Food Research International xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

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

Whey as a source of peptides with remarkable biological activities

Adriano Brandelli [a,*], Daniel Joner Daroit [b], Ana Paula Folmer Corrêa [a]

[a] Laboratório de Bioquímica e Microbiologia Aplicada, Departamento de Ciência de Alimentos, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre, Brazil
[b] Laboratório de Microbiologia, Universidade Federal da Fronteira Sul, Campus Cerro Largo, 97900-000 Cerro Largo, Brazil

ARTICLE INFO

Article history:
Received 15 January 2015
Accepted 25 January 2015
Available online xxxx

Keywords:
Bioactive peptides
By-product
Protein hydrolysis
Whey

ABSTRACT

The dairy industry generates increased amounts of whey from both cheese and casein production facilities. Whey presents an elevated content of lactose and proteins, which are associated with its high biological oxygen demand and decomposing potential. Despite its potential as pollutant, whey has been considered as a dairy by-product due to its nutritional, functional and bioactive properties. The use of enzyme technology may be an interesting strategy to convert whey into added-value products. The hydrolysis of whey proteins can generate bioactive peptides, which are described to perform physiological effects *in vivo*, such as antioxidant, antimicrobial, antihypertensive and antidiabetic activities. Bioactive peptides derived from whey proteins have been also associated with immunomodulatory, anticancer, opioid and hypocholesterolemic activities. This review presents a discussion on the main biological activities of peptides derived from whey proteins.

1. Introduction

Whey corresponds to the liquid fraction remaining after milk clotting and casein removal during cheese manufacturing. Whey is an abundant by-product of the dairy industry, resulting from either cheese or casein production. This by-product represents about 85–90% of milk volume and retains approximately 55% of milk nutrients (Siso, 1996; Smithers, 2008).

Whey contains the lactose and non-casein proteins of milk, and its elevated content of organic matter is associated with a high biochemical oxygen demand and potential for decomposition. Whey was considered the most important pollutant of the dairy industry, not only due to its high organic loading, but also due to its elevated volume (Walzen, Dillard, & German, 2001). However, the perception of whey as a pollutant has changed with the discovery of its functional and bioactive properties, being considered as an additional product of cheese manufacture (De Boer, 2014; Smithers, 2008).

Despite its elevated nutritional value, the use of whey *in natura* is limited due to its perishable characteristics and elevated dilution of its components. In this way, several technologies have been used to benefit this material. Thus, concentration of whey may be realized by heating and drying (evaporation, spray-drying, freeze-drying) or by reversed osmosis, whereas demineralization can be performed by ion exchange resins or electrodialysis. Membrane separation technologies have been equally used for obtaining protein ingredients from whey (Brans, Schröen, van der Sman, & Boom, 2004).

Alternatively, production of hydrolysates can be an interesting approach to add value to whey. Diverse protein hydrolysates obtained by enzymatic catalysis display biological activities, which are often associated with bioactive peptides. The bioactive peptides are inactive while encrypted in the sequence of original protein but can be released by (a) hydrolysis by digestive enzymes, (b) proteolytic microorganisms, and/or (c) the action of plant or microbial proteases (Korhonen & Pihlanto, 2006). Bioactive peptides are the focus of several investigations mostly related to antioxidant, antihypertensive and antimicrobial activities. Indeed, commercial proteases have been successfully tested for the production of bioactive hydrolysates from milk, including whey proteins. Intensive investigation of antioxidant and antihypertensive peptides derived from hydrolysis of bovine caseins has been performed in the last decades (Corrêa et al., 2011; Daroit et al., 2012; Hernández-Ledesma, García-Nebot, Fernández-Tomé, Amigo, & Recio, 2014; Phelan, Aherne, FitzGerald, & O'Brien, 2009). More recently, important amount of research including other species, such as ovine, caprine and camel, has confirmed the importance of milk proteins, mainly caseins, as source of bioactive peptides (Korhonen, 2009). Although less information is available about bioactive peptides derived from whey proteins, some important biological activities have been associated with protein hydrolysates derived from whey. Angiotensin I-converting enzyme (ACE)-inhibitory activity was observed in whey protein hydrolysates obtained from *Cynara cardunculus* protease (Tavares, Contreras, Amorim, Pintado, Recio and Malcata, 2011; Tavares, Monteiro, Possenti, Pintado, Carvalho and Malcata, 2011). Treatment of caprine whey proteins with gastrointestinal juice resulted in hydrolysates

