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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

from fatty acids, and 1464 cm^{-1} which corresponds to the C–H bend. The utility of these bands in the context of the present study was evaluated. For this purpose, the spectra of both powder and liquid milk samples were registered and compared with those obtained for the nylon membranes. The results showed that the best option was the band at 1745 cm^{-1}, as the spectra obtained for nylon membranes did not show any significant absorbance at this wavelength. Moreover, the signals in the other two regions were more affected by the presence of water in the membranes. This is illustrated in Fig. 1, which shows the registers obtained for two nylon membranes containing different levels of water. Except for the bands corresponding to the absorption of water (2750–3700 cm^{-1} and 1600–1680 cm^{-1} regions), no spectral differences were observed between them. On the other hand, the spectra recorded for the three types of nylon membranes used throughout the study were similar (data not shown).

3.2. Sample processing

The possibility of retaining milk fat on the nylon membranes was evaluated. Initial experiments were carried out with 0.45 μm membranes. Different working samples were prepared in order to produce fat contents in the 0.010–1.0 g/L range. Variable volumes of these samples were passed through membranes, and the retention of fat was evaluated by measuring the absorbance at 1745 cm^{-1}. In all instances, a significant band was detected at the wavelength corresponding to the absorption of fats. As an example, in Fig. 2 are shown the spectra recorder from liquid whole milk and from a membrane after passing through it 1 mL of the same milk diluted to give a fat content of 10 mg/L (dilution factor, 1:3600). In spite of the large dilution factor, the signals corresponding to the absorption of C=O group from fatty acids is significantly higher than that observed for the original milk. These results demonstrated that the membranes were very effective in retaining milk fat.

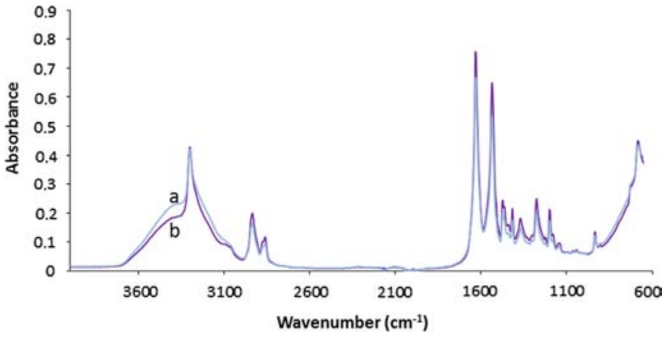

Fig. 1. Spectra recorder from 0.45 μm nylon membranes (47 mm diameter): (a) spectrum collected immediately after the passage of 10.0 mL of water; and (b) spectrum collected after passage of 10 mL of water and drying the membrane with air. For other experimental details, see text.

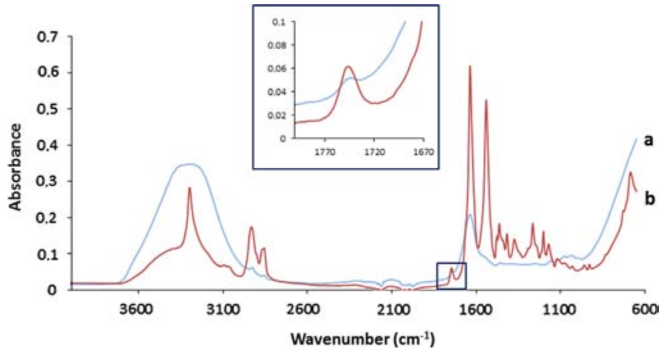

Fig. 2. Spectra recorder from: (a) liquid cow milk (fat content, 36 g/L); and (b) a 0.45 μm nylon (13 mm diameter) membrane after the passage of 1 mL of the solution resulting by diluting the same milk sample to give a fat content of 0.01 g/L (dilution factor 1:3600).

Variable volumes of the working samples were processed using 0.45 μm nylon membranes of either 47 mm or 13 mm diameter. The working samples were prepared from sheep powder milk, and the fat content varied in the 0.005–1.5 g/L range. Initial studies were carried out with membranes of 47 mm diameter. Aliquots of 50 mL of the samples were passed through the membranes by applying vacuum. It was observed that the absorbance at the working wavelength increased when increasing the content of fat in the samples up to concentrations of 0.050 g/L. For higher fat contents the absorbances remained approximately constant. On the other hand, the passage of the samples through the membranes became slower as the content of milk was increased; the sample with the highest content of fat assayed could not be totally filtered in 10 min. The reason is that high fat contents in turn involved high amounts of solid material, causing partial clogging of membranes. To overcome clogging, membranes with 10 μm pore diameter were tested. Although all working samples could be rapidly processed, the absorbances registered were significantly lower than those measured with the 0.45 μm membranes. These observations revealed that 10 μm membranes were not effective for retaining fat, probably because sonication applied during sample preparation resulted in fat globules with a diameter smaller than 10 μm [10]. Consequently, 10 μm membranes were discarded for further tests.

