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Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit

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ABSTRACT

The aims of the study were to optimize the production a fish protein hydrolysate (FPH) by enzymatic hydrolysis of sardine solid waste using crude pepsin, and to scale up the process in a bioreactor coupled to an ultrafiltration unit for product recovery. Results showed that the crude pepsin prepared by autolysis of the mucous membranes of a sheep stomach at optimal conditions (i. e. pH = 1.5–2 and incubation time of 6 h) could be satisfactory used for the enzymatic hydrolysis of fish solid waste. The optimal conditions for enzymatic reaction were: temperature 48 °C, and pH 1.5. The scale up of the enzymatic hydrolysis and the coupling of the reactor an ultrafiltration unit to concentrate the hydrolysate gave good results with a rejection coefficient for the protein hydrolysate product in the range of 90%. The volumetric concentration factor was 2.5, with a permeate flux of 200 L m^{-2} bar^{-1}. However, the results also suggest that the ultrafiltration product concentration process may be operating beyond the critical flux at which point irreversible membrane fouling occurs.

1. Introduction

Solid fish wastes have been utilized as a source of proteins and essential amino acids of high nutritional value during the last decade [1–4]. Several amino acids, for example, such as Tyr, Met, His, Lys, and Try are generally accepted as being antioxidants [5]. The current global fish production, including aquaculture, is approximately 140 million tons, of which 110 million were for human consumption [6]. While capture fisheries have remained at the same level for the last few years, aquaculture has been expanding. Oceans and seas provide close to 90% of the world's catches. This has remained relatively stable since the mid-nineties at between 80 and 86 million tons. About 25% of the total production (i.e. 35 million tons) is considered inedible (i.e. waste). However the nutritional value of the waste products is almost identical to that of the edible parts. The reprocessing of fish waste material has led to cheaper food products, with high nutritional value, good taste, and which is stable and thus good for storage [6].

During the last twenty years there have been numerous studies on the processing of solid wastes from fisheries to produce food of high nutritional quality [7]. The first processes used, based on chemical hydrolysis and extraction by organic solvent, gave poor results in terms of nutritional quality of the proteins and conservation of amino acids [7]. This last decade, much progress has been achieved in the application of biologic enzymatic processes to produce fish protein hydrolysate (FPH) [8,9]. Bioactive peptides can be found in fish products, such as Sardine muscle [10], Tuna muscle bonito [11] and Alaska Pollack Skin [12]. FPH may also have a role as a cardio protective nutrient in human health [13].

Many studies have been published on the optimization of enzymatic hydrolysis conditions by using response surface methodology [8,9], or on the biological activities of the FPH (i.e. bioactive peptides). Different enzymes have been used to generate bioactive peptides as anti hypertensive agents [14–16]. One drawback, however, is that the retail price of enzymes such as pepsin is high and is an obstacle in the commercialization of the fisheries waste processes [17]. The development of inexpensive enzymes for processing of fish waste products would be of great benefit to the food processing industry. Furthermore, FPH protein must be concentrated before storage and use. The concentration step must also minimize nutritional quality losses, by avoiding high temperatures and long processing periods [18–21]. The use of ultrafiltration, may be a promising approach in this regard [22].

The aims of the study were to optimize the production a fish protein hydrolysate (FPH) by enzymatic hydrolysis of sardine solid waste using crude pepsin, and to scale up the process in a bioreactor coupled to an ultrafiltration unit for product concentration. Specifically, the optimal conditions (i. e. incubation time, temperature, enzyme concentration and pH) for enzymatic activity on a standard substrate (i. e. bovine

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serum albumin) and on proteins from sardine solid waste were first determined using crude pepsin prepared from sheep stomach. With the best conditions for enzymatic activity determined, the solid wastes were hydrolysed in a laboratory pilot scale bioreactor batch mode. Finally, the enzymatic reactor was coupled to an ultrafiltration unit in order to concentrate the protein hydrolysate product.

2. Materials and methods

2.1. Sardine solid wastes

The Sardine solid wastes were collected from Dellys's fishery (Kabylia region) over a one year period. They were transported to the laboratory in sterile flasks under aseptic conditions at 4 °C. The solid waste consisted of a complex mixture containing fish heads, stomachs, and viscera. Heat inactivation of endogenous stomach enzymes (i.e. 100 °C for 30 min) was carried out and then the samples were stored at 20 °C until use.

2.2. Analysis of moisture, protein, fat and ash of mucous membrane

After evaporation of solvent extraction, the residue was dried and weighed. The dry weight of mucous membrane was expressed in percentage (%) and was calculated by the following relation:

$$X(\%) = (m_2/m_1).100 \quad (1)$$

Where, m_1 and m_2 are respectively the wet weight and dry weight of mucous membrane. The dry weight of mucous membrane was determined after 24 h under 105 °C. The concentration of proteins was determined by the Biuret method modified by Umeto [23]. This method allows a rapid determination of proteins which are assayed without mineralization. The technique is easier to implement and also is less expensive and has the advantage of good repeatability [24,25]. The fat content of the dried sample was determined by extraction with technical grade n-hexane or ether-petrol [5]. The total ash was determined by incineration of the dry residue in a muffle furnace (550 °C) during 24 h.

2.3. Preparation of crude enzyme

Pepsin was chosen as the proteolytic enzyme due to its great specificity for hydrolyses of polypeptides. Its optimal pH for enzymatic activity is in the acid range which minimizes bacterial contamination. The crude pepsin was prepared according to the experimental protocol developed by Lin Liu and Pigott [39]. Soon after sheep death, the stomach was emptied and then well cleaned. The mucous membrane was unstuck intact from the stomach and then was crushed into 2–5 mm chunks with a hammer and stored at 20 °C until use.

