# Ultrafiltration of Whey Concentrates to Manufacture *Requeijão*

Beatriz Monjardino de Brito de Azevedo
Master Degree in Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal
December 2015

## Abstract

In this work was investigated the possibility of using the protein concentrates, obtained by reverse osmosis and ultrafiltration, in the manufacturing of *requeijão*, a Portuguese traditional whey cheese. The optimization of the concentration process of the pre-concentrated whey by ultrafiltration, was made in view of the use of the concentrates obtained, with a concentration factor by volume (VCF) 2.0, to *requeijão* manufacturing. It was studied the influence of using concentrates, obtained by reverse osmosis and ultrafiltration, in the increase of *requeijão* production yield and was made the textural analysis of the produced *requeijões.* In the ultrafiltration trials, in total recirculation and concentration modes, a GR95PP membrane type (Alfa Laval) was used. This membrane proved to be adequate for the process due to a high permeation flux observed, a high apparent rejection coefficient to the protein and low apparent rejection to lactose. The protein concentrates (produced by ultrafiltration from concentrated whey by reverse osmosis) were used to produce *requeijão*. *Requeijão* was also produced by whole whey mixture and by concentrate of reverse osmosis before ultrafiltration. Comparatively to the traditional *requeijão* (produced by whole whey mixture), the *requeijão* produced by protein concentrates show similar levels of dry matter and cohesiveness, and significantly low levels of fat. However, it shows higher protein, lactose and magnesium contents, as well as higher hardness and adhesion, comparatively to the traditional *requeijão.* The use of protein concentrates allowed a significant increase in the *requeijão* manufacturing process yield.

**Keywords:** Whey from mixture of cow, goat and sheep milks; Reverse Osmosis (RO); Ultrafiltration (UF); Protein Concentrates; *Requeijão.*

## 1 Introduction

Whey is the main by-product obtained from cheese production and it can be classified according to their acidity degree in sweet whey or acid whey [1].

The whey represents the 85 to 95% of milk volume used in cheese production and it contains about 55% of milk nutrients, such as soluble globular proteins, lactose, mineral salts and vitamins, as well as varying amounts of lactic acid and not soluble nitrogen [1].

Currently, it's produced approximately 190 million tons of whey per year worldwide, which only 50% of this value is processed [2]. According to the statistical data of global production of whey, Portugal produced approximately 112 430 tons in 2013. This production resulted about 86 970 tons of liquid whey, 17 640 tons of whey powder and 7 830 tons of concentrate whey [3].

Due to the lack of appropriate technologies for its treatment, whey has been devaluated and discarded by many industries for decades [4], becoming a major threat to the environment, due to its high organic load and its difficult degradation. In order to combat this environmental problem and at the same time to take advantage of this by-product with commercial value, a number of studies have been made on possible investments in new products and the development of new processing technologies, such as membrane technologies, which makes this a product of future in food industry [5].

Nowadays, in some countries such as Italy, Spain and Portugal, part of the production of ovine whey is intended for the manufacture of cheeses such as *Ricotta*, *Requesón* and *Requeijão*, respectively, after being processed. The traditional process of production of requeijão leads to production of *sorelho* (deproteinated whey), which contains approximately 60% of dry matter initially present in the whey. In this dry matter predominates the presence of lactose and minerals, which make the *sorelho* a threat to the environment, due to its high organic load [6].

It is evident the importance of creating alternatives to the appropriate utilization of *sorelho*, especially for the production of *requeijão*, providing at the same time the possibility of both environmental gains as process profitability.

## 2 Experimental

The different steps performed during the present work are shown in **Figure 1.**

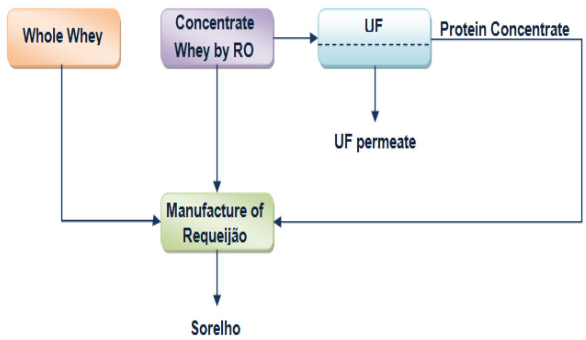


**Figure 1** – Schematic diagram of operations and fluids involved in experimental work.

### 2.1 *Whey from mixture of cow, goat and sheep milks*

The samples of whole whey and concentrate by RO were collected at Production Unit of *Torres Vedras* from Portuguese company *Queijo Saloio S.A.*

All samples were stored in a chamber cooled to 4$^o$C, after addition of $H_2O_2$ [7].

Samples of whole whey and concentrates whey were object of the following determinations: Acidity [NP-470 (1983)]; pH (Methrom pH meter); lactose (according to the equation $(\text{Lactose} = \text{total solids} - \text{Protein} - \text{ash})$; total solids [NP-475 (1983)], total nitrogen by the *Kjeldahl* method, being the protein is calculated from the determination of total nitrogen [NP-1986 (1991)]; ash [NP-477 (1983)], fat [determined by the Gerber method, according to NP-469 (1983)]; chloride by Charpentier-Volhard method, according to NP – 471 (1983), the content of other mineral elements, such as phosphorus, sodium, potassium, calcium and magnesium were determined from the ash residue, after treatment with HCl (6N), with heat and subsequent filtering; the mineral elements were determined in solution, in the Laboratório Químico Agrícola Rebelo da Silva (INIAV), by ICP-OES (Thermo, Unicam, Mod.ÍRIS Intrepid II XSP Radial).

