Application of Membrane Technology to Slaughterhouse Blood to Produce Edible Powdered Protein Mixture

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Abstract: Animal blood generated from slaughtered animals is often released into the environment resulting in significant pollution and also in the loss of a valuable protein source. This study aimed at developing a procedure that will allow for utilizing animal blood for protein powders production on an industrial scale. To meet this goal, hygienically collected animal blood was first treated with membrane technology: microfiltration (MF) or ultrafiltration (UF). A ceramic MF membrane and a PCI UF membrane were used. Average MF flux was 6.62 kg h^{-1} m^{-2} at transmembrane pressure of 2.5 bar. Average UF flux was 3.55 kg h^{-1} m^{-2} at transmembrane pressure of 4 bar. MF succeeded in separating the blood plasma proteins (permeate) from the red cell fraction (retentate). UF concentrated both the red cell and blood plasma proteins in a single sample (retentate). The volume ratio of retentate to permeate was 10:14 and 14:10, for the MF and UF, respectively. A membrane cleaning regime was developed. The treated blood fractions were then freeze dried and red and white protein powders were produced successfully. The potential of a SME (small-medium enterprise) to apply this procedure into practice is presented.

Keywords: Animal blood, ultrafiltration, microfiltration, freeze drying, red protein powder mixture, white protein powder mixture.

1. INTRODUCTION

The slaughtering of livestock for meat purposes generates a considerable amount of blood. Animal blood constitutes one of the most problematical by-products of the meat industry due to the large amount generated and its high polluting capacity. The polluting load generated by the world meat industry due to blood only, is estimated to have a biological oxygen demand (BOD) of about 1.7 million Mg per year, which is equivalent to the organic wastewater pollution caused by 11 million people [1]. In Korea, an amount of 70,000 Mg of animal blood is produced in slaughterhouses annually [2]. In Mexico, about 92,000 m^3 of blood were generated from cattle slaughtering in 1995, which represented a BOD load of 10^4 Mg [3]. The amount of blood produced per year in a country such as the UK is around 100,000 Mg, containing a large amount of solids (18%) and a high COD of 500,000 mg O_2 L^{-1} [4, 5].

On the other hand, provided it is hygienically collected, animal blood is a valuable protein source [6]. Blood contains about 18% protein [7]. Blood proteins are of high biological value, a possible source for biotechnology products, and hence are of potential high economic value [7, 4]. Proteins from blood could be used within the food industry, for example, as additives in dietetic or other products [8]. It is therefore necessary to consider the recovering of the proteins in the blood, both to minimize a sizable pollution hazard and to prevent the loss of a valuable protein source.

Processes that can be used for concentrating blood proteins without degrading their delicate and revenue producing properties include vacuum evaporation, freeze drying, gel filtration and ultrafiltration (UF) [9]. Of these, UF is a very simple process, having low energy requirements, and also allowing concentration, fractionation and purification to be carried out simultaneously [7]. The low operating temperatures of UF allow sensitive biological solutions, like blood serum, to be treated without the constituents being damaged or chemically altered [10, 11]. Membrane technology is an interesting method for protein purification and concentration, especially when the lipid mass fraction of the by-product remains low [12].

Only a limited proportion of the blood generated from slaughtered animals is utilized in Greece, whereas most of it is released into the environment. Hence, both to minimize the environmental hazard and to prevent the loss of a valuable protein source, it is necessary to develop a procedure that will permit the utilization of animal blood on an industrial scale. This study aimed at utilizing the edible blood from slaughterhouses through

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the production of red and white protein powders, by using membrane separation technology combined with advanced cryogenic drying technique.