The dry weight of mucous membrane was determined before its use. This parameter was used as reference to estimate the quantities of mucous membrane employed during the enzymatic hydrolysis experiments. The chopped mucosa was autolyzed by the pepsin enzymes by incubation at ambient temperature with addition of HCl solution. To determine the optimal conditions of the preparation of the crude pepsin by the sheep mucosa, the incubation time was varied from 6 h to 12 h and the pH from 0.5 to 2. This transformed the pepsinogen (the inactive form of the enzyme) into pepsin (the active form).

2.4. Crude enzyme activity

The stability of the crude enzyme was determined at 20 °C for pH 1, 1.5, 1.75 and 2. The pepsin activity tests were performed by adding 4 mL of crude pepsin solution to 1 mL of protein test solution. Bovine serum albumin (BSA), Sigma Chemical Co. (St Louis, USA) was employed as a reference protein and/or the Sardine wastes proteins. The mixture was maintained at a constant temperature for 10 min [26–28]. The reaction was stopped after 10 min by addition of 6 mL of trichloroacetic acid (TCA) at 4% (w/vol). After 5 min the solution was filtered (Whatman filter paper No. 3). The amino acids and peptides released in the filtrate were quantified by the Biuret method [23]. The performance of this enzyme was compared with that of commercial pepsin supplied by Sigma (enzymatic activity 980 units/mg protein).

2.5. Hydrolysis of solid sardine waste in batch mode

The enzymatic hydrolysis tests of the solid waste were performed on crushed samples. Before use the sample (100 g) was mixed with an equal volume of water (100 ml). After homogenization, the pH was adjusted with concentrated HCl and kept at a constant temperature of 48 °C in a water bath. The pepsin solution previously heated at the same temperature was poured with care into the mixture during the 4 h hydrolysis period under moderate stirring (300–400 rpm). Aliquots of 20 ml of reaction blend were withdrawn at regular intervals, and then mixed with 10 ml of trichloroacetic to stop the reaction. After 10–15 min the whole solution was filtered through Whatman filter paper No. 3. The concentrations of the amino acids and peptides contained in the filtrate were determined by the Biuret method [23].

The working volume of the bioreactor during the optimization of the enzymatic hydrolysis in batch mode was 2 L (Setric, Toulouse, France). The results of the enzymatic hydrolysis were expressed in terms of specific activity and specific yield. The specific activity was used to estimate the ratio of proteins hydrolysed weight with reference to the quantity of initial proteins content in the substrate per quantity of enzyme per hour. The specific yield was used to estimate the ratio of weight of proteins hydrolysed per amount of initial proteins and per amount of enzyme.

2.6. Hydrolysis of sardine solid waste by enzymatic membrane reactor at pilot scale

The pilot scale experiments were performed with a 60 L reactor in combination with a 130 S ultrafiltration pilot unit (Gamma filtration company, France) equipped with a Membralox-Ceraver module. This module (P19–40) was a multi-channel ceramic membrane composed of ultrafine porous ZrO_2 (0.05 μm) supported on coarse porous alumina (15 μm). The total filtration area of this module was 0.2 m^2. The ultrafiltration process is presented in Fig. 1. Two operating modes were utilized. The first mode (Fig. 1a) consisted of re-injecting the permeate into the reactor volume until a satisfactory hydrolysis yield was attained. Then the concentration process was started without permeate recirculation (Fig. 1b). Plots were made of solvent flux versus transmembrane pressure (P_a).

The influence of the hydrodynamic parameters, namely the tangential velocity and the transmembrane pressure was investigated during the product concentration experiment. The solute rejection of the ultrafiltration (UF) membrane was assessed. An optimal tangential velocity of 6 m/s, previously determined with a similar membrane during the optimization of the treatment of the fishery washing water by ultrafiltration [22], was utilized in this study.

After each ultrafiltration experiment, the following membrane cleaning operation was used; Distilled water at the temperature $T = 40 \pm 2$ °C was circulated in a closed loop in the apparatus for 10 min. The temperature used during the cleaning experiment was chosen by taking into account the maximum membrane operating temperature indicated by the supplier. The membrane was then washed with detergent for 20 min (10 min without trans-membrane pressure and 10 min with $\Delta P = 0.5 \times 105$ Pa). An ethanol–water mixture (10/90; v/v) was used to rinse the membrane without applied pressure for 15 min. Finally, pre-filtered water was used to rinse the membrane at room temperature at an applied pressure of 1×105 Pa.

Membrane fouling was assessed by running the UF process with pure solvent (i.e. distilled water) before the hydrolysate (FPH) concentration step (i.e. with a clean UF membrane) and then repeating the process with pure solvent after the UF membrane had been used for

