S7P16.WP

POULTRY PLASMATIC PROTEINS RECOVERY USING ULTRAFILTRATION MEMBRANES: STUDY OF THE OPERATING CONDITIONS

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INTRODUCTION

In the poultry industry the blood by-product, is considered to be a residue. This wastes about 12g of protein for each 100ml of blood, something that equals the quantity of protein present in a lean steak (Grossklaus, 1979). On the other hand, blood disposal is a significant pollution source, accounting for approximately half of the B.O.D. of the slaughterhouse effluent (Bourgeois, 1986).

Taking into account the close relationship between ecology and nourishment, and in the latter, the limited supply of proteins (Primo, 1987), we herein outline a blood recovery process for the poultry industry that mitigates environmental damage and increases the available protein supply.

Membranes technology has been implemented in food and chemical industries because in them a fundamental problem is the separation and/or concentration of substances, something which can consume about 80% of the energy used by the company. Due to its greater economic and technical efficiency, its wide range of working conditions and its cleanliness, ultrafiltration (UF) has experienced a significant rise over the last few decades as a profitable technical alternative to evaporation methods. As such, UF may be employed to concentrate proteins in plasma.

MATERIAL AND METHODS

Chicken blood was manually withdrawn from the sacrifice chain in recepticals containing an anticoagulant solution. We opted for the sodium citrates group because of their low price and acceptability as a food additive. To avoid blood coagulation two mixtures of sodium citrates were tested: one in 3:1 proportion (monosodium:trisodium) and other formed solely by trisodium citrate at a 3.8% concentration level, and in a 1:4 proportion with the blood. The 3:1 mixture was used at a 5% concentration level and in equal proportions with the blood as that applied in the previous case (above). Transportation of the anticoagulant-blood mixture (AB) took over four hours at refrigeration temperatures.

The AB mixture was centrifuged to 900G over a 20 minute period (Dirakaron, 1983) in a centrifugal Selecta-Meditronic. The plasma was ultrafiltrated in Millipore-Minitan S equipment. The module consists of a flat membrane with an effective area of 30cm^2. UF tests were accomplished with poliacrilonitrile membranes whose molecular thresholds (cut-offs) are 100kD and 40kD (IRIS 3026, Rhone-Poulenç). The parameters that were studied were feed-flow, cutt-off and feed pressure pursuant to a factorial design of 2^3, having the following high and low working levels: 900ml/m, 600ml/m; 100kD, 40kD; 1.4atm, 2.5atm. The temperature was maintained at a constant level of 10±0.5°C over 14 hours. A refrigeration unit EK-51 Haake, was employed which included a bath of etilenglicol:water (1:1), at an average temperature of -6°C and an initial volume, in the nourishment tank, of 375ml.

Before and after plasma ultrafiltration, water flux was measured to determine the extent of membrane fouling. Upon ending each work session membranes were cleaned. At the beginning of the operation and at each consecutive hour samples were taken to measure the permeate characteristics (i.e., volume, conductivity, pH, total solids and proteins) and feed characteristics (i.e., conductivity, pH and proteins). Microbiological analyses were carried out on the AB and concentrated plasma, incorporating potassium metabisulfite in different proportions in order to evaluate the process efficiency on an industrial scale.

RESULTS AND DISCUSSION

Effect of the anticoagulant on the pH of the plasma and concentrate

With the anticoagulant solutions tested it was observed that the pH of the plasma and the plasma concentrate, as well as in the permeate, varied in a manner that depended on the composition on the anticoagulant employed (Figure 1). By employing an appropriate mixture that inhibited the coagulation and regulated the plasma pH, control of the microbial populations could be achieved.

Separation

The centrifugation of the AB mixture gave two fractions: a precipitate formed by the cells and a supernatant formed by the plasma (50-60% of the total weight). The solid fraction can be employed by the pharmaceutical industry (e.g., to obtain histidine, leucine, fenilalanine, lysine, hemine, protoporfirin, oxyhemoglobin, etc.) or to obtain blood flour, blood coal and foamy compounds (Dirakaron, 1983). The liquid fraction obtained in this work had a protein content of 0.046 ± 0.011 g/ml (α=0.01).

Thermal stability tests

Thermostability tests were undertaken in order to determine the optimum working temperature. Coagulation was observed at 60°C (coagulation time: 1 minute) and 50°C (5 minutes). Coagulation processes were not observed at 45°C and 40°C; neither at one or five minutes.

Based on the results, and since the plasma is an easily alterable material, it was initially determined that a working temperature of 25°C would be suitable. Other researchers have noted that at this temperature, greater permeate flows are obtained. However, after four hours of UF processing, the plasma presented distasteful odours and anomalous colours; so it was decided to establish the working temperature of 10°C.

Protein concentration

In the bovine and swine blood protein recovery process, the concentration stage is currently accomplished using a tubular evaporator system (Jönsson, 1990). The statistical design selected to explore the influence of the parameters studied on the operation was a fractional 2^3 with two repetitions in each one x1: feed-flow; x2: cutt-off; x3: pressure; x4: interaction x1x2,x5: interaction x1x3,x6: interaction x2x3,x7: interaction x1x2x3,y1: the speed of protein concentration ($g.ml^{-1}/h$) and y2: average protein concentration in the permeate (g/ml).

Table 1 presents the calculated experimental lay-out together with the results for y1 and y2.