2. MATERIALS AND METHODS

2.1. Blood Material

Blood samples were supplied by a slaughterhouse located at Oichalia, Farkadona, in central Greece. The blood came from slaughtering of healthy sheep, pig and calves. Blood samples were taken in 25 L stainless tanks (AISI 304). Trisodium citrate 2-hydrate ($C_6H_5Na_3O_7.2H_2O$) was added to samples immediately to prevent coagulation, at the rate of 0.4% w/w. Samples were stored in the fridge (3 ± 1 °C) until the beginning of the filtration process, for a maximum of 24 h. Blood samples were treated using membrane technology, including microfiltration (MF) or ultrafiltration (UF).

2.2. Treatment with Microfiltration (MF)

Blood samples were microfiltered to separate the red cell fraction (retentate) and the blood plasma (permeate). The membrane for the microfiltration process used was a ceramic tubular MF membrane of tangential flow, with an active surface area of 0.24 m^2 (CMF19040 - 200 nm, Jiangsu Jiuwu Hi-Tech Co. Ltd, China). A stainless steel tubular module (CMV 1-30, Jiangsu Jiuwu Hi-Tech Co. Ltd, China) used to accommodate the membrane. A Watson-Marlow 620 Du peristaltic pump was used for blood pumping to the membrane. A pressure control valve was installed at the output of the membrane module. Two analog pressure indicators (glycerin manometers), within the range of 0-6 bar, were installed at the entrance and the exit of membrane module, respectively. Plastic flexible pipes were used to interconnect the rig elements. Figure **1** represents the experimental rig used for the MF process. Microfiltration was conducted at constant transmembrane pressure (2.5 bar).

The MF process commenced with membrane cleaning. Membrane cleaning regime is described in detail in paragraph 2.4. After the cleaning procedure was completed, MF of distilled water took place, aiming at recording a reference flux to be used as "control" for comparison with the flux obtained for blood MF at the same experimental conditions, and also to evaluate the effectiveness of the cleaning regime. Following this step, the filtration of blood samples started. Blood pumping to the MF membrane was at the range of 5 (at the beginning) to 7 kg min^{-1}. Blood velocity within the ceramic unit was between 0.35 and 0.49 m s^{-1}. Experimental conditions were being continuously recorded during the whole experimental process. The performance of the MF membrane was estimated by measuring permeate flow rate vs. time, which also measures the kinetics of membrane fouling [12]. MF flux was recorded by collecting the permeate for 5 min and weighing it. The MF flux was calculated in kg m^{-2} h^{-1}, using the following equation (Eq. 1), where *W* is the weight of the permeate (kg) collected within time *B* (h) from a surface area of *A* (m^2). In the case of the MF process, A equals to 0.24 m^2 and B to 1/12 h.

$$\text{Flux} = W/(A \times B) \qquad \text{(Eq. 1)}$$

The permeate was collected into a plastic tank, whereas the red cells fraction was concentrated and returned into the blood feed tank, where it was mixed with the remaining blood sample and then was circulated again for further concentration. The MF process was completed when the volume of the

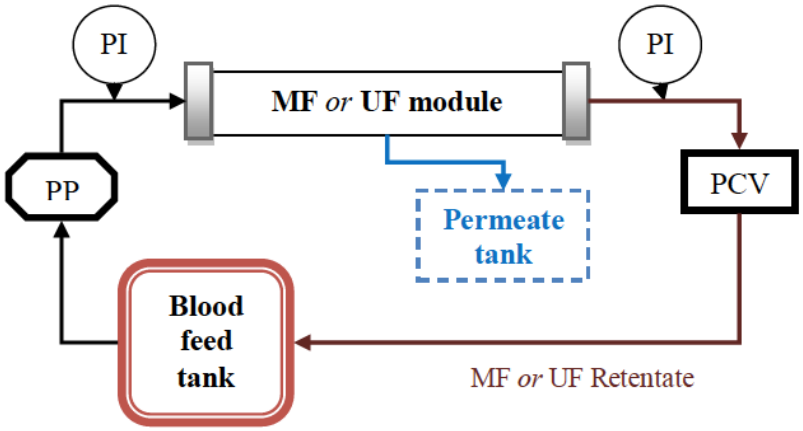

Figure 1: Schematic representation of the experimental rig (MF: microfiltration, UF: Ultrafiltration, PP: peristaltic pump, PCV: pressure control valve, PI: pressure indicator).

permeate was about the 1/3 of the volume of the initial blood sample. Once the blood MF process was completed, the membrane was cleaned again.

