Bioactive whey peptide particles: An emerging class of nutraceutical carriers

Ashkan Madadlou[1,*], Alireza Abbaspourrad[2,*]

[1]*Department of Food Science and Engineering, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran.*

[2]*Department of Food Science, College of Agriculture & Life Sciences, Cornell University, NY, USA.*

Corresponding authors: Alireza@cornell.edu; A.madadlou@ut.ac.ir

Abstract

Whey-based diets have been linked with prolonged life expectancy and improved physical performance. These observations based on numerous clinical and simulated studies are attributed to diverse biological activities of whey peptides. Recently, bioactive whey peptides were exploited for enveloping nutraceuticals and drugs in view of fabricating capsules that the carrier matrix is also bioactive.

Some of the most considered bioactivities of whey peptides including antihypertension, antioxidant, anti-obesity, anti-diabetes and hypocholesterolemic properties with corresponding underlying mechanisms are briefly discussed. Then, we overview the supramolecular and gelation-prompted encapsulation of nutraceuticals with whey proteins, followed by summarizing recent developments in utilization of synthetic peptides for gene and drug delivery. Finally, particulation of bioactive whey peptides are communicated.

Whey peptides may exert both biologically beneficial and technologically appreciated activities. Two procedures including desolvation and internal gelation have been so far employed for

bioactive peptides particulation. Crosslinking is a prerequisite to confer acid-induced cold-set gelation to bioactive peptides. It also increases peptides Fe^{3+}-reducing power. Surface activity of a population of peptides in whey protein hydrolysate may result in co-adsorption of the peptides together with small molecule surfactants onto oil-water interface, leading to modulated interfacial architecture and particle morphology.

Keywords

ACE-inhibitory; Whey protein isolate; Nanotube; Nanofibril; Nanoparticle.

1. Introduction

The co-product of cheese-making or casein production processes, whey, represents about 85−90% of milk volume and contains approximately 50% of milk total solids (Brandelli et al., 2015). It encompasses several compounds including the milk serum proteins, the κ-casein-derived glycomacropeptide, lactose, lipids, vitamins and minerals. The recognition of whey components, especially its proteins and their properties has transformed whey from a waste to a value-added dairy stream (Smithers, 2008). Whey proteins not only are extremely nutritious but also exhibit some technologically fascinating characteristics such as high solubility, heat-induced and cold-set gelation, foaming, emulsifying and water-binding. As well, whey proteins show certain physiological benefits which are attributed to special peptide sequences encrypted throughout the parent protein molecule. Nonetheless, certain amino acids that are released by digestion and existing ions in protein hydrolysates may also contribute (Table 1). A partially lyzed whey protein solution with medium degree of hydrolysis fed to exercising young rats caused a better physical performance compared with intact protein solution. This finding was supported by decreased levels of blood lactate immediately after exhaustion, increased concentrations of serum albumin, and reservoirs of muscle glycogen and whole-muscle protein, even at 48 h after exhaustion (Pimenta et al., 2006). Opioid, angiotensin I-converting enzyme (ACE)-inhibitory (Pihlanto-Leppälä, 2001), and immunomodulatory (Rodríguez-Carrio et al., 2014) activities are among the biofunctionalities observed for whey protein hydrolysates. Bioactive peptide sequences usually consist of 2–20 amino acid residues (Erdmann et al., 2008). Recently Moura et al. (2016) reported that the Leucyl-Valine peptide found in whey protein hydrolysate stimulated heat shock proteins response in rats. Heat shock proteins participate in

restoration and/or stabilization of damaged proteins induced by various stressors, thus maintain normal cellular function.

Bioactive peptides can be released *in vivo* through the gastrointestinal digestion. The peptides (namely ACE-inhibitory peptides) are then absorbed in the small intestine by carrier-mediated transport mechanism which allows peptides transfer across the cell membrane even against the concentration gradient and in trace quantities. Absorbed peptides are finally released from the basal surface of enterocytes into circulation (Yun et al., 2013). *In vitro* generation of bioactive peptides by microbial and enzymatic proteolysis processes to develop exotic foods (Wildman, 2001) such as beverage mixes (Sinha et al., 2007; Goudarzi et al., 2015) is also common. However, the utilization of the peptides in food products is challenging as some peptides exert bitterness to foods (Belem et al., 1999). Conjugation of bioactive peptides with reducing sugars through the Maillard reaction which was intended to form highly antioxidative nutraceutical ingredients and modify the emulsification and foaming properties of the peptides (Nooshkam and Madadlou, 2016 a,b) is considered a potential method for tuning peptides flavor. The gastrointestinal digestibility and absorbability of the Maillard conjugated peptides may nonetheless differ from those of non-conjugated sequences.

For accomplishing *in vitro* generation of bioactive peptides, diverse types of enzymes and microbial species have been employed. Wheys drained from the curds clotted by a crud extract containing serine endopeptidases from the Osage orange fruits showed both antioxidant and ACE-inhibitory capacities (Corrons et al., 2012). In another work, at a pilot scale study, centrifugally defatted and microfiltered whey was at the outset ultrafiltered to whey protein concentrate (WPC I), followed by diafiltration to remove lactose and minerals and increase

protein concentration. Afterwards, the diafiltered retentate (WPC II) was microfiltered to reduce microbial load and then hydrolyzed by an aqueous proteolytic extract of the plant *Cynara cardunculus* to an ACE-inhibitory hydrolysate. The subsequent ultrafiltration of the hydrolysate with a 20 kDa MW cut-off membrane separated non-hydrolyzed proteins from the peptide-rich permeate. The latter was finally fractionated using a 3 kDa MW cut-off nanofiltration membrane (Tavares et al., 2012). Chemical synthesis is another method for production of bioactive peptides to confirm and relate a specific physiological activity associated with a particular amino acid sequence (Clare and Swaisgood, 2000). Many whey protein-derived peptides possess multifunctional aptitudes. Adjonu et al. (2013) found that biologically multifunctional (ACE-inhibitory, antioxidant, and opioid activities) whey peptides demonstrate technological (nanoemulsification) abilities. Recently bioactive whey peptides were employed in fabrication of nutraceutical-confining particles. Herein, we briefly review the most considered health-supporting and anti-malady capacities of whey peptides, followed by summarizing and discussing the efforts taken to particulate the peptides.

2. Biological activity of whey peptides

2.1. Antihypertension property

Hypertension is greatly associated with cardiovascular disease and affects about one-fourth of the world's population. ACE (peptidyldipeptide hydrolase, EC 3.4.15.1) is an enzyme associated with the renin angiotensin system regulating peripheral blood pressure. It performs several functions including conversion of the decapeptide angiotensin-I to the octapeptide angiotensin-II which is a potent vasoconstrictor and catalyzing the degradation of the vasodilating nonapeptide bradykinin (Mullally et al., 1997). The enzyme inhibition by substrate competitors means

bradykinin stimulation (Eckert et al., 2013) and is therefore considered a therapeutic approach for lowering blood pressure (Madadlou et al., 2011). However, the exact *in vivo* functional mechanism of antihypertensive peptides remains raveled. Polar amino acids such as proline are frequently found in the sequence of ACE-inhibitory peptides (Hartmann and Meisel, 2007). A few of several peptides released from the glycomacropeptide, α-lactalbumin, and one from β-lactoglobulin by the plant proteases previously used in cheese-making in specific regions of Portugal showed strong *in vitro* ACE-inhibitory activity. Subsequent simulated gastrointestinal digestion although caused partial hydrolysis, did not affect the ACE-inhibitory of the corresponding *de novo* synthesized peptides. The diet based on whey cheese was therefore linked with the remarkable longevity of some Portuguese people (Tavares and Malacta, 2012). It has been shown that α-lactorphin [YGLF, α-lactalbumin f(50–53)] which is produced via the enzymatic hydrolysis of α-lactalbumin with pepsin and trypsin reduced blood pressure in a dose-dependent manner without effecting heart rate in spontaneously hypertensive rats, suggesting that opioid receptors were involved in its blood pressure depressing action (Hernández-Ledesma, Ramos, & Gomez-Ruiz, 2011). Chobert et al. (2005) found that more hydrophilic β-lactoglobulin peptides showed higher ACE-inhibitory activity. The sequence obviously dictates the potency of a peptide for ACE inhibition. β-Lactorphin [YLLF, β-lactoglobulin f(102–105)] shows the highest ACE-inhibitory activity compared to both α-lactorphin and β-lactotensin [HIRL, β-lactoglobulin f(146–149)] (Belem et al., 1999).