### 2.2 *Membranes and filtration unit*

A schematic illustration of the permeation installation is displayed in **Figure 2.**

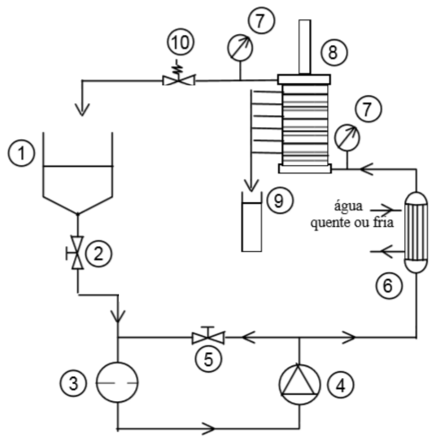


**Figure 2** - Schematic illustration of the permeation installation.

Legend:
1.Tank; 2.Valve; 3.Filter; 4.Cross-flow pump; 5.Safety valve; 6.Heat exchanger; 7.Pressure gauge; 8.Module; 9.Collecting permeate; 10.Pressure control valve

For whey concentration was used a membrane of type GR95PP, provided by *Alfa Laval*. All the permeation experiments were carried out in a plate and frame filtration unit, with a total membrane surface area of 0,072m$^2$. In all tests operated at a feed circulation velocity of maximum speed (0,94 m/s) [8] and a temperature of 25$^o$C.

The permeate flux was calculated according to the **Equation 1**

$$J_v(m.s^{-1}) = \frac{V_p(m^3)}{t(s)A_m(m^2)}$$

**Equation 1**

Where $V_p$ is the permeate volume collected during the time interval, t, and $A_m$ is the membrane surface area of permeation.

### 2.3 Manufacture of *requeijão*

The analytical methods used in the physicochemical characterization of *requeijões* were identical to those commonly used for cheese: total solids [NP 3544 (1987)]; ash [AOAC (1990)]; lactose [NP 2104 (1988)]; total nitrogen by the *Kjeldahl* method, being the protein is calculated from the determination of total nitrogen [NP-1986 (1991)]; fat [NP 2105 (1983)]; chloride by argentimetric, according to FIL088/ISO5943 (2006) and the mineral elements were determined from the ash, following the same methodology described for the case of whey

The textural properties of the *requeijões* were analyzed on a texturometer, TA-HDi type texture analyzer, Stable Microsystems, with stainless steel penetration probe, 5mm in diameter, which penetrated the sample to 50% of the height of the sample at a speed of 1 mm.s$^{-1}$.

## 3 Results and discussion

### 3.1 Characterization of the whole whey and concentrate whey by RO

The results of **Table 1** show that occurred, in general way, a increase of the components concentration of the whey after the concentration process by RO. Note only the fact that there is a reduction in the concentration of fat. This reduction is easily explained by the whey treatment previously made, immediately before being this to be processed by RO. As in most processes using membranes, the concentration by RO in the company, that provided the whey for this work, is preceded by a separation of the fat by centrifugation.

**Table 1 –** Average composition of the whole whey and concentrate whey bay RO

| Parameter | Whole Whey | Concentrate Whey by RO |
|---|---|---|
| pH | 6,38 | 5,71 |
| Acidity (ml NaOH N/L) | 14,8 | 54,70 |
| Protein (g/100g) | 0,67 | 2,13 |
| Ash (%) | 0,44 | 1,35 |
| Chloride (g NaCl/100g) | 0,32 | 0,59 |
| Fat (g/100g) | 0,75 | 0,30 |
| Total solids (%) | 6,25 | 17,00 |
| Lactose (%) | 4,39 | 13,22 |
| Calcium (mg/100g) | 30 | 97,67 |
| Phosphate (mg/100g) | 30 | 97,33 |
| Sodium (mg/100g) | 41 | 116,67 |
| Potassium (mg/100g) | 132 | 501,00 |
| Magnesium (mg/100g) | 7 | 21,33 |

### 3.2 Characterization of the ultrafiltration membrane

**Table 2 –** Characteristics of the ultrafiltration membrane.

| Membrane reference | Material | MWCO (Da) | $L_p$ (L/h.m$^2$.bar) |
|---|---|---|---|
| GR95PP | *Polyethersulphone* | 7500 | 1,21 |

### 3.3 Ultrafiltration in total recirculation mode: influence of pressure on the permeate flux of whey sample

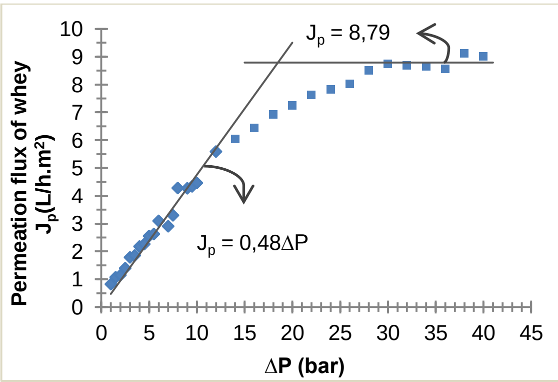


**Figure 3 –** Variation of permeation fluxes of concentrate whey by RO (Jp) with transmembrane pressure (ΔP). Membrane: GR95PP; Membrane surface area: 0,072 m$^2$; Temperature: 25ºC; a feed circulation velocity of maximum speed (0,94m/s).

