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Review

Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides

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ABSTRACT

In the last years, the identification and characterization of bioactive peptides have become emerging research subjects. Food peptidomics can be considered a subfield of the food proteomics focused on composition, interaction and properties of peptides present in a food matrix. On the basis of the description of recent works, the objective of this review is to highlight the increasing role of peptidomics as indispensable tool in the fields of discovery, bioavailability and monitoring of dairy bioactive peptides. The enhanced peptide identification, resulting from the valuable mass spectrometry development and the regular use of high-resolution techniques, supports the application of peptidomic approaches in the case of empirical bioactive peptide identification workflow. Bioinformatic-driven approaches have gradually gained importance through the wider application of in silico analysis, structure activity relationship models, chemometrics and peptide database management. Investigations of bioactive peptide modifications during digestion, whether it be selective or untargeted search using peptidomic tools have been discussed, as well as peptide changes along absorption, distribution, metabolism and elimination, including studies in cellular and animal models. Examples of application of peptidomics in the analysis of bioactive peptide occurrence in dairy products together with peptide monitoring during scaling up, industrial treatments and storage have been also described.

Contents

1. Introduction

Nutrition exerts an important life-long environmental impact on human health, and this interplay between nutrition and health has been known for centuries (Kussmann, Panchaud, & Affolter, 2010).

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The quality of a dietary protein source depends not only on the amino acid composition, their digestion, absorption, and availability for subsequent anabolism, but also on the peptides that are released (Awati et al., 2009). Many physiological functions in the organism are mediated by peptides, acting as neurotransmitters, hormones or antibiotics (Hruby & Balse, 2000). Because peptides from food sources can be structurally similar to these endogenous peptides, it is reasonable that they can interact with the same receptors and play a role as modifiers of food intake, growth factors, immune regulators, or antimicrobials in the host organism (Kamau et al., 2010; Meisel, 1998). Bioactive peptides can be released in vivo, during gastrointestinal digestion, by the action of host or microbial enzymes but they may also be originated in vitro, whether it be from ripening, fermentation (naturally occurring enzymatic reactions) or targeted food hydrolysis with selected enzymes. Besides, preparation of bioactive peptides can be performed using recombinant DNA technology or chemical synthesis (Hernández-Ledesma, Contreras, & Recio, 2011). Once they are released in the body, bioactive peptides may act as regulatory compounds with hormone-like activity, exhibiting a wide range of biological functions, including antihypertensive, antioxidant, opioid, antimicrobial, and immunostimulating activities (Hartmann & Meisel, 2007). Although other animal as well as plant proteins contain potential bioactive sequences, milk proteins are currently the main source of biologically active peptides. Thus, they account for most of the researches on bioactive peptides that apply peptidomics. This review will focus on studies dealing with peptides from milk and related dairy products.

The food peptidome can be defined as the whole peptide pool present in food products or raw materials, or obtained during processing and storage. Food peptidomics can be considered a subfield of the food proteomics focused on composition, interactions, and properties of peptides present in a food matrix (Gagnaire, Jardin, Jan, & Lortal, 2009). Some issues are common between the food proteomics and food peptidomics fields, like the occurrence of non-sequenced proteins, which makes mandatory to use de novo sequencing. This approach can be also helpful for identifying new single amino acid polymorphisms. Working with complex matrices is inherent to these fields as well. However, while in food proteomics a certain coverage is enough to find the selected protein, in the case of food peptidomics, peptides may be unique (variants or modifications) whereas many similar species can be found in the studied matrices. Furthermore, food peptides are released by the action of various unspecific and specific proteases, as opposed to the tryptic peptides generated in the proteomic experiments (Panchaud, Affolter, & Kussmann, 2012). An additional difficulty arises from the need to follow up these specific molecules not only in the food matrix but also upon ingestion and absorption.

