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An *in silico* approach to highlight relationships between a techno-functional property of a dairy matrix and a peptide profile

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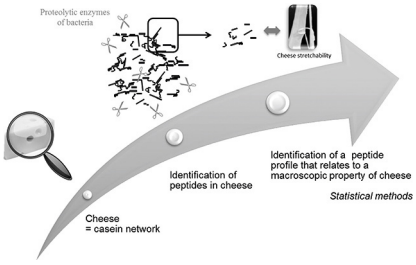
HIGHLIGHTS

- Proteolysis is strongly correlated with the stretchability of Emmental cheese.
- The type of protease affects the cheese stretchability.
- Peptide identification explains the modulation of the cheese stretchability.
- A peptide profile is related to a macroscopic property.

GRAPHICAL ABSTRACT



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ABSTRACT

Food products are complex matrices. They contain molecules from several families that interact with each other to generate specific structures and functionalities. These molecules may include proteins and peptides resulting from protein hydrolysis, *e.g.* in cheese. It is still difficult to establish a clear relationship between a pool of peptides and their techno-functionalities such as emulsifying, foaming, gelling or even texture properties within a complex matrix. Among the data usually known, there is the degree of hydrolysis that provides information about the average size of the peptides formed. A high degree of hydrolysis induces the production of a pool of small-size peptides (less than 20 amino acids). Advances in identification methods have made the identification of peptides present in food products possible. The size, charge, hydropathy index and isoelectric point of the peptide, as well as its secondary structure, can be calculated on the basis of the peptide sequence. The aim of this study was to determine which physicochemical and structural characteristics of peptides are involved in the cheese stretchability. This work is a re-evaluation, using multivariate exploratory analysis, of the experimental data obtained from a former study (Sadat-Mekmene et al., 2013) in which one typical functionality criterion of Swiss-type cheese (stretchability) was measured and peptides were identified. This methodology, based on Principal Component Analysis and Correspondence Analysis, is one way to establish a relationship between peptide characteristics and their techno-functional property within a complex dairy matrix. This statistical approach showed that the peptides predominantly involved in cheese stretchability were a mixture of both hydrophobic and hydrophilic peptides and that they are large enough to interact with each other and with native proteins. This approach could be applied to better understand the impact of peptides on various food matrices and food techno-functionalities.

Abbreviations: TN, total nitrogen; NCN, non-casein nitrogen; NPN, non-protein nitrogen.

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1. Introduction

Food products have a complex composition that includes various types of molecules such as proteins, lipids and carbohydrates as well as different types of minerals. Over time and depending to the process used, this composition may change, giving a specific final quality to a product in terms of texture and flavour. Among the main food components, proteins play an essential role in the structuring of food products and other types of matrices [1,2]. In some cases, the hydrolysis of proteins enhances their functional properties, *e.g.* solubility, foam or emulsion stability, gelling capacity and even texture properties [3–5]. Until now, relationships have mainly been established between the degree of hydrolysis (DH) of proteins and the enhancement of their functional properties in the final food product [6,7]. The DH includes not only the peptides produced but the residual native proteins as well. In relation to foam or emulsion capacity and similar interfacial related properties, many other parameters should also be taken into account, including peptide composition, which was determined in order to link the peptide composition to the emulsion and foam properties in dilute solutions [8,9]. Since peptides are poorly identified in food products, it is still difficult to establish a clear relationship between a peptide profile and the enhancement of a defined functional property or to know what types of peptides lead to a specific functionality, especially in complex matrices.

Stretchability, a parameter of texture quality increasingly demanded by the consumer, is one of the techno-functionalities of dairy products. Stretchability concerns cheeses such as Emmental, Mozzarella and Cheddar. The cheese stretchability depends on its gross composition, pH, mineralization and proteolysis. Stretchability varies between cheeses depending on cheese-making processes, cheese composition and the ripening conditions used [10].

In silico methods are tools that can be used to analyze protein structure–functional property relationships, among other things. They involve computer simulations that provide knowledge about how peptide or protein structures impact their functionalities. Such a computer simulation is a way to analyze some experimental results without carrying out additional experiments. Moreover, the functional property of one precise component in complex matrix can be studied in more depth and specifically extracted using certain software [11]. *In silico* methods can be used for preliminary studies and/or as a validation tool. As far as we know, the relationships between the resulting fragments of protein hydrolysis and the structural and mechanical properties of food systems have not yet been explored to the same extent.