**Figure 3** shows that the permeate flux increase linearly with the pressure, in the range of pressures 1 to 12 bar, and shows a limit level from 30bar. From 12bar it appears that increasing the permeate flux of whey with the pressure becomes less sharp, showing a small deviation from linearity. For a pressure range close to zero, the variation of permeation flux with the pressure can be described by the equation Jp = 0,48ΔP. For a range of pressures greater than 30bar, permeation flux variation with the pressure can be described by the equation Jp = 8,79, which represents a limit level of pressures. Note that for pressures greater than 12 and less than 30bar the limit flux is not reached, indicating that the whey can be processed by UF in a wider pressures, without showing much adverse effects the membranes. However, we decided to operate at a pressure of 12 bar, as it is contained in the nearest linearity of pure water permeability, which allows to increase the lifetime of membranes. As can be seen by the results, there is a tendency for occur fouling of the membranes, which is confirmed by the decrease of the slope of the linear regression obtained for range of high pressures.

### *3.4 Ultrafiltration in concentration mode: influence of VCF on the permeate flux of whey samples*

In the 1st UF test was used 5L and in the 2nd was used 7L of concentrate whey by RO. Both solutions were concentrated to a VCF of 2.

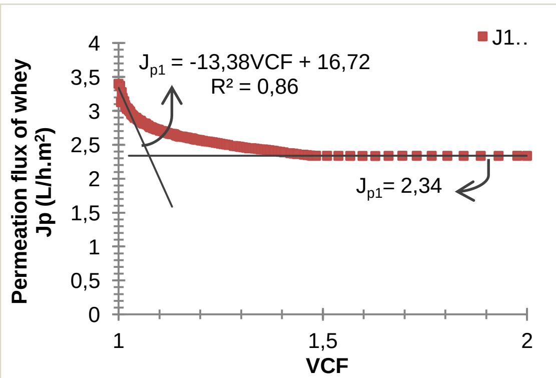


**Figure 4 –** Variation of permeation fluxes of concentrate whey by RO with VCF until 2.0, during the production of protein concentrates obtained by UF, pre-concentrate by RO. Membrane:GR95PP; Membrane surface area: 0,072 $m^2$; Pressure: 12 bar; Temperature: 25ºC; a feed circulation velocity of maximum speed (0,94m/s). Legend: J1 corresponds to permeate fluxes obtained in 1º test of UF of whey

**Figures 4** and **5** show that the permeate flux decrease with the VCF, as a consequence of the concentration polarization phenomena and/or fouling of the membranes, being the greater the intensity of these phenomena as the concentration increase, as would be expected.

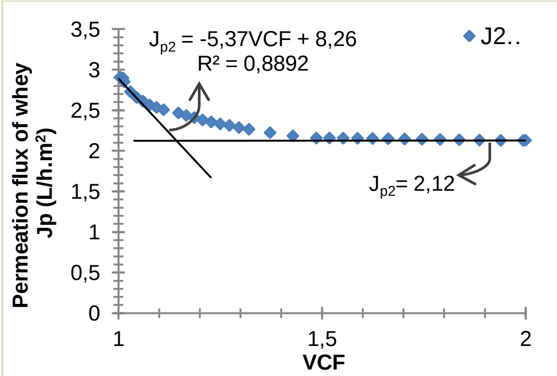


**Figure 5 -** Variation of permeation fluxes of concentrate whey by RO with VCF until 2.0, during the production of protein concentrates obtained by UF, pre-concentrate by RO. Membrane:GR95PP; Membrane surface area: 0,072 $m^2$; Pressure: 12 bar; Temperature: 25ºC; a feed circulation velocity of maximum speed (0,94m/s). Legend: J2 corresponds to permeate fluxes obtained in 2º test of UF of whey

Both graphics show two distinct zones. Initially, a linear zone, described by the equations Jp1 = -13,38VCF + 16,72 (see **Figure 4**) and Jp2 = -5,37VCF + 8,26 (see **Figure 5**). In this linear zone there is a lowering of permeation fluxes of the whey as the VCF increases. In the final experimental results, there is a limit of permeation fluxes, described by the equation $J_{p2}$= 0,0041VCF+ 2,12, which means the permeation fluxes reach a constant value. There are reductions of the permeation fluxes of about 31% and 27%, in the 1st and in the 2nd concentration test (VCF=2), respectively.