Although few have been identified *in vivo*, a hundred or so bioactive peptides are encrypted in whey proteins (Boutrou et al., 2015). The degradation of the major whey protein, β-lactoglobulin begins to occur only from the simulated small intestine phase but causes a 10-fold increase in the

ACE-inhibitory activity (Vermeirssen et al., 2004). Fermentation of caprine whey with 25 cheese microflora for 3 days yielded 14 peptide-rich hydrolysates among which 6 samples exhibited higher ACE-inhibitory activity (ranging from 31%–56%) than non-fermented whey (with 14% ACE-inhibitory activity). Five hydrolysates out six were obtained through aerobic fermentation and the peptides were mainly released from α-lactalbumin (Didelot et al., 2006). The peptides encoded in α-lactalbumin f(99–110) have been suggested as the main contributors to the ACE-inhibitory activity of α-lactalbumin hydrolysates. Several peptides in the tryptic digestate of β-lactoglobulin namely f(22–25), f(32–40) and f(81–83) have been identified with ACE-inhibitory activity. The tryptic β-lactoglobulin peptide f(142–148) showed a significant ACE-inhibitory activity with an IC_{50} value (the concentration that inhibits 50% of enzyme activity) of 42.6 μM (Pihlanto-Leppälä, 2001). Interestingly, the latter sequence was found together with f(32–40) in the duodenal effluents of mini-pigs fed raw milk (Barbé et al., 2014).

2.2. Antioxidant property

Reactive oxygen species (ROS) including superoxide anion radicals $(O_2•)^-$, hydroxyl radicals (HO•), hydrogen dioxide (HO_2•), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2•) which are generated by normal body reactions act at low levels as signaling molecules and activators of stress responsive survival pathways (Poljsak and Milisav, 2014). However, at elevated amounts ROS can cause cellular damage, destruction of protein structures and mutation of DNA (Tanzadehpanah et al., 2012). Efficient compounds and strategies are therefore sought by researchers to suppress the ROS activity, as well as, hinder the lipids autoxidation reactions in food commodities. Although non-hydrolyzed food proteins possess lipid oxidation-suppressing activity (based on thiobarbituric acid-reactive substances (TBARS) measurement), enzymatic

hydrolysis significantly increases this property (Peñ̲a-Ramos et al., 2003). Proteins and antioxidant peptides can be employed successfully for inhibiting ROS and polyunsaturated fatty acids autoxidation (Hernández-Ledesma et al., 2011). Amino acid residues containing sulfur and aromatic R-groups are involved at the antioxidant functionality of proteins and peptides through several mechanisms including scavenging free radicals, chelating prooxidative transition metals, reducing hydroperoxides, and modifying physical characteristics of food systems (Elias et al., 2008). Peptide amphiphilicity appears an important criterion for its antioxidant activity in foods as hydrophobic segment provides access to aploar zones (Erdmann et al., 2008). Soy protein isolate and whey protein isolate (WPI) hydrolysates decreased lipid oxidation extent in cooked pork patties (Peñ̲a-Ramos and Xiong, 2003). Moreover, in contrast to WPI hydrolysates prepared by pure pepsin, papain, trypsin, and chymotrypsin, the WPI hydrolyzed by commercially available crude enzymes decreased TBARS formation initiated by iron redox cycling using $FeCl_3$ and ascorbate in liposomes (Peñ̲a-Ramos & Xiong, 2001). Although a report exists that largest-peptides fraction (>45 kDa) showed higher TBARS inhibitory effect in comparison with lower-molecular-weight fractions and total whey protein hydrolysate (Peñ̲a-Ramos et al., 2003), most identified biologically active peptides are small sequences with molecular weight less than 2−3 kDa (Gómez-Ruiz et al., 2004; Nongonierma et al., 2013). The most antioxidative sequence identified in Ostrich egg white protein hydrolysate had a molecular weight of 1317 Da (Tanzadehpanah et al., 2012). Hernández-Ledesma et al. (2005) obtained numerous antioxidant peptides from α-lactalbumin and β-lactoglobulin via hydrolysis with various enzymes among which the sequence Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile released from β-lactoglobulin by the enzyme Corolase PP possessed a higher radical scavenging activity than that

of butylated hydroxyanisole (BHA). A fractionated whey protein hydrolysate prepared through 5-h digestion with Alcalase composed of peptides with weights >40 kDa, 2.8−40 kDa, 0.1−2.8 kDa and <0.1 kDa among which the fraction with 0.1−2.8 kDa exhibited the highest free radical scavenging activity (Peng et al., 2009).

2.3. Anti-obesity and anti-diabetes properties

Both obesity and diabetes are world-wide crises; it is expected that by 2030 approximately 552 million people throughout the world will be affected by diabetes (Diabetes Atlas, 2011). In the United States, overweight prevalence in American children is anticipated to double by 2030 (Wang et al., 2008). Accordingly, efficient strategies are required to be commenced to control these threatening issues. As alternatives with higher safety to anorectic (appetite suppressant) drugs, the food industry makes attempts to produce commodities with satiating properties (Madadlou et al., 2016). It has been shows that substantial protein intake at breakfast causes weight loss. This effect is prominent by milk proteins, in particular whey proteins. A 12-week study demonstrated that consumption of 65 g whey protein concentrate (WPC) dissolved in water 30 min before lunch exerted stronger beneficial effects than soy protein isolate preloads. WPC preloads not only decreased appetite but also reduced total calorie intake and had the potential to decrease body weight, body mass index, waist circumference, and body fat mass while increasing lean muscle of overweight and obese men (Tahavorgar et al., 2014). Milk protein hydrolysates show higher *in vivo* insulinotropic effects than non-hydrolyzed proteins (Morifuji et al., 2010). It was found in a placebo-controlled double-blind randomized study that ingestion of 0.2 g whey protein hydrolysate/kg body weight or 0.4 g whey protein isolate/kg body weight by male patients with Type 2 diabetes markedly promoted insulin secretion and

decreased plasma glucose response. Plasma insulin concentration increased significantly by administering 0.4 g protein hydrolysate/kg body weight concomitant with returning the glucose level to the normal range 2 h after the meal (Goudarzi and Madadlou, 2013). Bioactive whey peptides that are released from precursor proteins during the gastrointestinal digestion are argued to serve as inhibitors of dipeptidyl peptidase IV (DPP-IV) in the proximal gut, preventing the degradation of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). This results in an increased half-life of the incretins GIP and GLP-1 (Nongonierma and FitzGerald, 2013) which stimulate postprandial glucose-dependent insulin secretion. Such mechanisms lead to prolonged satiety and reduced food intake. A potent DPP-IV inhibitory peptide corresponding to β-lactoglobulin f(78–82) was identified in a whey protein hydrolysate digested with trypsin (Silveira et al., 2013). Fractionation results indicate that free amino acids and hydrophilic peptides may be majorly involved in the insulinotropic effect of whey protein hydrolysates (Nongonierma et al., 2013). Ultrafiltration by 5 and 2 kDa cut-off membranes increased the DPP-IV IC_{50} value of an *in vitro* digested WPI with a pancreatic enzyme preparation. Combination of sitagliptin, a DPP-IV inhibitory drug and whey peptides enhanced DPP-IV inhibition, suggesting that a holistic strategy combining conventional clinical drugs, dietary manipulations and physical activity may cause management of Type 2 diabetes (Nongonierma and FitzGerald, 2013). Indeed, the competence of DPP-IV inhibitory peptides to decrease blood glucose level has been confirmed for a β-lactoglobulin tryptic digestate in an *in vivo* study on mice (Uchida et al., 2011).

In addition to peptides, branched-chain amino acids specifically leucine may also contribute to weight loss by up-regulating (activating) mammalian target of rapamycin (mTOR) signaling

pathway that affects mitochondrial biogenesis and protein synthesis, thereby elevating hormone expression/secretion and thermogenesis (Jakubowicz and Froy, 2013). Leucine can as well increase expression/activity of the silent information regulator transcript 1 (SIRT1), an NAD^+-sensitive protein deacetylase implicated in inducing fat oxidation, improving insulin sensitivity and reducing oxidative stress (Hirahatake et al., 2014). The involved mechanisms can potentially attenuate postprandial blood glucose excrusion over the day and improve glucose homeostasis in Type 2 diabetics (Jakubowicz and Froy, 2013). Besides comprising nutritious protein, whey contains calcium that can assist in decreasing body fat accumulation. Calcitophic hormones like 1,25-dihydroxycholecalciferol and parathyroid hormone which respond to low calcium diets trigger calcium influx and thus increase adipocyte intracellular calcium content. The latter in turn upsurges lipogenic gene expression and *de novo* lipogenesis and hinders lipolysis. Diets high in calcium suppress the calcitrophic hormones, thereby inhibiting adipose tissue lipid storage (Ha and Zemel, 2003).

