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# Application of Infrared Spectroscopy to the Monitoring of Lactose and Protein From Whey After Ultra and Nano Filtration Process

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**Abstract.** Whey is produced during cheese manufacture, some of its constituents are lactose and proteins. In different countries such constituents are separated for use as raw material; some of the processes to separate these components are by ultra and nano-filtration, however most common methods for the determination of lactose and proteins are not accurate. This paper shows that infrared spectroscopy is a good alternative for the quantification of lactose and proteins after ultra- and nano-filtration processes. Linear calibration curves were obtained with this analytical technique for aqueous solutions containing lactose or protein in the range 0 to 20%; after 20% lactose, the solution becomes saturated. Infrared spectroscopy is a rapid and precise method that could be successfully used to quantify these compounds and follow the ultra- and nano-filtration process applied to purify lactose and proteins from whey.
**Keywords:** FT-IR, lactose, proteins, whey, ultra-filtration, nano-filtration.

**Resumen.** El suero se produce durante la elaboración de queso, algunos de sus componentes son lactosa y proteínas. En diferentes países estos constituyentes son separados para usarse como materias primas; algunos procesos usados para su separación son por ultra y nano filtración, sin embargo, la mayoría de los métodos comerciales para cuantificar lactosa y proteínas no son exactos. En este trabajo se demuestra que la espectroscopía infrarroja es un buen método para cuantificar lactosa y proteína después de los procesos de ultra y nano filtración. Las curvas de calibración que se prepararon con esta técnica analítica midiendo soluciones acuosas de lactosa y proteína en el intervalo de 0 a 20% de estos componentes, mostraron buena linealidad; después del 20% la solución de lactosa se satura. La espectroscopia infrarroja mostró ser un método rápido y preciso que puede ser usada para cuantificar esos compuestos y dar seguimiento a los procesos de ultra y nano filtración aplicados para purificar lactosa y proteínas del suero.
**Palabras clave:** FT-IR, lactosa, proteínas, suero, ultra filtración, nano filtración.

## Introduction

Whey is a yellow-green liquid separated from the curd during manufacture of cheese [1], it has long been considered by the dairy industry as a waste by-product, and it has been discarded with an environmental consequence [2]. This aqueous solution contains nearly all the lactose originally present in the milk, as well as some fat, protein, and inorganic salts [3-4]. The high cost of disposal and the need to reduce environmental pollution have prompted considerable efforts to increase use of cheese whey components. Some developing processes for recovering the whey components include electro-dialysis, formation of complexes, ethanol precipitation, sephadex gel filtration, and membranes [5-7]. Many membrane technologies, such as ultra-filtration (UF; lower than 100 angstroms or 150 KDa molecular weight) and nano-filtration (NF; lower than 10 angstroms or 20 KDa molecular weight), have been applied by the dairy industry to concentrate whey components and reuse the water in its processes [8]. NF is a process used to separate mineral salts from lactose, having previously removed the proteins by UF. Both, proteins and lactose, can be used as raw materials to prepare a variety of products [9].

Lactose measurements have been done using different methods. Polarimetry, based on the measurement of specific rotation of the polarized light due to the asymmetric carbon of lactose, has as main disadvantages the interference from other optically active components and the non differentiation between carbohydrates. Gravimetry is a very simple and cheap procedure, however, it can be affected by interference from all reducing carbohydrates and there is not differentiation between them. HPLC is a direct method, allowing differentiation between carbohydrates but it is somewhat expensive. Additionally, some of these methods are tedious and time-consuming due to long sample preparation [10-11]. For many years, the reference method for determining protein content has been the Kjeldahl method; however, as milk also contains other sources of nitrogen, calculated protein values can be overestimated. Proteins are also commonly quantified by colorimetric methods like Bradford assay. Using this method, non-accurate values are often obtained because it is linear over a short range, and Bradford reagent can be inhibited by several compounds such as detergents [12].

Infrared spectroscopy is a rapid, inexpensive, and sensitive technology used for the high-throughput analysis of food components without requiring special skills from users. This technique expresses typical vibration modes of covalent bonds in molecules, and thus, contains quantitative information about all the constituents that absorb IR radiation, including proteins, sugars and fats. The middle infra-red spectral region, between

4000 and 400 $cm^{-1}$ (MIR), is especially attractive because measurements in this range provide direct information concerning the specific constituents in the sample, as well as their characteristic molecular structure. In particular, Fourier transform infrared (FT-IR) spectroscopy in attenuated total reflectance (ATR) sampling mode is commonly used by specializing laboratories in milk analysis to obtain information related to composition and conformation of their components. However, this is an indirect method that requires instrument calibration with milk samples that have reference values established by reference chemistry methods [13-14].

