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Concentration and purification of blue whiting peptide hydrolysates by membrane processes

Laurent Vandanjon [a,*], Ragnar Johannsson [b], Maryse Derouiniot [c], Patrick Bourseau [a], Pascal Jaouen [c]

[a] Université de Bretagne Sud, LETEE, Rue Saint-Maudé, BP 92116, 56321 Lorient cedex, France
[b] Icelandic Fisheries Laboratory (IFL), Skulagata 4, 101 Reykjavik, Iceland
[c] Université de Nantes, GEPEA, UMR CNRS 6144, Boulevard de l'Université, BP 406, 44602 Saint-Nazaire cedex, France

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Abstract

This study aims to evaluate the performances of the membrane processes during the treatment of blue whiting peptide hydrolysates.

Ultrafiltration with high molecular weight cut-off (MWCO) can be used for the separation between peptides and non-hydrolyzed proteins. A membrane of MWCO 20 kDa was tested on hydrolysates containing 11.4 g of peptides per liter of solution. Steady fluxes were satisfactory (100 l/h/m^2 at 12 bars and 15 °C) but the nitrogenized matter retention is approximately 30%, which leads to a considerable peptide loss in the permeate.

In addition, ultrafiltration with intermediate MWCO is considered for the enrichment of fractions of peptides of a definite molecular weight range. Two membranes of MWCO 4 and 8 kDa were selected; allowing easy separation between peptides of high and low molecular weight. Moreover, they show variable rejection rates according to classes of molecular weight, which gives hope for a possible fractionation in the range of 1000–7000 Da.

Lastly, the nanofiltration enables the concentration of peptides of low molecular weight by avoiding an excessive concentration of salts. The results with a membrane of MWCO 300 Da are very encouraging because the retention of peptides bigger than 1000 Da is total so that the concentration of the retentate up to about 300 g/l can be reached.

Keywords: Ultrafiltration; Nanofiltration; Fractionation; Hydrolysates; Marine peptides

1. Introduction

This study lies within the scope of the European Integrated project SEAFOODPlus. It is a part of a work aiming to produce biologically active molecules from marine by-products by innovating processes to benefit human health.

In this frame, the membranes, according to their molecular weight cut-off (MWCO), can be used at different levels:

- Separation of peptides and non-hydrolyzed proteins or proteolytic enzymes, with UF membranes of high MWCO, approximately 20 kDa (Bouhallab & Touze, 1995; Lajoie et al., 2001),
- Fractionation of peptide hydrolysates according to their molecular weight (MW) (the latter being generally linked to the biological activity) with UF membranes of intermediate MWCO, approximately 4000–8000 Da (Beelin Cheang & Zydney, 2004; Jao & Ko, 2002),
- Concentration of peptide solutions (initial mixture or selected fractions), with NF membranes of low MWCO, approximately 200–300 Da (Fenton-may et al., 1971; Tessier et al., 2006),

* Corresponding author. Tel.: +33 2 97 87 45 32; fax: +33 2 97 87 45 88.
E-mail address: Laurent.Vandanjon@univ-ubs.fr (L. Vandanjon).

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Nomenclature

C_f	concentration in peptides in the retentate after concentration
C_i	concentration in peptides in the initial solution
COD	chemical oxygen demand
C_p	concentration in peptides in the permeate
J	permeation flux
L_P	hydraulic permeability
MCF	mass concentration factor
MEB	membrane enzymatic bioreactor
MW	molecular weight
MWCO	molecular weight cut-off
NF	nanofiltration
NTK	total nitrogen Kjeldahl method
PA	polyamid
PES	polyethersulfon
PL	peptides lost
PS	polysulfon
RR	rejection rate
SE-HPLC	size exclusion high performance liquid chromatography
UF	ultrafiltration
V_f	final volume of the concentrate at a concentration C_f
V_i	initial volume of solution at a concentration C_i
V_p	volume of permeate at a concentration C_p
VRF	volume reduction factor

