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Whey protein hydrolysates as a source of bioactive peptides for functional foods – Biotechnological facilitation of industrial scale-up

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ABSTRACT

Whey proteins, which possess the highest nutritional quality of all food proteins, are an optimal source of functional food ingredients. Enzymatic hydrolysis of whey proteins liberates fragments that can promote health benefits in the immune, cardiovascular, nervous and gastrointestinal systems. Industrial production of peptide-based food ingredients requires overcoming several challenges in product development to achieve economically viable downstream processes. We suggest hybrid strategies currently utilized in biopharmacy that are based on computational modelling combined with heuristics and mechanistic modelling, thus minimizing the time- and cost-intensive *trial and error* approach. Additionally, we propose an application of a cost performance indicator during early decisions at the laboratory scale, which can lead to optimal downstream processing of bioactive, peptide-based food ingredients. This review summarizes the requirements of industrial processes regarding peptide release and stability, depending on several process parameters, and considers some enrichment techniques for whey-derived peptides that are potentially applicable to industry.

1. Introduction

Functional foods (also called nutraceuticals, pharmafoods and designer foods) are made from natural ingredients and offer, in addition to their nutritional value, a specific health advantage due to the functional *in vivo* activity of a supplemented food additive (McIntosh et al., 1998). Japan was the first country to introduce so-called Foods for Specified Health Uses (FOSHU) into the market in 1991 (Yamada, Sato-Mito, Nagata, & Umegaki, 2008). These types of functional foods are divided into three categories: (1) standardized FOSHU, (2) reduction of disease risk FOSHU, and (3) qualified FOSHU. Currently, these categories are based on evidence of eleven different health claims, e.g., gastrointestinal regulation, lower blood cholesterol, glucose level reduction or dental health (Maeda-Yamamoto, in press). Yamada et al. (2008) and Maeda-Yamamoto (in press) provided circumstantial information about the necessary documentation for the approval of products as FOSHU by the Japanese Office of Health Policy on Newly Developed Foods, highlighting that legal recognition as FOSHU requires a detailed review of scientific evidence, including human clinical studies, animal studies, and *in vitro* metabolic and biochemical data.

Food proteins are suggested to be a high-quality source of protein because they are a natural ingredient in functional foods. Typical food proteins derived from plant and animals are meat, milk, egg, fish, soy, and wheat, amongst others. Whey proteins have the highest nutritional quality (Protein Digestibility-Corrected Amino Acid Score (PDCAAS) = 1) of the dietary protein sources and even greater biological value than the milk protein casein. Whey proteins are rich in human-essential amino acids (AAs), branched-chain amino acids

Abbreviations: FOSHU, Foods for Specified Health Uses; PDCAAS, protein digestibility corrected amino acid score; BCAAs, branched-chain amino acids; β-LG, β-lactoglobulin; TMP, total milk protein; α-LA, α-lactalbumin; BSA, bovine serum albumin; BLF, bovine lactoferrin; GMP, glycomacropeptides; IgG, immunoglobulin G; BOD, biological oxygen demand; COD, chemical oxygen demand; ACE, angiotensin-converting enzyme; DH, degree of hydrolysis; WPI, whey protein isolate; WPC, whey protein concentrate; BLP, *Bacillus licheniformis* protease; UHPH, ultrahigh-pressure homogenization; STTT, short-time thermal treatment; US, ultrasonication; MW, microwave; VIP, virtual intermediate peptide; GIT, gastrointestinal tract; CPP, critical process parameters; DPP-IV, dipeptidylpeptidase IV; BU, binding unit; SU, stimulating unit; SUMO, small ubiquitin-related modifier; LAB, lactic acid bacteria; NSLAB, non-starter lactic acid bacteria; CCP, corn cob powder; PES, polyethersulfone; HPLC, high-pressure liquid chromatography; MS, mass spectrometry; FDA, Food and Drug Administration; AAs, amino acids; ACPs, anticancer peptides; NK cells, natural killer cells; DoE, Design of Experiments; QSAR, quantitative structureactivity relationships; AMP, antimicrobial peptide; SVM, support vector machines; RF, random forest; ANN, artificial neuronal networks; DA, discriminant analysis; MBPD, Milk Bioactive Peptide Database; CAMP, Collection of Anti-Microbial Peptides; DPP-4, dipeptidyl peptidase-4; PPI, purification performance index; SCI, separation cost indicator; QSPR, quantitative structureproperty relations; SDS, sodium dodecyl sulphate; AC, activated carbon; EDUF, electrodialysis-ultrafiltration; CFEMF, cross-flow electro-membrane filtration; MC, membrane chromatography; MAC, membrane adsorption chromatography; MWCO, molecular weight cut-off; IMER, immobilized enzyme reactors; EOPO Co-polymer (UCON)/Phosphate, poly(ethylene glycol-ran-propylene glycol) monobutyl ether/phosphate