### *3.5 Physico-chemical characterization of protein concentrates and permeates*

**Table 5** shows that the pH of the concentrates are slightly down and the acidity increased, after the concentration process by UF. Regarding protein, ash, fat, lactose and total solids there is an increased concentration of these components in both the first and second test of UF, as would be expected, since the tests were conducted under the same conditions, to the same VCF. Among the minerals analyzed in concentrates (Table 5), calcium and phosphorus were those which concentration increased during the process of UF. Despite the concentration of chlorides (as NaCl), sodium and potassium decrease with the concentration process, and they still continue to be the predominant components. This fact is due to the addition of sodium chloride in the production of the cheese that originated the whey of mixture of cow, goat, and sheep milks used as a raw material of the concentration UF process.

**Table 3 –** Average composition of protein concentrates obtained during the UF processing of the pre-concentrate whey by RO (FCV=2).

| Parameter | Concentrate whey by RO | Concentrate whey by $UF_1$ | Concentrate whey by $UF_2$ |
|---|---|---|---|
| pH | 6,07 | 5,89 | 5,82 |
| Acidity (ml NaOH N/L) | 44,40 | - | 58,30 |
| Protein (g/100g) | 2,14 | 2,81 | 2,52 |
| Ash (%) | 1,34 | 1,00 | 1,36 |
| Chloride(g NaCl/100g) | 0,62 | 0,54 | 0,50 |
| Fat (g/100g) | 0,30 | 0,55 | 0,53 |
| Total solids (%) | 16,89 | 18,32 | 19,20 |
| Lactose (%) | 13,11 | 13,96 | 14,80 |
| Calcium (mg/100g) | 100,00 | 101 | 127 |
| Phosphate (mg/100g) | 98,50 | 96 | 111 |
| Sodium (mg/100g) | 120,50 | 125 | 102 |
| Potassium (mg/100g) | 418,50 | 364 | 376 |
| Magnesium (mg/100g) | 21,50 | 21 | 26 |

Legend: Concentrate whey by RO – corresponds to the pre-concentrate whey by RO, used as a raw material for the tests of concentration by UF; concentrate whey by $UF_1$ - corresponds to the concentrate whey obtained in the 1st test by UF (FCV=2); concentrate whey by $UF_2$ - corresponds to the concentrate whey obtained in the 2nd test by UF (FCV=2).

Comparing the results of the permeate compositions (see **Table 6**) with the respective concentrates (see **Table 5**), it shows that the protein concentration is very low. This result confirms the effectiveness of the membrane used on UF process, in terms of protein rejection, since the main objective of this operation is to obtain protein concentrates.

**Table 4 -** Average composition of permeates obtained during the UF processing of the pre-concentrate whey by RO (FCV=2).

| Parameter | Permeate $UF_1$ | Permeate $UF_2$ |
|---|---|---|
| pH | - | 5,10 |
| Acidity (ml NaOH N/L) | - | 39,90 |
| Protein (g/100g) | 0,28 | 0,20 |
| Ash (%) | 1,30 | 1,11 |
| Chloride (g NaCl/100g) | 0,64 | 0,60 |
| Fat (g/100g) | 0,00 | 0,00 |
| Total solids (%) | 7,63 | 8,37 |
| Lactose (%) | 6,05 | 7,06 |
| Calcium (mg/100g) | 46 | 63 |
| Phosphate (mg/100g) | 59 | 76 |
| Sodium (mg/100g) | 114 | 104 |
| Potassium (mg/100g) | 408 | 368 |
| Magnesium (mg/100g) | 11 | 14 |

Legend: Permeate $UF_1$ – corresponds to the permeate obtained in the 1st test of concentration by UF (FCV=2); Permeate $UF_2$ - corresponds to the permeate obtained in the 2nd concentration test by UF (FCV=2).

The concentration of lactose in permeates is about half of the lactose contained in the protein concentrates obtained. The total solids content has a behavior similar to that observed for lactose, i.e, permeates contains about half of the total solids content presented by the respective concentrates. This result is not surprising, since the total solids consist mainly of lactose. For calcium and phosphorus, the concentration of these ions in permeates is significantly lower than the concentration of these in whey concentrates. This behavior may be a result, for example, the formation of complexes of these with proteins, especially calcium, as described in the literature [9], [10]. On the other hand, potassium and chloride ions contains identical concentrations in both permeates as in the respective concentrates, which shows that the membrane that was used is permeable to these minerals. For sodium and magnesium, the concentration of these ions in permeates is significantly lower than the concentration of these in whey concentrates. For the ash content (%), it shows up that these are identical, in the respective permeates and in the concentrates. This fact was due most likely to the high permeability of the membrane to potassium, verified by the results obtained. Finally, it shows that permeates resulting from the ultrafiltration process are fat-free.

*3.6 Physico-chemical characterization of produced requeijões*

Several conclusions can be drawn based on results shown in **Table 7**.

The protein content of the *requeijões* produced from the various protein concentrates (RO, UF1 and UF2) are higher than those determined in the cheese produced from the whole whey from mixture of cow, goat and sheep milks (traditional method). Since the proteins were preferentially retained by the UF membranes, the concentrates produced are enriched in this component.

