LWT - Food Science and Technology 53 (2013) 331–337

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Front face fluorescence spectroscopy and multi-way data analysis for characterization of milk pasteurized using instant infusion

Anni B. Hougaard, Anders J. Lawaetz, Richard H. Ipsen*

Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form
22 October 2012
Accepted 9 January 2013

Keywords:
Milk
Heat treatment
Front face fluorescence spectroscopy
PARAFAC

ABSTRACT

Front face fluorescence spectroscopy was applied for characterization of milk pasteurized using instant infusion at six different temperatures, ranging from 72 °C to 120 °C. Comparisons were made with raw milk and milk subjected to high temperature short time (HTST) pasteurization at 72 °C for 15 s as well as with a more intensive pasteurization at 85 °C/30 s. Fluorescence excitation emission matrices (EEMs) were measured covering excitations from 250 nm to 350 nm and emissions in the range from 260 nm to 500 nm. The different heat treatments were separated based on three components by parallel factor analysis (PARAFAC) of the EEMs. Two components were suggested to be related to protein and one component to be vitamin A. Different models were needed for skim milk and non-standardized milk. Front face fluorescence spectroscopy, which requires no sample preparation, in combination with PARAFAC appears to be a powerful tool for discrimination of different milk heat treatments.

1. Introduction

Heat treatment of milk is applied to ensure consumer safety and obtain a prolonged shelf life as compared to raw milk. The standard heat treatment for fresh milk products is high temperature short time (HTST) pasteurization at 72 °C for 15 s, or a combination of time and temperature providing at least the same heating effect, performed in a plate heat exchanger.

Instant infusion is a recently developed direct heat treatment technology characterized by very high heating and cooling rates and short holding times, and it has been suggested that instant infusion could be used for gentle pasteurization of milk (Fredsted, Rysstad, & Eie, 1996). In order for a pasteurization treatment to qualify as being more gentle than, e.g., HTST pasteurization, it should result in less chemical changes in the milk at comparable microbiological inactivation levels.

Fluorescence spectroscopy is a rapid and sensitive technique and the properties of fluorescent compounds are highly dependent on the environment surrounding the compound (Lakowicz, 2006; Strasburg & Ludescher, 1995), which suggests that measurement of changes in fluorescence may be used in characterization of the changes that a certain treatment induces in a sample. Fluorescent compounds can be found both as intrinsic constituents or added as probes for a specific reaction or compound (Lakowicz, 2006). Milk contains several intrinsic fluorophores, with the aromatic amino acids tryptophan and tyrosine, vitamin A and riboflavin, as the most dominant ones (Christensen, Becker, & Frederiksen, 2005). A number of studies have used the changes in the fluorescence of these compounds in analysis and characterization of milk and dairy products during processing and storage (Boubellouta & Dufour, 2008; Christensen, Povlsen, & Sørensen, 2003; Christensen et al., 2005; Dufour & Rioblanc 1997; Herbert, Riaublanc, Bouchet, Gallant, & Dufour, 1999; Karoui, Dufour, & De Baerdemaeker, 2007; Kulmyrzaev, Levieux, & Dufour, 2005). Fluorescent compounds may also be formed during processing of milk, e.g., products of the Maillard reaction, and can be used in evaluation of processing severity (Birlouez-Aragon et al., 1998; Birlouez-Aragon, Sabat, & Gouti, 2002).

Parallel Factor Analysis (PARAFAC) has proven to be successful in evaluating applications with Fluorescence Excitation Emission Matrices (EEM's) (Andersen & Bro, 2003; Christensen et al., 2005; Stedmon & Bro, 2008). Under the right circumstances and applied correctly, the method can provide estimates of the true underlying excitation and emission spectra as well as the relative concentrations of the fluorophores in the measured samples.

The aim of the present study was to explore the potential of front face fluorescence spectroscopy combined with multi-way analysis for characterization of heat treatment of milk performed by instant infusion pasteurization at different heat treatment temperatures and compared with heat treatments performed in a plate heat exchanger.

* Corresponding author. Tel.: +45 35333225; fax: +45 35333190.
E-mail address: ri@life.ku.dk (R.H. Ipsen).

0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.01.010

2. Materials and methods

2.1. Instant infusion pasteurization

Two batches of milk were obtained from a dairy factory (Rødkærsbro Mejeri, Arla Foods, Rødkærsbro, Denmark) on two consecutive days. Each batch was 3000 L, one batch was skim milk, which had also been microfiltrated (membrane pore size 1.4 μm) as part of the skimming process, and the other batch was raw, non-standardized milk. Pilot scale instant infusion equipment (APV (SPX), Silkeborg, Denmark) with a flow rate of 500 L/h was used for the instant infusion pasteurizations. The holding time was 0.2 s and the pasteurization temperatures used were 72 °C, 80 °C, 90 °C, 100 °C, 110 °C and 120 °C. The pasteurizations were performed by starting at 120 °C and then gradually changing the temperature downwards. Two replicates were made of each processing series with water flushing and re-sterilization of the equipment in between. Samples were drawn aseptically into bags containing approx. 4 kg of milk. Furthermore, two reference treatments were applied; a standard HTST pasteurization at 72 °C for 15 s and a more intensive pasteurization at 85 °C for 30 s, both these treatments were performed in a pilot scale plate heat exchanger (APV, Silkeborg, Denmark). Reference samples were filled into sterile ½ L bottles under laminar air flow. The unprocessed skim milk and raw milk were also sampled, leading to a total of 30 samples.

