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Rapid analysis of effluents generated by the dairy industry for fat determination by preconcentration in nylon membranes and attenuated total reflectance infrared spectroscopy measurement

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ABSTRACT

This paper describes a new approach for the determination of fat in the effluents generated by the dairy industry which is based on the retention of fat in nylon membranes and measurement of the absorbances on the membrane surface by ATR-IR spectroscopy. Different options have been evaluated for retaining fat in the membranes using milk samples of different origin and fat content. Based on the results obtained, a method is proposed for the determination of fat in effluents which involves the filtration of 1 mL of the samples through 0.45 μm nylon membranes of 13 mm diameter. The fat content is then determined by measuring the absorbance of band at 1745 cm^{-1}. The proposed method can be used for the direct estimation of fat at concentrations in the 2–12 mg/L interval with adequate reproducibility. The intraday precision, expressed as coefficients of variation CVs, were $\leq$ 11%, whereas the interday CVs were $\leq$ 20%. The method shows a good tolerance towards conditions typically found in the effluents generated by the dairy industry. The most relevant features of the proposed method are simplicity and speed as the samples can be characterized in a few minutes. Sample preparation does not involve either additional instrumentation (such as pumps or vacuum equipment) or organic solvents or other chemicals. Therefore, the proposed method can be considered a rapid, simple and cost-effective alternative to gravimetric methods for controlling fat content in these effluents during production or cleaning processes.

1. Introduction

The dairy industry is considered one of the biggest producers of effluents. Although these effluents mostly contain the major milk components, their environmental impact can be of relevance because of the high content of organic matter and nutrients, and also because the degradation of certain compounds, i.e. fats, may be slow [1]. In addition, these wastes also reflect the contamination associated with cleaning and disinfection processes which are usually performed by means of cleaning in place (CIP) systems. Therefore, the characterization of the effluents generated by these factories is important not only from an environmental point of view, but also to optimize CIP processes and to test the efficiency of new detergent formulations [2].

The fat content has been extensively used in the evaluation of the environmental impact of the effluents produced by dairy industry [1,3]. A rapid in-real time response is considered necessary for an efficient control of both production and cleaning processes. Rapid analytical methods would help to reduce the residues by increasing the efficiency in the use of raw materials, water, cleaning products and other resources [4,5]. Current methods used for the determination of fat in waters entail gravimetric measurement after the extraction of the fat into an organic solvent [1–3]. However, gravimetric methods involve long time for extraction and solvent evaporation steps, and they are clearly unsuitable for real-time monitoring purposes. Therefore, there is a real need for the development of rapid and simple procedures that can be used for monitoring the fat content in these kinds of effluents.

IR spectroscopy either in the near or mid regions, often combined with multivariate statistical methods, has been extensively used in the dairy industry in order to determine the major components (fat, lactose, protein) of milk, whey, cheese and other products, with important advantages such as rapidity and minimum sample preparation. This technique has been extensively used to establish the nutritional value of dairy products and to detect milk adulteration, as well as to predict microbial spoilage [6–12]. IR is also considered an efficient technique for real-time control of production, i.e. transesterification of fat blends [13]. However, in the analysis of fat in effluents previous extraction steps would be necessary to reach the desired sensitivity as concentrations below g/L can be expected.

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Among the different alternatives proposed to increase the sensitivity in spectroscopic methods, the employment of extraction membranes is very attractive, especially if the analytes can be directly monitored on the membrane [14]. The utility of this approach in IR based methods for determination of oils and grease matter (alkanes, diesel oil and gasoline) in water matrices has been demonstrated [15,16]. For this purpose, the samples were filtered through PTFE membranes; in the analysis of volatile compounds the membranes were exposed to the headspace of the samples. After extraction, the membranes with the retained compounds were held in a sampler holder so that the light could pass through them, and the IR spectra were registered in transmission mode. More recently, the determination of traces of contaminants (some volatile organic compounds) preconcentrated on polymer membranes by ATR IR has been reported [17]. However, to the best of our knowledge, this option has never been tested for determination of fat in effluents generated by the dairy industry.