**Table 5 –** Physico-chemical characterization of *requeijões* produced from the whole whey and the protein concentrates obtained by RO and RO/UF.

| Parameter | $R_{SI}$ | $R_{OI}$ | $R_{UF1}$ | $R_{UF2}$ |
|---|---|---|---|---|
| pH | - | - | - | - |
| Acidity (ml NaOH N/L) | - | - | - | - |
| Protein(g/100g) | 9,85 | 12,36 | 13,39 | 13,08 |
| Ash (%) | 1,57 | 3,28 | 1,92 | 1,87 |
| Fat (g/100g) | 20,75 | 3,00 | 3,25 | 1,75 |
| Total solids (%) | 34,18 | 33,41 | 33,58 | 33,69 |
| Lactose (%) | 2,01 | 14,77 | 15,02 | 16,99 |
| Calcium (mg/100g) | 386 | 605 | 293 | 258 |
| Phosphate (mg/100g) | 245 | 424 | 221 | 198 |
| Sodium (mg/100g) | 39 | 215 | 142 | 157 |
| Potassium (mg/100g) | 171 | 538 | 449 | 455 |
| Magnesium (mg/100g) | 31 | 64 | 39 | 38 |

Legend: $R_{SI}$ - *requeijão* produced from the whole whey; $R_{OI}$ - *requeijão* produced from the concentrate whey by RO; $R_{UF1}$- *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); $R_{UF2}$- *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

The content of lactose in the *requeijões* produced from the protein concentrates is much higher than those determined in the control *requeijão* (RSI), according to the contents of lactose much higher in the *requeijões* obtained from the concentrate whey, since the ultrafiltration and RO membranes show a partial retention similar for the contents of total solids. The contents of total solids are similar in all produced *requeijões*, however it is lower for those obtained from protein concentrates, showing a higher retention of moisture, probably because of higher contents of lactose and the respective influence on the aggregation process of protein [11]. Combining this with the above, it should be noted that the higher protein content of these *requeijões* should be mainly due to the composition of the respective raw material. The concentrations of fat in the different *requeijões* are significantly higher than the verified for the control *requeijão*, as compared with the *requeijão* produced from protein concentrates. This is not surprising, since the control *requeijão* was produced from whole whey and the remaining was obtained from protein concentrates, in which the contents of fat was reduced during the RO and UF processes, according to the results shown in the **Tables 1** e **5.** The contents of ash (%) are significantly lower than the ones verified for the *requeijão* OI ($R_{OI}$). Perhaps this result occurred due to the effect of minerals permeation through the membrane, and was overlapped for the reduction of volume during the concentration process by UF. Comparing the results obtained for the ash contents of control *requeijão* with the ROI, it was verified that the last one shows a content much more high than the verified for the ROI, due to the effect of concentration by RO. The concentrations of mineral elements in the whey concentrates are a result of the essential characteristics of the membranes used, especially represented by the contents in the respective permeates. The sodium, potassium and magnesium show higher concentrations than the verified for the *requeijões* produced from concentrates. On the other hand, the contents of calcium and phosphorous are significantly higher in the control *requeijão* (RSI) and in the *requeijão* produced from concentrate whey by RO (ROI), comparing with the contents of these in the *requeijões* RUF1 and RUF2. The different behavior of the analysed minerals may be occurred due to the fact, for example, of the calcium ($Ca^{2+}$) participate in the formation of the gel that originated the *requeijão,* affecting either protein limit concentration for the gel formation, as well as temperature and the gel time [12]. Furthermore, the fact of, during the process of concentration by UF, some part of these component have been permeate through the membrane, making that the concentration of these in the *requeijão* produced is lower than the determinate in the control *requeijão* and in the ROI, although the results shown in the **Table 5** do not show concentrations of calcium and phosphorus lower than the verified for the concentrates whey by UF.

Firstly, it can be seen by analyzing the results of **Table 8**, that the *sorelhos* resulting from the manufacture of *requeijões* have low protein concentrations (g/100g), and as expected, these concentrations increase in the same way that the checked for the respective *requeijões* (see **Table 7**). It is concluded that most of the protein concentration was retained in the respective *requeijão*, during the manufacturing process. This result is positive and it means that is made a proper use, in terms of nutritional value of this component in the final product. It is also important to analyze the lactose content (%) of the respective *sorelhos* (see **Table 8**) since the final destination of this fluid is to be discarded into the environment, after their proper treatment. It is critical that the resulting *sorelho* of *requeijão* production does not present high organic load, in order to reduce the environmental impact.

**Table 6 -** Physico-chemical characterization of *sorelhos* resulting from the manufacturing of *requeijões.*

| Parameter | $S_{SI}$ | $S_{OI}$ | $S_{UF1}$ | $S_{UF2}$ |
|---|---|---|---|---|
| pH | 6,00 | 5,38 | 5,83 | 5,82 |
| Acidity (ml NaOH N/L) | 13,3 | 54,9 | 50,1 | 52,7 |
| Protein(g/100g) | 0,54 | 1,63 | 1,88 | 2,00 |
| Ash (%) | 0,61 | 1,88 | 1,63 | 1,69 |
| Fat (g/100g) | 0,00 | 0,00 | 0,00 | 0,00 |
| Total solids (%) | 6,11 | 21,34 | 21,37 | 21,75 |
| Lactose (%) | 4,96 | 17,83 | 17,86 | 18,06 |
| Calcium(mg/100g) | 27 | 64 | 124 | 132 |
| Phosphate (mg/100g) | 33 | 102 | 127 | 133 |
| Sodium (mg/100g) | 57 | 231 | 154 | 176 |
| Potassium (mg/100g) | 220 | 594 | 495 | 502 |
| Magnesium (mg/100g) | 9 | 30 | 32 | 33 |

Legend: $S_{SI}$ corresponds to the *sorelho* resulting from the manufacture of *requeijão* produced from the whole whey; $S_{OI}$ corresponds to the *sorelho* resulting from the manufacture of *requeijão* produced from the concentrate whey by RO; $S_{UF1}$ corresponds to the *sorelho* resulting from the manufacture of *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); $S_{UF2}$ corresponds to the *sorelho* resulting from the manufacture of *requeijão* produced from the concentrate whey by RO/UF (2nd test UF2, FCV=2).