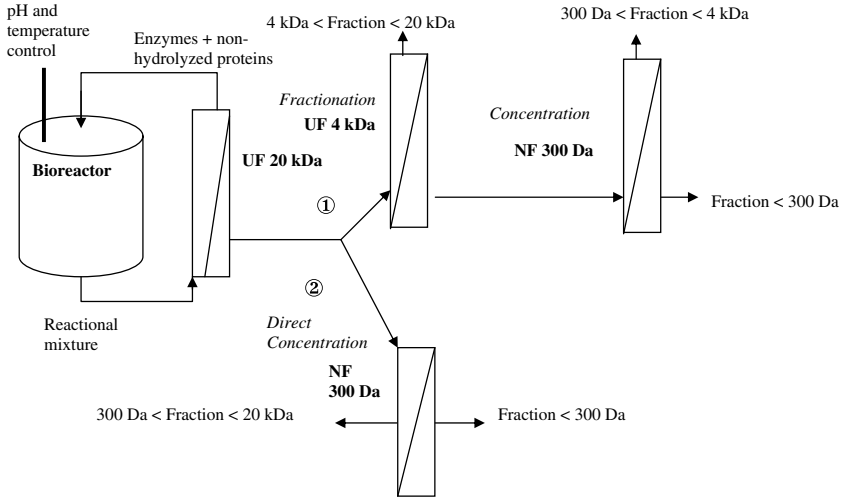

Fig. 1. A MEB for the concentration and the fractionation of marine peptides.

- Possible purification of solutions (desalination, partial deodorization…) by NF in a diafiltration mode (Simon, Vandanjon, Levesque, & Bourseau, 2002; Vandanjon, Bourseau, & Jaouen, 2005).

The objective of this paper is to evaluate the performances of ultrafiltration (UF) and nanofiltration (NF) membranes in the case of a future production, in batch or continuous mode, of blue whiting peptide hydrolysates in a membrane enzymatic bioreactor (MEB). Two different configurations are possible: direct concentration of peptides or fractionation before concentration (Fig. 1).

2. Material and methods

2.1. Raw material

Blue whiting is mainly used for the production of fish meal and fish oil. Recently it has been used for the production of surimi (Trondsen, 1998). Good quality surimi can be made from fresh chilled fish and therefore, most of the process takes place onboard the fishing vessels. The surimi process onboard vessels is rather expensive due to extensive water usage and increasing oil prices. Experiments are in progress to overcome this problem of prolonging the storage period to make on-land processing for human consumption possible.

Novel methods for protein isolation from pelagic fish species have been put forward, where finely minced muscular proteins are dissolved at high pH (around pH 11) and insoluble material removed using centrifugal techniques. The isolated muscular proteins are thereafter collected by decreasing the pH to about pH 5.6, which is the approximate isoelectric point for muscular proteins (Batista, 1999; Hultin et al., 2000).

These methods can give better yield than traditional or improved surimi processes and possibilities for on-line further processing with enzymatic methods such as protein hydrolyses.

Trials with hydrolysates of blue whiting protein isolates using above methods have shown interesting antiproliferative activity in a recent publication (Picot et al., 2006).

Proteins to be hydrolyzed are obtained from frozen blue whiting (PRIMEX Company, Iceland), headed and gutted, minced and prewashed with three parts of tapwater (with low content of carbonates) and centrifuged at 3000*g*. Washing water was removed using an Alfalaval NX409 decanter centrifuge whose flow was adjusted at about 1.2 m^3/h. After the mince was dissolved in six parts of water at pH 10.8, solid phase was removed using a disk centrifuge at 6000*g*. The pH was adjusted to the isoelectric point at pH 5.6 and the precipitate collected using a decanter centrifuge at 3000*g*. In disk stack centrifuge and decanter centrifuge, the dwelling time is only a matter of seconds.

The precipitated proteins were then hydrolyzed at 55–57 °C for 106 min with Protamex (Novozymes A/S Denmark) 98 g/1000 kg and 20 ml/1000 kg of alkalase (Novozyme A/S Denmark). The enzymes were added simultaneously to the protein isolate when reaction temperature was reached. The optimum pH of Protamex is between pH 7 and 8 but at pH 5.6 the activity is above 90% at 50 °C. The alkalase is about at 70–80% of its maximum performance at pH 5.6.

