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Electrophoretic characterization of protein interactions suggesting limited feasibility of accelerated shelf-life testing of ultra-high temperature milk

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ABSTRACT

Accelerated shelf-life testing is applied to a variety of products to estimate keeping quality over a short period of time. The industry has not been successful in applying this approach to ultra-high temperature (UHT) milk because of chemical and physical changes in the milk proteins that take place during processing and storage. We investigated these protein changes, applying accelerated shelf-life principles to UHT milk samples with different fat levels and using native- and sodium dodecyl sulfate-PAGE. Samples of UHT skim and whole milk were stored at 20, 30, 40, and 50°C for 28 d. Irrespective of fat content, UHT treatment had a similar effect on the electrophoretic patterns of milk proteins. At the start of testing, proteins were bonded mainly through disulfide and noncovalent interactions. However, storage at and above 30°C enhanced protein aggregation via covalent interactions. The extent of aggregation appeared to be influenced by fat content; whole milk contained more fat than skim milk, implying aggregation via melted or oxidized fat, or both. Based on reduction in loss in absolute quantity of individual proteins, covalent crosslinking in whole milk was facilitated mainly by products of lipid oxidation and increased access to caseins for crosslinking reactions. Maillard and dehydroalanine products were the main contributors involved in protein changes in skim milk. Protein crosslinking appeared to follow a different pathway at higher temperatures (≥40°C) than at lower temperatures, making it very difficult to extrapolate these changes to protein interactions at lower temperatures.

Key words: storage, aggregation, protein interactions, ultra-high temperature, whole and skim milk

INTRODUCTION

Production of UHT milk involves heating the milk to a high temperature (usually 130 to 140°C) for 3 to 5 s, followed by aseptic packaging to produce a commercially sterile product with minimal changes in quality (Holland et al., 2011). Thermal treatment enables storage of UHT milk at room temperature for up to 9 mo, eliminating the need for refrigeration in the distribution chain. Its long shelf life at room temperature has made UHT milk an important food product from nutritional, technological, and economic points of view. However, high temperature treatment induces changes such as whey protein denaturation, Maillard reaction, and mineral imbalances, which may lead to changes in protein–protein interactions and result in physical storage instabilities, including sedimentation of proteinaceous material at the bottom of the storage container, gel formation (age gelation), or both (Andrews and Cheeseman, 1972; McMahon, 1996; Deeth and Lewis, 2016). Proteolysis, another underlining mechanism of gelation, also involves protein network formation (i.e., interaction or linking between proteins or their fragments; Deeth and Lewis, 2016). Irrespective of mechanism, gelation or sedimentation appears to be governed and preceded by changes in the extent and nature of interactions among milk proteins.

Changes in protein interactions primarily involve noncovalent interactions, via either weak bonding (such as hydrophobic, van der Waals, or electrostatic interactions) or covalent crosslinking with other proteins through formation of disulfide bonds, advanced Maillard products (**AMP**), and dehydroalanine (Singh, 1991; Friedman, 1999; Wang et al., 2010; Holland et al., 2011). In addition to protein–protein linking, the role of protein–lipid interactions in the initiation and buildup of sediment or gel has not been studied extensively. However, several reports have reported on the lower extent of proteolysis (López-Fandiño et al., 1993; Garcia-Risco et al., 1999), and the creation of products of Maillard reaction (AMP; Valero et al., 2001) in stored

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UHT milk with higher fat content indicates that fat may play some role in the extent of protein interactions during storage.