**Table 8** shows that the *sorelhos* resulting from the production of *requeijões* RO, UF1 and UF2 show high levels of lactose and a bit higher than those obtained in the respective *requeijões* (see **Table 7**). The *sorelho* resulting from the production of *requeijão* SI shows a low content of lactose, comparing to the remaining *sorelhos*. On the other hand, the *sorelho* SI shows a content of lactose about two times higher than the respective *requeijão* (requeijão SI). Since the total solids are composed mainly by lactose, is not surprising the fact that they show the same proportion of the lactose in the respective *sorelhos* The *sorelhos* are fat free, a fact that is also relevant in order to its further treatment. For mineral elements, *sorelhos* presented, in general, lower concentrations compared to the respective *requeijões.* Except for the sodium and potassium that is present in similar concentrations in the *requeijões* and respective *sorelhos.*

### *3.7 Textural characterization of requeijões produced*

**Table 7 –** Textural characterization of *requeijões* produced from the whole whey and the protein concentrates obtained by RO and RO/UF

| Average± σ | Hardness(N) | Adhesion (N.s) | Cohesiveness |
|---|---|---|---|
| $R_{SI}$ | 5,872±0,820 | -443,450±36,672 | 0,031±0,002 |
| $R_{OI}$ | 2,172±0,157 | -254,776±29,211 | 0,021±0,002 |
| $R_{UF1}$ | 8,664±0,445 | -560,367±47,184 | 0,031±0,002 |
| $R_{UF2}$ | 7,169±0,908 | -413,638±52,941 | 0,028±0,003 |

Legend: $R_{SI}$ - *requeijão* produced from the whole whey; $R_{OI}$ - *requeijão* produced from the concentrate whey by RO; $R_{UF1}$- *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); $R_{UF2}$- *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

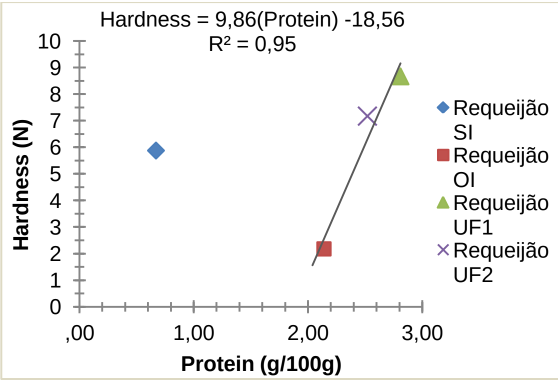


**Figure 6 –** Variation of *requeijões* hardness with protein concentration (g/100g), present in whole whey and protein concentrates that originated the respective *requeijões.*

Legend: *Requeijão* SI - *requeijão* produced from the whole whey; *Requeijão* OI - *requeijão* produced from the concentrate whey by RO; *Requeijão* UF1 - *requeijão* produced from the concentrate whey by RO/UF (1st test

UF1, FCV=2); *Requeijão* UF2 - *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

**Table 9** e **Figure 6** show that the *requeijões* produced from protein concentrates, obtained in the UF1 and UF2 tests, show an higher hardness than the *requeijão* produced from the whole whey (control *requeijão*). On the other hand, the *requeijão* produced from the protein concentrate obtained by RO shows not only a lower hardness, probably reflecting the effect of higher protein levels (apparently insignificant levels representing 3 to 4 times greater) but also an effect of higher levels of lactose and minerals. Focusing only on the *requeijões* RO, UF1, and UF2, it shows that the hardness of the respective *requeijões* increases linearly with protein concentration (g/100g), in the respective protein concentrates from which they originated, according to the expression Hardness = 9,86(Protein)- 18,56 (see **Figure 6**). A preliminary approach, these results show that the hardness is related to the protein concentration, which can be supported by previous studies [13].