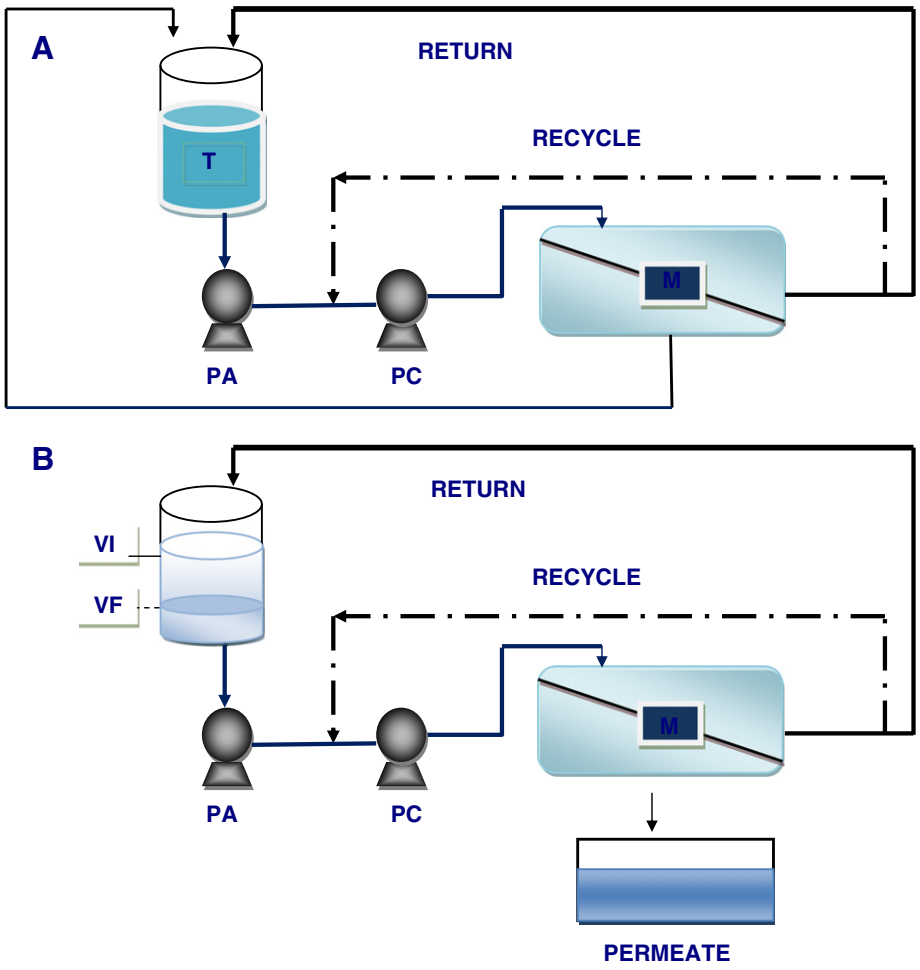

Fig. 1. Schematic representation of ultrafiltration process (UF): (A) with recycling of permeate and (B) without recycling of permeate. T: feed tank; PC: recycling pump; PA: Feed pump; M: Ultrafiltration module; V_i: Initial volume and V_f: Final volume.

FPH concentration. Plots were made of solvent flux (Jv) versus transmembrane pressure Pa. Any reduction in flux would give an indication of membrane fouling.

To measure the efficiency of the UF in concentrating the FPH, an apparent rejection coefficient (R_o) was utilized [29]. This parameter using the initial (C_o) and permeate (C_p) solute concentration was determined by the following equation:

$$R_o = \left(1 - C_p/C_o\right) \times 100 \quad (2)$$

All experiments were run in triplicate.

3. Results and discussion

3.1. Characterization of solid waste

The solid waste fish mixture contained on average 20% dry matter and 13.7% protein (Table 1). The slightly elevate standard deviation obtained for the fats (6.3 ± 1.5) may be due to seasonal variation with the natural fish being less fatty during winter compared to the summer. The protein concentration was similar to other data in the literature [23,26].

3.2. Optimization of crude pepsin enzymatic activity

3.2.1. Specific activity and stability of crude pepsin at various pH

The results presented in Fig. 2, indicate that for values of pH greater than 1, the specific activity of the crude enzyme remained relatively constant in the range 25–38 $gg^{-1}\,mg^{-1}\,h^{-1}$ during 8 days. On the other hand, for pH = 1, the enzyme activity decreased from 57 to 38 $gg^{-1}\,mg^{-1}\,h^{-1}$, about 33%, during the two first days of storage, after which it stabilized to a steady state value around 38 $gg^{-1}\,mg^{-1}\,h^{-1}$. These results suggest that self digestion of enzymatic preparations, to activate the pepsinogen to crude pepsin, continues at low temperatures (inside the refrigerator), especially at low pH (pH 1). From a economic standpoint since at pH 1 the enzyme had the greatest activity, especially during the first two days better activity, it would be best to prepare crude pepsin at this pH for any commercial application.

3.2.2. Effect of temperature on crude enzymatic activity

The optimal value of the temperature was determined by using two enzyme substrates: BSA (bovine serum albumin) and solid fish waste. The influence of the temperature on enzyme performance (Fig. 3) indicates that the optimal value of the temperature occurred around 48 °C for the enzymatic specific activity of the crude pepsin with the fish protein

Table 1
Composition of fish solid waste.

Constituent	Range of values (% wt)	Annual average ± SD (% wt)
Dry matter	19.2–21.3	20.1 ± 0.8
Moisture% D.M.	78.2–81.6	80 ± 0.5
Ashes% D.M.	4.1–5.2	4.8 ± 0.4
Fats% D.M.	2.1–9.2	6.3 ± 1.5
Proteins% D.M.	13.1–14.2	13.7 ± 0.3

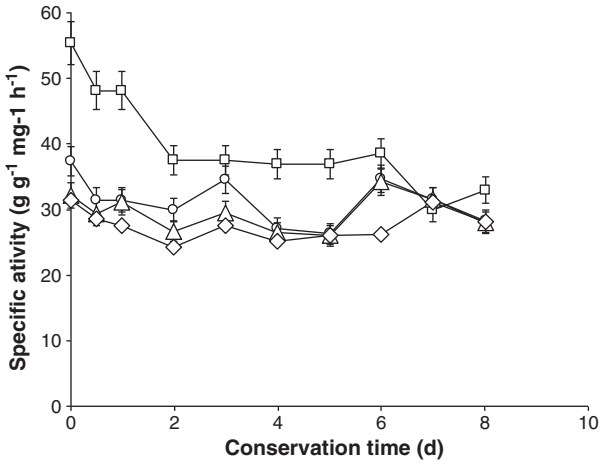

Fig. 2. Evolution of the specific activity of the crude pepsin at various pH during the conservation. With BSA as substrate; (□) pH = 1; (o) pH = 1,5; (Δ) pH = 1,75 and (◊) pH = 2.

