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Hexose-derived glycation sites in processed bovine milk

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ABSTRACT

Milk products are consumed by many people on a daily basis, which demands sophisticated technical processes to guarantee the microbiological safety and to retain the nutritional value. The heating during pasteurization and ultra high temperature (UHT) treatment triggers diverse chemical reactions, such as the reaction of sugars and amino groups of proteins typically termed protein glycation. The glycation by lactose as dominant sugar in milk has been recently investigated, whereas the contribution of hexoses remains open. We identified first hexose-derived glycation sites in raw milk, colostrum, three brands of pasteurized milk, three brands of UHT milk, five brands of infant formula, and one brand of lactose-free pasteurized and UHT milk using tandem mass spectrometry and electron transfer dissociation. In total, we could identify 124 hexosylated tryptic peptides in a bottom-up proteomics approach after enriching glycated peptides by boronate affinity chromatography, which corresponded to 86 glycation sites in 17 bovine milk proteins. In quantitative terms glycation increased from raw milk to pasteurized milk to UHT milk and infant formula. Lactose-free milk contained significantly higher hexosylation degrees than the corresponding regular milk product. Interestingly, the glycation degrees varied considerably among different brands with lactose-free UHT milk and infant formula showing the highest levels.

Biological significance: The established proteomics strategy enables the identification and relative quantification of different protein glycation types in diverse milk products ranging from raw milk to milk powders. This will allow detailed in vitro studies to judge positive or negative aspects when consuming differently processed milk products including lactose-free milk that is obligatory for people with lactose intolerance but is increasingly consumed by the general population assuming health benefits. The established analytics will also permit studying the influence of each technical processing step on the glycation degrees and thus offers the possibility to reduce glycation early during production, as obvious from the variation among different brands. Special attention should be given to the high hexose- and lactose-derived glycation levels found in infant formula, although it is still controversially discussed if protein glycation has a negative biological impact.

1. Introduction

Thermal processing of milk, such as pasteurization and ultra high temperature (UHT) treatment, reduce the number of viable bacteria to ensure the microbiological safety of milk products for longer periods of time [1]. However, heating denatures proteins or protein complexes and triggers chemical reactions, such as oxidation or non-enzymatic glycosylation (often referred to as glycation) of proteins and lipids [2]. The aldehyde group of lactose and the ε-amino group of a lysine residue can react to form the Amadori product lactulosyllysyl [3]. Amadori products and the consecutively or alternatively formed advanced glycation endproducts (AGEs) can trigger immune responses (food allergies) [4,5] with extensive glycation degrees even compromising the nutritional value of milk proteins [2]. Moreover, Maillard reactions can influence protein functions including reduced enzyme activity, altered receptor binding, and changes in the protein stability [6,7].

Early products of the Maillard reaction have been usually evaluated by quantifying furosine by HPLC or protein lactosylation by mass spectrometry (MS). Recent studies [8–14] have correlated the number of lactosylation sites and their relative quantities to the harshness of the thermal treatment procedures. However, milk contains also other further sugars including hexoses like glucose and galactose [15] that contribute significantly to the Maillard reaction. Furthermore, lactose can isomerize to lactulose at elevated temperatures, which can further degrade via β-elimination to produce galactose, tagatose, saccharinic acids, and other low molecular weight compounds [7,16].

Hexoses, in particular D-galactose and D-glucose, are more reactive towards amines than lactose [17,18]. For instance, D-galactose is more reactive to bovine serum albumin than D-glucose and D-lactose when incubated at 60 °C [18]. This observation is especially important in view of the growing demand for lactose-free milk products, which

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were first produced for people with lactose intolerances [19,20], but are nowadays consumed by a larger part of the population. Therefore, bovine milk is typically pasteurized before lactose is enzymatically cleaved into D-glucose and D-galactose followed by a second thermal treatment (pasteurization or UHT) to ensure the microbiological safety [21,22]. Thus, lactose-free milk is more prone to glycation as two thermal processes are combined and, more importantly, the more reactive reducing monosaccharides glucose and galactose are present during the second heating. Thus, skim milk powder with hydrolyzed lactose contains less available lysine than regular skim milk powder [23]. This was confirmed for whey milk proteins by MALDI-TOF-MS indicating higher glycation degrees in lactose-free milk than in regular milk [24]. Furthermore, UHT milk with hydrolyzed lactose contains significantly more furosine than conventional UHT milk [25]. However, detailed studies on hexose-derived glycation sites in regularly processed and lactose-free milk products are missing, to the best of our knowledge.