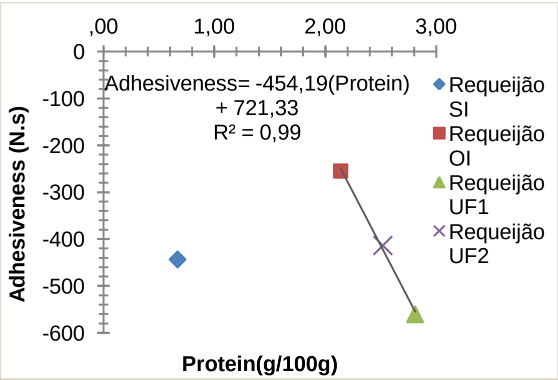


**Figure 7 -** Variation of *requeijões* adhesiveness with protein concentration (g/100g), present in whole whey and protein concentrates that originated the respective *requeijões.*

Legend: *Requeijão* SI - *requeijão* produced from the whole whey; *Requeijão* OI - *requeijão* produced from the concentrate whey by RO; *Requeijão* UF1 - *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); *Requeijão* UF2 - *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

**Table 9** and **Figure 7** show that the adhesiveness of the *requeijão* produced from protein concentrate obtained by RO is significantly lower than that obtained for the remaining *requeijões*. On the other hand, the *requeijões* produced from the whey concentrate by RO/UF present an adhesiveness similar to the control *requeijão* (SI). *Requeijões* UF1 and UF2 have discrepant values of adhesiveness, which may be related to the production process of the same, as well as the different composition presented of protein concentrates from which they originated, particularly in regard to the protein content. Comparing only the *requeijões* RO, UF1, and UF2, it is found that the adhesion of the respective *requeijões* decreases linearly with protein concentration (g/100g) in the respective protein concentrates from which they originated, according to the expression Adhesiveness= -454,19(Protein)+721,33.

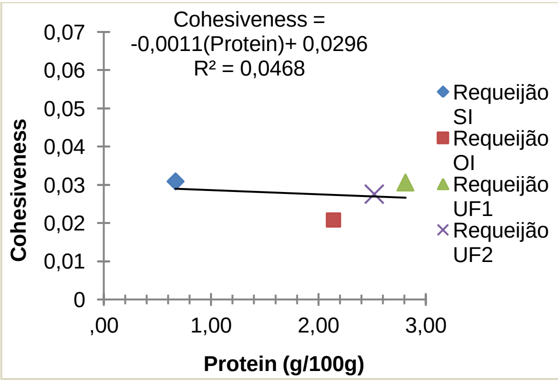


**Figure 8 -** Variation of *requeijões* cohesiveness with protein concentration (g/100g), present in whole whey and protein concentrates that originated the respective *requeijões.*

Legend: *Requeijão* SI - *requeijão* produced from the whole whey; *Requeijão* OI - *requeijão* produced from the concentrate whey by RO; *Requeijão* UF1 - *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); *Requeijão* UF2 - *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

The different *requeijões* contain cohesiveness values very similar to the cohesiveness of *requeijão* SI (control) (see **Table 9** and **Figure 8**). According to the slope obtained by linear regression (near zero), Cohesiveness = -0,0011(Protein)+0,0296, it can be conclude that the protein concentration has no effect on cohesion presented by products. Therefore, cannot be established any correlation between the cohesiveness and protein concentration (g/100 g), present in the whole whey and in the protein concentrates that originated the respective *requeijões.*

### 3.8 *Yield of the manufacturing process of requeijão*

According to the results of **Table 10**, the concentration by the two processes considered to be allowed at least duplicate the yield which is an advantage.

**Table 8 –** Experimental parameters used for the determination of the yield of the manufacturing process of the *requeijões.*

| Components | $R_{SI}$ | $R_{OI}$ | $R_{UF1}$ | $R_{UF2}$ |
|---|---|---|---|---|
| Mass of *Requeijão* (g) | 153,72 | 155,63 | 205,8 | 249,59 |
| Volume of spent whey (L) | 3 | 1,35 | 2 | 2 |
| η(%) | 5,1 | 11,5 | 10,3 | 12,5 |
| η(% dry basis) | 1,8 | 3,9 | 3,5 | 4,2 |

Legend: $R_{SI}$ - *requeijão* produced from the whole whey; $R_{OI}$ - *requeijão* produced from the concentrate whey by RO; $R_{UF1}$- *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); $R_{UF2}$- *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

**Table 9 –** Retention of whey components (%) in the *requeijão* according to the type of whey.

| Components (%) | $R_{SI}$ | $R_{OI}$ | $R_{UF1}$ | $R_{UF2}$ |
|---|---|---|---|---|
| Protein | 75,0 | 66,6 | 49,0 | 64,7 |
| Total solids | 27,6 | 22,8 | 18,9 | 22,1 |
| Lactose | 2,3 | 13,0 | 11,1 | 14,3 |

Legend: $R_{SI}$ - *requeijão* produced from the whole whey; $R_{OI}$ - *requeijão* produced from the concentrate whey by RO; $R_{UF1}$- *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); $R_{UF2}$- *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

**Table 11** shows that there is a greater efficiency in the use of the protein, in processing from whole whey, with 75% retention of protein present in the whey, being lower than in *requeijões* obtained from the concentrated whey by RO/UF. On the other hand, the retention of lactose, which follows the water content of the *requeijão,* is significantly higher than in *requeijões* produced by protein concentrates. These data indicates that it's possible to improve the technological process in order to better use the available protein in concentrate whey, with the advantage of providing a growth in the global yield of the transformation which, as it's evident in **Table 10**, is significantly higher in manufacturing from concentrated whey.

**Figure 9** shows that the yield of the manufacturing process of the *requeijão* increase linearly with increasing concentration of protein (g/100g), present in whey, according to the expression η= 3,04(Protein) + 3,67; providing the prospect of an important advantage about energy consumption, since one of *requeijão* manufacturing constrains is the energy consumption involved in the production process.

