Analytic determinations of minerals content by XRF, ICP and EEA in ultrafiltered milk and yoghurt

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Abstract—The application of ultrafiltration for milk concentration allows obtaining a raw material for yoghurt elaboration, with total solids increased. The products have special characteristics due to different ratios proteins/lactose can be obtained, as well as they have nutritional properties with beneficial effects in human health. In this study the content of calcium, potassium, phosphorous, iron, zinc, magnesium and sodium in milk, its distribution during ultrafiltration and in the fermented product have been studied by means of X-ray fluorescence spectrometry (XRF). The results show that XRF technique has been successfully used for determination of minerals content in dairy products, still for trace species. The minerals measures were validated with other analytical techniques with high accuracy and versatility as inductively coupled plasma spectrometry (ICP) and atomic emission spectrometry (EEA). Furthermore the proteins content was increased 13% and lactose content has been reduced in 30%, in compared with some commercial products.

Keywords — Ultrafiltration, milk, yoghurt, mineral content.

I. INTRODUCTION

Cow milk and dairy products supply essential inorganic elements for humans, and constitute the most important source of bioavailable calcium in our diet (Closa et al., 2003; De la Fuente et al., 2003). There is good evidence that a number of milk minerals play important roles in bone metabolism and bone mass (Cashman and Flynn, 1999; Cashman, 2006; Flynn, 2003). Scholz-Ahrens and Schrezenmeir (2006) have recently demonstrated that dairy products have hypotensive effect of fat-reduced and milk minerals have positive effect on components of the metabolic syndrome.

The knowledge of milk and milk products benefices has increased in the time and this tendency has driven the manufacturers to develop and produce a wide variety of products with different characteristics. Fractionated milk components enable a more constant quality and will lead to a more efficient and diverse use. Membrane technology makes it possible to improve the quality of traditional dairy products, to create new food staffs, to a much greater extent for human nutrition (Cheryan and Alvarez, 1995; Brans et al., 2004). It is known that ultrafiltration (UF) membranes allow passage of lactose, water and some salts while retaining the proteins, fats and some of the insoluble or bound salts. This behavior was employed in order to obtain a concentrate with high protein content and total solids adequate to manufacture yoghurt (Alvarez et al., 1998). The product obtained additionally has reduced the lactose values respect to commercial yoghurts, which benefices to people with intolerance to lactose (Somkuti and Holsinger, 1997). Calcium, phosphorus, potassium, iron and zinc partition and redistribution during ultrafiltration process is measured in this work employing an analytic technology fast and sensitive between the ranges studied, as the X-ray fluorescence spectrometry (XRF), which alloy a direct analysis upon the sample, without previous treatment (Deluigi et al., 2003). The mineral determinations were compared employing other methodologies as inductively coupled plasma spectrometry (ICP) and atomic emission spectrometry (AES), with these techniques concentrations of magnesium and sodium were also determined. Accuracy and versatility for dairy samples were determined to evaluate the efficiency of the methodologies employed. The results let to characterize the mineral composition of yoghurt manufactured by fermentation of a skim- milk concentrate and they are compared with commercial products.

II. MATERIALS AND METHODS

A. Raw Material

Partially skim-milk (1.5% fat, 3.5% protein, 4.8% lactose) (MILKAUT, Industry Argentina), was used.

B. Analytical

Raw material, concentrate, permeate and fermented products were analyzed in duplicate according to standard methods (Hart and Fisher, 1991; Casado, 1991). Density (ρ) was measured with a digital densimeter DA-110 Density/Specific (Metter, USA) and pH was measured using a pH digital (Orion model 720A, USA). The total protein content was calculated by determination of total nitrogen by the Kjeldahl method using Tecator accessories for Kjeltec Systems I and Digestion Systems DS6, USA (AOAC 15017). The fat content was measured with the Rosse-Gottlieb method (AOAC 15029). Lactose was determined by reaction with potassium iodate-chloramine T (FIL 28A/1974). Total solids were determined by weighty difference, drying in an oven at 70 ± 1°C (AOAC 15014). For ash determination, samples were weighted into porcelain crucibles and incinerated in a muffle furnace (Indef model 132, Argentine) with a temperature programmer to reach 520°C (AOAC 15016).

