Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 536 (2005) 153–158
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Fluorescence characteristics of several whey samples subjected to different treatments and conditions

José A. Murillo Pulgarín*, Aurelia Alañón Molina, M. Teresa Alañón Pardo

Department of Analytical Chemistry and Foods Technology, University of Castilla-La Mancha, 13071 Ciudad Real, Spain

Received 2 September 2004; received in revised form 22 December 2004; accepted 22 December 2004
Available online 30 January 2005

Abstract

The total fluorescence spectra of several wheis, obtained from the corresponding mammals' milk, subjected to different treatments and conditions, were recorded.

Whey was obtained after two treatments of the milk: precipitation of insoluble protein fraction at pH 4.6, and separation of the soluble fraction, whey, using a filter paper.

Wheis corresponding to: cow's milk (whole, skimmed or partially skimmed) at different heat treatments (raw milk, Pasteurization and UHT treatment), goat's milk, sheep's milk and human milk, were studied. In all cases, two fluorescent bands, in the UV region, were obtained.

It was also studied if some chemical conditions (whey dilution, pH and ethanol concentration) modify or not the intensity and the position of the two fluorescent bands.

A liquid chromatographic study with fluorescence detection showed that the two fluorescent amino acids tyrosine and tryptophan, which are present at soluble fraction of milk, are responsible for the intrinsic fluorescence of whey.

Keywords: Fluorescence; Liquid chromatography (LC); Milk; Whey

1. Introduction

The total fluorescence of a complex system or and a fluorescent compound, is characterized by three parameters: the excitation wavelength, the emission wavelength and the fluorescence intensity. A three-dimensional plot is therefore required for a complete description of the fluorescence. It may be presented as a so-called excitation/emission matrix [1].

The study of the total fluorescence of human serum was described by Wolfbeis and Leiner [2] in 1985. In 1987, Leiner et al. [3] described the total fluorescence of human urine. Both biological fluid, are composed of numerous organic substances but only some of them are fluorescents.

Milk is a complex fluid exhibiting simultaneously emulsion, colloidal and solution phases. The aqueous phase of milk, whey, contains the following major proteins: β-lactoglobulin, α-lactoalubumin, inmunoglobulines and seroalbumins, while α_{s1}- and α_{s2}-caseins, β-casein and κ-casein, are retained in the colloidal phase; the amino acids composition of all these proteins includes at least one tryptophan residues [4]. Depending on their structures, each proteins exhibits, following excitation in the region 280–295 nm, a characteristic fluorescence emission spectrum defined by its maximum emission wavelength and the tryptophan quantum yield [5]. Also milk is rich in aqueous-soluble vitamins (B-complex and C) as in fat-soluble vitamins (A, D, E and K). Vitamin A occurs in more than one form but is generally found as retinol. Because of its alcohol group, retinol readily forms esters. In milk, almost all the vitamins occur in the palmitate or acetate ester forms. Vitamin A is located in the core and in the membrane of the fat globule [6]. Due to its conjugated double bounds, retinol is a good fluorescent probe with excitation and emission wavelengths at about 330 and

* Corresponding author.
E-mail address: joseantonio.murillo@uclm.es (J.A. Murillo Pulgarín).

0003-2670/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2004.12.087

450 nm, respectively. The fluorescence properties of retinol change as a function of the sample composition. A very weak fluorescence is observed for aqueous solution of retinol, but its quantum yield is drastically enhanced in an apolar medium [7]. Milk also contains a great quantity of carbohydrates such as lactose, glucose and galactose, being lactose the majority of them and easily extracted from the whey.

Several analytical chemical methods have been described to characterize milk samples. Tedious and time-consuming chemical methods are often replaced by more rapid and non-invasive spectroscopic methods. In that sense, Dufour and Riaublanc [8] used a variable angle front-face fluorescence method to distinguish between raw, heated, homogenised and homogenised + heated milks. Later, Birlouez-Aragón et al. [9] described a rapid fluorimetric method to estimate the heat treatment of milk by the simultaneous quantification of two complementary indicators of the heat treatment (furosine and β-lactoglobulin concentrations).