In another set of experiments, different volumes of the working samples were processed through 0.45 μm filters of 13 mm diameter using a 10.0 mL glass syringe connected to the filter holder. Samples were passed through the membranes by applying manually positive pressure. The sample volumes tested were 1.0, 5.0 and 10.0 mL. It was observed that for a given working solution, the absorbance increased by increasing the sample volume. However, the increment was less significant for samples with the higher fat contents. This is illustrated in Fig. 3 which shows the variation of the absorbance as a function of the sample volume for two of the concentrations assayed, 2.0 and 8.0 mg/L. As observed, no significant increment of the absorbance was obtained by increasing the fat content when passing either 5.0 or 10.0 mL of the samples through the membranes. These results suggest that for such concentrations and sample volumes the membrane became saturated. A sample volume of 1 mL was considered a good compromise between sensitivity and variation of the response to concentration.

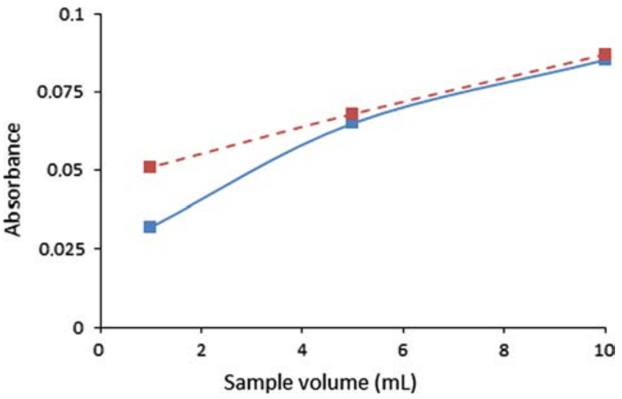

Fig. 3. Effect on the absorbance of the volume of sample when using 0.45 μm nylon membranes of 13 mm diameter: dotted line, sample containing 2 mg/L of fat; dashed line, sample containing 8 mg/L of fat.

The absorbance measured by processing 50 mL of a sample through 47 mm membranes or by passing 1.0 mL of the same sample through 13 mm membranes were similar (see Fig. 4). However, taking into account that the objective of the study was the development of a method capable of providing fast information, processing 1 mL of the samples with membranes of 13 mm diameter was considered a better option. The reasons for this choice were that the entire method was more rapid (the filtration step only required about 0.5 min), and also because no special instrumentation such as a vacuum pump or filtration equipment was necessary for sample treatment.

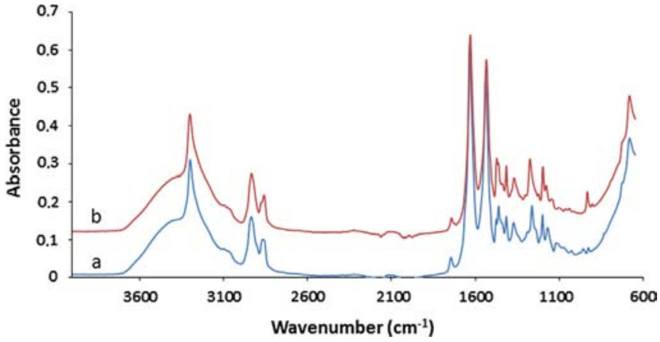

Fig. 4. Spectra recorder for a sample with a fat content of 0.010 g/L using 0.45 μm nylon membranes: (a) 1 mL of sample passed through 13 mm diameter membranes; and (b) 50 mL of sample passed through 47 mm diameter membranes.

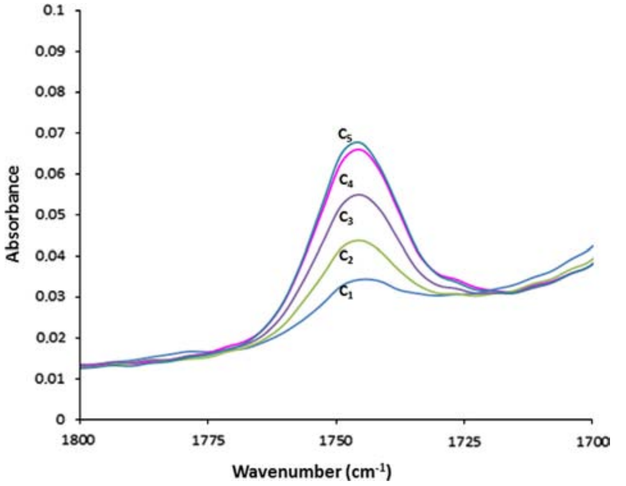

Fig. 5. Spectra collected for under the selected working conditions for samples containing different concentrations of fat: C_1—2 mg/L, C_2—4 mg/L; C_3—8 mg/L; C_4—12 mg/L; and C5—16 mg/L.