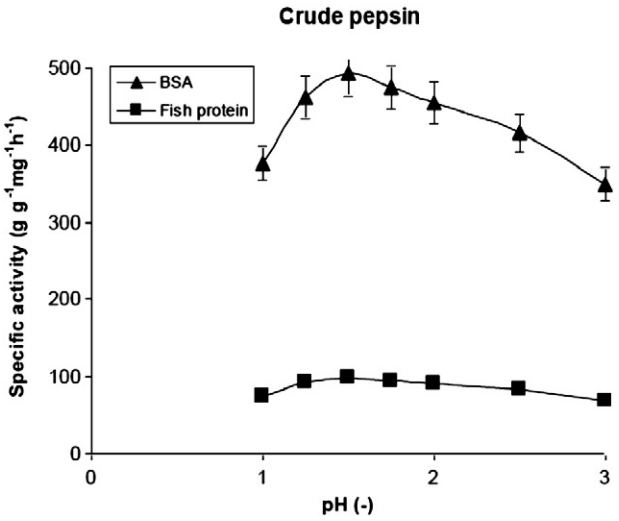

Fig. 4. Change of in specific activity of crude pepsin as a function of pH. Ω = 350 rpm. (▲) BSA as substrate; C_E = 0,1 g/l (■) Fish protein as substrate; C_E = 0,5 g/l.

as substrate. For the BSA maximal specific activity was obtained at the same temperature (48 °C). In a related study Bleustein [26] reported that it was possible to work with BSA at 50 °C without thermal denaturation occurring. The latter occurs at temperatures near 65 °C [27].

3.2.3. Comparison of crude and commercial pepsin

The enzymatic activity of the crude pepsin previous prepared at optimal conditions was compared to commercial pepsin. Both pepsin solutions were tested at their optimal temperature which pH ranging from 1 to 3. The results indicated that the greatest activity for the solution of crude pepsin was obtained at ph 1.5 for both substrates (BSA and fish proteins) (Fig. 4). The commercial pepsin exhibited a maximal specific activity enzymatic at ph 1.5–2 for fish proteins (results not shown).

In summary, the results suggest that the crude pepsin prepared by autolysis of the mucous membranes of sheep stomach may be effectively used for the enzymatic hydrolysis of fish solid waste. The optimal conditions for the enzymatic hydrolysis are pH = 1.5 and temperature 48 °C.

3.3. Pilot scale bioreactor study combined with hydrolysate concentration by ultrafiltration

The experiments at pilot scale were run under the optimal conditions previously determined. The enzymatic reactor membrane was operated at a constant concentration mode (Fig. 1 (a)). Results, presented in Fig. 5, indicate that the specific yield with time was similar to the results determined at the laboratory scale, with a relatively constant specific yield of about 50 g/g.mg after 3 h. However, performance decreased in scale up; the specific yield dropped from 65% for the lab scale to 50% for the pilot scale (Fig. 5). This slightly lower yield may be attributable to the hydrodynamic parameters of the working volume of the bioreactor which could be more difficult to control with an increase in the volume (e.g. poorer mixing). Experiments at the laboratory scale confirmed that under similar experimental conditions, the mixing parameters are a significant operational parameter for the effectiveness of this enzymatic process for both substrates (results not shown). All experiments were run in triplicate and showed differences of less than 6%.

In the current study, the enzymatic membrane reactor was operated in permeate recycling during the first 4 h until an apparent steady state was reached. After this period, permeate recycling was stopped and the hydrolysate was concentrated by ultrafiltration.

The influence of the transmembrane pressure (Pa) on the permeate flux during ultrafiltration of the FPH is shown in Fig. 6(a), lower curve. There was an increase in flux (J_v) with transmembrane pressure (Pa).

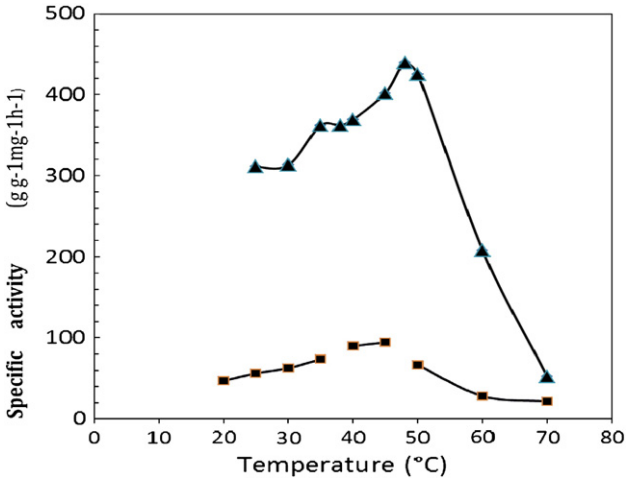

Fig. 3. Effect of temperature on the specific activity of crude pepsin. Ω = 350 rpm, C_E = 0,1 g/l, (■) BSA; (▲) Fish protein.