2.3. Treatment with Ultrafiltration (UF)

Blood samples were concentrated using ultrafiltration technology. The membrane for the UF process used was a tubular UF membrane of tangential flow, with an active surface area of 0.9 m^2 (ESP04 - PCI Membranes, USA), produced from polysulphone material. Its molecular weight cut off was of 4.000 Da. A tubular stainless steel module, with a capacity of 18 membranes, twin-entry, length 1.2 m (PCI Membranes, USA) used to accommodate the membranes. A Watson-Marlow 620 Du peristaltic pump was used for blood pumping to the membrane. A pressure control valve was installed at the output of the membrane module. Two analog pressure indicators (glycerin manometers), within the range of 0-6 bar, were installed at the entrance and the exit of membrane module, respectively. Plastic flexible pipes were used to interconnect the rig elements. Figure **1** represents the experimental rig used for the UF process.

Ultrafiltration was conducted at constant transmembrane pressure of 1, 2, 3 and 4 bar. The temperature of the retentate ranged between 13-15 °C. The permeate flux versus time was measured. UF flux was recorded by collecting the permeate for 5 min and weighing it. The UF flux was calculated in kg m^{-2} h^{-1}, using the Equation 1, where *W* is the weight of the permeate (kg) collected within time *B* (h) from a surface area of *A* (m^2). In the case of the UF process, A equals to 0.9 m^2 and B to 1/12 h.

The UF process commenced with membrane cleaning. Membrane cleaning regime is described in detail in paragraph 2.4. After the cleaning procedure was completed, UF of distilled water took place, aiming at recording a reference flux to be used as “control” for comparison with the flux obtained for blood UF, at the same experimental conditions. Following this step, the filtration of blood samples started.

The permeate (virtually water) was collected into a plastic tank, whereas the red cells and blood plasma proteins were concentrated and returned into the blood feed tank, where it was mixed with the remaining blood sample and then was circulated again for further concentration. The UF process was completed when the volume of the permeate was about the 2/3 of the volume of the initial blood sample. Once the blood UF process was completed, the membrane was cleaned again.

2.4. Membrane Cleaning Regime

Once the filtration process (MF or UF) was completed, the membranes were cleaned in order to prevent the reduction of membrane permeability due to particle depositions onto membrane surface, and also to investigate the potential of restoring the membrane permeability to its original condition.

The membrane cleaning procedure lasted for about 4 h, involving liquid circulation under zero transmembrane pressure, and included the following stages: i) membrane rinsing with water at temperature in the range of 40-60 °C until the solution and the pollutants were completely removed, ii) fulfilling the feed tank with water of 48 °C, iii) addition of detergents for alkaline cleaning (P3–ultrasil: 69, 67, 02, which were added gradually in this specific order) and washing for 45 min (the first 10 L of the permeate were removed in order not to be circulated), iv) rinsing with water at temperature in the range of 40-60 °C until the solution and the pollutants were completely removed, v) fulfilling the feed tank with water of 48 °C, vi) addition of P3–ultrasil 75 for acidic cleaning and washing for 15 min (the first 10 L of the permeate were removed), vii) rinsing with water at temperature in the range of 40-60 °C, until the solution and the pollutants were completely removed, viii) fulfilling the feed tank with water of 48 °C, ix) addition of P3–ultrasil 110 for alkaline cleaning and washing for 30 min (the first 10 L of the permeate were removed), x) final rinsing with water at temperature of 20 °C until the solution and the pollutants were completely removed, xi) fulfilling the feed tank with water of 20 °C, xii) flux measurement, xiii) addition of preservative metabisulphite, stirring for 20 min, and finally closing module input and output taps to prevent membrane drying out. A schematic representation of the cleaning procedure described above is given in Figure **2**.