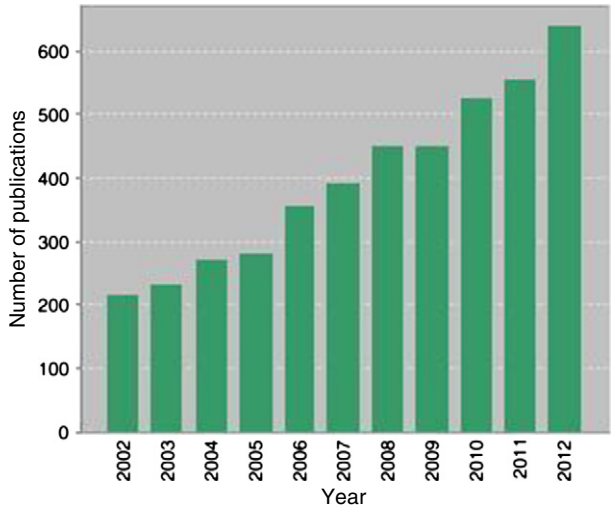

Fig. 1. Number of publications including the topic "bioactive peptide" in the 2002–2012 period. Web of Science®.

In biomarker proteomics, identification and quantification rely on several peptides that can unambiguously be inferred back to one parent protein sequence accounting for the key biological activity. By contrast, bioactive peptides are the active molecules per se and their identification relies on the detection of this particular sequence in its full length (Panchaud et al., 2012). Furthermore, their quantification is needed when the effective dose has to be calculated. Peptidomics can also have a role in biomarker search. For instance, a peptidomic approach has permitted to identify peptide panels which discriminate between two bacterial causes of infection in bovine mastitis, as it has been recently reported (Mansor et al., 2013). In human milk, the identification of the complete set of peptides naturally occurring suggests that protein cleavages in the mammary gland are not random events and represents the first step in understanding where and when milk peptides exert specific functions (Dallas et al., 2013).

In the past ten years, the identification and characterization of bioactive peptides have become emerging research subjects as shown by the increasing number of publications with the term "bioactive peptides" as topic (Fig. 1). As a result of its analytical versatility and power for structure elucidation and, to a lesser extent, quantification, mass spectrometry (MS) has developed into the major contributor to proteome and peptidome-wide assessment in food (Kussmann et al., 2010). This is a review in the fields of discovery, bioavailability, and monitoring of dairy bioactive peptides emphasizing those contributions where peptidomics has constituted an indispensable tool to achieve the objective.

2. Peptide discovery

Nowadays, the research focused toward the discovery of new dairy bioactive peptides continues mostly based on empirical strategies, including advanced analytical techniques as MS in different configurations. Despite the current prevalence of empirical approaches, in the last years emerging bioinformatic tools have achieved an increasing importance in the discovery of dairy bioactive peptides, through the prediction of their biological activity and the optimization of the empirical procedure.

2.1. Empirical approaches

The use of high-resolution separation techniques along with the enhanced peptide identification, resulting from the combination of MS methods and databases, support the present peptidomics status regarding the empirical discovery of dairy bioactive peptides. Usually, the empirical approach involves a series of steps: 1) release of the bioactive sequences; 2) initial screening for a targeted bioactivity; 3) purification and separation; 4) further determination of biological activity; 5) peptide identification by MS; 6) in vitro and in vivo validation of biological activity. A representative example of this workflow is found in the discovery of casein (CN)-derived peptides with antihypertensive properties (Contreras, Carrón, Montero, Ramos, & Recio, 2009). Initially, peptic hydrolysis of CN was carried out, its angiotensin-converting enzyme (ACE)-inhibitory activity was monitored at different intervals of time and the antihypertensive activity was also verified in an animal model. Once the activity was determined, the hydrolysate was firstly subjected to ultrafiltration and later fractionated by semi-preparative reverse phase high performance liquid chromatography (RP-HPLC). Those chromatographic fractions with relevant ACE-inhibitory activity were analyzed by RP-HPLC coupled to tandem MS (MS/MS) and the peptides comprised in the active fractions were identified. Finally, the ACE-inhibitory activity was verified for selected peptides, besides the verification of their in vivo antihypertensive activity.

In addition to in vitro enzymatic hydrolysis, the release of bioactive peptides from the parent dairy proteins may occur during gastrointestinal