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Review

Bioprocess challenges to the isolation and purification of bioactive peptides

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ABSTRACT

Food protein-derived bioactive peptides (BPs) have been reported to trigger certain physiological responses in the body, thereby influencing health positively. These peptides have attracted high research and consumer interests due to their huge potential of use in functional foods and other dietary interventions of disease control and health promotion. However, successful product development is limited by the fact that current manufacturing processes are either difficult to scale up, high in cost, or have the potential to affect the structure-activity properties of these peptides. To overcome these challenges, we have proposed in this review, the use of an integrated '-omics' approach comprising *in silico* analysis and '-omics' techniques (such as peptidomics) to respectively forecast and validate the biological and physico-chemical properties of the peptides. This information is then used for the rational design of suitable purification steps for peptides of interest. Downstream purification could also be undertaken by liquid chromatography using monolithic adsorbents physico-chemically engineered (using results of *in silico* analysis) for rapid isolation of peptides. By coupling the high throughput and predictive capability of '-omics' to the enhanced convective hydrodynamics of monolithic columns, it becomes feasible, even at preparative scale, to produce BPs that meet the requirements of high purity, potency, and cost-effectiveness.

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Abbreviations: ACE, angiotensin-I-converting enzyme; DH, degree of hydrolysis; EMF, electromembrane filtration; MC, membrane chromatography; QSAR, quantitative structure–activity relationship; QSPR, quantitative structure–property relationship; HILIC, hydrophilic interaction liquid chromatography.

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1. Introduction

Over the years, it has become a known fact that health and nutrition are intricately linked. Not only do food nutrients supply the necessary biomolecules for various metabolic activities, but, in some cases, food nutrients are able to trigger certain desirable physiological responses in the body. Food proteins and hydrolysates thereof are amongst the most well studied bioactive molecules (Danquah and Agyei, 2012). Bioactive peptides have been defined as protein hydrolysates which, upon entry and absorption into the body, have the ability to induce certain desirable and physiologically measurable 'hormone-like' activities (Korhonen and Pihlanto, 2006). Some biological functions induced by these peptides include immunomodulatory, cytomodulatory, opiate, antihypertensive, antimicrobial, antithrombotic and metal-chelation activities (Möller et al., 2008). As natural products of food origin, bioactive peptides have a huge potential in health-promoting functional foods and therapeutic products (Korhonen, 2009). However, this potential is not being realized as a result of certain bioprocess challenges. The lack of commercially-viable processes for large-scale production of bioactive peptide has been a major hindrance to the percolation of bioactive peptides in marketable products (Agyei and Danquah, 2011). Further, basic research is lacking on the optimization of purification techniques tailored specifically for producing bioactive peptides at industrial scale (Korhonen and Marnila, 2013). The goal of peptide purification is not merely to achieve products of high purity and high recovery levels, but also, the purification technique must be economically viable (Desai et al., 2000). These challenges need to be overcome *via* the development of innovative, high throughput, and cost-effective platforms for the bioprocessing bioactive peptide. This review gives an overview on bioactive peptides, their production and functionalities; and also discusses the challenges encountered during the downstream purification of these peptides. A cascade of processing techniques that could offer some advantages over existing ones has also been proposed. Specifically, we propose (a) the use of '-omics' techniques (*e.g.* foodomics) and high throughput *in silico* simulation models to predict the properties of peptides that can be generated from a protein of interest. This prior knowledge aids the determination of suitable purification techniques that can be employed for specific peptides of interest. In addition, we propose (b) liquid chromatography using monolithic adsorbents specially designed for small sized and charged molecules (such as bioactive peptides). Some challenges encountered with the use of these proposed techniques and possible ways of overcoming them have also been presented.

2. Bioactive peptides: an overview

2.1. *Production of bioactive peptide*

Bioactive peptides are often inactive in the parent protein molecules and are released by processes such as (a) microbial fermentation of proteins by proteolytic microbes, (b) proteolysis by enzymes from plants or microorganisms, and (c) proteolysis by gastrointestinal enzymes. These production routes have been captured in Fig. 1. Each of these production alternatives has its own merits and demerits. For example, processes (a) and (b) are the only ones which can be pursued for the purposes of industrial scale production. Putatively, the production of bioactive peptide *via* (c) is to be expected, however, the amounts produced through such uncontrolled means may not be sufficient to induce the needed biological effect, especially in adult humans (Gauthier et al., 2006). Pathway (a) has been widely used in the past decades, especially for releasing bioactive peptides from dairy products (Korhonen and Pihlanto, 2006). In fact, there are several commercial products of the market that rely on this production method (Korhonen and Marnila, 2013). However, because fermented products usually contain other biomolecules such as live and dead bacteria cells, exopolysaccharides, and/or bacteriocins – all of which could exert some form of biological functions – it is inconclusive to assume that observed bioactivities is due to peptides released during fermentation (Martínez-Augustin et al., 2014).

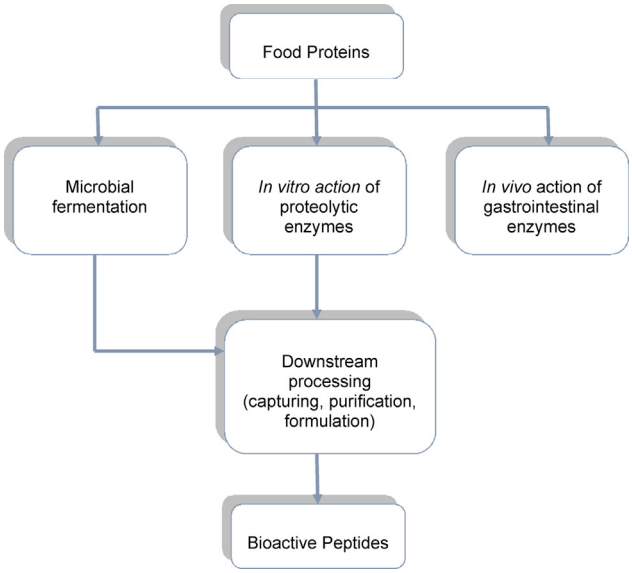

Fig. 1 – Production of bioactive peptides.
***Source*: Adapted from Danquah and Agyei (2012).**

2.2. *Bioactivities and applications*

As a result of the physiological activities they trigger, bioactive peptides have huge potential applications in the major