2.5. Freeze Drying

Blood proteins were expected to be recovered in the MF permeate (blood plasma proteins) MF retentate (red cell fraction proteins) and UF retentate (both blood plasma and red cell fraction proteins). The UF permeate did not contain any protein, as it was indicated by refractometry (portable digital refractometer, type “pocket”, supplied by ATACO, Japan).

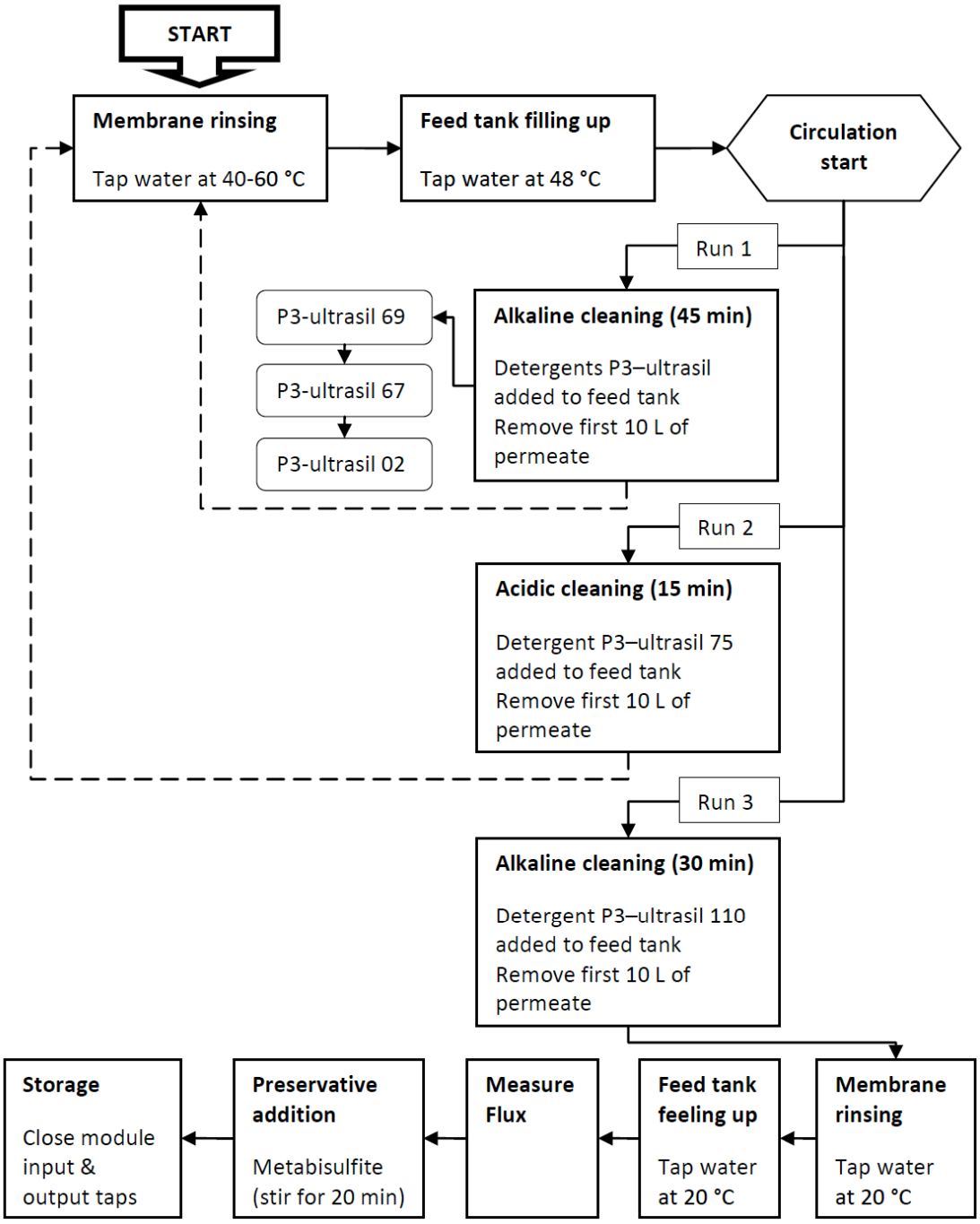

Figure 2: Schematic representation of the membrane cleaning procedure.

The permeate and the retentates were collected in plastic bottles, and were stored in the freezer at -23 ± 1 °C immediately after the filtration was completed. The samples remained in the freezer for a maximum of 48 h, till the freeze drying process started. Freeze drying was used for protein recovery.

The freeze dryer used was a Scanvac Cool safe 110-4 (LaboGene, Danemark). The frozen blood plasma and the red cell fraction were placed in separate flasks of the freeze dryer. Flasks capacity was 600 mL. Flasks were filled with 500 mL of sample. Freeze drying was at condenser temperature of -110 °C and vacuum pressure of 0.001 bar.

3. RESULTS AND DISCUSSION

3.1. Microfiltration Flux

The microfiltration process lasted in total 900 min. At the time point of 900 min, the volume ratio between