The resulting suspension was centrifuged at 3000 g for 10 min and the supernatant collected. The supernatant was either used directly (45 g dry matter/l) or was freeze-dried for further measurements.

2.2. Membrane processes

A UF/NF Microlab40 pilot plant (VMA Industrie) (Fig. 2) of a maximum capacity of 5 l (launching tank 4.3 l + dead volume 700 ml) was used.

The pilot plant was equipped with tubular organic membranes of a surface area of 0.033 m^2 (manufacturer PCI):

MT04 in Polyamid/Polyethersulfon (PA/PES) of MWCO 300 Da (NF),
MTP04 in modified Polyethersulfon (PES) of MWCO 4 kDa (UF of intermediate MWCO),
MT68 in Polysulfon (PS) of MWCO 8 kDa (UF of intermediate MWCO),
MT120 in Polysulfon (PS) of MWCO 20 kDa (UF of high MWCO).

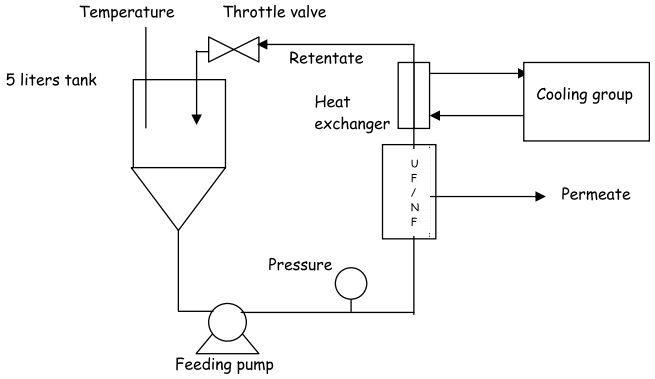

Fig. 2. Ultrafiltration/nanofiltration pilot plant.

2.3. Techniques of analysis

The inorganic-matter contents of the raw hydrolysate and of the various fractions have been characterized by static and dynamic light scattering measurements. The latter were made using an ALV-5000 multi-bit multi-τ correlator in combination with a Malvern goniometer and a Spectra Physics laser operating with vertically polarized light with wavelength $\lambda = 532$ nm. The range of scattering wave vectors covered was $3.0 \times 10^{-3} < q < 3.5 \times 10^{-2}$ nm^{-1} with $q = (4\pi n/\lambda)\ \sin(\phi/2)$, ϕ being the angle of observation and n the refractive index. The temperature was controlled by a thermostat bath and was set at 20 °C.

Light scattering involves measuring the amount of light scattered by a solution at some angle relative to the incident laser beam. The scattering intensity as well as a cross-correlation function $G_2(t)$ is measured as a function of angle from 10° to 150°. In dynamic light scattering, one measures the time dependence of the light scattered from a very small region of solution, over a time range from tenths of a microsecond to milliseconds. These fluctuations in the intensity of the scattered light are related to the rate of diffusion of molecules in and out of the region being studied (Brownian motion), and the data can be analyzed to directly give the diffusivity D (m^2/s) of the particles (or molecules) doing the scattering. When multiple species are present, a distribution of diffusivity is seen. Traditionally, rather than presenting the data in terms of diffusivity, the data are processed to give the "size" of the particles (radius or diameter). The relation between diffusivity and particle size is based on theoretical relationships for the Brownian motion of spherical particles, originally derived by Einstein. The "hydrodynamic diameter" or "Stokes radius", R_h, derived from this method is the size of a spherical particle that would have a diffusion coefficient equal to that of the protein, and the data is commonly presented as the fraction of particles as a function of their diameter. The Stokes–Einstein relation links D and R_h as follows:

$$D = \frac{kT}{6\pi\eta R_h}$$

Measurement of the proteins and peptides has been done by the modified reaction of Biuret which is fast and easy to use (colorimetric reaction) (Umemoto, 1966). A standardization of this method by measurement of total nitrogen with the Kjeldahl method (NTK) (ISO International Norm, 1984) has been carried out. The approximative content of proteins was obtained by multiplying the quantity of nitrogen by 6.25.