* Corresponding author at: Av. Bento Gonçalves 9500, ICTA-UFRGS, 91501-970 Porto Alegre, Brazil. Fax: +55 51 3308 7048.
E-mail address: abrand@ufrgs.br (A. Brandelli).

http://dx.doi.org/10.1016/j.foodres.2015.01.016
0963-9969/

showing antimicrobial activity (Almaas et al., 2011). The inhibition of dipeptidyl peptidase IV (DPP-IV) by a whey protein hydrolysate generated with food-grade pancreatic enzyme was also described (Nongonierma & FitzGerald, 2013a).

Considering the great potential of whey as a source of bioactive peptides, an effective knowledge on the production and characteristics of these peptides would be very relevant. In addition, taking into account that whey is an ingredient in several food formulations, a whey-derived product rich in bioactive peptides would be an interesting added-value product. The investigation on bioactive peptides generated by enzymatic hydrolysis of whey has a great potential to generate novel products and biotechnological processes. Therefore, this article presents a current evaluation on the major biological activities associated with hydrolysates of whey proteins and some specific bioactive peptides derived from whey proteins.

2. Whey derived products

Hydrolysis of whey proteins has been employed to modify solubility, viscosity, emulsifying and foaming properties, as well as to improve nutritional properties. Special attention has been devoted to the utilization of whey-derived products in sports medicine. It has been suggested that protein hydrolysates providing mainly di- and tripeptides are superior to intact (whole) proteins and free amino acids in terms of skeletal muscle protein anabolism (Manninen, 2009). Thus, consumption of whey-derived products may allow amino acids to be more rapidly absorbed than whole proteins, maximizing nutrient delivery to muscle tissues.

Proteolytic enzymes derived from several sources have been employed in the hydrolysis of whey proteins (Siso, 1996; Zhang, Wu, Ling, & Lu, 2013). As the microorganisms can be cultivated through controlled and well established methods, microbial proteases have been pointed as interesting biocatalysts to the production of protein hydrolysates in commercial scale. Indeed, commercial proteases of microbial origin have been successfully tested for production of hydrolysates from whey proteins (Butré, Wierenga, & Gruppen, 2012).

Hydrolysates of whey protein are considered as ideal ingredients in the formulation of human milk substitutes due to their high nutritional value, low bitterness and low antigenicity. Allergic reactions are often associated with specific sequences of β-LG, the major whey protein. Thus, bacterial proteases have been used for production of hydrolysates with reduced allergenicity. The commercial alkaline protease form *Bacillus licheniformis* Protex 6L was used to hydrolyze whey proteins in a continuous membrane reaction system, resulting in hydrolysates with an estimated 99% reduction of antigenicity (Guadix, Camacho, & Guadix, 2006). Different combinations of proteolytic enzymes, namely trypsin, neutrase, papain and protease S, were tested on the production of low-allergenic whey-derived products. The combination of trypsin with either neutrase or papain was the most effective in the removal of β-LG, producing low molecular mass peptides with reduced antigenic properties (Shin et al., 2007). Hydrolysis of goat acid whey with pepsin was performed in an ultrafiltration membrane reactor. A diversity of peptides were identified in hydrolysates, mostly derived from α-LA, due to the resistance of β-LG towards pepsin. A broad range of peptides, from dipeptides to large peptides containing disulfide bridges, were detected among hydrolysis products (Bordenave, Sannier, Ricart, & Piot, 2000). Thus, improvement of functional and bioactive properties of acid whey can be achieved using this methodology.