The aim of this research was to apply and validate ATR-FT-IR spectroscopy in the middle infrared spectral region, for monitoring lactose and proteins in each step of the recovery of the whey components from UF and NF techniques.

## Results and discussion

### Calibration curves

In order to demonstrate that infrared spectroscopy can be used to determinate the lactose concentration during the UF and NF processes, a calibration curve was prepared by measuring aqueous solutions of lactose at different concentrations in triplicate. Calibration curve was acquired using FT-IR integrated intensities obtained from several dilutions of lactose. Fig. 1 shows the FT-IR spectra of lactose in the interval 1220-800 $cm^{-1}$. All these spectra correspond to lactose aqueous solutions at concentrations from 1 to 45%. Fig. 2 shows the integrated intensity values of the FT-IR absorption (in the 1190-930 $cm^{-1}$ region) plotted as a function of lactose content and fitted by means of a linear regression. The integrated intensity values for solutions with less than 20% lactose showed a linear dependence according to equation 1:

$$Y = 0.55\,X - 0.26 \qquad (1)$$

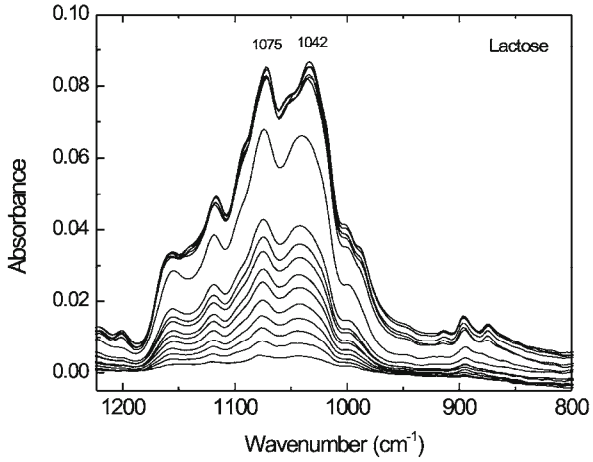


**Fig. 1.** FT-IR spectra of lactose.

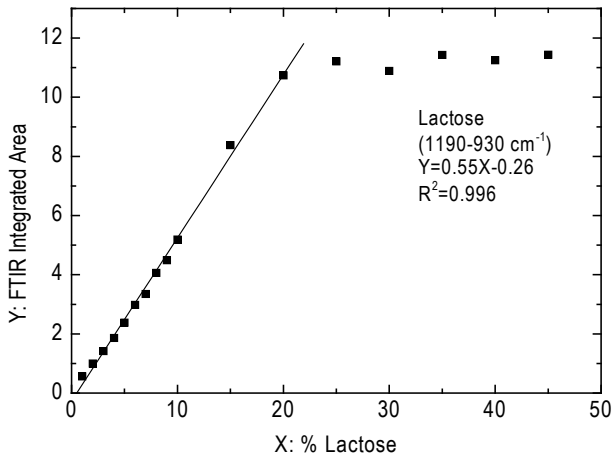


**Fig. 2.** Linear regression of lactose calibration curve.

— Where, $Y$ is the integrated intensity, and $X$ is the lactose concentration.

However above this concentration (from 20 to 45%), the solution was saturated and the infrared absorption intensity did not change anymore, as shown in Fig. 2.

NF permeate samples have a too small quantity of lactose that cannot be determined using the curve of Fig. 2. This fact is illustrated in Fig. 3, where the FT-IR spectra of solutions containing 1 and 2% of lactose are compared with the NF permeate samples. As observed the spectra of samples from NF process have the lower intensity in the lactose region. Therefore, equation 1 was used to calculate lactose only during the UF process.

A similar procedure was followed for the determination of protein. FT-IR spectrum of protein has well defined signals in the 1700-1481 $cm^{-1}$ region. Fig. 4 shows the protein FT-IR spectra acquired from aqueous solutions of protein at several concentrations, from 1 to 20%. The integrated intensity values of FT-IR absorption were correlated with the protein content and the experimental dependence was fitted with a linear regression (see Fig. 5). The region between 1700 and 1480 $cm^{-1}$