2.4. Hypocholesterolemic property

Certain dietary proteins impose cholesterol-lowering effects. A hypocholesterolemic diet supplemented with various protein resources caused significantly higher blood serum and liver cholesterol for rats on casein diet than for those on soy protein isolate and whey protein concentrate (Jacobucci et al., 2011). Indeed, bovine casein has been reported to tend to elevate blood cholesterol level because probably of its high ratios of methionine-glycine and lysine-arginine. In contrast, soy, fish and whey proteins alter the plasma profile from atherogenic to cardioprotective (Erdmann et al., 2008). Whey protein hydrolysates exert hypocholesterolemic effects. The significantly higher levels of steroids in faeces of rats fed β-lactoglobulin tryptic

digestate compared with those fed casein tryptic digestate was attributed to induction of micellar cholesterol solubility or augmented taurocholate binding (Hartmann and Meisel, 2007). A two-year trial of 30 g/day whey protein supplemented beverage in healthy elderly women indicated that although it did not reduce the hepatic steatosis or the prevalence of fatty liver disease, can prevent worsening of hepatic steatosis associated with weight gain (Ooi et al., 2015).

3. Whey protein particles

There has been an increasing demand by consumers for clean-label and nutraceutical-enriched foods in recent years which has motivated the food industry to implement rational tactics for meeting this requirement. The low bioavailability of most bioactives is challenging. It is caused by a diversity of factors such as susceptibility to environmental and processing foes (oxygen, heat, light, etc.), poor solubility in food matrices (e.g. phytosterols are poorly soluble at both water and oil), enzymatic degradation and polymerization (e.g. polyphenol oxidase-induced browning), instability at the gastrointestinal condition, and etc. (McClements, 2015). Encapsulation is an efficient strategy to protect nutraceuticals and augment their bioavailability.

Unique techno-functional advantages of whey proteins including amphiphilicity, gel formation and inexpensiveness has allowed researchers to load both water- and lipid-soluble bioactive substances within either an individual fraction of whey proteins (e.g. β-lactoglobulin) or a mixture of whey protein fractions. Binding study of vitamin D_3 to α-lactalbumin indicated that hydrophobic interactions play a major role but one hydrogen bonding between serine and vitamin D_3 also occurs (Fig. 1). The binding caused conformational changes of protein to a more hydrophobic surface and random coil structure (Delavari et al., 2015). Beta-lactoglobulin binding with several bioactive molecules including retinol, vitamin D_2, fatty acids, phenolic compounds,

and cholesterol has been confirmed. Beta-lactoglobulin nanostructures generated through heat denaturation associated with riboflavin with an association efficiency of around 26% and protected it during *in vitro* gastric digestion (Madalena et al., 2016). The protein contains at least two binding pouches which allow each β-lactoglobulin molecule to bind two different ligands simultaneously (Teng et al., 2015). Utilization of pure protein fractions for delivery of nutraceuticals appears far from commercialization. As well, more systematic studies are required to develop industrially exploitable whey protein-based carriers that rely not only on simple binding but implicate complementary mechanisms. In addition to being used for direct binding with bioactives, whey proteins have been employed via complexing with other biopolymers for encapsulation purposes. In a recent attempt, electrostatic complexation of β-lactoglobulin with egg white lysozyme produced self-assembled spherical co-precipitates that successfully encapsulated vitamin D_3 (Diarrassouba et al., 2015). The electrostatically stabilized biopolymeric complexes are inherently vulnerable to changes in the ionic strength and pH value of the surrounding environment, which limits the applicability of the complexes in real food systems.

Bulk heat-induced (Gunasekaran et al., 2007; Zand-Rajabi and Madadlou, 2016) and cold-set (Abaee and Madadlou, 2016) hydrogels are among the versions of whey protein carriers that covalent crosslinking contributes. Curcumin as lipophilic and caffeine as hydrophilic model cargos were loaded in lactoferrin-glycomacropeptide nanohydrogels (≈150 nm) produced by heat gelation (Bourbon et al., 2016). The emulsification-internal gelation of whey proteins is a robust method for fabrication of shape-controlled nutraceutical-confining particles. Aqueous nanodroplets microemulsified in an organic phase can serve as nanoreactors for synthesis of

protein particles with controllable size and shape via gelation. Zhang and Zhong (2010) were the first who formed heat-gelled whey protein nanoparticles using microemulsified nanodroplets as templates. Transglutaminase-induced crosslinking of whey protein molecules prior to microemulsification enhanced the heat stability of subsequently generated particles (Zhang and Zhong, 2009). A similar thermal procedure was later employed for production of caffeine-carrying whey protein nanoparticles (Madadlou et al., 2014). To avoid destruction of thermo-labile bioactive ingredients by heat, a cold-set gelation route was developed for phenolic extracts encapsulation within whey protein nanoparticles. Accordingly, an aqueous phase composed of heat-denatured WPI, a phenolic extract, glucono-δ-lactone (GDL) and calcium carbonate was microemulsified in a mixture of sunflower oil and sorbitan monooleate. Gluconic acid generation caused gradual cold gelation of whey proteins inside the nanodroplets templates (Sadeghi et al., 2014).

4. Peptidic particles

4.1. Synthetic peptide-based vehicles

Peptide synthesis is a common practice in areas of inorganic and organic nanomaterials chemistry. Due to being constructed of amino acids, synthetic peptides are generally considered to be biocompatible and biodegradable (Ouboter et al., 2013). They may serve as ligands at the surface of drug-carrying particles for targeting intestinal epithelium. Goblet cell-targeting nanoparticles for the oral delivery of insulin were decorated with a CSKSSDYQC (CSK) targeting peptide. This function of synthetic peptides resembles that of lectins. Lectins are ubiquitous in nature and found in grains, legumes, dairy, nuts and most potatoes. They are carbohydrate-binding proteins which can target the glycocalyx of the intestinal enterocytes and

the mucus layer, thereby increasing nanoparticles transportation across the intestinal mucus (Yun et al., 2013).

As peptidic carrier, synthetic poly(L-lysine)-based block copolymers have been used for insulin encapsulation (Pippa et al., 2015). Polypeptides are also used as non-viral vehicles with ability to escape from endosomes for transferring nucleic acids into cells. Reducible cysteine-flanked linear lysine and arginine-rich peptides underwent dimethylsulfoxide-mediated oxidative polycondensation to a mixture of oligomers. The peptidic oligomers were then complexed with DNA to protect DNA against nucleases and cell surface glycosaminoglycans, as well as, enhance transfection. The reducible peptide plyplexes (i.e. polymer-DNA complexes) are finally expected to cleave in the intracellular reducing environment (Kiselev et al., 2013). In addition to oxidative polycondensation, ultraviolet irradiation can be used for peptides polymerization. Polymerization of self-assembled diacetylene peptide amphiphiles were performed by UV irradiation. Compared with linear peptide amphiphiles, the polymerization efficiency of branched peptide amphiphiles into nanofibers was lower due likely to the imperfect molecular packing (Hsu et al., 2008).

Vesicular structures composed entirely of pure peptides are rare. Peptosomes are the vesicles formed by synthetic peptide amphiphiles. It was demonstrated that a simple 7–8 residue amphiphilic peptide could self-assemble into nanotubes and nanovesicles (Barros et al., 2016). Unlike vesicular structures which provide much larger volume for hydrophilic payloads but smaller volume for hydrophobic payloads, peptide beads as multicompartment supramolecular assemblies with hierarchical organization of micelle-like structures offer almost equal space for both types of cargos (Ouboter et al., 2011). The self-assembly ability of amphiphilic peptides was exploited to fabricate organic/inorganic composites. An amphiphile peptide was C-

terminally modified with a L-cysteine and linked to gold nanoparticles. Subsequent water content increment induced self-assembly of the peptide-coated gold nanoparticles, leading to formation of composite peptide-gold superstructures (Ouboter et al., 2013). Loligomers, synthetic peptides composed of a branched polylysine core harboring identical arms represent simple and versatile molecular vehicles with potential applications in intracellular delivery of drugs (Brokx et al., 2002). Branched amphiphilic peptide capsules (BAPCs) are structurally similar to liposomes that are supramolecular structures where a water-filled cavity is surrounded by a peptide bilayer (Barros et al., 2016). BAPCs are comprised of equimolar proportions of two branched peptide sequences bis(FLIVI)-K-KKKK and bis(FLIVIGSII)-K-KKKK that self-assemble to form bilayer delimited capsules (Sukthankar et al., 2014).