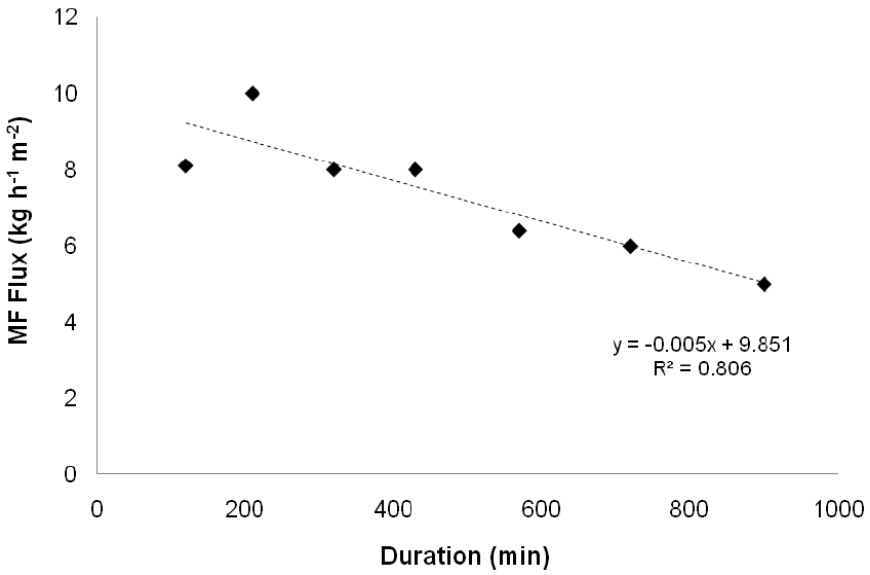

Figure 3: Microfiltration flux fluctuation with time, under constant transmembrane pressure of 2.5 bar.

the permeate (blood plasma) and the retentate (red cell fraction) was 10:14. The colour of the retentate was red, whereas the permeate was colourless. After these 900 min, the permeate started getting red coloured and the flux was substantially decreased.

MF flux fluctuation with time, under constant transmembrane pressure of 2.5 bar, is presented in Figure **3**. Temperature fluctuated from 13.0 °C to 14.1 °C during the 900 min of the MF process. Figure **3** shows a higher flux at 210 min than at 120 min, and after the 210 min, a continuous decrease in the flux with time. This higher flux observed at 210 min, in comparison to the 120 min point, is attributed to the fact that that the initial blood flow rate to the MF module (from time 0 to 120 min) was 5.0 kg min^{-1}, whereas after that time point the flow rate was 7.0 kg min^{-1}. The average flux value for the whole 900 min was 6.62 kg h^{-1} m^{-2}, which is relatively high, allowing for potential industrial application which will meet the requirements of a small slaughterhouse, with low costs.

