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Short communication: Peptide profiling in cheeses packed using different technologies

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ABSTRACT

Peptides released during the shelf life of cheeses packaged using 2 different technologies, vacuum packaging (VP) and modified-atmosphere packaging (MAP), were identified by on-line reverse phase-HPLC-tandem mass spectrometry. A total of 22 peptides from the N-terminal domain of α_{S1}-casein (CN) and 26 from β-CN were identified, the latter more evenly distributed over the whole sequence. Peptides were monitored during the shelf life of these cheeses when stored at 4°C, revealing that the peptide profile changed significantly with the storage time. Qualitative differences between VP and MAP cheeses were only found for 3 α_{S1}-CN peptides, which were absent in MAP cheeses. Semiquantitative analysis of peptides revealed some differences between cheeses packaged using different technologies. However, evolution of peptides during storage followed a common trend in both types of cheeses. In addition, the presence of certain peptides, which had been previously described because of their potential bioactivity, is illustrated. For instance, some of the identified peptides had been previously reported as antihypertensive peptides, such as peptide α_{S1}-CN (1–9) or β-CN f(201–209).
Key words: cheese, vacuum packaging, modified-atmosphere packaging, cheese bioactive peptide

Short Communication

Proteolysis is a phenomena occurring during cheese ripening comprised of numerous biochemical reactions that contribute to the flavor and texture of the final product. In the primary proteolysis, indigenous milk enzymes and those present in the coagulant play an important role. During secondary proteolysis, a great variety of peptides are released from the milk CN fraction by proteolytic enzymes which mainly belong to microorganisms that participate in cheese manufacturing (primary and secondary starters, as well as adventitious microflora; Fox, 1989).

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In recent decades, food packaging has undergone major technological development, partly in response to consumer demand for preservative-free food and in control-packaging methods to preserve quality and food safety. Changes in consumer preferences have affected buying habits and a growing interest in sliced and ready-to-eat products has been observed. These types of goods have a high value of convenience, but are also susceptible to physical and chemical changes. Vacuum packaging (**VP**) of cheese retards molds growth; however, the product may undergo changes in color, flavor, and texture (Romani et al., 2002) or show excessive surface humidity due to the migration of water from the inside to the surface (Pantaleão et al., 2007). Therefore, VP is not suitable for all kinds of cheeses, as it may lead to some structural and visual changes in the product. Consequently, alternative methods of packaging have been proposed, such as modified-atmosphere packaging (**MAP**). This technique appears suitable for cheese packaging, taking into account physicochemical and microbiological criteria (Dermiki et al., 2008), especially when using a gas mixture combination of 30% CO_2 to 70% N_2, which is able to extend the shelf life in terms of microbiological stability, keeping the sensory characteristics of Provolone cheese (Favati et al., 2007) or Greek whey cheese (Papaioannou et al., 2007) intact.

When cheese is packed to extend its shelf life, the effect of packaging on the ripening process should be evaluated. Some studies focused on the evaluation of proteolysis of VP cheese during ripening have been carried out using total nitrogen and ripening index (Tarakci and Kucukoner, 2006). The application of reverse phase-HPLC analysis (Sousa et al., 2001; Poveda et al., 2003) or HPLC-tandem mass spectrometry (**HPLC-MS/MS**; Piraino et al., 2007) has been proposed as a reliable tool to evaluate proteolysis in cheese. However, to our knowledge no comparative studies have been carried out on peptide profile changes occurring along the shelf life of cheese subjected to different packaging techniques.

In this study, HPLC-MS/MS detection has been used to identify the peptides released and evaluate the peptidic profile changes during the shelf life of cheeses packaged using 2 different technologies. Based on previ-

ous studies, the presence, origin, and potential bioactivities of these peptidic sequences are also discussed.

Semihard cheeses samples, provided by a national dairy manufacturer, were made out of mixed pasteurized sheep, cow, and goat milk with 2 different packaging systems (VP for cheese wedges and MAP for sliced cheeses). Prior to cheese packaging, the curd was processed using different molds, depending on the final format, and subsequently ripened for 20 d under controlled temperature and humidity conditions

Cheese samples were received immediately after manufacturing and stored at 4°C for the duration of the study: 0, 30, 60, and 90 d for the MAP samples, and 0, 30, 60, 90, and 150 d for the VP samples. In both cases the maximum number of storage days refers to the shelf-life of the respective cheese type.

Water-soluble extracts were obtained at each selected time according to the method described by Gómez-Ruiz et al. (2002). Supernatants were ultrafiltered on a 3,000 Da cut-off ultrafiltration membrane (Pall Corporation, Ann Arbor, MI). The 3,000 Da permeates were freeze-dried and kept at −20°C until their analysis.