In the present work we have evaluated the possibility of determining fat content in effluents produced by dairy plants using nylon membranes as a support for the retention of fat and registration of ATR IR spectra. Parameters that affect the sensitivity such as pore and diameter of the membranes, and sample volume have been optimized using powder and liquid milks of different origin and fat content. The possibility of applying this methodology under conditions of CIP processes has also been investigated. On the basis of the results obtained a new method is proposed for the quantification of fat at low mg/L levels. The utility of the method has been tested by analysing the effluents produced by a dairy pilot plant.

2. Experimental

2.1. Apparatus

ATR-FTIR spectra were recorded using a Cary 630 FTIR spectrometer equipped with a diamond ATR sampling accessory from Agilent Technologies (Böblingen, Germany). Spectra were recorded in the frequency range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1}. For data collection a MicroLab FTIR software from Agilent Technologies was used; for data processing a ResolutionsPro software (Agilent Tecnologies) was used. Prior to each sample measurement a background scan was made against the air, and 8 scans were averaged for each sample (a higher number of scans did not significantly improve the signal to noise ratio).

2.2. Reagents and materials

Nylon membranes of 47 mm diameter and 10.0 μm and 0.45 μm pore diameter were obtained from Teknokroma (Barcelona, Spain), and nylon membranes of 13 mm diameter and 0.45 μm pore diameter were purchased from Sartorius Stedim (Goettingen, Germany). Nitric acid (69%) and sodium dodecylsulphate were obtained from Panreac (Barcelona, Spain), and sodium hydroxide was purchased from J.T. Baker (Deventer, Holland). Detergent formulations containing polymeric cationic and non-ionic surfactants were supplied by Betelgeux S.L. Company.

2.3. Milk samples

Two powder milks and two liquid commercial milks of different origin and fat content were used throughout the study (Table 1). Stock samples were daily prepared by diluting the appropriate amount of the milk samples with distilled water to give a total amount of fat of 0.5 g/L, according to the fat content declared by the producer. For powder milks, the resulting suspensions were sonicated into an ultrasonic bath for about 5 min for homogenization. Unless otherwise stated, working samples were prepared by diluting the stock samples with distilled water.

2.4. Sample processing

In studies carried out with membranes of 47 mm diameter the samples were passed through the membranes by vacuum. For this purpose a vacuum filter assembly (Supelco, Bellefonte, USA) connected to a vacuum pump was used. Then the membranes were removed from the assembly and placed into the spectrophotometer to collect the spectra so that the upper side of the membrane was in contact with the ATR crystal.

In studies with the 13 mm diameter membranes, the samples were filtered using a luer-lock glass syringe of 10 mL connected to a 13 mm diameter stainless steel filter holder (Whatman, Kent, England). The samples were manually filtered by passage through the nylon membranes mounted in the filter holder. After extraction, the holder was separated from the syringe and opened. The nylon membranes were then removed from the holder, and placed on the spectrometer for recording the spectrum.

For simplicity, all assays were carried out in triplicate at ambient temperature (the goal of this study was to develop a simple method easy to implement for routine analysis).

2.5. Analysis of real samples

The proposed method was applied to analyze effluent samples that were collected from a pilot plant aimed at the production of cheese. Samples were collected at different stages of a CIP process used to clean the pasteurizer, tanks and pipes: three samples during a first rinsing step with water at 90 °C, three samples during an intermediate rinsing step with water at 85 °C, two samples during a cleaning step using water at 78 °C with detergent (alkali), and two samples during the final rinsing with water (45 °C). After the arrival to the laboratory, the samples were stored at 4 °C until analysis. Before the analysis each sample was shaken vigorously by hand for 30 s. Each sample was analysed in triplicate and at room temperature.

3. Results and discussion

3.1. Selection of the measurement wavelength

The main absorption bands in the mid-IR region that have been utilised for the quantification of fat in milk and dairy products are 2874 cm^{-1}, a band related to the acyl chain on fatty acids, 1745 cm^{-1}, which is due to the stretch vibration of C═O group

Table 1
Commercial milk samples used throughout the study.

Sample	Origin	Fat content
Powder milk	Sheep	39 g/100 g of sample
Powder milk	Cow	24 g/100 g of sample
Liquid whole milk	Cow	36 g/L
Liquid semi-skimmed milk with high fibre content	Cow	13 g/L