Measurements of total fluorescence spectra are considered to be a potentially useful tool for pattern of definitions. This procedure was already applied to serum [2] and urine [3], as mentioned above.

The focus of this paper is the establishment of patterns for the intrinsic fluorescence of whey. Total fluorescence spectra were recorded by the use of FTOTAL program [10] which allows us to obtain information on a fluorescent compound through the isometric representation of the three-dimensional spectrum and in the form of a level curve.

2. Experimental procedures

2.1. Instruments

Fluorimetric measurements were performed on an Aminco Bowman Series 2 luminescence spectrometer, connected to software which operates on the Windows'98. The instrument uses a continuous xenon lamp for fluorescence measurements. Quartz glass cuvettes with a pathlength of 1 cm × 1 cm were used.

Thermostatic equipment and a Crison Model 2001 pH-meter with a glass-saturated calomel combination electrode were also used.

The chromatographic system consisted on a Waters 2690 liquid chromatograph, fitted with a Novapack C18 column (4 μm, 300 mm × 3.9 mm). The system was equipped with a temperature-controlled oven and a syringe-loading sample injector. Detection was made using a fluorimetric detector (λ_{ex} = 282 nm/λ_{em} = 331 nm).

2.2. Software

The AB2 program allows the instrument operation to obtain excitation and emission spectra, total luminescence spectra and time trace, such as decay curves and time resolved curves. The data processing was done with a home-made software, the FTOTAL program [10]. Using this program, total fluorescence spectra can be plotted as isometric projection and as contour maps or level curves where the excitation wavelength is *x*-axe, the emission wavelength is *y*-axe and intensity signals are shown as continuous lines between equal luminescence points.

The program also allows auto scaling and removing light scattering. In that way, all the fluorescent characteristics of the compounds can be appreciated. Moreover, spectra can be processed by means of mathematical operations, derived or smoothed.

One of the main advantages of this program is the possibility to perform any bi-dimensional spectrum (excitation, emission, conventional synchronous, constant energy synchronous, linear variable angle synchronous and non-linear variable angle synchronous spectrum) from any trajectory in the total luminescence spectrum.

2.3. Reagents

Experiments were performed with analytical reagent grade chemicals, pure solvents and Milli-Q water. Acetic acid, sodium acetate, ethanol and chlorhidric acid were supplied by Panreac (Barcelona, España).

Standards of tyrosine (Tyr) as hydrochloride, typtophan (Trp) as hydrochloride and phenylalanine (Phe) as hydrochloride, were obtained from Sigma–Aldrich (Steinheim, Germany).

Milk of different mammals (cow, sheep, goat) at different heat treatments (raw milk, Pasteurization and UHT treatment) with different cream content (whole, skimmed and partially skimmed) were supplied by some commercial marks. Human milk was obtained from fasting and healthy women who had just given birth to a child.

2.4. Procedure

2.4.1. Sample whey obtaining

Whey is obtained by precipitation of the protein fraction of the milk at pH 4.6: 10 mL of milk were introduced into a 100 mL calibration flask, 75 mL of Milli-Q water at 40 °C and 1 mL of the acetic acid solution (10%, p/v) were added to the calibration flask. The content of the flask was smoothly mixed and left to settle 10 min. Next, 1 mL of the sodium acetate 1 M was added mixing again the flask and diluted to volume with water. The flask must be left to cool down.

Once protein fraction has been precipitated, soluble phase, whey 1:10, was separated using a filter paper. Whey must be stored in a dry container at low temperature.

2.4.2. Total fluorescence spectra recording

Four milliliters of the whey were introduced into a 10 mL calibration flask that was diluted to volume with water giving a 1:25 final dilution. Standard fluorescence cuvette was filled with that solution.