3.3. Linearity, reproducibility and sensitivity studies

The proposed method was applied to milk samples of different origin and fat content. For each kind of milk five concentrations were assayed, and for each of the concentrations three measurements were made. To optimize the calibration, different ways to measure the signal were tested: band height at 1745 cm^{-1}, band height after subtraction of blank spectrum (the spectrum of a nylon membrane after passing 1 mL of water through it), and band height with two points baseline correction using different pairs of wavelengths. Best linearity was obtained by using the absorbance after correction between 1717 cm^{-1} and 1779 cm^{-1}. In Fig. 5 are depicted the registers obtained for samples containing from 2 to 16 mg/L of fat, according to the fat content declared by the producer. A good correlation was obtained between the corrected absorbance and the concentration of fat within the 2–12 mg/L interval. However, increasing the concentration of fat from 12 mg/L to 16 mg/L resulted in a slight increment of the absorbance (see also Fig. 5). The calibration equations obtained from the different kinds of milk in the 2–12 mg/L interval are listed in Table 2. In all instances the slopes of the calibration equations were statistically equivalent, which means that the method is not affected by the matrix. On the other hand, the results obtained are consistent with the amount of fat declared by producers of the milk tested. The minimum concentration that could be measured with satisfactory reproducibility was 2 mg/L. This value corresponds to the limit of quantification (LOQ) of the method. The limit of detection (LOD) was established as the concentration which provided three times the noise of the baseline of a blank (membrane after passage of 1 mL of water) [15,18]. The LOD was found to be 0.5 mg/L.

Precision studies were carried out through the consecutive processing of three replicates of samples containing fat contents of 4 mg/L and 12 mg/L. The results are also listed in Table 2. As observed from this table, the intraday coefficients of variation for absorbance measurements ranged from 4% to 11%. Additionally, the interday precision was evaluated by processing samples prepared from sheep powder milk in four different days. The concentrations assayed were also 4 mg/L and 12 mg/L. The interday CVs observed were $<20\%$. Thus the achieved precision was considered adequate.

3.4. Application of the method

At present, there is an increasing demand for rapid and cost-effective methods for characterizing industrial effluent, not only

Table 2
Linearity and reproducibility data obtained for the different kinds of milk samples tested.

Sample type	**Linearity calibration equation, $y=a+bx$ ($n=12$)**	**Reproducibility CV (%) ($n=3$)**	
		4 mg/L	**12 mg/L**
Powder milk (sheep)	$a\pm S_a$: 0.02 ± 0.01 $b\pm S_b$: $(1.91\pm0.01)10^{-3}$ R^2: 0.990	11	7
Powder milk (cow)	$a\pm S_a$: 0.009 ± 0.009 $b\pm S_b$: $(2.0\pm0.1)10^{-3}$ R^2: 0.992	4	9
Liquid whole milk	$a\pm S_a$: 0.017 ± 0.006 $b\pm S_b$: $(1.98\pm0.01)10^{-3}$ R^2: 0.992	7	10
Liquid semi-skimmed milk with high fibre content	$a\pm S_a$: 0.002 ± 0.002 $b\pm S_b$: $(1.9\pm0.2)10^{-3}$ R^2: 0.990	11	10

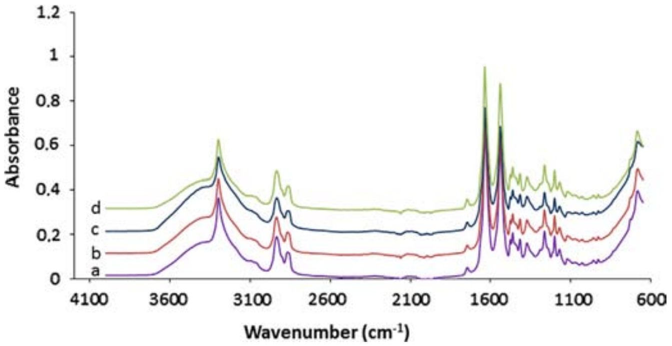

Fig. 6. Spectra collected under the selected working conditions from a sample containing 10 mg/L of fat, prepared in: (a) water; (b) 0.5% HNO_3 (m/v); (c) 2% NaOH (m/v); and (d) 0.4 g/L of SDS.