Thus, we studied hexose-derived glycation sites in raw milk and different brands of pasteurized milk, UHT milk, infant formula, lactose-free pasteurized milk, and lactose-free UHT milk. The glycation sites were identified by nanoRPC-ESI-MS/MS [collision induced (CID) and electron transfer dissociation (ETD)] after enriching the tryptic digests by boronate affinity chromatography. Afterwards, the identified 124 glycated peptides corresponding to 86 glycation sites in 17 proteins were relatively quantified using targeted nanoRPC-ESI-MS/MS.

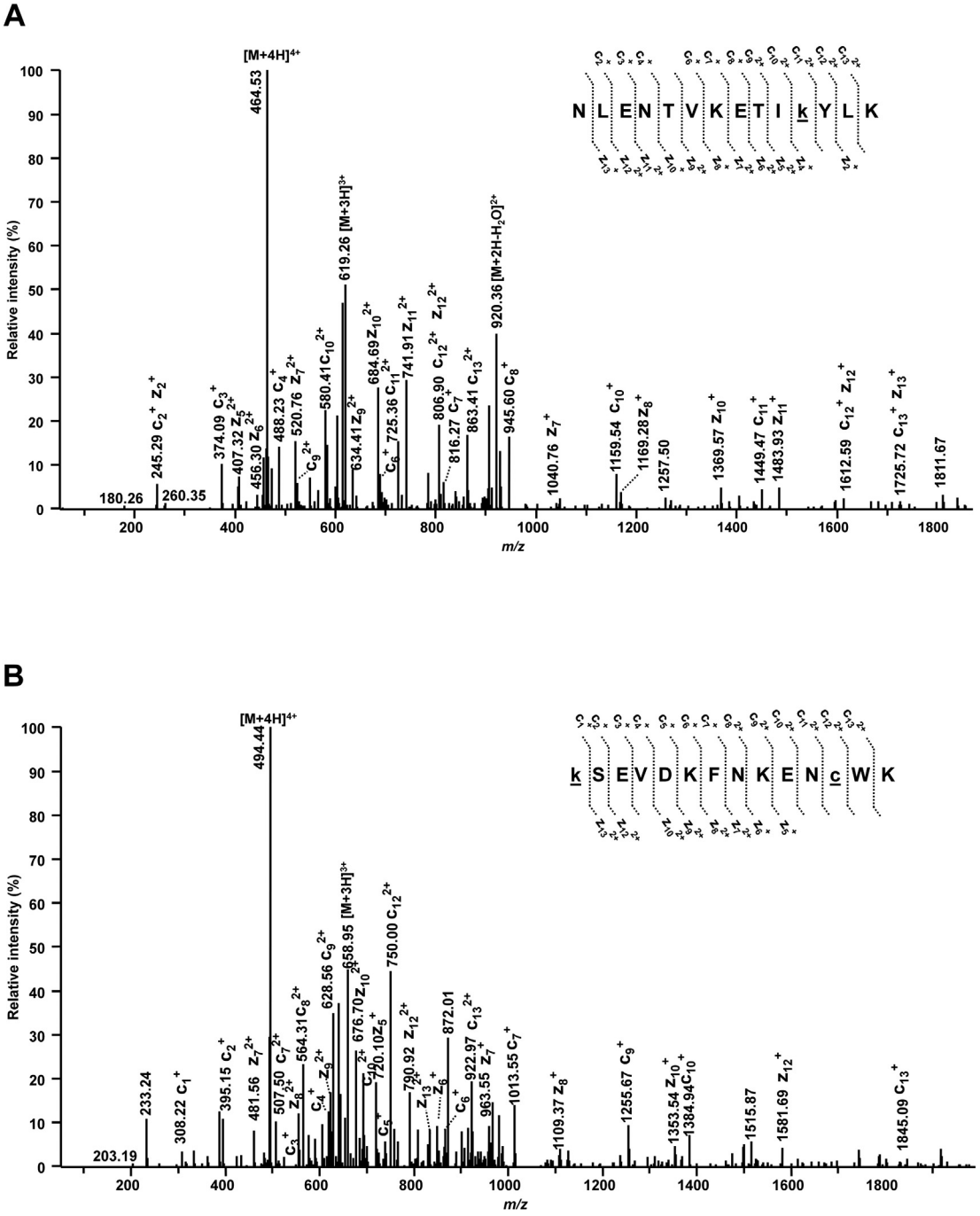

Fig. 1. ETD tandem mass spectra recorded for precursor ions at *m/z* 464.51 (A) and *m/z* 494.24 (B) corresponding to GLYCAM (110–123) glycated at Lys120 and xanthine dehydrogenase (980–1003) glycated at Lys980. Proteins were precipitated from ULF, digested, enriched with BAC, and analyzed by targeted nRPC-ESI-MS/MS.

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