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Method article

Two alternative chromatography methods assisted by the sulfonic acid moeity for the determination of furosine in milk

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A B S T R A C T

N^6-(2-(2-Furanyl-2-oxoethyl))-L-lysine (furosine) is a deteriorative reaction product that is produced during heat treatment and storage of milk. This compound affects the quality of commercial dairy products. Accurate determination of furosine is necessary as it may serve as a measure of the degree of protein degradation in dairy products.

In this article, two HPLC based methods (1. a novel ion-pairing reagent 2. a strong cation exchange column) are proposed to quantify furosine. These methods were optimized and validated for their application to analyze fluid milk and dried milk powder.

- Two methods that can be used for routine milk quality control, including heat damage and adulteration, were developed.
- Compared to previous methods, the modified procedures herein using aromatic sulfonic acids (a pairing agent or covalently bound to a matrix on a strong cation exchange column) provide less expensive and more sensitive determinations.
- The identification and quantification of the furosine chromatographic signal was successfully achieved during analysis of commercial and spiked samples.

A R T I C L E I N F O

Method name: Furosine in powdered and fluid milk
Keywords: Furosine, Milk, DAD/PDA detector, *p*-toluenesulfonic acid, Ion-pair liquid chromatography, Strong cation exchange chromatography, Adulteration

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Method details

Background

Furosine is a compound formed in the early stages of the Maillard reaction (i.e., non-enzymatic browning reaction) [1,2]; it is formed after the hydrolysis of protein-bound lactosyl-lysine, commonly produced by heat exposure of milk. The lack of furosine in fresh milk means that its presence provides a marker indicating the application of heat treatment or prolonged storage [1,2]. Hence, it is classified as a Type II indicator (in the time-temperature integrators class) and thus is a suitable measurement of the nutritional quality and biological value of protein of a dairy product [1,3].

Miscellaneous methods for the determination of furosine in dairy include capillary zone electrophoresis [4] as well as a front-face fluorescence method [5], and recently, a stable isotope dilution assay coupled with tandem mass spectroscopy was reported for the simultaneous detection of several Maillard reaction products including furosine [6].

The most common methods for the determination of furosine are based on high- performance liquid chromatography (HPLC). For example, a previous report utilizing HPLC with an acetate buffer was applied to assay furosine in a variety of dairy products (and interestingly, dry dog food) [7]. In this regard, the ISO 18329 IDF 193 reference method [8] indicates the use of potassium chloride/acetic acid as furosine pairing agent. However, the technique fails to state, explicitly, which HPLC column was used for the separation. Few papers have used this approach [9–11], and other methods have been developed since.

Previously, sodium heptane sulfonate has been used as pairing agent by several research groups to aid the measurement of furosine in commercial milk [12], whipping cream [13] and retail whipping cream, coffee cream and condensed milk [14]. Ion exchange columns with post-column ninhydrin derivatizations have been used as an additional way to detect furosine in dried skimmed milk [15] and rumen undegraded protein [16]. Recently, an improved methodology was published (based on a modification from HPLC to UHPLC of an already established method [17]) to evaluate “heat load” in extended shelf milk samples, thus significantly reducing the analysis time during commercial milk assays [3].

Herein we report modifications of method ISO 18,329:2004(E). IDF: 193:2004(E). We substituted acetic acid and potassium chloride in the mobile phase for a solution of *p*-toluenesulfonic acid (TsOH) which interacts simultaneously both with the furosine and the C_8 column. Alternatively, we used a strong cation exchange column (SCX, based on polymer bound sulfonic acid) for furosine determination. These modifications were optimized and validated, resulting in two reproducible and accurate approaches for the determination of furosine.

Reagents

Furosine analytical standard was attained from Polypeptide Laboratories (hydrochloride, 99.4%, SC494, Strasbourg, France). Hydrochloric acid (HCl, ACS reagent, 37%), TsOH (402885, ACS reagent, $\geq$ 98.5%) and sodium 1-heptane sulfonate (H2766, HSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN, LiChrosolv®), and methanol (MeOH, ACS reagent) were acquired from Merck Millipore (Merck KGaA, Darmstadt, Germany). Ultra-High Pure Nitrogen was purchased from Praxair Technology Inc. (Danbury, Connecticut, USA). Ultrapure water [type I, 0.055 μ S cm^{-1} at 25 °C, 5 μg L^{-1} TOC] was obtained using an A10 Milli-Q Advantage system and an Elix 35 system (Merck KGaA, Darmstadt Germany).

Liquid chromatography equipment

A modular HPLC system (Shimadzu Prominence, Shimadzu Corporation, Kyoto, Kyoto Prefecture, Japan) equipped with a degasser (DGU-20A5), quaternary pump (LC-20AT), an autosampler (SIL-20 A HT), a system controller (CBM-20 A), a column oven (CTO-20 A), a photodiode array detector