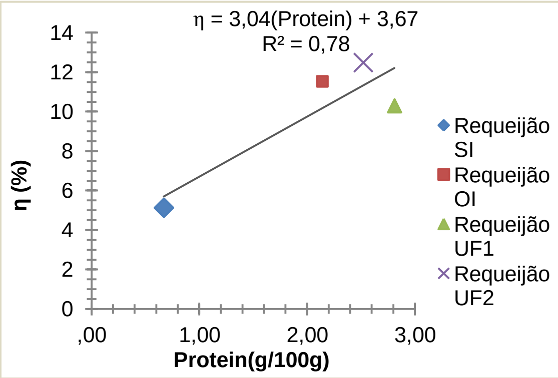


**Figure 9 –** Graphic representation of the variation of the yield (η) of the manufacturing of *requeijão* with the protein concentration (g/100g), presents in the whole whey and in the concentrates whey that origin to the respective *requeijões.*

Legend: *Requeijão* SI - *requeijão* produced from the whole whey; *Requeijão* OI - requeijão produced from the concentrate whey by RO; *Requeijão* UF1 - *requeijão* produced from the concentrate whey by RO/UF (1st test UF1, FCV=2); *Requeijão* UF2 - *requeijão* produced from the concentrate whey by OI/UF (2nd test UF2, FCV=2).

## 4 Conclusions

Protein concentrates were produced by ultrafiltration of whey from mixture of cow, goat and sheep milks, pre-concentrate by RO, with a higher concentration of protein, compared to the whole whey and protein concentrates by RO. This result shows the importance and the interest of the production of protein concentrate from whey, although the pre-concentrate by RO is not particularly suitable in terms of product recovery, due to the high content of lactose obtained by the subsequent UF process.

In production of *requeijões*, it was concluded that the use of protein concentrates can increase significantly (duplicate) the yield of the production process of the *requeijão,* which is directly proportional to the protein concentration of the concentrate whey that origin to the respective *requeijão*. The *requeijões* produced from the protein concentrates show higher levels of protein, lactose, sodium, potassium and magnesium and fat contents significantly lower when compared to the *requeijões* produced from the whole whey, showing the influence of the composition of the whey/raw material.

The textural properties of the *requeijões* obtained from concentrate whey reflected a lot of the elements of the composition of the *requeijão*, and they differ from the properties of the *requeijão* produced from whole whey, which could mean, eventually, some difficulty at the level of acceptance of the product, leading to the need to find formulations of the composition of the whey that would optimize the composition and texture properties of the final product.

**Acknowledgements**

I would like to thank my thesis supervisors, Prof. Dra. Maria Norberta Correia de Pinho and Prof. Dr. António Pedro Louro Martins for all their knowledge and support.

I also thank my family and friends for always supporting me.

**References**

[1] I. Y. Mizubuti, "Soro de leite: composição, processamento e utilização na alimentação," pp. 80–94, 1994.

[2] C. Baldasso, T. C. Barros, and I. C. Tessaro, "Concentration and purification of whey proteins by ultrafiltration," *Desalination*, vol. 278, no. 1–3, pp. 381–386, 2011.

[3] Milk Market Observatory, "Annual Production Series of Dairy products," 2014.

[4] Portal Lacteo, "Soro de leite é foco de pesquisa para alimentos funcionais," 2012. [Online]. Available: http://www.portalacteo.com.br/noticias/soro-de-leite-e-foco-de-pesquisa-para-alimentos-funcionais-2917/. [Accessed: 06-Apr-2015].

[5] A. R. Prazeres, F. Carvalho, and J. Rivas, "Cheese whey management: A review," *J. Environ. Manage.*, vol. 110, pp. 48–68, 2012.

[6] C. D. Pereira, O. Diaz, and A. Cobos, "Valorization of by-products from ovine cheese manufacture: Clarification by thermocalcic precipitation/microfiltration before ultrafiltration," *Int. Dairy J.*, vol. 12, no. 9, pp. 773–783, 2002.

[7] R. . Campbell, E. J. Kang, E. Bastian, and M. . Drake, "The use of lactoperoxidase for the bleaching of fluid whey," *J. Dairy Sci.*, vol. 95, pp. 2822–2900, 2012.

[8] A. Macedo, E. Duarte, and M. Pinho, "The role of concentration polarization in ultrafiltration of ovine cheese whey," *J. Memb. Sci.*, vol. 381, no. 1–2, pp. 34–40, 2011.

[9] A. J. Antunes, *Funcionalidade de Proteínas do Soro de Leite Bovino*. Manole, 2003.

[10] M. Cheryan, *Ultrafiltration and Microfiltration Handbook*. Taylor & Francis Routledge, 1998.

[11] J. M.Garrett, R. A.Stairs, and R. G. Annett, "Thermal Denaturation and Coagulation of Whey Proteins: Effect of Sugars," *J. Dairy Sci.*, 1988.

[12] P. Cayot and D. Lorient, *Structures et technofonctions des protéines du lait*. Arilait Recherches, 1998.

[13] A. T. Z. N. Macedo, "Fraccionamento de Lactossoro de Ovelha por Tecnologias de Membranas e Estudo das Possíveis Utilizações dos Concentrados Obtidos," 2010.