Distilled water MF flux was 15 kg h^{-1} m^{-2}, prior to blood microfiltration. Following blood microfiltration and membrane cleaning, the water flux was 13.5 kg h^{-1} m^{-2}, which means a reduction of 10% to membrane permeability. It is obvious that the membrane permeability was not fully restored with the proposed cleaning regime. That was mainly because of the low flow rate of the cleaning agents through the membrane, caused by the use of the particular peristaltic pump. Higher flow rates during the cleaning process are necessary for better membrane cleaning.

3.2. Ultrafiltration Flux

The ultrafiltration process lasted in total 435 min at the transmembrane pressure of 4 bar. After the time point of 435 min, the permeate started getting red coloured. At the time point of the 435 min, the volume ratio between the permeate and the retentate was 14:10. As anticipated, the UF process was more efficient in concentrating the blood sample. The colour of the retentate was red, whereas the permeate was colourless.

UF flux fluctuation with time is presented in Figure **4**, for the different transmembrane pressures used (1, 2, 3 and 4 bar). Average temperature was 13.5 °C. Figure **4** shows that UF flux is decreasing with time. The filtration flux decreases faster at the highest pressure. This finding is in good agreement with other work [7]. Figure **4** also indicates higher flux with higher transmembrane pressure. The effect of the transmembrane pressure on the flux is more pronounce within the first 3 hours of the UF process. The average flux values for the whole 435 min of the process for each transmembrane pressure are presented in Table **1**. The average UF flux at the transmembrane pressure of 4 bar (3.55 kg h^{-1} m^{-2}) is considered satisfactory for industrial application to small-medium slaughterhouses (treating about 3,000 kg of blood per day).

Feed flow rate was differentiated with time. Table **2** shows the feed flow rate fluctuation with time and the feed velocity developed within the membranes. Feed flow rate was not stable and this was mainly attributed

Table 1: Average Permeate Flux Value for the whole Duration of the Ultrafiltration Process (435 min), at the Different Transmembrane Pressures Used

Pressure (bar)	Average flux (kg h^{-1} m^{-2})
1	1.45
2	1.90
3	2.90
4	3.55

to the low levels of feed velocity. A feed velocity at a minimum of 7 m s^{-1} is expected to give better performance and a more steady flow through the small diameter holes of the membrane.

Once blood ultrafiltration was completed, the membrane was cleaned as described in section 2.4. After membrane cleaning, the flux of the distilled water was equal to the initial value (prior to blood filtration), suggesting that membrane permeability was fully restored. The detergent Ultrasil has been used in other work [13] for cleaning PCI membrane from slaughterhouse effluents (Ultrasil 10 or 11, concentration of 15 g L^{-1}, at 40 °C for 1.5 h), and they also observed that membrane permeability was restored to 100%. The efficiency of this cleaning agent was attributed to the active enzymes contained in Ultrasil.

3.3. Protein Powder by Freeze Drying

Freeze drying final products were red protein powder and white protein powder. The red powder resulted from the red cell fraction, which was collected as MF retentate, and also, from both the red cell and blood plasma collected as UF retentate (Figure **5** - left). The white powder resulted from the blood plasma collected as MF permeate (Figure **5** - right).