Measurements of calcium, potassium, iron, zinc and phosphorus contents were performed using a Philips
PW 1400 wavelength-dispersive X-ray spectrometer (the Netherlands). The spectra were obtained employing a LiF 200 (2d = 4.028 Å) analyzer crystal, a 0.15 mm collimator and in step scan mode, during periods of time long enough to obtain high-resolution spectra. X radiation can remove inner orbital electrons of the atoms and the outer shell electrons can fill the vacancies with the consequent emission of characteristic X-rays (Evans, 1995). The spectra, originating from electron transitions between the valence band and the inner shells provides a powerful technique for obtaining information on the mineral composition (Markowicz, 1993).

The analysis were performed directly on milks and yoghurts (diluted with bi-distilled water), without any previous treatment of the sample which represents a great advantage. The qualitative analysis was performed to obtain the adequate operational conditions to the measures. The quantitative analysis was performed with the aggregate pattern method so the matrix and the original state of the samples were conserved. In the case of phosphorus was determined by a technique that requires a few mg of powdered sample, which are compressed to produce pellets. This is a rapid, very low cost of instrumentation and simple approach to specimen preparation for the analysis by XRF spectrometry and determining major, minor and trace elements (D’Angelo et al., 2002).

The results for calcium, potassium, iron and zinc were compared with determination performed with another analytic technique of high accuracy as inductively coupled plasma spectrometry (ICP). Moreover magnesium concentration was measured, this metal was too light to be determined in a direct way by XRF.

The equipment employed was a sequential inductively coupled plasma spectrometer [BAIRD (Bedford, MA, USA) ICP 2070]. The 1m Czerny Turner monochromator had a holographic grating with 1800 grooves mm⁻¹. Patrons of each ion were performed to the analytical measures and the corresponding long wave. ICP is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its chemical character. Although each element emits energy at multiple wavelengths, in the ICP technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of the element in the analysed sample (Skoog et al., 1995).

For this determination, the organic fraction must be separated from the mineral fraction to avoid interference in the signal readout. So, samples were mineralised by dry ashing in a muffle. This procedure was performed by weighing five grams of sample into a porcelain crucible and dried on to store at 50 °C. After charring samples were incinerated in a muffle at 450 °C for 24h, until obtain white ashes. The ashes were transferred to capsules of Teflon and an acid disintegration with regal water was carried out. The remainder was re-taken with HNO₃ and warmed it during 20 minutes, the procedure was repeated until total dissolution of the samples. All chemicals were of reagent grade.

The potassium determination was furthermore carried out with a conventional methodology for alkaline metals as the atomic emission spectrometry (AES), sodium ion was also measured. The potassium and sodium determination was carried out by flame emission spectrometer (Metrolab 315, USA) at wave-lengths of 766.5 and 589 nm, respectively. Atomic emission spectroscopy uses quantitative measurement of the optical emission from excited atoms to determine analyte concentration (Skoog et al., 1995). The samples were pre-treated in the same way that was described for the ICP technique.

C. Ultrafiltration Equipment and Procedures

The experimental set-up is shown in Fig. 1. A Pellicon Cassette (Millipore, USA) with modified polysulfone membranes, with a cut-off of 10 kDa, was employed for milk ultrafiltration. Total membrane area was 0.47 m². Applied transmembrane pressure and re-circulation rate were controlled by valves and the feed rate, supplied by a variable speed pump (Procom, USA) from a feed tank provided with a thermostat. The pressure applied through the membrane was measured with two manometers and the flow of the permeate was determined by measuring the filtrate volume collected during a certain period of time. In order to protect the membrane from fouling (mainly fats), the solutions were passed through a polypropylene filter ( Pall Corporation, USA) of 5-10 µm.

Milk concentration by UF was carried out as usual by continuously removing the permeate stream. The solids content was measured in the concentrate stream and the process was stopped when the desired concentration was achieved. Temperature, re-circulation rate, transmembrane pressure, pH and permeate flow rate were continuously recorded. It was selected to operate approximately at room temperature (25 ± 2 °C) and low pressure, ± 1 bar, thus a good permeate flux evading

tation of pure water was reached. All the process was performed in duplicate for statistic analysis.

D. Yoghurt manufacturing

Milk concentrated by UF was fermented to manufacture yoghurt. The samples were put into sterilized bottles and heated at 72 ± 1°C during one minute. After the concentrated milk was pasteurized, it was cooled to 38 ± 0.2°C, inoculated with 50 ml of *S. thermophilus* and *L. bulgaricus* (Yo-Flex, YF-L702, Argentine), and kept at 38 ± 0.2°C. During the fermentation stage the acidity development was studied, and the process was stopped (cooling to 4°C) when the product reached a pH between 4.2 and 4.6.