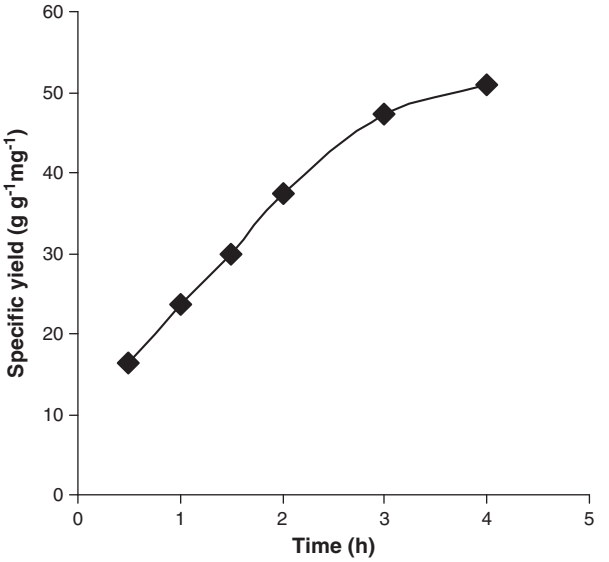

Fig. 5. Enzymatic hydrolysis of fish proteins at pilot scale. pH = 1.5, T = 48 °C, Rws = 1/1, E/S = 2/100 and Ω = 350 rpm.

A maximum permeate flux of 350 $dm^3\ m^{-2}\ h^{-1}$ occurred at Pa = 2 bars (i.e. 2×10^5 Pa). The permeate flux was independent of the transmembrane pressure beyond this point. This is normally termed the critical flux, J_{vcrit}, [30,31] and is due to concentration polarization of solute (e.g. fish protein hydrolysate) at the membrane surface. A fouling layer, however, will form between the polarization layer and the membrane surface when the applied pressure exceeds the critical pressure (ie $P_{a\ crit}$ = 2 bar in Fig. 6a). Any increase in pressure results in a temporary increase in permeate flux. However, at the same time there is a precipitation of solute at the membrane surface (i.e. gel layer formation; Fig. 6b) which increases the overall membrane resistance and thus lowers the permeate flux. Therefore the two effects cancel each other out and we see the flux becoming independent of pressure beyond this critical point (i.e. Pa = 2 bar and $J_{vcrit} = 350\ L.m^{-2}.h^{-1}$).

While the concentration polarization layer is reversible, the results shown in the top two curves of Fig. 6a (i.e. pure solvent flux for original clean membrane and for membrane after use in hydrolysate concentration) suggest the formation of an irreversible bound (i.e. permanent) fouling or gel layer on the membrane surface. For example, at Pa = 2 bar, the pure solvent flux, Jv, for the clean (unused) UF membrane is 1455 $dm^3\ m^{-2}\ h^{-1}$ (upper curve). At the same pressure the pure solvent flux for a fouled membrane (i.e. permanent layer) is reduced to 600 $dm^3\ m^{-2}\ h^{-1}$ (middle curve) due to an additional resistance caused by the irreversible fouling layer (i.e. fish protein hydrolysate and solid waste). Now, during the UF of the fish protein hydrolysate (lower curve in Fig. 6a) there is an additional resistance due to the formation of a reversible concentration polarization layer (i.e. protein) on top of the permanent fouling layer. This reduces the permeate flux even further to 350 $dm^3\ m^{-2}$.

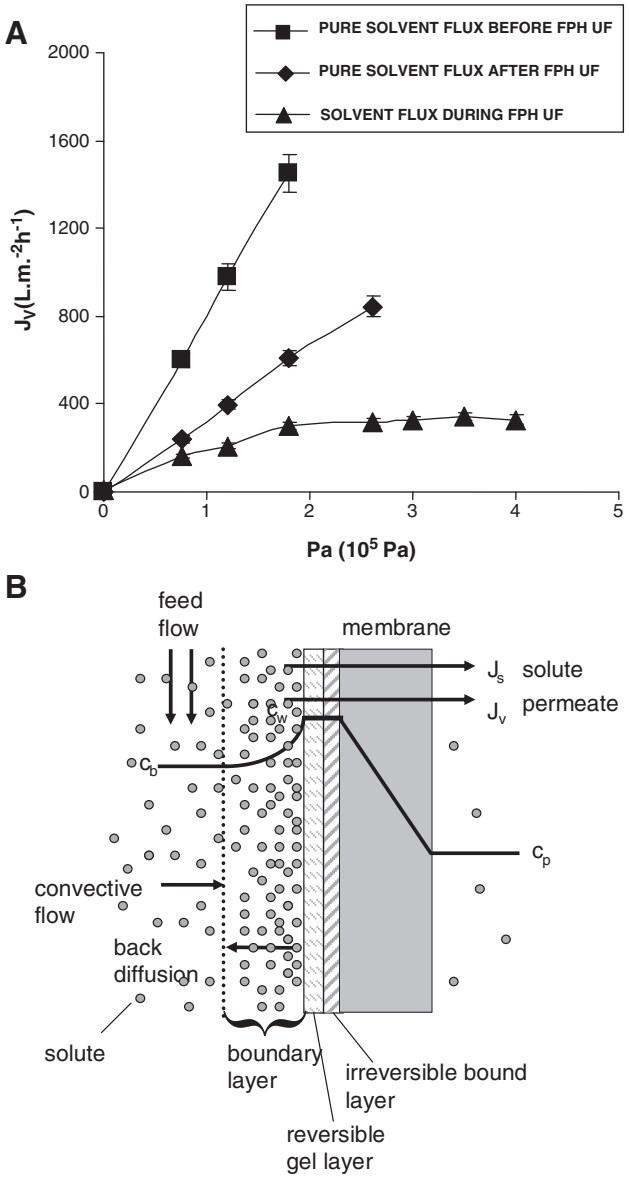

Fig. 6. a. Ultrafiltration of the hydrolysate after 4 h operation with recycling permeate mode, U = 6 m/s, T = 48oC (■) Solvent curve before ultrafiltration, (▲) Hydrolysate Ultrafiltration (♦) Solvent curve after hydrolysate ultrafiltration. b. A schematic representation of concentration polarization and fouling at the membrane surface [30].