Chemical oxygen demand (COD) was measured by the standardized method with $K_2Cr_2O_7$ (ISO International Norm, 1986).

Size exclusion high performance liquid chromatography (SE-HPLC), with a separation column Superdex Peptides HR30, was used for the separation of peptides between 1000 and 7000 Da (standards of 1000, 4000 and 7000 Da).

Viscosity of solutions was determined with a rheometer Physica MCR500 using a mobile CC28.7 of 28.72 μm diameter and 1 μm concentricity.

2.4. Experimental procedure for membrane experiments

Before measuring initial flow with water, the membranes were conditioned by means of an alkaline solution P3-Ultrasil 11 (natrium hydroxyde at 5 g/l with emulsifying agents, 40 °C, 30 min) then nitric acid (3 g/l, 20 °C, 20 min) according to the recommendations of the membranes manufacturer.

2.4.1. Tests in total recycling mode of the retentate and the permeate

Experiments were carried out with 2 l of a solution at a concentration ranging from 4.8 to 11.4 g peptide/l (obtained by dilution of the mother solution at 45 g/l or by dissolution of the freeze-dried powder in pure water at moderate temperature).

The temperature was maintained at 15 °C (cooling group Fisons), tangential velocity was 2.5 m/s ($Re = 11{,}000$) and the pressure was selected between 8 and 35 bars according to the optimal range of use of the membranes. Steady flux, COD and peptides rejection rates (RR) were measured for each change of pressure.

RR was calculated as the ratio:

$$1 - \frac{\mathrm{COD}_{\mathrm{permeate}}}{\mathrm{COD}_{\mathrm{retentate}}} \text{ for the COD} \quad \text{and}$$

$$1 - \frac{\mathrm{NTK}_{\mathrm{permeate}}}{\mathrm{NTK}_{\mathrm{retentate}}} \text{ for the peptides.}$$

2.4.2. Tests in concentration mode

The concentration of 4 l of peptide solution with an initial concentration $C_i = 45$ g/l was carried out by NF under the following conditions: tangential velocity = 2.5 m/s, temperature = 40 °C, pressure = 35 bars. The evolution of the permeate flux and peptide RR were measured in the course of concentration.

2.4.3. Cleaning procedure

After each test in recycling or concentration mode, a chemical cleaning of the membranes was carried out until the initial water flux was recovered.

Each sequence of cleaning is composed of a step of alcaline washing (pH 12) with Ultrasil 11 (with or without the addition of H_2O_2) for 30 min at 50 °C, followed by rinsing with water until neutrality, cleaning with nitric acid (pH 1.2) for 20 min at 20 °C, and then rinsing with water again.

3. Results and discussion

3.1. Ultrafiltration with a high MWCO membrane (20 kDa)

The UF can be considered for the separation of peptides and non-hydrolyzed proteins or proteolytic enzymes in the case of a MEB (Pouliot, Gauthier, & L'heureux, 2000). Former studies (Jaouen, 1989; Jaouen, Morancais-bothorel, & Quemeneur, 1992) showed that UF membranes of MWCO close to 20 kDa retain proteins satisfactorily if the membrane material is well chosen. On the basis of these results, a membrane in PS of MWCO 20 kDa whose water permeability is $L_p = 35$ l/h/m^2 and peptide transmission is high was selected. Its performances, measured on a 11.4 g peptide/l solution, are summarized in Table 1.

It is worth quoting that steady flux at 10 bars reaches nearly 100 l/h/m^2 at 15 °C. These values, though satisfactory, can be almost doubled if ultrafiltration is carried out at 40 °C.

The rejection of nitrogenized matter is close to 30% whatever the pressure applied. That means that a considerable part of the peptides (undoubtedly those of high MW) present in the initial mixture are retained.

In addition, it should be noticed that the cleaning of membrane MT120 was particularly difficult. Two cleaning sequences made it possible to recover only 70% of the initial flux. Additional cleaning sequences did not generate the recovery of more than 85% of the initial flux. It can be concluded from these results that the regeneration of the membrane must be absolutely improved by using more specific cleaning reagents (Bird & Bartlett, 2002). It will also be possible to choose another membrane material whose adsorption rate is low and resistance to a tough cleaning process is high.

