10</td>
<td>Corn oil/octadecane</td>
<td>162</td>
<td>Qian &amp; McClements, (2011)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation</td>
<td>10</td>
<td>1</td>
<td>Corn oil</td>
<td>181</td>
<td>Ahmed et al., (2012)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miglyol® 812</td>
<td>174</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tributyrin</td>
<td>1981</td>
<td></td>
</tr>
<tr>
<td>Sodium caseinate (SC)</td>
<td>HPVH</td>
<td>40</td>
<td>3.6</td>
<td>α-tocopherol/low melting triacylglycerols</td>
<td>293–304</td>
<td>Relkin et al., (2009); Relkin et al., (2008)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation</td>
<td>0.05–0.3</td>
<td>0.5–5</td>
<td>β-carotene in hexane</td>
<td>17</td>
<td>Chu et al., (2007)</td>
</tr>
<tr>
<td></td>
<td>Microfluidisation</td>
<td>5</td>
<td>1–10</td>
<td>Corn oil/octadecane</td>
<td>179</td>
<td>Qian &amp; McClements, (2011)</td>
</tr>
<tr>
<td>Pea protein</td>
<td>HPVH</td>
<td>8, 10</td>
<td>3</td>
<td>Sunflower oil</td>
<td>184–218</td>
<td>Donsì et al., (2012)</td>
</tr>
<tr>
<td>Soy protein (SPI)</td>
<td>Microfluidisation</td>
<td>0.1</td>
<td>1</td>
<td>β-carotene in hexane</td>
<td>196</td>
<td>Chu et al., (2007)</td>
</tr>
<tr>
<td>Maize germ protein</td>
<td>Combined aqueous extraction-ultrafiltration method</td>
<td>5</td>
<td>3</td>
<td>Maize germ oil bodies</td>
<td>155</td>
<td>Nikiforidis et al., (2011)</td>
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</tbody>
</table>
Table 5: Bioactive peptides from whey proteins

<table>
<thead>
<tr>
<th>Bioactivity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Antioxidant</td>
<td>Gauthier et al., (2006); Kilara &amp; Panyam, (2003); Kim et al., (2007);</td>
</tr>
<tr>
<td>Mineral binding</td>
<td>Kong et al., (2012); Korhonen, (2009); Madureira et al., (2010);</td>
</tr>
<tr>
<td>Antimicrobial / anti-bacterial</td>
<td>Théolier et al., (2013)</td>
</tr>
<tr>
<td>Anti-appetising</td>
<td></td>
</tr>
<tr>
<td>Cytomodulatory</td>
<td></td>
</tr>
<tr>
<td>Immunomodulatory</td>
<td></td>
</tr>
<tr>
<td>Anti-thrombotic</td>
<td></td>
</tr>
<tr>
<td>Anti-gastric</td>
<td></td>
</tr>
<tr>
<td>Hypcholesterolemic</td>
<td></td>
</tr>
<tr>
<td>Anti-diabetes</td>
<td>Silveira et al., (2013)</td>
</tr>
<tr>
<td>Opioid</td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitory</td>
<td></td>
</tr>
<tr>
<td>Anti-hypertensive peptides</td>
<td>Hernández-Ledesma et al., (2011); Korhonen &amp; Pihlanto, (2006); Li et al.,</td>
</tr>
<tr>
<td>Protein source</td>
<td>Enzyme used</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Quinoa</td>
<td>Alcalase</td>
</tr>
<tr>
<td>Atlantic cod</td>
<td>Protamex™</td>
</tr>
<tr>
<td>Yellow stripe</td>
<td>Alcalase</td>
</tr>
<tr>
<td>trevally (Selaroides lepotelepis)</td>
<td>Flavourzyme</td>
</tr>
</tbody>
</table>