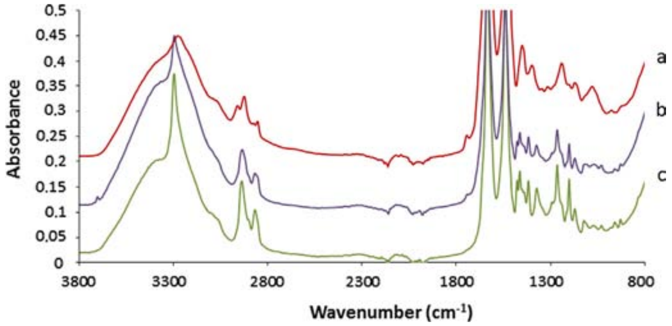

Fig. 7. Spectra collected under the selected working conditions from effluent of a dairy pilot plant at different stages of a CIP process: (a) intermediate rinse, (b) washing, and (c) final rinse.

for environmental purposes but also for reducing the production and maintenance costs. The proposed method can be directly applied to quantify fat in effluents generated by the dairy industry at fat contents within the 2–12 mg/L range. According to the literature these are typical contents of fat in the effluents of dairy plants, but in some studies values of several hundred mg/L have been reported [1]. The proposed method can be easily adapted for such high concentrations by sample dilution with water.

The potential application of the method for controlling CIP process was also evaluated. In the dairy industry CIP processes typically involve the sequential use of chemical alkaline and acidic washing steps in the presence of surfactants, and eventually other additives (chelating agents, emulsifying compounds and others) [4]. It should be remarked that fast methods are available to study the effectiveness of CIP systems through the determination of detergent residues [19]. However, the determination of fat still involves liquid–liquid extraction followed by solvent evaporation and mass measurement [2,3].

To evaluate the possible influence of pH on the response, different samples were prepared using 0.5% HNO_3 (m/v) or 2% NaOH (m/v) as the solvent, and the results were compared with those obtained for samples containing an equivalent amount of fat but prepared in water. The effect of the presence of surfactants was also evaluated. For this purpose working solutions were prepared by dilution of the stock solution with response in the presence of SDS at a concentration of 4 g/L. In Fig. 6 are shown the spectra recorded under the different conditions assayed. The differences in the absorbances obtained under the tested conditions with respect to those registered for samples prepared in water varied from -10% to $+4\%$. Taking into account the reproducibility values of Table 2, it was concluded that the differences in the absorbances at 1745 cm^{-1} were not significant.

The spectra of different polymeric cationic and non-ionic surfactants typically used in dairy industry were tested, including different tertiary alkylamines, salts of quaternary ammonia, alkyl polyglucosides and linear alcohol etoxylates. None of these compounds presented significant absorption at the working wavelength (data not shown).

Finally, the proposed method was applied to analyse the effluents generated at different stages of a CIP process in a pilot plant aimed at the manufacturing of cheese. As an example, in Fig. 7 are shown the spectra obtained for some samples analysed. One of the register (7a) corresponds to the sample which contained the highest fat content. This sample was collected during the intermediate rinsing step, and it had to be diluted with water (factor dilution, 1:10) to adjust the fat content to the linear working interval. The fat content found for this sample was (79 ± 7) mg/L ($n=3$). The other two samples were collected during the wash (7b) and final rinsing (7c) steps; the spectra showed that these samples contained fat levels below the LOQ and below the LOD, respectively. Some of the samples analysed contained a significant amount of suspended solids. However, these samples could be easily passed through the membranes, and reproducible absorbances were measured after an effective sample homogenization.

4. Conclusions

In this study, a method has been developed for the detection and quantification of fat in effluents generated by the dairy industry. The method entails manual filtration of 1 mL of the samples through 13 mm diameter nylon membranes (0.45 μm pore diameter), and direct monitorization of fat on the membrane surface by FTIR in ATR mode. Organic solvents and additional equipment such as delivering pumps or vacuum systems are totally avoided during sample preparation. In addition, neither prior washing of the membranes nor exhaustive drying steps to eliminate water before measurement are required. Therefore, the proposed method is very simple, rapid and cost-effective.

According to the literature, the major part of the pollution caused by dairy industries comes from CIP systems [2]. As demonstrated in the above sections, the proposed method is suitable for determining the fat content in the waters generated during CIP processes. Therefore, it can be considered a useful tool to evaluate the effectiveness of CIP systems, which may help to optimize the consumption of water and cleaning products, as well as to reduce the generation of wastes.

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