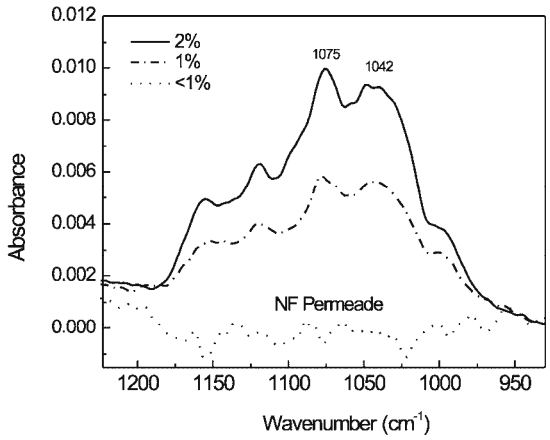


**Fig. 3.** FT-IR spectra of 1 and 2% lactose solution, and NF permeate.

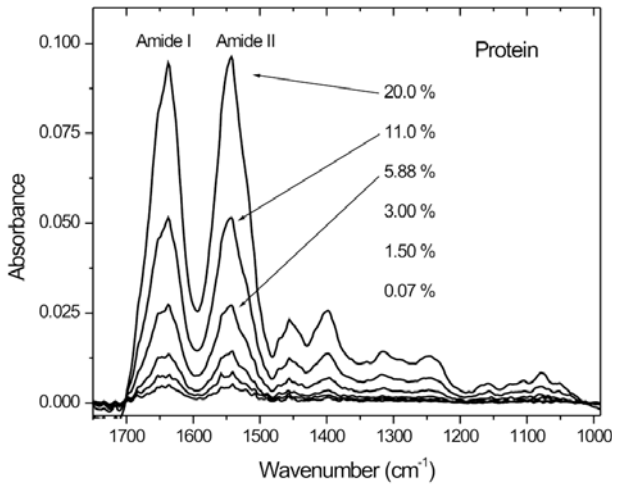


**Fig. 4.** FT-IR protein spectra.

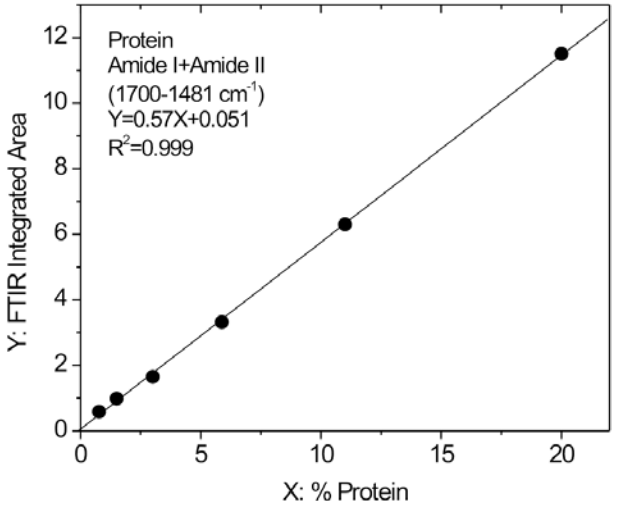


**Fig. 5.** Linear regression of protein calibration curve.

was chosen for obtaining the integrated intensity values, which showed a linear dependence with protein content according to equation 2:

$$Y = 0.57\,X + 0.051 \qquad (2)$$

— Where, $Y$ is the integrated intensity, and $X$ is the protein concentration.

## Whey

First of all, ten samples of fresh whey were analyzed with Milkoscan S-54B equipment; Table 1 shows the content of proteins and lactose in the fresh whey. The composition for both compounds is similar to that reported by Armstrong [15], who found a content of 0.8% protein and 4.9% lactose. This analysis shows that whey has an important quantity of proteins and lactose to consider its purification.

**Table 1.** Composition of fresh whey (n = 10).

| Component | Percentage ± standard deviation |
|---|---|
| Protein | 1.26 ± 0.35 |
| Lactose | 5.48 ± 0.71 |

