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Title:

Peptidomics in Extended Shelf Life Dairy Products for Assessment of Proteolysis in relation to Product Quality

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Abstract:

Whey is used as an ingredient in long shelf life sports drinks due to its high nutritional value. However, some liquid Whey Protein Isolate (WPI) based products develop insoluble material during shelf time; a phenomenon referred to as “unwanted protein aggregation”. In many cases this deterioration can be avoided, by increasing the heat treatments from 95°C/180s to 120°C/20s. A fluorescence amine assay that monitors the level of free amino terminals (Larsen et al., 2004) has shown that the products subjected to the mild heat treatment have an increase in amino terminals during storage which is more than twice as high as the increase observed in the more harsh heat treated products. This indicates a presence of proteolytic activity from heat stable proteases in products having undergone both types of heat treatments, but also a heat treatment dependent inactivation of the respective proteases.

Therefore a mass spectrometry (MS) based peptidomic approach was initiated to characterize the peptide fraction of the WPIs, with the aim of identifying a relation between the peptide profiles and the product quality of the re-dissolved WPIs during storage and furthermore to deduce information about the proteolytic activity and the identity of the proteases.

Redissolved WPIs were adjusted to pH 3 to simulate the sports drinks conditions and subsequently subjected to heat treatments. After two weeks of storage at room temperature the peptide fractions of the samples were isolated using centrifugal filter units with a cutoff of 10kDa and 3kDa and the permeates were desalted and concentrated on handmade chromatographic columns as described by Gobom et al, (1999). By means of MALDI-TOF-TOF, this peptide fraction was subjected to MS/MS and mass searches were carried out using the SwissProt database.

A number of peptides were identified in the samples. These matches peptides reported to originate from bovine derived proteases, such as plasmin, cathepsin D and elastase (Considine et al., 2004; Hurley et al., 2000; Le Bars and Gripon, 1993), as well as bacterial proteases from *Lactobacillus helveticus* and thermolysin (Robert et al., 2004), or industrially applied proteases such as chymosin (Reid et al., 1997). Further studies will be made to profile these peptide fractions - and hopefully identify a link between the presence and/or quantity of specific peptides, proteases and the quality of the products.

Reference List

Considine, T., Á. Healy, A. L. Kelly, and P. L. H. McSweeney. 2004. Hydrolysis of bovine caseins by cathepsin B, a cysteine proteinase indigenous to milk. *International Dairy Journal* 14(2):117-124.

Hurley, M. J., L. B. Larsen, A. L. Kelly, and P. L. H. McSweeney. 2000. Cathepsin D activity in quarg. *International Dairy Journal* 10(7):453-458.

Larsen, L. B., M. D. Rasmussen, M. Bjerring, and J. H. Nielsen. 2004. Proteases and protein degradation in milk from cows infected with *Streptococcus uberis*. *International Dairy Journal* 14(10):899-

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Le Bars, D., and J. Gripon. 1993. Hydrolysis of α_s1 -casein by bovine plasmin. Lait 73(4):337-344.

Reid, J. R., T. Coolbear, J. S. Ayers, and K. P. Coolbear. 1997. The action of chymosin on κ -casein and its macropeptide: Effect of pH and analysis of products of secondary hydrolysis. International Dairy Journal 7:559-569.

Robert, M. C., A. Razaname, M. Mutter, and M. A. Juillerat. 2004. Identification of Angiotensin-I-Converting Enzyme Inhibitory Peptides Derived from Sodium Caseinate Hydrolysates Produced by *Lactobacillus helveticus* NCC 2765. J. Agric. Food Chem. 52(23):6923-6931.