Exploring the progression and extent of changes in protein interactions by applying full-length shelf-life tests would be time- and resource-intensive. Accelerated shelf-life tests could be a suitable alternative for predicting storage changes in products such as UHT milk. In fact, by accelerating the rate of deterioration of sensory attributes by exposing the product to elevated storage temperatures, shelf life has been predicted in a comparably short time (Richards et al., 2014). Besides deterioration in sensory attributes, protein–protein interactions are also enhanced at higher storage temperatures (Al-Saadi and Deeth, 2008). Therefore, using elevated storage temperatures, protein interactions could also be accelerated, and the results could then be extrapolated to predict aggregation mechanisms at lower storage temperatures using a rapid method of determination such as Fourier transform infrared spectroscopy, avoiding lengthy real-time analysis. However, as has been previously reported, higher storage temperatures ($\geq$40°C) inhibit age gelation (Kocak and Zadow, 1985; McMahon, 1996; Deeth and Lewis, 2016). The reasons for this are not known, but a few suggestions have been postulated. First, an increased rate of proteolysis at higher temperatures results in extensively degraded proteins, which are no longer able to form a stable gel matrix (Datta and Deeth, 2001). The second hypothesis is that accelerated Maillard browning reactions at higher temperatures block lysine residues, which would likely participate in protein–protein interactions leading to gelation (Samel et al., 1971; Gaucher et al., 2008). Third, higher rates of chemical crosslinking via Maillard and other reactions prevents the release of βκ complex from the micelle, which is the starting point for the onset of age gelation (McMahon, 1996). Furthermore, high storage temperatures slow gelation but result in increased nondispersible sediment (Nieuwenhuise and van Boekel, 2003). Thus, accelerated shelf-life testing in UHT milk could only be used to predict sedimentation at room temperature.

The present study was aimed at establishing the feasibility of using elevated temperatures during storage of UHT milk with different fat contents to predict changes in the interactions of milk proteins that may lead to sedimentation at room temperature.

MATERIALS AND METHODS

Materials

Commercial UHT whole (full cream) milk (**WM**) and skim milk (**SM**) were provided by a local manufacturer (Murray Goulburn Co-operative Co. Ltd., Victoria, Australia). All milk packs originated from the same batches. Both SM and WM packs were produced on the same day using an established process on an indirect tubular processor (SPX Flow Technology, Mulgrave, Australia) with a 9,000 L/h capacity at 138°C for 6 s. The composition of the WM as reported by the manufacturer was 33 g/L protein, 34 g/L fat, 53 g/L sugars, 0.55 g/L sodium, and 1.2 g/L calcium. The composition of the SM was 34 g/L protein, 1 g/L fat, 53 g/L sugars, 0.55 g/L sodium, and 1.2 g/L calcium.

The electrophoresis chemicals were obtained from Bio-Rad Laboratories (Richmond, CA). The reducing agent 2-mercaptoethanol, bovine milk standards α-LA, β-LG, and BSA were purchased from Sigma (Castle Hill, NSW, Australia). Prestained SDS-PAGE standard (SeeBlue Plus2) was procured from Thermo Fisher Scientific (Scoresby, Victoria, Australia).

Storage of UHT Milk

The UHT milk packs were stored at room temperature (20°C) and 3 elevated temperatures (30, 40, and 50°C) for 28 d in incubators (Thermoline Scientific Pty Ltd., Wetherill Park, NSW, Australia). The elevated storage temperature served to accelerate the development of storage instabilities. Milk packs were analyzed on the first day of delivery (d 0), and then at two 2-wk intervals (14 and 28 d) during storage for change in the interactions of milk proteins.

Electrophoretic Analysis of Changes in Protein Interactions

We investigated changes in the interactions of milk proteins during storage of UHT milk using gel electrophoresis—native- and SDS-PAGE under reducing and nonreducing conditions, as previously described (Dissanayake et al., 2013), with some minor modifications. For native-PAGE, milk from each pack was diluted with the native sample buffer [0.11 *M* Tris-HCl buffer (pH 6.8), 8.8% (vol/vol) glycerol, 2.22% (vol/vol) of 0.4% (wt/vol) bromophenol blue solution]. A working volume of 10 μL was then loaded on 12.5% gels and run for 85 min at 210 V and 70 mA in a Protean II xi cell (Bio-Rad Laboratories), filled with tank buffer solution (0.025 *M* Tris, 0.19 *M* glycine, pH 8.3). Gels were rinsed in MilliQ water for 15 min and stained using staining solution (0.15% Coomassie Brilliant Blue R250 dye, 72% isopropanol, and 3% acetic acid) by slowly shaking for 1 h. The stain was completely removed, and the gel was destained by shaking it slowly in destaining solution (10% isopropanol, 10% acetic acid) overnight on a shaking platform.