Reverse phase-HPLC-MS/MS analyses of the permeates <3,000 Da were carried out on an Agilent HPLC system (Agilent Technologies, Waldbronn, Germany) connected on line to an Esquire-LC quadrupole ion trap instrument (Bruker Daltonik GmbH, Bremen, Germany). A Mediterranea Sea18 15 × 0.21 cm column was used in the experiments (Teknokroma, Barcelona, Spain). Auto MS(n) analyses used a signal threshold of 10,000, a voltage ramp from 0.35 to 1.4 V for the fragmentation of precursor ions, and an isolation width of 4.0 m/z. The estimated amount of peptides (in arbitrary units) in each sample was calculated by extracting their corresponding characteristic ions (molecular ion or doubly charged ion, when present); duplicate samples were prepared for each time and package condition, and were individually analyzed.

The peptide profile of the different cheeses was studied by evaluating both the UV spectra and the mass spectra after analysis by liquid chromatography. Essentially no differences in the UV spectra were found between the 2 types of packaging along the storage times (results not shown). A more detailed MS analysis revealed some differences in the peptide profile of cheeses packaged with both systems. A total of 48 peptides were identified, 22 sequences belonging to α_{S1}-CN and 26 sequences to β-CN. No peptides were detected from α_{s2}-CN or κ-CN. This can be linked to their lower content compared with α_{S1}- or β-CN. In addition, the fragment incorporated to the curd derived from κ-CN, para-κ-CN, has been described to be rather resistant to proteolysis. As cheeses were manufactured with different types of milk some of the sequences could belong to different species (cow, goat, or sheep). Figure 1 shows the α_{S1}-CN-derived peptides identified in VP and MAP cheeses, their presence during the storage time, and their intensity obtained from the HPLC-MS analysis at 0 d of MAP cheese. Most of the identified peptides from α_{S1}-CN were released from the N-terminal segment. The peptides that showed the highest intensities at 0 d corresponded to α_{S1}-CN f(1–16), f(17–23), and f(24–32), although many other peptides that also belong to these 3 regions were identified. In cheese, chymosin rapidly cleaves at Phe_{23}-Phe_{24} in cow milk and Phe_{23}-Val_{24} in sheep and goat milk, giving 2 major fragments as a result: α_{S1}-CN f(1–23) and f(24–199) (McSweeney and Fox, 1993). Further hydrolysis by cell envelope-associated proteinases and endopeptidases of starter and nonstarter bacteria release several peptides from the fragment α_{S1}-CN f(1–23). As shown in Figure 1, the same sequences were identified regardless the packaging method used; the only exceptions were the α_{S1}-CN peptides RPKHPIK, LPQEVLN, and PFPEVF, which were absent in MAP cheeses. Additional differences between the 2 packaging methods were also notable at the quantitative level. For example, the evolution of some of the most abundant α_{S1}-CN peptides during the storage time in VP and MAP cheeses is shown in Figure 2. In some cases, higher peptide intensities during storage time were detected in VP cheeses compared with MAP cheeses, especially at longer storage times (90 d). This is the case of the peptides α_{S1}-CN f(24–32) and α_{S1}-CN f(25–32; Figure 2). However, some peptides reached higher concentration in MAP cheeses compared with VP cheeses, as can be observed for the peptide α_{S1}-CN f(1–16). Concerning their evolution during the storage time, some peptides [α_{S1}-CN f(17–23), α_{S1}-CN f(24–32), and α_{S1}-CN f(25–32)] behaved similarly regardless the packaging technology, whereas others followed different patterns [α_{S1}-CN f(1–9) and α_{S1}-CN f(17–22)].

In contrast to α_{S1}-CN, peptides from β-CN were more evenly identified over the whole sequence. For β-CN peptides, no qualitative differences were found between VP and MAP cheeses (Figure 3). Those peptides that reached the highest concentrations in the water-soluble extract corresponded to β-CN f(1–6), f(44–52), f(45–52), and f(47–52; Figure 4). It is notable that, in general, for β-CN peptides differences were more pronounced at longer storage times (90 d) and peptide evolution during storage was similar for both packaging techniques. At 90 d, peptides β-CN f(7–14) and f(44–52) reached higher levels in VP cheeses than MAP cheeses, but the opposite was found for β-CN f(46–52), f(47–52), and f(74–82).

Some of the peptides identified in the water-soluble extracts of these cheeses have been previously described because of their potential to exert biological activities. For instance, α_{S1}-CN f(1–9) with sequence