4.2. Whey peptide-based nanostructures

Naturally occurring peptides and the peptides released from proteins may be utilized as building blocks of nano and microstructures. The layer-by-layer assembly technique using silica nanoparticles (SiO_2) as the template was taken for preparation of protamine/heparin nanocapsules (Fig. 2). Protamine is an FDA-approved peptide and consists mainly of arginine residues which render it a suitable substrate for trypsin. The resulting capsules are applicable in enzyme-responsive drug delivery (Radhakrishnan et al., 2015). Whey protein nanofibrils are eminent examples of peptide-based structures with natural basis. They are conventionally formed by heating whey protein solutions for several hours at acidic condition. An enzymatic proteolysis may also be carried out prior to heat-driven fibrillation (Gao et al., 2013). It is now generally accepted that the nanofibrils are constructed by the self-assembly of polypeptides released from parent protein molecules by either acidic or enzymatic hydrolysis rather than intact proteins

(Akkermans et al., 2008). Accordingly, the competence of potentially bioactive peptides to form nanofibrils was investigated. Whey proteins were at first hydrolyzed to *in vitro* highly antioxidant peptides, then fibrillated by heating at pH 2.0. Fibrillation extent of antioxidant peptides was much lower than that of whey proteins. Nonetheless, fibrillation increased the antioxidant activity and β-sheet content of protein hydrolysate. It also increased the foam stability of the antioxidant whey protein hydrolysate (Mohammadian and Madadlou, 2016).

Tubular structures formation by food peptides has been observed. Limited proteolysis of α-lactalbumin by a serine protease from *Bacillus licheniformis* resulted in formation of micron-long and nano-thickness tubes in the presence of Ca^{2+} at neutral pH (Figure 3a). Calculations based on by X-ray scattering data indicated that the cylinder diameter and the cavity diameter of nanotubes were 19.9 and 8.7 nm, respectively (Graveland-Bikker et al., 2006). A minimum concentration of 30 mg/mL of α-lactalbumin is required to progress peptide growth and nanotubes formation (Graveland-Bikker et al., 2004). A very recent report informs that non-pure albumin fraction of wheat bran can form tubular nanostructures (with inner diameter of 100 nm) through hydrolysis by the endoproteinase from *Staphylococcus aureus* V 8 (EC 3.4.21.19) which specifically cleaves Asp–Glu bonds in the presence of Ca^{2+}. It was postulated that hydrolysis increased the possibility of intermolecular interactions by generating new negatively charged carboxyl groups provided by the amino acids Asp and Glu, as well as, destabilizing the α-helical structure of albumins. The co-extracted arabinoxylans from wheat bran could function as template for directing self-assembly of partially lyzed protein molecules (Chaquilla-Quilca et al., 2016). Albumin-based nanotubes potential for utilization in bioactives delivery has been suggested (Livney, 2010).

The application of bioactive peptides as building units of carriers for nutraceuticals is still in its infancy. Such an elaboration generates particles that not only convey nutraceutical cargos but are also bioactive by themselves. There are few reports that *in vitro* antioxidant peptides have been exploited for nutraceuticals confinement. Caffeine was encapsulated within antioxidant whey peptide particles assembled through antisolvent (ethanol) addition method (Bagheri et al., 2014a). In a similar manner, the antisolvent method (desolvation with water in this case) was used to assemble a strongly hydrophobic synthetic peptide sequence into solid spherical particles with dimeters around 500 nm (Dittrich and Meier, 2010). Particulation of antioxidant whey peptides by ethanolic desolvation was as well employed for gallic acid encapsulation (Nourbakhsh et al., In press). Enzymatic crosslinking of whey peptides with transgluaminase prior to ethanolic desolvation increased the size homogeneity of particles, reinforced particles structure and decreased the release rate of caffeine from the peptidic particles in simulated gastric fluid. It also inclined the ferric (Fe^{3+}) reducing power activity of peptides (Bagheri et al., 2014a) which was ascribed to enhanced electron donation tendency of peptides due to multimerization and higher count of COO^{-} groups relative to NH_3^{+} that brought about higher attractive interactions between peptides and Fe^{3+} cations (Nourbakhsh et al., 2016a). Likewise, sodium hydroxide-catalyzed pre-crosslinking of antioxidant whey peptides as alternative to enzymatic crosslinking yielded smaller particles and caused a higher encapsulation efficiency of gallic acid (Nourbakhsh et al., In press). Post-particulation coating of the caffeine-loaded antioxidant peptidic particles with alginate via spray drying augmented particles stability against gastric digestion. This protective effect was significantly amplified by Ca^{2+}-crosslinking of the

alginate coat so that only around 15% of the loaded caffeine within the peptidic nanoparticles released upon simulated gastric digestion (Bagheri et al., 2014b).

In a recent study, transglutaminase-induced crosslinking was applied to boost the gelation proficiency of extensively hydrolyzed whey protein solution so that the hydrolysis-born antioxidant peptides could particulate upon acidification toward their isoelectric point inside microemulsified nanodroplets (Nourbakhsh et al., 2016a). Accordingly, the acid-induced cold-set gelation property of whey proteins and enzymatically crosslinked hydrolysate (i.e. antioxidant whey peptides) was utilized for developing a one-pot procedure through which gallic acid encapsulation and protein/peptides particulation occurred simultaneously. As illustrated schematically in Figure 4, in this route, the recovery of phenolic acid by surfactant reverse micelles existing in the organic membrane of an emulsion or microemulsion liquid membrane from the exterior aqueous phase (for example, a wastewater) into interior aqueous compartments, induced pre-heat-denatured whey proteins/crosslinked peptides *in situ* gelation and subsequent particle formation (Nourbakhsh et al., 2016b). Figure 3 demonstrates microscopic images of whey peptide and whey protein particles. It is observed that whey peptide nanoparticles were orthorhombic and cubic which was ascribed to existence of rod shape nanodroplets in microemulsion, as well as, surface activity of at least a portion of hydrolysate peptides that consequently localized at the oil-water interface together with sorbitan monooleate, thereby engineering droplets shape to platelet-like entities (Nourbakhsh et al., 2016a). Dual functionality including bioactivity and emulsion stabilization has already been discussed for whey protein hydrolysates (Adjonu et al., 2014). It is noteworthy that a peptide should have a minimum of 21 amino acid residues to show good emulsifying properties. The so-called amino acid fraction of a

trypsin-digested β-lactoglobulin hydrolysate which composed mostly (≈87%) of peptides with molecular weights less than 2 kDa showed very poor interfacial tension diminishment efficacy; whereas, the fraction enriched in high molecular weight peptides compared to both the so-called amino acid fraction and total hydrolysate decreased the interfacial tension more efficiently (Turgeon et al., 1992). Adjonu et al. (2014) proposed to fractionate large surface active sequences from whey hydrolysates to employ in accompaniment of co-emulsifiers at formation of emulsions and nanoemulsions. But, the lone application of large peptides that possess good emulsification capability would result in loss of antioxidant fragments. Application of whole hydrolysate at fabrication of peptidic particles via water-in-oil emulsification-internal gelation method sounds a rational manner to enjoy benefits of both bioactive and surface active peptides. Nonetheless, utilizing such a mixed hydrolysate at preparation of oil-in-water emulsions is inappropriate. The preferential adsorption of amphipathic large peptides to oil−water interface and amassing of short antioxidant peptides within the continuous aqueous phase will limit the effectiveness of antioxidant sequences for preventing lipids oxidation which proceeds mainly at droplets interface. Accumulation of low-molecular-weight peptides in the continuous aqueous phase of emulsion can also trigger instability mechanisms such as depletion flocculation (McClements, 2015), leading eventually to emulsion breakdown. As well, short peptides may interact with other water-soluble compounds that exist in the aqueous phase of emulsion and undergo complexation to less antioxidative conjugates.