This work is a re-examination, using multivariate statistical methods, of the experimental data obtained from a former study [12] and some other parameters calculated for the peptides. In a previous study, stretchable cheeses were manufactured and peptides were identified in the aqueous extract of each cheese. The aim of the present study was to understand which physicochemical and structural characteristics of peptides could be involved in cheese stretchability. To fulfil this purpose, a methodology based on statistical methods was developed to establish the relationships between the physicochemical and structural characteristics of peptides produced during processing, and cheese stretchability. The characteristics of peptides identified in the cheese aqueous extract were then calculated. A combination of multivariate exploratory analyses such as Principal Component Analysis (PCA) and Correspondence Analysis (CA) was then used in order to link the typology of the peptides with the cheese composition and stretchability.

2. Methodology

2.1. Cheese making and cheese analyses

The data used here were collected from a former study [12] and are summarized in Fig. 1. Some proteolysis measurements were made on cheeses based on standard protocols used in the dairy sector. They include the percentage of residual caseins and nitrogen fractions that give a general idea of the size of the peptides: (i) the amount of total nitrogen (TN), which includes the amount of protein nitrogen and the amount of non-protein nitrogen; (ii) the non-casein nitrogen/total nitrogen ratio (NCN) in the cheese, which represents the percentage of soluble nitrogen [13]; (iii) the non-protein nitrogen/total nitrogen ratio (NPN) which represents the small soluble peptides and the amino acids [14] and (iv) the percentage of non-protein nitrogen that is soluble ((NCN-NPN)/NCN), which represents the small peptides and soluble amino acids.

2.2. Cheese stretchability

The stretchability of each cheese was measured at various periods of ripening. Cheese stretchability was assayed by a method involving vertical traction of the cheese melted at 82 °C, according to Richoux et al. [15]. The length of strands of heated cheese was measured at the breaking point of the stretched strand.

2.3. Peptide extraction and peptide identification

Peptides were extracted from cheeses with water at day 1(D1), day 13 (D13), day 27 (D27) and day 41 (D41). The peptides present in the cheeses were separated by reversed-phase HPLC for both the first (F1) and the second (F2) cheese batches. Three fractions were collected as a function of the elution time. Peptides were eluted from the most hydrophilic to the most hydrophobic: (i) fraction A was collected from 2 to 22 min and contains peptides from 9 to 20 amino acids with a mean of 13 amino acids; (ii) fraction B was collected from 22 to 42 min and contains peptides from 6 to 45 amino acids with a mean of 15 amino-acids; (iii) fraction C was collected from 42 to 65 min and contains peptides from 6 to 44 amino acids with a mean of 19 amino-acids. These fractions were quantified according to their respective peak areas on the chromatographic graph.

For F1, peptides from the three fractions were identified by MS/MS at three crucial points of cheese ripening: D1 (at the beginning of the cheese ripening), D13 (when the stretchability is minimal) and D41 (when stretchability is the greatest). All together, 30 peptides were identified in the fraction A, 406 peptides in the fraction B and 139 peptides in the fraction C.

Throughout the text, LH1 refers to *Lactobacillus helveticus* ITGLH1 and LH77 refers to *L. helveticus* ITGLH77. The cheeses are designated according to the following combination: “name of the starter strain – day of peptide quantification and/or peptide identification”: LH1-D1, LH1-D13, LH1-D27, LH1-D41, LH77-D1, LH77-D13, LH77-D27 and LH77-D41.

2.4. Physicochemical and structural characteristics of peptides

Peptide characteristics such as size, hydropathy index, isoelectric point (pI) and number of positive and negative charges at neutral pH were calculated on the basis of the peptide sequence using the ProtParam method of Gasteiger et al. [16], available from the ExPASy website [17].

The isoelectric point represents the pH at which the peptide net charge is null. The number of both positive charges and negative charges was calculated at pH 7. The hydropathy index of the peptide is the sum of the hydropathy index of each amino acid