3.2. Ultrafiltration with intermediate MWCO membranes (MWCO 8 kDa and 4 kDa)

3.2.1. Performances of membranes

Many studies showed that the biological activity of peptides are related to their MW (Berot, Popineau, Compoint, Blassel, & Chaufer, 2001; Guerard, Dufosse, De la broise, & Binet, 2001; Guerard, Guimas, & Binet, 2002; Jeon et al., 1999). Small-size peptides often present an intense biologi-

Table 1
Performances of the membrane MT120 (20 kDa) in total recycling mode for different pressures ($\mathrm{NTK}_{\mathrm{retentate}} = 1820$ mg/l; $\mathrm{COD}_{\mathrm{retentate}} = 12.90$ g/l)

Pressure (bars)	Flux (l/h/m^2) at 15 °C (or 40 °C)	$\mathrm{NTK}_{\mathrm{permeate}}$ (mg/l)	$\mathrm{RR}_{\mathrm{nitrogen}}$ (%)	$\mathrm{COD}_{\mathrm{permeate}}$	$\mathrm{RR}_{\mathrm{COD}}$ (%)
8	$J_{2h} = 75$	1281	29.6	10.46 g O_2/l	18.9
10	$J_{2h} = 95$	1280	29.7	10.28 g O_2/l	20.4
12	$J_{2h} = 100$ (196 at 40 °C)	1260	30.8	9.82 g O_2/l	23.9

Table 2
Performances of MWCO 8 kDa and MWCO 4 kDa membranes in total recycling mode for different pressures

Pressure (bars)	Flux (l/h/m^2) at 15°C	$RR_{Nitrogen}$ (%)	RR_{COD} (%)
MT68 (MWCO 8 kDa)			
8	$J_{2h} = 25$	79.3	–
9	$J_{2h} = 36$	87.6	–
10	$J_{2h} = 46$	86.9	58
MTP04 (MWCO 4 kDa)			
12	$J_{2h} = 29$	78.7	–
16	$J_{2h} = 46$	81.5	–
20	$J_{2h} = 57$ (105 at 40 °C)	80.7	78.6

cal activity (Fruitier, Garreau, Lacroix, Cupo, & Piot, 1999). Thus, it seems interesting to select purified fractions of peptides of close MW in order to better target their action (Hajjou, Smine, Guerard, & Le gal, 1995).

UF membranes of MWCO ranging between 1 and 10 kDa seem to correspond to this type of peptide fractionation (Pouliot, Wijers, Gauthier, & Nadeau, 1999).

Performances, in term of permeation flux and retention, of both the UF membranes MT68 of MWCO 8 kDa ($L_p = 16.7$ l/h/m^2/bar) and MTP04 of MWCO 4 kDa ($L_p = 10.8$ l/h/m^2/bar) have been tested with a solution at 4.8 g peptide/l. Results are presented in Table 2.

Fluxes are relatively moderate for the two membranes but they can be clearly improved while working at 40 °C (105 l/h/m^2 at 20 bars with membrane MTP04).

As expected, the reduction in COD is higher for the membrane of MWCO 4 kDa (78.6%) than for the membrane of MWCO 8 kDa (58%), as the total rejection rate of peptides is of the same order of magnitude (80–85%) for the two membranes. It can be supposed that the small differences of rejection of peptides according to their MW are not detected by the NTK method.

In order to link the rejection rate of peptides according to their MW, the HPLC chromatograms of the raw hydrolysate and of the two UF membranes permeates (MWCO 4 kDa and MWCO 8 kDa) have been established. These chromatograms are shown in Fig. 3. They clearly indicate that the two membranes behave differently. Whereas, the two membranes completely retain peptides of high MW, the maximum molecular weights they retain correspond quite well to the nomimal membranes MWCO given by manufacturer (4000 and 8000 Da). It also appears that the lower 4000 Da MWCO membrane better retains the peptides of MW up to 800–1000 Da (peptides of lower MW seem retained in the same way).