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(BCAAs) and sulfur-containing AAs, and they promote metabolic regulation and protein folding (Smithers, 2015).

The health benefits of whey proteins may have been known for hundreds of years or even longer, as whey was part of treatments for illness in folk medicine (Hoffmann, 1961) and is still used by various societies for food preservation and illness treatment (Gupta, 2012; Pieroni & Giusti, 2008; Pieroni & Gray, 2008; Sikarwar & Kaushik, 1993). In the period from the 17th to the early 19th century, whey became popular as a medicinal drink and an inexpensive alternative bath ingredient (Smithers, 2015).

The precise molecular components of whey are known. In addition to lactose, minerals and vitamins, the health benefits of whey are related to whey proteins, including enzymes and glycomacropeptide (GMP) (Anand, Som Nath, & Chenchaiah, 2013). The main components of bovine whey proteins are β-lactoglobulin (β-LG, 3.3 g/L total milk protein (TMP)) and α-lactalbumin (α-LA, 1.2 g/L TMP), and components in minor concentrations include bovine serum albumin (BSA, 0.3 g/L TMP), bovine lactoferrin (BLF, 0.1 g/L TMP), immunoglobulins (0.5–1 g/L TMP), lactoperoxidase (0.03 g/L TMP), and proteinaceous GMP (1.2 g/L TMP) (Korhonen, 2009a; Korhonen, 2012; Madureira, Tavares, Gomes, Pintado, & Malcata, 2010). The enzymatic action of chymosin releases proteinaceous GMP from milk κ-casein during cheese-making, cleaving the C-terminal region of κ-casein between AA residues 105 and 106 and therefore leading to the precipitation of caseins, thus forming cheese (Thomä-Worringer, Sørensen, & López-Fandiño, 2006). GMP is a glycophosphopeptide that is 64 AAs in length and lacks the aromatic AAs Phe, Tyr and Trp; for this reason, it is recommended in medical food design (Ney & Etzel, 2017) and showed prebiotic and anti-inflammatory effects in mice (Sawin et al., 2015). Yadav et al. (2015) noted that in addition to their chemical function (e.g., aiding emulsification or gelling) and nutraceutical characteristics, whey proteins have known physiological benefits, including vitamin binding, antibacterial activity and immunomodulatory effects, amongst others.

Whey proteins with *in vivo* biological functions, such as positive influences on the cardiovascular, digestive, endocrine, immune, and nervous systems, are therefore optimal for use as a functional food additive. However, many of the bioactivities of whey proteins are encrypted within their native protein sequences and can thus be liberated only by protein fragmentation, e.g., through enzymatic hydrolysis (Pihlanto-Leppälä, 2000). The natural production of these bioactive whey peptides occurs via (I) gastrointestinal digestion of milk proteins by digestive enzymes and (II) food processes, such as the fermentation of milk or the ripening of cheese by lactic acid bacteria (LAB) (Korhonen, 2009b).