5. Concluding remarks and future trends

Whey protein hydrolysate has been successfully particulated for encapsulating water-soluble nutraceuticals. Particulation of crosslinked bioactive whey peptides through gel formation within

(micro)emulsified (nano)droplets and subsequent coating with acid-insoluble biomaterials such as alginate yields capsules of high resistance to gastric digestibility. Surface decoration of bioactive peptide capsules with lectins (as natural cell recognition molecules) would facilitate intestinal absorption. Hybrid particles comprised of whey protein-derived peptides and synthetic peptide amphiphiles may open an avenue for exploring fascinating biomedical applications such as intracellular endosomal free delivery of radical scavenging peptides. It is mandatory to identify bioactive peptide sequences in whey protein hydrolysates and investigate the self-assembly, gel formation and emulsion stabilization capacity of these fractions of peptides for rational design of bioactive carriers.

References

Abaee, A., and Madadlou, A. (2016). Niosome-loaded cold-set whey protein hydrogels. *Food Chem.* **196**: 106–113.

Adjonu, R., Doran, G., Torley, P., and Agboola, S. (2014). Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities. *Journal of Food Engineering*, 122, 15–27.

Adjonu, R., Doran, G., Torley, P., and Agboola, S. (2013). Screening of whey protein isolate hydrolysates for their dual functionality: Influence of heat pre-treatment and enzyme specificity. *Food Chem*. **136**: 1435–1443.

Akkermans, C., Venema, P., van der Goot, A. J., Gruppen, H., Bakx, E. J., Boom, R. M., et al. (2008). Peptides are building blocks of heat-induced fibrillar protein aggregates of β-lactoglobulin formed at pH 2. *Biomacromolecules*. **9**: 1474–1479.

Bagheri, L., Madadlou, A., Yarmand, M., and Mousavi, M. E. (2014a). Potentially bioactive and caffeine-loaded peptidic sub-micron and nanoscalar particles. *J. Functional Foods*. **6**: 462–469.

Bagheri, L., Madadlou, A., Yarmand, M., & Mousavi, M. E. (2014b). Spray-dried alginate microparticles carrying caffeine-loaded and potentially bioactive nanoparticles. *Food Res. Int.* **62**: 1113–1119.

Barbé, F., Le Feunteun, S., Rémond, D., Ménard, O., Jardin, J., Henry, G., Laroche, B., and Dupont, D. (2014). Tracking the in vivo release of bioactive peptides in the gut during digestion:

mass spectrometry peptidomic characterization of effluents collected in the gut of dairy matrices fed mini-pigs. *Food Res. Int.* **63**: 147–156.

Barros, S. M., Whitaker, S. K., Sukthankar, P., L.Avila, A., Gudlur, S., Warner, M., Beltrão, E. I. C., John M., and Tomich, J. M. (2016). A review of solute encapsulating nanoparticles used as delivery systems with emphasis on branched amphipathic peptide capsules. *Arch. Biochem Biophys*, **596**: 22–42.

Belem, M. A. F., Gibbs, B. F., and Lee, B. H. (1999). Proposing sequences for peptides derived from whey fermentation with potential bioactive sites. *J. Dairy Sci.* **82**, 486–493.

Bourbon, A. I., Cerqueira, M. A., and Vicente, A. A. (2016). Encapsulation and controlled release of bioactive compounds in lactoferrin-glycomacropeptide nanohydrogels: Curcumin and caffeine as model compounds. *J. Food Eng.* **180**: 110–119.

Boutrou, R., Henry, G., and Sanchez-Rivera, L. (2015). On the trail of milk bioactive peptides in human and animal intestinal tracts during digestion: A review. *Dairy Sci. Technol.* **95**: 815–829.

Brandelli, A., Dariot, D. J., and Corrêa, A. P. F. (2015) Whey as a source of peptides with remarkable biological activities. *Food Res. Int.* **73**: 149−161.

Brokx, R. D., Bisland, S. K., and Gariépy, J. (2002). Designing peptide-based scaffolds as drug delivery vehicles. *J. Control. Release*. **17**: 115–123.

Chaquilla-Quilca, G., Balandrán-Quintana, R. R., Azamar-Barrios, J. A., Ramos-Clamont Montfort, G., Mendoza-Wilson, A. M., Mercado-Ruiz, J. N., Madera-Santana, T. J., López-Franco, Y. L., and Luna-Valdez, J. G. (2016). Synthesis of tubular nanostructures from wheat

bran albumins during proteolysis with V8 protease in the presence of calcium ions. *Food Chem.* **200**: 16–23.

Chobert, J-M., El-Zahar, K., Sitohy, M., Dalgalarrondo, M., Métro, F., Choiset, Y., & Haertlé, T. (2005). Angiotensin I-converting-enzyme (ACE)-inhibitory activity of tryptic peptides of ovine β-lactoglobulin and of milk yoghurts obtained by using different starters. *Lait*, 85, 141–152.

Clare, D. A., and Swaisgood, H. E. (2000). Bioactive milk peptides: A prospectus. *J. Dairy Sci.* **83**: 1187–1195.

Corrons, M. A., Bertucci, J. I., Liggieri, C. S., López, L. M. I., and Bruno, M. A. (2012). Milk clotting activity and production of bioactive peptides from whey using *Maclura pomifera* proteases. *LWT-Food Sci. Technol.* **47**: 103–109.

Delavari, D., Saboury, A. A., Atri, M. S., Ghasemi, A., Bigdeli, B., Khammari, A., Maghami, P., Moosavi-Movahedi, A. A.,, Haertl, T., and Goliaei, B. (2015). Alpha-lactalbumin: A new carrier for vitamin D3 food enrichment. *Food Hydrocolloid.* **45**: 124–131.

Diarrassouba, F., Remondetto, G., Garrait, G., Alvarez, P., Beyssac, E., and Subirade, M. (2015). Self-assembly of b-lactoglobulin and egg white lysozyme as a potential carrier for nutraceuticals. *Food Chem.* **173**: 203–209.

Didelot, S., Bordenave-Juchereau, S., Rosenfeld, E., Fruitier-Arnaudin, I., Piot, J.-M., and Sannier, F. (2006). Preparation of angiotensin-I-converting enzyme inhibitory hydrolysate from unsupplemented caprine whey fermentation by various cheese microflora. *Int. Dairy J.* **16**: 976–983.

Dittrich, C., and Meier, W. (2010). Solid peptide nanoparticles – structural characterization and quantification of cargo encapsulation. *Macromolecular Biosci.* **10**: 1406−1415.

Eckert, E., Zambrowicz, A., Pokora, M., Polanowski, A., Chrzanowska, J., Szoltysik, M., Dąbrowska, A., Różański, H., and Trziszka, T. (2013). Biologically active peptides derived from egg proteins. *World. Poultry Sci. J.* **69**: 375–386.

Elias, R. J., Kellerby, S. S., and Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Cr. Rev. Food Sci.* **48**: 430−441.

Gao, Y.-Z., Xu, H.-H., Ju, T.-T., and Zhao, X.-H. (2013). The effect of limited proteolysis by different proteases on the formation of whey protein fibrils. *J. Dairy Sci.* **96**: 7383–7392.

Goudarzi, M., and Madadlou, A. (2013). Influence of whey protein and its hydrolysate on prehypertension and postprandial hyperglycaemia in adult men. *Int. Dairy J.* **33**: 62−66.

Goudarzi, M., Madadlou, A., Mousavi, M. E., and Emam-Djomeh, Z. (2015). Formulation of apple juice beverages containing whey protein isolate or whey protein hydrolysate based on sensory and physicochemical analysis. *Int. J. Dairy Technol.* **68**: 70−78.

Gómez-Ruiz, J.A., Recio, I., and Belloque, J. (2004). ACE-inhibitory activity and structural properties of peptide Asp-Lys-Ile-His-Pro [*β*-CN f(47–51)]: study of the peptide forms synthesized by different methods. *J. Agric. Food Chem.* **52**: 6315–6319.

Graveland-Bikker, J. F., Fritz, G., Glatter, O., and de Kruif, C. G. (2006). Growth and structure of *α*-lactalbumin nanotubes. *J. Appl. Crystallogr.* **39**: 180–184.

Graveland-Bikker, J. F., Ipsen, R., Otte, J., and de Kruif, C. G. (2004). Influence of calcium on the self-assembly of partially hydrolyzed a-lactalbumin. *Langmuir*. **20**: 6841–6846.

Gunasekaran, S., Ko, S., and Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *J. Food Eng.* **83**: 31–40.

Hartmann, R., and Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. *Curr. Opin. Biotech.* **18**: 163–169.

Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., and Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from α-Lactalbumin and β-Lactoglobulin. Identification of active peptides by HPLC-MS/MS. *J. Agric. Food Chem.* **53**, 588−593.