3.4. Industrial Application

Despite the relatively high energy consumption involved, the freeze drying technique is recommended for high added value products, such as the red protein powder. Considering a small-medium enterprise

Table 2: Feed Flow Rates and Velocity Fluctuation with Time

Time (min)	Flow rate (kg min^{-1})	Feed velocity (m s^{-1})
90	5.8	0.6
165	4.6	0.5
255	3.5	0.4
435	7.0	0.7

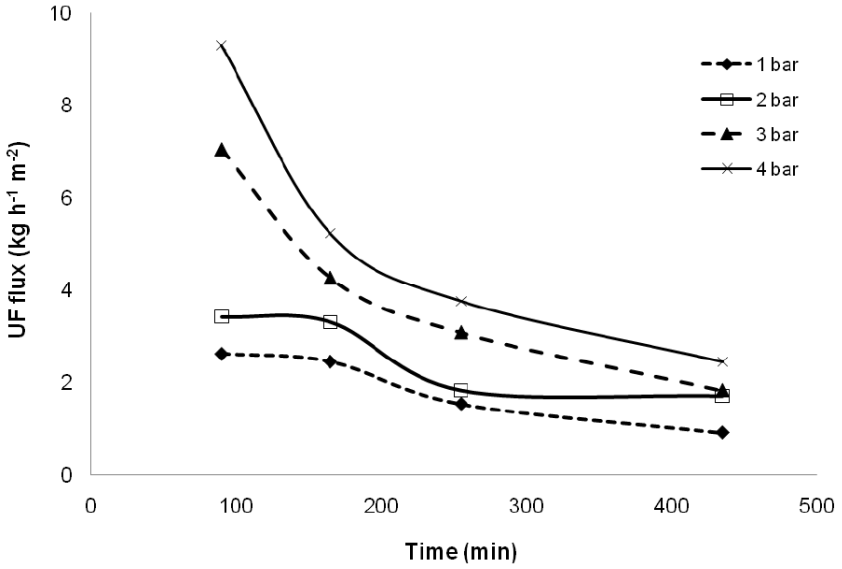

Figure 4: Ultrafiltration permeate flux fluctuation with time, for the different transmembrane pressures used.

Figure 5: Left: The freeze dried red cell and blood plasma fraction (red protein powder from the UF retentate), and right: the freeze dried blood plasma (white protein powder from the MF permeate).

(SME), as are the majority of the slaughterhouses in Greece, with an average peak production of approximately 3,000 kg of blood per day, the treatment of blood on site, by ultrafiltration and freeze drying, may decrease the pollution load in effluents discharged to the environment and also provide extra income to the industry from the protein powder. For a common slaughterhouse, a UF unit with membrane active surface of 100 m^2, will be able to produce 1.000 kg of condensed red cell and blood plasma fraction within 8 hours. Feed flow rate is suggested to be at a level of 10 m s^{-1}. An industrial freeze dryer with a drying capacity of 600 L per 24 hours for the drying of the retentate will be sufficient to produce the red powder, which will then be sterilized by a UV disinfection device, and finally will be packed in 5-25 kg bags, ready for sale. This way, the SME will be able to protect the environment on the one hand, and to gain extra revenue from a previously wasted material.

4. CONCLUSIONS

Microfiltration process was successful in separating the blood plasma and the red cell fraction of the animal blood, resulting in a ratio of 10:14 between blood plasma (permeate) and red cell fraction (retentate). On the other hand, the Ultrafiltration process resulted in concentrating both the red cell and blood plasma proteins in the retentate, whereas the permeate was protein free. The ratio between retentate and permeate was 14:10 for the ultrafiltration. The average MF flux value was 6.62 kg h^{-1} m^{-2} at transmembrane pressure of 2.5 bar and the whole process lasted for 900 min. The average UF flux was 3.55 kg h^{-1} m^{-2} at the transmembrane pressure of 4 bar and the whole process lasted for 435 min, which has potential for industrial application to small-medium slaughterhouses (treating about 3,000 kg of blood per day).

The cleaning regime used was successful in restoring the MF and UF membranes to their original permeability, hence suggesting that the proposed regime may be potentially applied in industrial scale.

The freeze drying of the UF and MF retentates resulted in red protein powder, whereas the freeze drying of the MF permeate in white protein powder.

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