Diverse proteases and procedures have been employed to generate whey-derived products with different degrees of hydrolysis (DH) and bioactivities. Whey proteins can be hydrolyzed by either digestive enzymes, plant or microbial proteases, and then generate peptides that may display a number of physiological roles (Fig. 1). Enzymatic hydrolysis of whey protein concentrate (WPC) was performed with pancreatin, Protamex or Alcalase 0.6L, to produce hydrolysates with 20% DH. Alcalase showed the lowest specificity for β-LG. Considering the protein content from WPC the pancreatin hydrolytic system was the most efficient, since only 4.7% of non-hydrolyzed protein remained in the final hydrolysate, against 8.0 and 9.8% for Alcalase and Protamex, respectively (Pacheco, Amaya-Farfan, & Sgarbieri, 2002). The pancreatin and Protamex hydrolysates showed higher ability to stimulate hepatic glutathione synthesis when administered in mice diets (Pacheco & Sgarbieri, 2005). Naik, Mann, Bajaj, Sangwan, and Sharma (2013) investigated the effect of enzyme/substrate (E/S) ratio, pH and T on DH, antioxidant and ACE-inhibitory activities of WPC hydrolyzed by commercial trypsin. The E/S ratio and pH had a major influence on DH. The resultant hydrolysates were subjected to ultrafiltration, and the permeate and retentate obtained were collected separately and evaluated for bioactivities. Majority of low molecular mass peptides contributed for higher ACE-inhibitory and antioxidant activity from the permeate fraction (Naik et al., 2013). Commercial pancreatin and papain were used to hydrolyze WPC under different E/S ratios. The hydrolysates were subjected to ultrafiltration or not, resulting in 16 different peptide formulations. ACE-inhibitory activity was evaluated and the greatest values were obtained with pancreatin at an E/S ratio 0.5/100, either in the presence or in the absence of ultrafiltration (Silvestre et al., 2012).

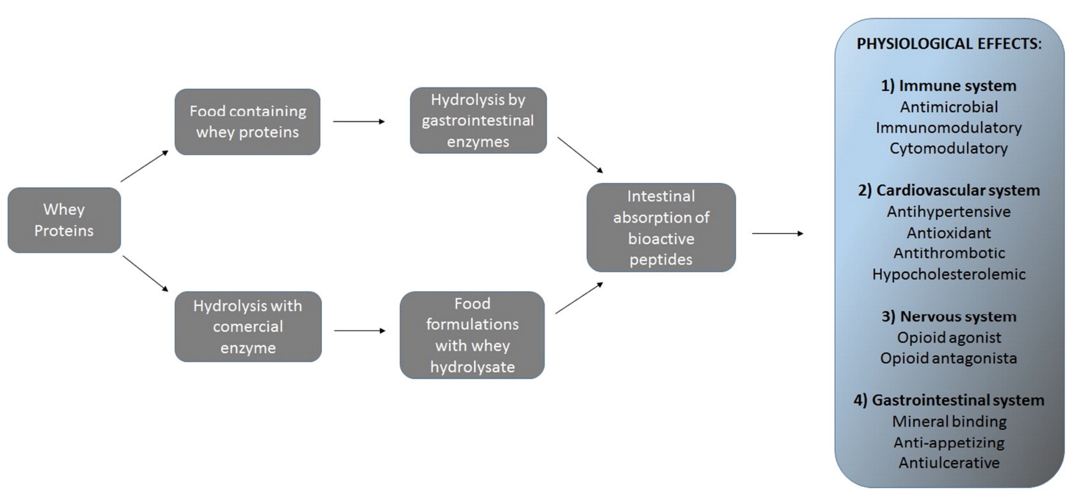

Fig. 1. Physiological effects of bioactive peptides derived from whey proteins. Whey proteins can be hydrolyzed by gastric and/or pancreatic proteases, or by commercial enzymes of plant or microbial origin to release encrypted bioactive peptides causing several physiological effects.

Please cite this article as: Brandelli, A., et al., Whey as a source of peptides with remarkable biological activities, *Food Research International* (2015), http://dx.doi.org/10.1016/j.foodres.2015.01.016