Hernández-Ledesma, B., Ramos, M., and Gomez-Ruiz, J. A. (2011). Bioactive components of ovine and caprine cheese whey. *Small Ruminant Res.* **101**: 196–204.

Hirahatake, K. M., Slavin, J. L., Maki, K. C., and Adams, S. H. (2014). Associations between dairy foods, diabetes, and metabolic health: Potential mechanisms and future directions. *Metabolism*. **63**: 618–627.

Hsu, L., Cvetanovich, G. L., and Stupp, S. I. (2008). Peptide amphiphile nanofibers with conjugated polydiacetylene backbones in their core. *J. AOCS* **130**: 3892−3899.

Diabetes Atlas, 5th ed. (2011). International Diabetes Federation, www.idf.org

Jacobucci, H. B., Sgarbieri, V. C., Dias, N. F. G. P., Borges, P., and Tanikawa, C. (2001). Impact of different dietary protein on rat growth, blood serum lipids and protein and liver cholesterol. *Nutr. Res.* **21**: 905–915.

Jakubowicz, D., and Froy, O. (2013). Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. *J. Nutr. Biochem.* **24**: 1–5.

Kiselev, A., Egorova, A., Laukkanen, A., Baranov, V., & Urtti, A. (2013). Characterization of reducible peptide oligomers as carriers for gene delivery. *Int. J. Pharm.* **441**: 736–747.

Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. *Cur. Opin. Colloid & Interface Sci.* **15**: 73–83.

Madadlou, A., Rakhshi, E., and Abbaspourrad, A. (2016). Engineered emulsions for obesity treatment. *Trends Food Sci. Tech.* **52**: 90−97.

Madadlou, A., Sheehan, D., Emam-Djomeh, Z., and Mousavi, M. E. (2011). ACE-inhibitory peptides from casein hydrolyzed with nanoencapsulated protease. *J. Sci. Food Agric.* **91**: 2112−2116.

Madalena, D. A., Ramos, O. L., Pereira, R. N., Bourbon, A. I., Pinheiro, A. C., Malcata, F. X., Teixeira, J. A., and Vicente, A. A. (2016). In vitro digestion and stability assessment of β-lactoglobulin/riboflavin nanostructures. *Food Hydrocolloid.* **58**: 89–97.

Mohammadian, M., and Madadlou, A. (2016). Characterization of fibrillated antioxidant whey protein hydrolysate and comparison with fibrillated protein solution. *Food Hydrocolloid.* **52**: 221–230.

Morifuji, M., Ishizaka, M., Baba, S., Fukuda, K., Matsumoto, H., Koga, J., et al. (2010). Comparison of different sources and degrees of hydrolysis of dietary protein: effect on plasma

amino acids, dipeptides, and insulin responses in human subjects. *J. Agric. Food Chem.* **58**: 8788–8797.

Moura, C. S., Lollo, P. C. B., Morato, P. N., Risso, E. M., and Amaya-Farfan, J. (2016). Functional effects of milk bioactive peptides on skeletal muscle of rats. *Food Res. Int.* **84**: 18–25.

Mullally, M. M., Meisel, H., and FitzGerald, R. J. (1997). Angiotensin-I-converting enzyme inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. *Int. Dairy J.* **7**: 299–303.

Nongonierma, A. B., and FitzGerald, R. J. (2013). Dipeptidyl peptidase IV inhibitory properties of a whey protein hydrolysate: Influence of fractionation, stability to simulated gastrointestinal digestion and foodedrug interaction. *Int. Dairy J.* **32**: 33–39.

Nongonierma, A. B., Gaudel, C., Murray, B. A., Flynn, S., Kelly, P. M., Newsholme, P., and FitzGerlad, R. J. (2013) Insulinotropic properties of whey protein hydrolysates and impact of peptide fractionation on insulinotropic response. *Int. Dairy J.* **32**: 163−168.

Nooshkam, M., and Madadlou, A. (2016). Maillard conjugation of lactulose with potentially bioactive peptides. *Food Chem.* **192**: 831−836.

Nooshkam M., and Madadlou, A. (2016). Microwave-assisted isomerisation of lactose to lactulose and Maillard conjugation of lactulose and lactose with whey proteins and peptides. *Food Chem.* **200**: 1−9.

Nourbakhsh, H., Emam-Djomeh, Z., Madadlou, A., Mousavi, M. E., Moosavi-Movahedi, A. A., and Gunasekaran, S. (In press). Antioxidant peptidic particles for delivery of gallic acid. *J. Food Process. Preserv.* doi:10.1111/jfpp.12767.

Nourbakhsh, H., Madadlou, A., Emam-Djomeh, Z., Wang, Y.-C., Gunasekaran, S. (2016a). One-pot nanoparticulation of potentially bioactive peptides and gallic acid encapsulation. *Food Chemistry*, 210, 317−324.

Nourbakhsh, H., Madadlou, A., Emam-Djomeh, Z., Wang, Y.-C., Gunasekaran, S., and Mousavi, M. E. (2016b). One-Pot procedure for recovery of gallic acid from wastewater and encapsulation within protein particles. *J. Agric. Food Chem.* **64**: 1575–1582.

Ooi, E. M., Adams, L. A., Zhu, K., Lewis, J. R., Kerr, D. A., Meng, X., Solah, V., Devine, A., Binns, C. W., and Prince, R. L. (2015). Consumption of a whey protein-enriched diet may prevent hepatic steatosis associated with weight gain in elderly women. *Nutr. Metab. Cardiovas.* **25**: 388–395.

Ouboter, D. B., Schuster, T. B., Mantion, A., & Meier, W. (2011). Hierarchical organization of purely peptidic amphiphiles into peptide beads. *J. Phys. Chem.* **115**: 14583−14590.

Ouboter, D. B., Schuster, T. B., Sigg, S. J., & Meier, W. P. (2013). Self-assembled peptide beads used as a template for ordered gold nanoparticle superstructures. *Colloid Surface B.* **112**: 542–547.

Peña-Ramos, E. A., and Xiong, Y. L. (2001). Antioxidative activity of whey protein hydrolysates in a liposomal system. *J. Dairy Sci.* **84**: 2577−2583.

Peña-Ramos, E. A., and Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Sci.* **64**: 259−263.

Peña-Ramos, E. A., Xiong, Y. L., and Arteaga, G. E. (2003). Fractionation and characterisation for antioxidant activity of hydrolysed whey protein. *J. Sci. Food Agric.* **84**: 1908−1918.

Peng, X., Xiong, Y. L., and Kong, B. (2009). Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chem.* **113**: 196−201.

Pihlanto-Leppälä, A. (2001) Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides. *Trends Food Sci. Tech.* **11**: 347−356.

Pimenta, F. M. V., Abecia-Soria, M. I., Flá via Auler, F., and Amaya-Farfan, J. (2006). Physical performance of exercising young rats fed hydrolysed whey protein at a sub-optimal level. *Int. Dairy J.* **16**: 984−991.

Pippa, N., Kalinova, R., Dimitrov, I., Pispas, S., and Demetzos, C. (2015). Insulin/poly(ethylene glycol)-block-poly(L‑lysine) complexes: Physicochemical properties and protein encapsulation. *J. Phys Chem. B.* **119**: 6813−6819.

Poljsak, B., and Milisav, I. (2014). Oxidized forms of dietary antioxidants: Friends or foes? *Trends Food Sci. Tech.* **39**: 156−166.

Radhakrishnan, K., Thomas, M. B., Pulakkat, S., Gnanadhas, D. P., Chakravortty, D., and Raichur, A. M. (2015). Stimuli-responsive protamine-based biodegradable nanocapsules for enhanced bioavailability and intracellular delivery of anticancer agents. *J. Nanopart. Res.* **17**: 341.

Rodríguez-Carrio, J., Fernández, A., Riera, F. A., and Suárez, A. (2014). Immunomodulatory activities of whey b-lactoglobulin tryptic-digested fractions. *Int. Dairy J.* **34**: 65–73.

Sadeghi, S., Madadlou, A., and Yarmand, M. (2014). Microemulsification–cold gelation of whey proteins for nanoencapsulation of date palm pit extract. *Food Hydrocolloid.* **35**: 590–596.

Silveira, S. T., Martínez-Maqueda, D., Recio, I., and Hernández-Ledesma, B. (2013). Dipeptidyl peptidase-IV inhibitory peptides generated by tryptic hydrolysis of a whey protein concentrate rich in b-lactoglobulin. *Food Chem.* **141**: 1072–1077.