See Fig. 6B for a schematic representation of the fouling process. A key phase in membrane separation processes is the transition from concentration polarization to fouling. This occurs at the critical flux. Song [31] indicated that in most theories developed, the limiting or critical flux is based on semi-empirical knowledge rather than being predicted from fundamental principles. To overcome this shortcoming, he developed a mechanistic model, based on first principles, for predicting the limiting flux. Similar to the critical flux results of Chen et al. [32] and the limiting flux of Koltuniewicz and Noworyta [33], Song [31] showed that there is a critical pressure for a given suspension. When the applied pressure is below the critical pressure, only a concentration polarization layer exists over the membrane surface. A fouling layer, however, will form between the polarization and the membrane surface when the applied pressure exceeds the critical pressure (i.e. Pa = 2 bar in the current study). The UF of FPH therefore be operated just below the critical flux in order to maximize productivity while minimizing membrane fouling. To lessen the inclination to irreversible fouling it is important to operate an ultrafiltration plant/unit below the critical flux.

The solute rejection coefficient should be included in defining the optimum conditions for the ultrafiltration process. The apparent solute rejection coefficient, R_o, depended strongly on the transmembrane pressure (Fig. 7). Indeed, R_o was 18% at $P_a = 1.10^5$ Pa and increased to 90% at $P_a = 4.10^5$ Pa. Similar results were obtained by Mameri et al. [22] during the treatment of fishery washing water by an ultrafiltration unit with Ceraver membranes; the rejection coefficients obtained were in the range of 60 to 90%. The current results also suggest the formation of a fouling layer on the membrane surface by the increase in slope of the curve above 2 10^5 Pa (Fig. 7). This fouling layer was originally described by Watanabe et al., in 1984 [34] as a dynamic membrane and later by Song [31], Goosen et al. [30] Chen et al. [32] and Koltuniewicz and Noworyta [33] in terms of concentration polarization and irreversible gel layer formation (Fig. 6a and b).

The variation in J_v and R_o as a function of time and at a pressure of 4×10^5 Pa and at the same mixing speed and temperature as in Fig. 6a during the UF concentration studies are presented in Fig. 8. The permeate flux rapidly reached a steady state value of 200 $L\ m^{-2}\ bar^{-1}$, after an equilibrium time estimated about 12 min. This again suggests the formation of a fouling (gel) layer at the membrane surface. The thickness of the layer increases with time, thus increasing the resistance to mass transfer as shown by the decrease in permeate flux, J_v. At 12 min a critical flux is reached at which point the flux becomes independent of both time and pressure [30–33].

The increase in the rejection coefficient during the concentration experiment reached a maximum value of R_o = 90%. As a result a reduction in hydrolysate volume by a factor of 2.5 was obtained at the end of the experiment. In a related study, Watanabe et al., [34] examined the effect of pore size of a ceramic support on the self-rejection characteristics of what they termed a dynamic membrane formed with water-soluble proteins in waste water. Microscopic observations showed that a large part of this membrane contained a fouling layer on the uneven surface of the ceramic support. This concept is similar to the concentration polarization and gel layer formation on the membrane surface [29–33].

The hydraulic permeability of the membrane before and after enzymatic hydrolysis of fish proteins was also determined. The permeability

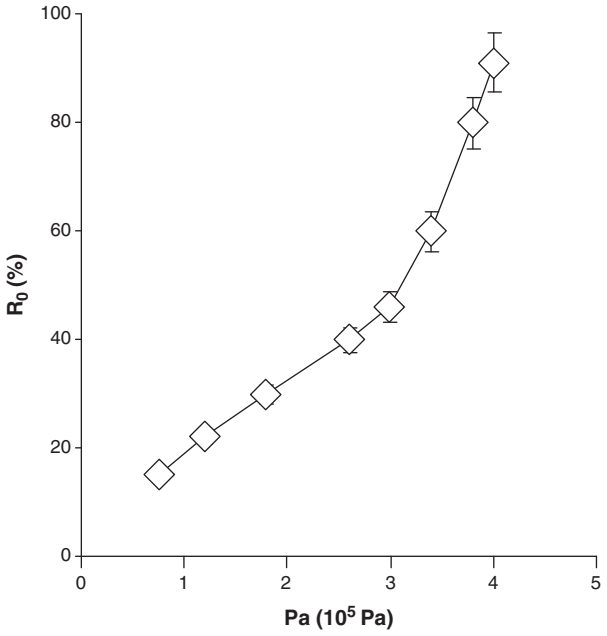

Fig. 7. Rejection coefficient plotted against average transmembrane pressure (P_a).