III. RESULTS AND DISCUSSION

A. Ultrafiltration Stage

The degree of concentration or partition reached through an ultrafiltration process is usually expressed as volume concentration ratio (VCR), defined as the quotient between initial feed volume and concentrate volume (Cheryan, 1986). It was found that with a VCR = 1.5, it can be obtained a concentrate with an adequate total solid (TS) content: 13.05 ± 0.05 % w/w to yoghurt manufacture, with a good consistence, whereas proteins content was increased up to 5.00 ± 0.15 %. Once the process was stopped the samples were refrigerated for subsequent analyses.

The membrane rejection of proteins was very high, as was expected taking into account that the membrane cut-off was 10 kD, lower than the molecular weights of most of the milk proteins (between 14-65 kD). The small quantities of nitrogen detected in the permeate could be due to the small size of the peptides produced as a result of the milk proteolysis or due to the experimental error. The diameter of the fat globules in raw milk is between 0.1 and 15 µm, with an average of approximately 3.4 µm, thus the UF process also concentrates fat (Brans et al., 2004). The molecular size of lactose is much lower than the membrane pore diameter and therefore the membrane should not retain the lactose and so it freely permeates through the UF membrane.

The quantitative measurement of calcium, potassium and phosphorus by XRF in the feed, permeate and concentrate are presented in Fig. 2.

The results show the degree of mineral partition by the UF process. It was verified that milk concentration by UF increased the calcium colloidal proportionally to the amount of protein. Since at the pH of the raw milk (6 ± 0.3) approximately two thirds of the calcium are associated with casein micelles (De la Fuente et al., 2003). In this sense different models of micellar structure have been proposed, which describes that the micelles are formed by spherical sub-micelles which are associated between them by “colloidal” calcium and magnesium phosphate (Schmidt, 1982). This result in a stabilization of calcium, phosphorus, and magnesium by the proteins micelles, and thus these elements are concentrated with the proteins by the UF process founding in the permeate a smaller content, corresponding to free mineral. The experimental values of calcium partition obtained by XRF have been validated employing ICP determination which is shown in Fig. 3, where it is also shown the magnesium concentration by UF.

Potassium and sodium are not fixed to the proteins, so they can freely distribute between the permeate and the concentrate, this fact can be see for potassium in Figs. 2, 3 and 4 and for sodium in Fig. 4.

Iron and zinc partitions by UF are showed in Fig. 5 and 6 respectively. It can be see that these minerals are also concentrated by UF, in agreement with literature, which informs the ability of the casein to bind Fe and Zn (Hekmat and McMahon, 1998; Wong and Kitts, 2003). More than the half of the Fe content and the 75% of Zn are fixed in the caseins micelles. Furthermore the emulsified fat globules, which are concentrated by UF, con
Figure 5. Determination of Fe by XRF and ICP. (Milk, Concentrate, Permeate).

Figure 6. Determination of Zn by XRF and ICP. (Milk, Concentrate, Permeate).

tents 1% phospholipid that fix Fe and Zn in their membranes (Goursaud, 1991; De la Fuente et al., 1996; Swaisgood, 1996; Closa et al., 2003).

B. Fermentation Stage

Table 1 shows the results of physicochemical properties of the final yoghurt with the working UF conditions.

In the yoghurt, the protein/lactose ratio increases from 0.67 ± 0.06 for different commercial skim yoghurts to 1.3 ± 0.05 for the VCR employed in this UF process. The coagulum firmness has been increased and remains stable, in addition, the proteins had undergone a milder thermal treatment comparing with traditional way to manufacture yoghurts (Cheryan, 1986). The reduction of lactose content is significant in comparison with commercial products. This is beneficial for large segments of world’s population, that are lactase-deficient people, limited to intake nutritious dairy foods (Somkutti and Holsinger, 1997). The fat content in the fermented product contributes to the final flavour, mouth feel and texture. Fats concentrated by UF do not have variations during fermentation, assuming that the lactic ferment has little lipases and the milks ones are destroyed in the pasteurization step, because of they are thermolabiles (Romero Estévez, 1988).