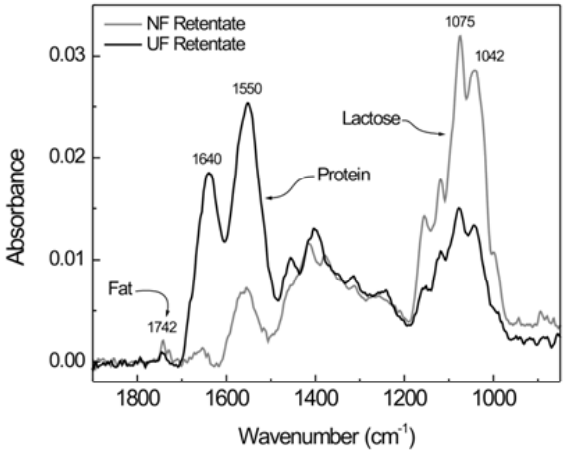


**Fig. 6.** FT-IR UF retentate and NF retentate spectrum.

## Ultra-filtration and nano-filtration of whey

Figure 6 shows the FT-IR spectrum of UF (continuous line) and NF retentate (dotted line), where the well-defined signals in the 930-1190 $cm^{-1}$ region correspond to lactose, and signals in 1500-1700 $cm^{-1}$ are associated to the amide group. According to results calculated via equations 1 and 2, when the UF process occurs lactose concentration in the retentate is lower and when NF process occurs, proteins concentration is now lower. Changes in concentration of lactose and proteins are clearly observed in the infrared spectrum, showing that FT-IR technique is sensible to changes produced during the ultra and nano-filtration. Table 2 shows the concentrations of lactose and proteins in the different fractions. Analysis of UF retentate showed that proteins were more concentrated in this fraction, but some lactose was also retained; whereas, after NF, almost 100% of remaining lactose was retained, as well as the residual proteins of small molecular weight. Permeate of NF did no reported protein or lactose in significant amounts.

**Table 2.** Calculation of lactose and protein in the different fractions

| Process | Lactose: integrated area (FTIR) | % Lactose[1] | Protein: integrated area (FTIR) | % Protein[2] |
|---|---|---|---|---|
| UF retentate | 2.04 | 4.18 | 3.261 | 5.6 |
| NF retentate | 3.93 | 7.61 | 0.698 | 1.1 |
| NF permeate | — | <1.0 | 0.137 | 0.15 |

[1] Calculated using Y = 0.55 X – 0.26; [2] Calculated using Y = 0.57X + 0.051.

## Conclusions

FT-IR spectroscopy was used to monitor the lactose and proteins concentration, during the UF and NF of whey. In particular, infrared spectroscopy was very useful to measure vibrational frequencies of lactose and proteins in order to quantify them. The proposed FT-IR method has some advantages over other methods, FT-IR is a rapid and precise analytical technique, the non-necessity of reagents and the ease of use. The values obtained by FT-IR did not depend on the analyst like in colorimetric methods; also is capable to detect both components of whey, in the range necessary for the daily industry.

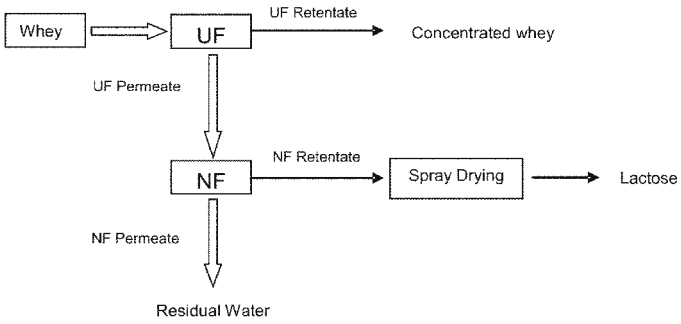


**Fig. 7.** Experimental process.

## Experimental details

### Sample of whey

Whey was obtained from a dairy factory in Tlaxcala, Mexico; ten samples were analyzed with a MilkoScan (S-54B, FOSS Electric A/S, Hilleroed, Denmark) equipment to evaluate the initial content of protein and lactose, results are reported as an average.

### Calibration curves

Serial dilutions of lactose reagent purchased from Sigma-Aldrich were prepared in triplicate considering aqueous solutions at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 and 45% lactose; solutions were measured by FT-IR to make a calibration curve for the determination of lactose concentration in the studied samples.

Bovine serum albumin from Sigma-Aldrich was used to prepare serial dilutions at 1, 2, 6, 12 and 20% protein in triplicate; these solutions were analyzed by FT-IR to make a calibration curve for the determination of protein concentration in samples.

### Ultra-filtration process

Whey was ultra filtered using a multi-step equipment NIRO (RO, model R, GEA, Wisconsin, EUA), at an inlet pressure 5.5 kg/cm$^2$, whey flux 9.31 L/s, inlet pressure 53.4 lb/in$^2$, temperature 25-45 $^o$C, air pressure 80 psig and using a 10 KDa membrane. After this process, permeate (no retained by the membrane) was then processed by NF. Retentate (part retained by the membrane) was analyzed with FT-IR.

### Nano-filtration process

The permeate material from UF was processed by NF. This procedure was carried out by using a NF CETA equipment at flux 1.2 L/min, work pressure 100 psi, temperature 35 $^o$C and using a 0.2 KDa membrane. The retentate and permeate samples were kept to be analyzed with FT-IR.

Fig. 7 shows the schematic diagram of the procedure followed to separate lactose and proteins from whey.

### FT-IR measurements of lactose and proteins from whey

Samples of lactose and proteins from UF and NF process were analyzed with a FT-IR (Bruker model Vertex 70) in ATR sampling mode; using an integration time of 300 seconds to acquire the infrared vibration spectra in the middle region (4000-400 cm$^{-1}$).

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