All samples were cooled and stored at max. 5 °C and preserved by addition of 0.2 mg/mL sodium azide when the bags/bottles were opened.

2.2. Measurement of fluorescence excitation-emission matrices

Fluorescence excitation-emission matrices (EEM) were measured on the preserved samples one week after heat treatment. Measurements were performed on a Perkin Elmer LS55 luminescence spectrometer (Perkin Elmer, Waltham, Massachusetts, USA) equipped for front face fluorescence measurements with a sample holder in an angle of app. 60° to the light source. The EEMs were obtained by using excitations at every 5 nm from 250 nm to 330 nm and every 10 nm from 330 nm to 350 nm. For every excitation wavelength, emission spectra were collected from 10 nm above the excitation wavelength, to avoid most of the 1st order Rayleigh scatter, and up to 500 nm with 0.5 nm intervals. Slit width was set to 3.0 nm for both excitation and emission, and the scan speed was 1200 nm/min. The spectra were corrected for the wavelength dependent excitation intensity by an internal reference detector. Milk samples were stored at 5 °C until measurement, and measured at room temperature. Duration of measurement was approx. 10 min/sample. Measurements were performed in a 10 × 10 mm quartz cuvette, and all samples were measured in triplicate.

2.3. Data analysis

The fluorescence EEM's are arranged in a $(I \times J \times K)$ three way data array with I being the number of samples measured (objects), J the number of emission wavelengths, and K the number of excitation wavelengths. The data is analysed by the multi-way method PARAFAC. PARAFAC can be seen as a generalisation of the well-known multivariate method Principal Component Analysis (PCA) into higher orders (Bro, 1997).

The data array is decomposed into a number of latent PARAFAC components, by minimizing the sum of squared residuals e in the PARAFAC model (Eq. (1))

$$X_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

where a_{if}, b_{jf} and c_{kf} are the ith element of the loading vectors for the fth PARAFAC component. In the ideal case fluorescence data has a true trilinear structure and hence this is often a reasonable approximation for the measured data. For the correct number of components the solution of the PARAFAC model is unique, and will then give true estimates of the true underlying profiles of the variables. This makes PARAFAC perfect for fluorescence spectroscopy when applied on EEMs. The loadings and scores can be treated as estimates of the excitation and emission spectra, and relative concentrations of the fluorophores in the samples respectively (Andersen & Bro, 2003; Bro, 1997).

This fruitful relation between PARAFAC and flourescence has made it a very popular tool for analysis of EEM data with many applications in such diverse areas as food quality analysis environmental monitoring of drinking water quality, and cancer diagnostics (Christensen, Nørgaard, Bro, & Engelsen, 2006; Lawaetz et al., 2012a, 2012b; Stedmon et al., 2011). Theoretical and practical aspects of PARAFAC modelling of fluorescence data can be seen in Andersen and Bro (2003) and Bro (1997).

The two datasets were of size (45 × 482 × 19) (objects × emission wavelengths × excitation wavelengths) for the skim milk and (47 × 482 × 19) for the non-standardized milk, due to retest of two samples of non-standardized milk. Before modelling the data, remaining information from 1st and 2nd order Rayleigh scatter were replaced with missing values, and for computational reasons the missing values in the area from 20 nm below the 1st order Rayleigh scatter and 20 nm above the 2nd order Rayleigh scatter were replaced with zeros (Andersen & Bro, 2003). Initial PARAFAC models with one to four components were made. Non-negativity constraints on all three modes were applied (Andersen & Bro, 2003). For both datasets three-component models were chosen as suitable based on core consistency (Bro & Kiers, 2003) and inspection of loadings. For the skim milk dataset, an improved model was found by removing the emission wavelengths from 260 to 285 nm. For the non-standardized milk dataset, two outliers were removed based on evaluation of model residuals and Hotellings T^2, leaving both datasets of size (45 × 482 × 19). All data analyses were performed in MATLAB v. R2008B (Math Works Inc) with the PLS_Toolbox v. 5.0.1 (Eigenvector Research Inc).

3. Results and discussion

3.1. Fluorescence excitation-emission matrices

In Fig. 1, examples of fluorescence EEMs obtained on skim milk (Fig. 1a) and non-standardized milk (Fig. 1b) are shown. When these EEMs are compared, one common main peak with excitation maximum around 290 nm and emission maximum around 340 nm is evident, which is suggestive of protein fluorescence. Fluorescence from proteins is due to emission from the aromatic amino acids and is usually dominated by tryptophan (Lakowicz, 2006). Furthermore, in Fig. 1b, one smaller peak is seen, with excitation maximum around 325 nm and a double peak emission structure with maxima around 410 nm and 435 nm. Since this peak is only visible in the non-standardized milk and not the skimmed milk, this fluorophore must be related to the presence of milk fat. A number of studies with fluorescence measurements on dairy products have reported an emission maximum around 410 nm, and a corresponding excitation maximum around 325 nm for the fat soluble vitamin A (Dufour & Riaublanc, 1997; Karoui & Dufour, 2003; Karoui et al., 2007). Pure vitamin A in solution has