A problematic aspect of whey is its increasing volume as a by-product, which became detrimental to the environment with industrialization and expansion of the manufacture of dairy products in the 20th century (Smithers, 2015). For the production of 1 kg of cheese, approximately 9 kg of cheese whey accrues as an effluent (Prazeres, Carvalho, & Rivas, 2012). Bovine whey contains 20% of the milk protein content, the entire milk lactose content (~5%), and traces of fat (0.1%) and mineral salts (0.46–10%). Approximately 99% of this organic matter is biodegradable, resulting in a high biochemical oxygen demand (BOD; > 30.000 mg O_2/L for sweet whey and 35.000 mg O_2/L for acid whey) and chemical oxygen demand (COD; > 60.000 mg O_2/L for sweet whey and ~80.000 mg O_2/L for acid whey). Biological treatment of whey is difficult because of its acidic pH (3.8–6.5), low alkalinity, and sodium, free ammonia, potassium and volatile fatty acid contents (Prazeres et al., 2012; Smithers, 2015). Yadav et al. (2015) reviewed possible methods to transform the proteinaceous part of effluent whey into bioprotein, functional proteins and bioactive peptides; however, as mentioned before, nearly the entire lactose content of whey remains in the whey permeate and thus requires further effluent post-treatment steps. Innovative treatment technologies, such as the production of D-tagatose from lactose as an alternate sweetener (D-tagatose contains only 30% of the energy content of sucrose), could result in the high-grade transformation of the effluent whey (Jayamuthunagai, Srisowmeya, Chakravarthy, & Gautam, 2017).

The elevated nutritional value of bovine whey proteins, together with their technological properties and distinguished health benefits in the form of hydrolysed peptides, have transformed whey into a high-value raw material for the food industry with particular applications in the production of functional foods (Yadav et al., 2015). Several recent studies have confirmed the applicability of health-promoting peptides to functional foodstuffs: Chatterjee, Kanawjia, and Khetra (2016) showed that the addition of a tryptic whey protein hydrolysate to Indian sweetened yogurt significantly increased its angiotensin-converting enzyme (ACE) inhibition and antioxidant activity, indicating the maintenance of peptide stability during food processing and within the food matrix. Yu, Amorim, Marques, Calhau, and Pintado (2016) reported as a lead for peptide functionality and stability that a low-molecular-weight (< 1 kDa) whey peptide extract of a mixture of cow, goat and ewe whey stimulated the growth of probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) in Wistar rats. Graves et al. (2016) found that an anticancer pentapeptide derived from rice bran was stable for 6 months when stored in spray-dried orange juice and received acceptance from consumers regarding its sensory properties. Hafeez et al. (2014) proposed the use of bioactive peptides from milk proteins, including whey proteins, to increase the functionality of fermented milk products and reviewed the three known production process strategies: (a) peptide release directly into fermented milk products, (b) supplementation of fermented milk products with peptides produced outside of the product and (c) production of bioactive peptides using recombinant DNA technology.

Generally, natural digestion of whey proteins in the gastrointestinal tract via the consumption of whey protein-containing products is not sufficient to achieve a positive health effect; as a consequence, the bioactive peptides responsible for the health benefits must be enriched by industrial processing of hydrolysed whey (Gauthier, Pouliot, & Saint-Sauveur, 2006). The ongoing development in the past two decades of technologies for the production of whey bioactive peptide-containing foods on the industrial scale has resulted in several products on the international market (Hettiarachchy, Sato, Marshall, & Kannan, 2011; Korhonen, 2009b). Table 1 lists bioactive peptides that are derived from whey proteins and are used as ingredients in functional foods. Regarding the industrial production of peptide-based food ingredients, membrane filtration techniques (ultrafiltration and nanofiltration) are the only methods that enrich food ingredients, such as whey hydrolysates, with bioactive peptides in an economically viable way (Agyei, Ongkudon, Wei, Chan, & Danquah, 2016; Korhonen, 2009b). More effective and specific enrichment of bioactive peptides, including purification, within downstream processes requires chromatography steps, which are cost intensive and time consuming when applied using conventional laboratory methods. These processes are expensive, difficult to apply or affect the secondary structure of peptides, resulting in the possible alteration or elimination of their bioactive characteristics; therefore, industrial scale-up is not economically viable for the food industry. Thus, the lack of economically profitable production processes decelerates the development of products that contain bioactive peptides (Agyei & Danquah, 2011; Agyei et al., 2016). Furthermore, basic research purification processes must be adapted to meet the requirements of an economically viable process at the industrial scale (Korhonen & Marnila, 2013). New purification technologies in the food industry are less focused on products with high-grade, pure bioactive peptides and are more focused on the development of economically viable enrichment techniques (Agyei et al., 2016).

This review article provides a brief overview of the conventional downstream process steps of bioactive whey peptide research developed under laboratory conditions compared to the actual flow path of industrial production. Furthermore, we discuss current critical process steps that can affect the release of bioactive peptide sequences and