of the membrane was calculated by deducing the slope of the lines obtained by plotting the change of the permeate flux against the average transmembrane pressure during the filtration of prefiltered water (data not shown). Assuming that solute adsorption onto an ultrafiltration membrane leads to a permeability variation resulting from modification of the average pore radius from r_{po} to r_{p1}, then the Poiseuille's equation [35]. can be written as:

$$J_v = N\pi r_{p1}{}^4/8\eta e \quad \Delta P = L_{p1}DP \tag{3}$$

$$L_{p1} = N\pi r_{p1}{}^4/8\eta e \tag{4}$$

$$L_{p0} = N\pi r_{p0}{}^4/8\eta e \tag{5}$$

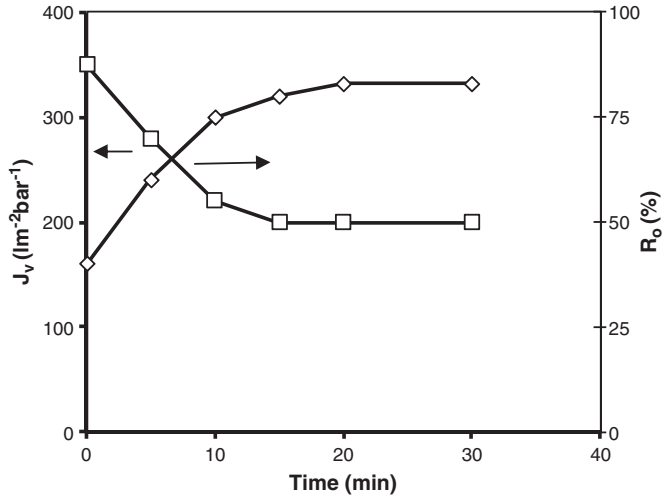

Fig. 8. Hydrolysate concentration experiment with permeate flux and solute rejection plotted against time $P_a = 4\ 10^5$ Pa, U = 6 m/s and T = 48 °C. (□) Jv and (◊) R_o.

where N is the number of pores per unit membrane area, e is the membrane thickness (m), η is the solvent viscosity (Poiseuille) and the reduced pore radius (r_{p1}) may be expressed as:

$$r_{p1} = r_{po}\left(L_{p1}/L_{p0}\right)^{0,25} \tag{6}$$

$r_{po} = 5\ 10^{-8}$ m = pore radius before ultrafiltration and the calculated value after ultrafiltration $r_{p1} = 4\ 10^{-8}$ m indicate a decrease in pore size which would increase solute rejection as well as decrease the permeate flux.

The difference between r_{p0} and r_{p1} can be explained by the fact that the decrease in the initial pore radius (r_{p0}) was due to the adsorption of various solutes on the surface of the pores of the membrane. With increasing solute concentration at the ceraver membrane surface (i.e. concentration polarization), we can speculate that protein aggregates became more numerous and larger. As Watanabe et al., [34] explains, after a certain time, a dynamic equilibrium is reached between protein aggregates in the solution and those on the membrane pore surface. This state may be called a gel or dynamic membrane layer (see Fig. 6b).

The issue of lipids or fats in the substrate needs to be addressed. Lipids would have an interfering effect during hydrolysis Lipid levels varied according to season from a low of 2 wt.% in the winter to a high of 9 wt.% in the summer (Table 1). Shenouda and Pigott [36] reported that in summer, the hydrolysis rate was estimated at 86% with a lipid content of the solid fish waste ranging from 1 to 1.5%. The rate dropped to 76% in winter when the fat content was estimated at 4%. This is explained by the formation of lipid-protein complexes which are difficult to hydrolyze.

To determine the amino acids present in the hydrolysate product and its nutritional quality, the hydrolysate was analyzed by HPLC [37] (Table 2). Eight key amino acids were found in the concentrated product namely: leucine, isoleucine, valine, lysine, methionine, tyrosine, phenylalanine and tryptophane. These amino acids, for example, are necessary for the daily food intake to assure normal human growth [38].

4. Conclusions

This study has shown that crude pepsin prepared by autolysis of the mucous membranes of a sheep stomach at optimal conditions (i. e. pH = 1.5–2 and incubation time of 6 h) could be satisfactory used for the enzymatic hydrolysis of solid fish waste. The optimal conditions for enzymatic reaction were: temperature 48 °C, and pH 1.5. The scale up of the enzymatic hydrolysis and the coupling of the reactor to an ultrafiltration unit to concentrate the hydrolysate gave good results

Table 2
Concentration of amino acids in hydrolysate [37]. All the results are expressed in g kg^{-1}, except tryptophan in ppm.

Amino acid	
Aspartic acid	0.10
Threonine	0.06
Serine	0.06
Glutamic acid	0.30
Proline	0.37
Glycine	0.6
Alanine	1.44
Cystine	0
Valine	0.91
Methionine	0.14
Isoleucine	0.64
Leucine	1.34
Tyrosine	0.15
Phenylalanine	0.46
Lysine	0.42
Histidine	0.05
Arginine	0.21
Tryptophan with acid hydrolysis	68

with a maximum rejection coefficient for the protein hydrolysate product in the range of 90%. The volumetric concentration factor was 2.5, with a permeate flux of 200 $L\,m^{-2}\,bar^{-1}$. However, the results also suggest that the product concentration process may be operating beyond the critical flux at which point the flux becomes independent of time and pressure. To reduce the tendency to irreversible fouling it is essential to operate the plant/unit below the critical flux. Further work is needed in this area.