Smithers, G. W. (2008) Whey and whey proteins−From 'gutter-to-gold'. *Int. Dairy J.* **18**: 695−704.

Sinha, R., Radha, C., Prakash, J., and Kaul, P. (2007). Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. *Food Chem*. **101**: 1484−1491.

Sukthankar, P., Avila, L. A., Whitaker, S. K., Iwamoto, T., Morgenstern, A., Apostolidis, C., Liu, K., Hanzlik, R. P., Dadachova, E., and Tomich, J. M. (2014). Branched amphiphilic peptides capsules: Cellular uptake and retention of encapsulated solutes. *BBA-Biomembrane.* **1838**: 2296–2305.

Tahavorgar, A., Vafa, M., Shidfar, F., Gohari, M., and Heydari, I. (2014). Whey protein preloads are more beneficial than soy protein preloads in regulating appetite, calorie intake, anthropometry, and body composition of overweight and obese men. *Nutr. Res.* **34**: 856–861.

Tanzadehpanah, H., Asoodeh, A., and Chamani, J. (2012). An antioxidant peptide derived from Ostrich (Struthio camelus) egg white protein hydrolysates. *Food Res. Int.* **49**: 105−111.

Tavares, T., Amorim, M., Gomes, D., Pintado, M. E., Pereira, C. D., and Malcata, F. X. (2012). Manufacture of bioactive peptide-rich concentrates from whey: Characterization of pilot process. *J. Food Eng.* **110**: 547–552.

Tavares, T., and Malacta, F. X. (2012). The Portuguese Paradox: Why do some inhabitants of Portugal appear to live so long when their diet is based on whey cheese? *Food Chem.* **131**: 727–729.

Teng, Z., Xu, R., and Wang, Q. (2015). Beta-lactoglobulin-based encapsulating systems as emerging bioavailability enhancers for nutraceuticals: a review. *RSC Adv.* **5**: 35138–35154.

Turgeon, S. L., Gauthier, S. F., Mollé, D., and Léonil, J. (1992). Interfacial properties of tryptic peptides of β−lactoglobulin. *J. Agric. Food Chem.* **40**: 669−675.

Uchida, M., Ohshiba, Y., and Mogami, O. (2011). Novel dipeptidyl peptidase-4-inhibiting peptide derived from β-lactoglobulin. *J. Pharmacol. Sci.* **117**: 63–66.

Vermeirssen, V., van der Bent, A., Camp, J. V., van Amerongen, A., and Verstraete, W. (2004). A quantitative in silico analysis calculates the angiotensin I converting enzyme (ACE) inhibitory activity in pea and whey protein digests. *Biochimie.* **86**: 231–239.

Wang, Y., Beydoun, M. A., Liang, L., Caballero, B., and Kumanyika, S. K. (2008). Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity*. **16**: 2323−2330.

Wildman, R. E. C. (2001). Nutraceuticals: A brief review of historical and teleological aspects. **In**: Handbook of Nutraceuticals and Functional Foods. Wildman, R. E. C. ed., CRC Press, New York.

McClements, D. J. (2015). Food Emulsions: Principals, Practices, and Techniques. 3rd edition, CRC Press, New York.

Yun, Y., Cho, Y. W., & Park, K. (2013). Nanoparticles for oral delivery: Targeted nanoparticles with peptidic ligands for oral protein delivery. *Adv. Drug Deliver. Rev.* **65**: 822–832.

Zand-Rajabi, H., and Madadlou, A. (2016). Caffeine-loaded whey protein hydrogels reinforced with gellan and enriched with calcium chloride. *Int. Dairy J.* **56**: 38–44.

Zhang, W., and Zhong, Q. (2009). Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by sequential enzymatic cross-linking and thermal pretreatments. *J. Agric. Food Chem.* **57**: 9181–9189.

Zhang, W., and Zhong, Q. (2010). Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by thermal pretreatment. *Food Chem.* **119**: 1318–1325.

Table 1. Some health-promoting and technologically important functionalities of whey protein hydrolysates, along with their molecular mechanisms and attributed effect.

Functionality	Mechanism of action	Attributed effect	Reference
Anti-lactic acidosis, Increased storage of muscle glycogen and protein	Not-known	Improving physical performance	Pimenta et al. (2006)
Antihypertension	ACE[1] inhibition	Longevity	Tavares and Malacta (2012)
Anti-oxidation	ROS[2] inhibition, Prooxidants chelation, Hydroperoxides reduction	Suppressing DNA mutation; protecting cell proteins structure; preventing food lipids and proteins oxidation	Tanzadehpanah et al. (2012); Elias et al. (2008)
Anti-obesity and anti-diabetes	DPP-IV[3] inhibition (by peptides); mTOR[4] activation (by BCAAs[5]); SIRT1[6] activation (by leucine); inactivating calcitrophic hormones (by calcium)	Prolonging satiety; decreasing food intake, blood glucose level and oxidative stress; improving glucose homeostasis and insulin sensitivity; inhibiting adipose tissue lipid storage	Nongonierma and FitzGerald (2013); Uchida et al. (2011); Silveira et al. (2013); Jakubowicz and Froy (2013); Hirahatake et al., (2014); Ha and Zemel (2003)
Hypocholesterolemic	Micellar cholesterol solubility and taurocholate binding	Preventing worsening of hepatic steatosis	Hartmann and Meisel (2007); Ooi et al. (2015)
Restoration of damaged proteins	Stimulation of heat shock proteins response	Maintain normal cellular function	Moura et al. (2016)

[1]Angiotensin-I converting enzyme.

[2]Reactive Oxygen Species.

[3]Dipeptidyl peptidase IV.

[4]Mammalian target of rapamycin (mTOR) signaling pathway.

[5]Branched chain amino acids.

[6]Silent information regulator transcript 1.

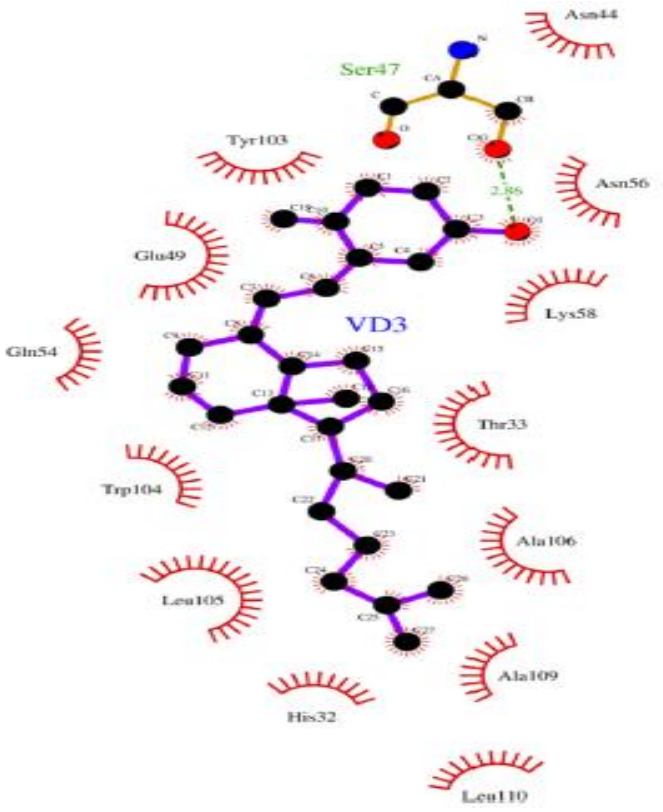

Fig. 1. Schematic illustration of vitamin D_3 binding to the hydrophobic pocket of α-lactalbumin (Source: Delavari et al., 2015).