Designating "E(n)" to be the effect of each independent variable, defined as the increase in the average magnitude of the highest response variable to the independent variable (X(n)), the following principal effects of the variables and their interactions were found:

E(1): 8.431E-4 E(3): -4.6088E-4 E(5): -2.095E-4 E(7): 2.063E-4

E(2): 1.734E-4 E(4): -4.607E-4 E(6): 4.743E-5

The confidence interval of the "n" effects for α=0.01 is:

$$E(n) \pm t8\ (S2/4)1/2 = E(n) \pm 7.65695E\text{-}6$$

As a consequence of this, it can be observed that all of the effects are meaningful in the studied ranges noting that "0" is not included in the confidence interval. The feed-flow has the greatest influence on the concentration speed, probably due to the influence of flow on the development of a turbulent regime that hinders membrane fouling and that favours the cross-membrane medium transfer. High cut-offs also favoured the concentration speed. This is explained by the favouring of membrane transfer of matter generally using a high cut-off membrane but a high cut-off membrane influences a smaller proportion of the feed-flow. The pressure negatively affected the membrane performance in this study by contributing more fouling. The interaction of feed-flow and cut-off resulted in an unfavourable result for the concentration speed, but favoured the matter transfer generally and possibly increased the protein loss at the high permeation levels. The interaction of feed-flow and pressure yielded similar results since high pressures tend to favour fouling while a total greater throughput speed tends to contribute to a greater speed of fouling. The interaction cut-off and pressure favoured, very slightly, the concentration speed which was likely due to the effect of molecular size. Finally, the triple interaction (X(7)) favoured a higher concentration speed.

The following equation, valid for the conditions covered in this work, expresses the concentration speed as a function of the independent variables studied:

$$y = y_o + (4.2155E\text{-}4)\ x_1 + (8.6720E\text{-}5)\ x_2 - (7.330E\text{-}5)\ x_3 - (2.3040E\text{-}4)\ x_1x_2 - (1.0475E\text{-}4)\ x_1x_3 + (2.3715E\text{-}5)\ x_2x_3 + (1.0315E\text{-}4)\ x_1x_2x_3 \quad (1)$$

where $y_o = 2.5144E^{-3} \pm 9.3150E^{-4}$ (for α=0.01).

Substituting x_1, x_2 and x_3 for (Qb-750)/150, (Cm-70)/30 and (P-1.95)/0.55 respectively, the following expression (model) for the speed of protein concentration was derived:

$$Vc = 2.5144E\text{-}3 + 2.8103E\text{-}6\ (Qb - 750) + 2.8907E\text{-}6\ (Cm\text{-}70) - 1.3327E\text{-}4\ (P\text{-}1.95) - 5.1200E\text{-}8\ (Qb\text{-}750)\ (Cm\text{-}70) - 1.2697E\text{-}6\ (Qb\text{-}750)\ (P\text{-}1.95) + 1.4394E\text{-}6\ (Cm\text{-}70)\ (P\text{-}1.95) + 4.1677E\text{-}8\ (Qb\text{-}750)\ (Cm\text{-}70)\ (P\text{-}1.95) \quad (2)$$

where
VC = speed of protein concentration for membrane surface of 30cm^2 and to 10°C (g ml^{-1}.h^{-1})
Qb = pump flow rate (ml m^{-1})
Cm = Molecular count (kD)
P = Pressure (atm).

These equations will permit an estimate of the optimum set of conditions for the operation to be determined, for the condition that lie within the range of conditions studied. Also, they will be useful towards the establishment of the suitable scaled-up membrane surfaces for full industrial applications.

Hygienic quality

Inventories of total bacteria to 30°C/two days and of Enterobacteriaceae to 37°C/one day were carried out. A decrease in the number of Enterobacteriaceae was observed when 0.05% potassium metabisulfite was added, whereas the total inventory was only considerably reduced with the addition of 0.5% potassium metabisulfite.

Application of the product to the sausage manufacturing

Although solubility decreased with the concentration, we believe that the primary limiting factor is the possible existence of unwanted or strange flavours (Dirakaron, 1983). To prove this, sausages were manufactured in a commercial formulation in which 1 and 3% concentrated plasma was added. This was compared to a control sausage without added plasma. Laboratory staff did not detect the presence of strange flavours during the test.

CONCLUSION

The combination of different citrates (as pH regulators), the use of potassium metabisulfite and a working temperature of 10°C assure the hygienic condition of the protein ultrafiltrate of poultry blood. All the principal variables (i.e., feed-flow, cut-off and pressure) and their interactions produce effects that are meaningful in terms of the performance of the membrane system. This is particularly true for the feed-flow rate.

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Table 1. Experimental lay-out.

x1	x2	x3	x4	x5	x6	x7	y1	y2
-1	-1	-1	+1	+1	+1	-1	1.712E-3	11E-3
+1	-1	-1	-1	-1	+1	+1	3.432E -3	22E-3
-1	+1	-1	-1	+1	-1	+1	2.505E -3	14E-3
+1	+1	-1	+1	-1	-1	-1	2.891E -3	17E-3
-1	-1	+1	+1	-1	-1	+1	1.475E -3	8E-3
+1	-1	+1	-1	+1	-1	-1	3.012E-3	8E-3
-1	+1	+1	-1	-1	+1	-1	2.220E -3	7E-3
+1	+1	+1	+1	+1	+1	+1	1.978E -3	16E-3

S2: 2.79697E-11