References

[1] H.G. Kristinsson, Ba Rasco, Crit. Rev. Food Sci. Nutr. 40 (2000) 43–81.
[2] P. Chowdhury, T. Viraraghavan, A. Srinivasan, Bioresour. Technol. 101 (2010) 439–449.
[3] F. Guerard, S. Maqsood, S. Benjakul, Food Chem. 119 (1) (2010) 123–132.
[4] N. Bhaskar, T. Benila, C. Radha, R.G. Lalitha, Bioresour. Technol. 99 (2) (2008) 335–343.
[5] A. Bougatef, N. Nedjar-Arroume, L. Manni, R. Ravallec, A. Barkia, D. Guillochon, M. Nasri, Food Chem. 118 (2010) 559–565.
[6] FAO Fisheries, Aquaculture production, 2008, pp. 16–23.
[7] F. Shahidi, J.K.V. Arachchi, Y.-J. Jeon, Trends Food Sci. Technol. 10 (1999) 37–51.
[8] F. Guerard, M.T. Sumaya-Martinez, D. Laroque, A. Chabeaud, L. Dufossé, Process. Biochem. 42 (2007) 1486–1491.
[9] S. Nilsang, S. Lertsiri, M. Suphantharika, A. Assavanig, J. Food Eng. 70 (4) (2005) 571–578.
[10] T. Matsui, H. Matsufuji, E. Seki, K. Osajina, M. Nakashima, Y. Osajima, Biosci. Biotechnol. Biochem. 57 (6) (1993) 922–925.
[11] N. Yamamoto, M. Ejiri, S. Mizuno, Curr. Pharm. Des. 9 (16) (2003) 1345–1355.
[12] H. Wu, H.L. He, X.L. Chen, C.Y. Sun, Y.Z. Zhang, B.C. Zhou, Process. Biochem. 43 (2008) 457–461.
[13] H. Wergedahl, B. Liaset, O.A. Gudbrandsen, E. Lied, M. Espe, Z. Muna, S. Mork, R.K. Berge, J. Nutr. 134 (6) (2004) 1320–1327.
[14] H. Fujita, B.S. Yamagami, K. Ohshima, Nutr. Res. 21 (2001) 1149–1158.
[15] W.K. Jung, E. Mendis, J.Y. Je, P.J. Park, B.W. Son, H.C. Kim, Y.K. Choi, S.K. Kim, Food Chem. 94 (2006) 26–32.
[16] J.F. Poulin, M. Araya-Farias, J. Amiot, L. Bazinet, Desalination 200 (2006) 620.
[17] J.K. Lee, J.K. Jeon, H.G. Byun, Food Chem. 125 (2011) 495–499.
[18] F. Shahidi, X.Q. Han, J. Synowiecki, Food Chem. 53 (3) (1995) 285–293.
[19] B. Liaset, R. Norvedt, E. Lied, M. Espe, Process Biochem. 37 (2002) 1263–1269.
[20] M. Martin, D. Porter, in: G. Charalambous (Ed.), Food Flavours: Generation Analysis and Process Influence, Elsevier, Amsterdam, 1995, p. 1395.
[21] J.R. Couper, W.R. Penney, J.R. Fair, S.M. Walas, Chem. Process Equip. 2 (2010) 357–393.
[22] N. Mameri, D. Abdessemed, D. Belhocine, H. Lounici, C. Gavach, J. Sandeaux, R. Sandeaux, J. Chem. Technol. Biotechnol. 67 (1996) 169–175.
[23] S. Umeto, Bull. Jpn. Soc. Fish. 32 (1996) 427–435.
[24] R.E. Beyer, Anal. Biochem. 129 (2) (March 1983) 483–485.
[25] R. Parvin, S.V. Pande, T.A. Venkitasubramanian, Anal. Biochem. 12 (1965) 219–229.
[26] C.B. Bleustein, M. Sennett, R.T. Kung, D. Felsen, D.P. Poppas, R.B. Stewart, Lasers Surg. Med. 27 (2000) 465–470.
[27] Qi Wang, Yi-Xin Guan, Shan-Jing Yao, Zi-Qiang Zhu, J. Supercrit. Fluids 56 (1) (February 2011) 97–104.
[28] R.E. Vega, J.G. Brennan, J. Food Eng. 8 (1988) 201–215.
[29] M.F.A. Goosen, S. Sablani, M. Dal Cin, M. Wilf, Sep. Sci. Technol. 46 (2011) 14–26.
[30] M.F.A. Goosen, S. Sablani, H. Al-Hinai, S. Al-Obeidani, R. Al-Belushi, D. Jackson, Sep. Sci. Technol. 39 (2004) 1–37.
[31] L. Song, J. Membr. Sci. 144 (1998) 173–185.
[32] V. Chen, A.G. Fane, S. Madaeni, I.G. Wenten, J. Membr. Sci. 125 (1997) 109–122.
[33] A. Koltuniewicz, A. Noworyta, Ind. Eng. Chem. Res. 33 (1994) 1771–1779.
[34] A. Watanabe, T. Ohtani, H. Horirita, H. Ohya, S. Kimura, Food Eng. Proc. Appl. 2 (1986) 225–336.
[35] A.G. Fane, C.J.D. Fell, A.G. Waters, J. Membr. Sci. 9 (1981) 245–262.
[36] Y.K.S. Shenouda, G.M. Pigott, J. Food Sci. 39 (1974) 726–734.
[37] H. Grib, Separation and concentration of amino acids by membrane technology, Thesis of doctorate, September 2002, National Polytechnic School, Algeria.
[38] W.E.M. Lands, Proc. Nutr. Aust. 15 (1990) 60–66.
[39] L. Lin Liu, G.M. Pigott, J. Food Sci. 46 (1981) 1569–1572.