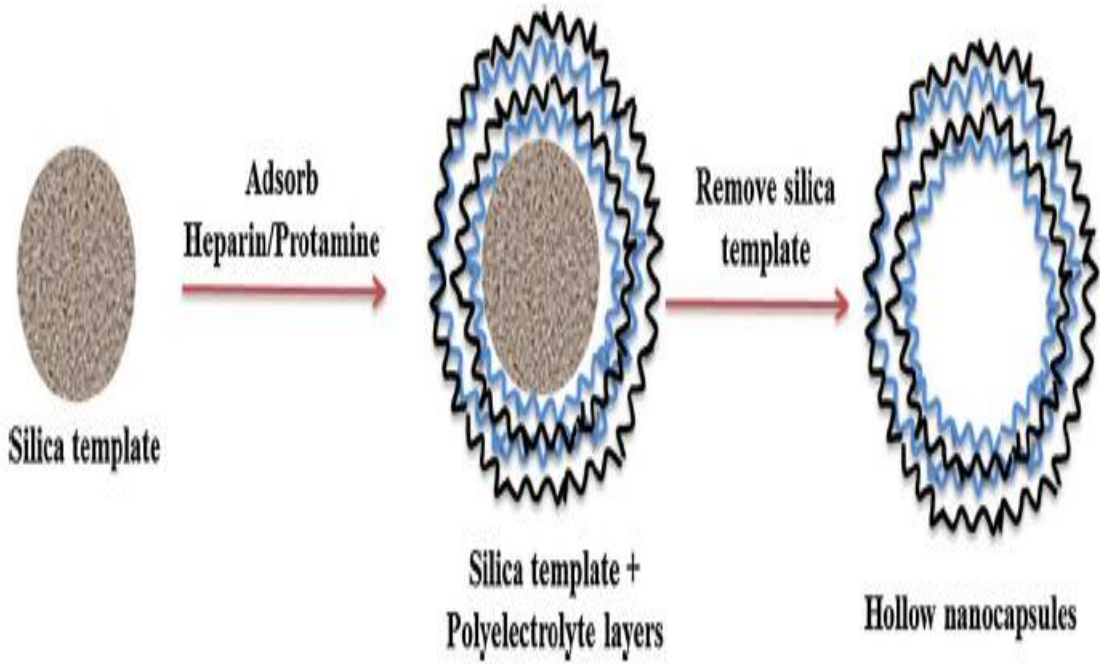

Fig. 2. Schematic illustration of protamine/heparine particles prepearation via layer-by-layer deposition technique using silica nanoparticles as the template (Source: Radhakrishnan et al., 2015).

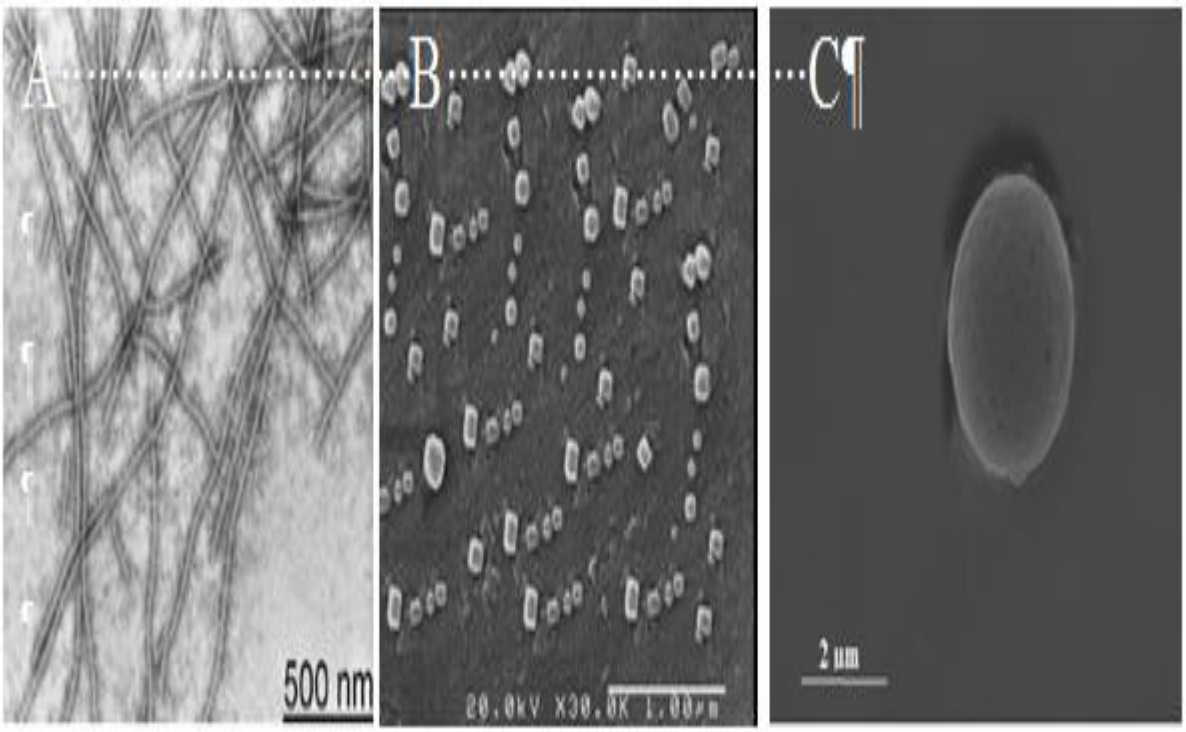

Fig. 3. (A) Transmission electron microscopy images of self-assembly of partially hydrolyzed α-lactalbumin to nanotubes (Graveland-Bikker et al., 2006); (B) Field emission scanning electron microscopy image of caffeine-free particles formed by desolvation of enzymatically crosslinked antioxidant whey peptides (Bagheri et al., 2014a); and (C) Scanning electron microscopy image of microparticles composed of whey protein/crosslinked whey peptides (Nourbakhsh, Madadlou, Emam-Djomeh, Wang, Gunasekaran, & Mousavi, 2016).

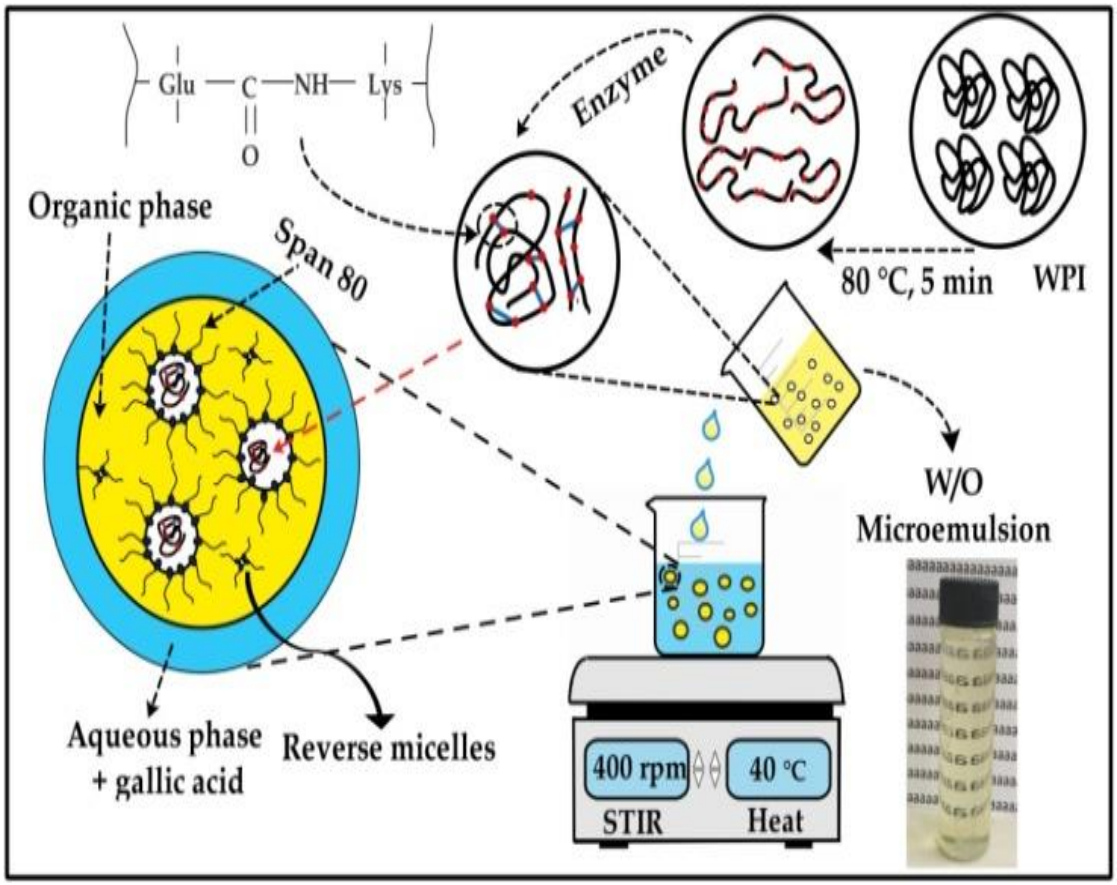

Fig. 4. Schematic illustration of the one-pot procedure employed for simultaneous recovery of gallic acid from an exterior aqueous phase into (micro)emulsified droplets and particulation of whey proteins/crosslinked antioxidant peptides.