The analytical determinations of minerals in the yoghurt obtained from the UF concentrated compared with commercials products are given in Table 2. The results show that phosphorus and magnesium contents have been incremented in the yoghurt manufactured a 10% for the first and a 20% for the second, respect to the commercial products. A similar behavior is observed for the calcium content has been increased approximately 50 % respect to commercial samples in a proportional protein concentration by UF. The measured value by the XRF technique is always lightly superior that the ones obtained by ICP. This fact can be due to that the direct determination prevents the loss associated to samples pre-treatment.

Potassium content is lower in the yoghurt obtained in comparison with commercial product, due to this ion in not concentrated by the UF process as we have seen in the previous section. Again the measured value by XRF is higher than the measured in the same sample with the other techniques employed.

Iron and zinc are concentrated in the UF step, as we have seen, but the iron content in our yoghurt is a 30% minor while zinc content is increased a 60%, respect to commercial samples. In both cases the amounts determined using XRF and ICP are rather similar, denoting the high sensibility of the XRF to direct determination even for trace minerals.

Table 1 Properties of the final yoghurt obtained compared with commercial products (mean ± S.D).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Commerical skim yoghurt</th>
<th>Yoghurt UF</th>
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<tbody>
<tr>
<td>Density (g/ml)</td>
<td>1.0382 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>pH</td>
<td>1.0382 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>TS (% w/w)</td>
<td>1.038 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>Ash (% w/w)</td>
<td>1.038 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>Fat (% w/w)</td>
<td>1.038 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>Protein (% w/w)</td>
<td>1.038 ± 0.002</td>
<td>1.040 ± 0.013</td>
</tr>
<tr>
<td>Lactose (% w/w)</td>
<td>1.038 ± 0.002</td>
<td>1.040 ± 0.013</td>
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Table 2 Comparatives mineral concentrations (ppm) of yoghurts measured by different analytical techniques. *

<table>
<thead>
<tr>
<th>Elements</th>
<th>XRF</th>
<th>ICP</th>
<th>EEA</th>
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<tbody>
<tr>
<td>Ca</td>
<td>1350 (0.015%)</td>
<td>2132 (0.014%)</td>
<td>1382 (0.02%)</td>
</tr>
<tr>
<td>K</td>
<td>2866 (0.01%)</td>
<td>1800 (0.04%)</td>
<td>2063 (0.025%)</td>
</tr>
<tr>
<td>P</td>
<td>1686 (0.037%)</td>
<td>1770 (0.05%)</td>
<td>1477 (0.054%)</td>
</tr>
<tr>
<td>Fe</td>
<td>1.2 (1.25%)</td>
<td>0.85 (1.2%)</td>
<td>1.3 (1.8%)</td>
</tr>
<tr>
<td>Zn</td>
<td>2.5 (3%)</td>
<td>4.2 (2.6%)</td>
<td>2.7 (1.9%)</td>
</tr>
<tr>
<td>Na</td>
<td>4.5</td>
<td>4.2 (2.6%)</td>
<td>4.13 (0.26%)</td>
</tr>
<tr>
<td>Mg</td>
<td>103 (0.8%)</td>
<td>125 (0.43%)</td>
<td>537 (0.07%)</td>
</tr>
</tbody>
</table>

*Average value from six determinations, values of relative SD percentage.
The analytical values obtained for the analyzed metals in milk and yoghurt were in good agreement with those reported by others researchers whom employed atomic absorption spectroscopy technique (Buttriss, 1997, Sánchez Segarra et al., 2000; Closa et al., 2003). While phosphorous has been determined with an UV-visible spectrophotometer (De la Fuente et al., 2003). However it is known that the metal concentration in milk can vary due the factors influencing its secretion from the mammary gland, such as breed of animal, season of year, feeding and factors related to animal handling by humans (Moreno Rojas et al., 1994).

IV. CONCLUSIONS

In this work X-ray fluorescence spectrometry (XRF) has been applied to give information of mineral content in skim milk, in both stream of milk ultrafiltration: percentages because classic methods needs a sample preparation in dairy samples. This procedure is an important advance pending of their ability to blind the caseins. The mineral content of calcium, phosphorus, magnesium, iron and zinc showed the mineral nutrient partition by the UF process methodologies such as ICP and EEA. The results compared and completed employing others analytical techniques and for a more complete nutritional and dietary evaluation of the dairy products.

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REFERENCES


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