This difference in the behaviour of the two membranes can be (roughly) quantified by considering the assumption that the response factors of the various classes of molecules are quite the same (the response factor being the ratio of the surface area under the chromatogram to the mass of eluted peptides). Then, the total retention rate of the molecules for a given membrane can be estimated from the ratio of the total surface area under the permeate to the total surface area under the retentate:

$$RR\ (\%) = [1 - (\text{Permeate area}/\text{Retentate area})] \times 100.$$

That leads to RR = 68% for the MWCO 8 kDa membrane and RR = 81% for the MWCO 4 kDa membrane. These values are appreciably different from those obtained by NTK measurements, in particular for the MWCO 8 kDa membrane (86.9% at 10 bars).

Following the same assumption, a retention has been calculated for three classes of molecules corresponding respectively to MWs of 1000, 4000 and 8000 Da (see Table 3):

$$RR\ (\%) = [1 - (\text{Permeate peak height}/\text{Retentate peak height})] \times 100.$$

Table 3 suggests that HPLC profiles can be an interesting tool to calculate the retention of molecules by a membrane with respect to the size of the molecule. These results also indicate it would be possible to separate peptides of MW higher than 7000 Da from peptides of low MW (lower than 1000 Da) with membranes MT68 or MTP04 functioning in a diafiltration mode. Moreover, the combination of

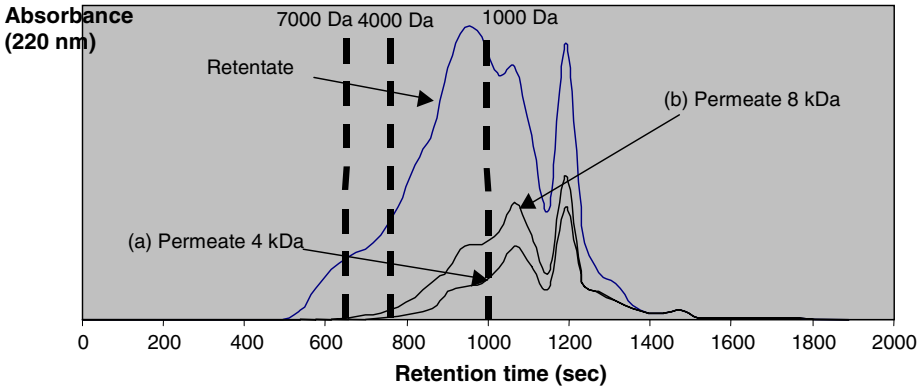

Fig. 3. HPLC profiles of the retentate (4.8 g/l) and the permeates fractionated by membranes MWCO 4 kDa (a) (at 20 bars) and MWCO 8 kDa (b) (at 10 bars) – (measurements were duplicated).

Table 3
Estimate of the RR of membranes MT68 (8 kDa) and MTP04 (4 kDa) according to the MW of the solutes (MWs are determined from the correlation between retention times in the column and MW of the standards)

MW (Da)	$RR_{peptides}$ MT68 (%)	$RR_{peptides}$ MTP04 (%)
ca. 7000	98	100
ca. 4000	92	99.5
ca. 1000	71	78

membranes with physicochemical processes such as selective precipitation by modification of ionic strength or pH (Bothorel-morancais, 1992) or the use of charge effects (Ebersold & Zydney, 2004) could improve the separation in the range 1000–7000 Da.

3.2.2. Dynamic light scattering measurements

A solution containing 2 g/l of the MT68 (MWCO 8 kDa) retentate in distilled water was measured directly and after filtration through a 0.45 μm filter. The total scattering intensity and the intensity cross-correlation function ($G_2(t)$) were determined over a range of scattering angles between 10° and 150°. From these data, the q-dependence of the experimental intensity I_r can be determined, q being the scattering wave vector. Fig. 4 shows I_r with respect to q for both unfiltered and filtered (0.45 μm) hydrolysates.

The large particles are visible in the unfiltered solution. The solution was easily filtered through 0.45 μm indicating that the concentration of the large particles was low. The filtered solution (triangles on Fig. 4) showed much less angle dependency than the unfiltered solution (circles), indicating smaller particles. Mean hydrodynamic radius of the filtered solution was determined: radius was determined in the filtered solution over a range of q-values and it was independent of q with an average value of 34 nm.

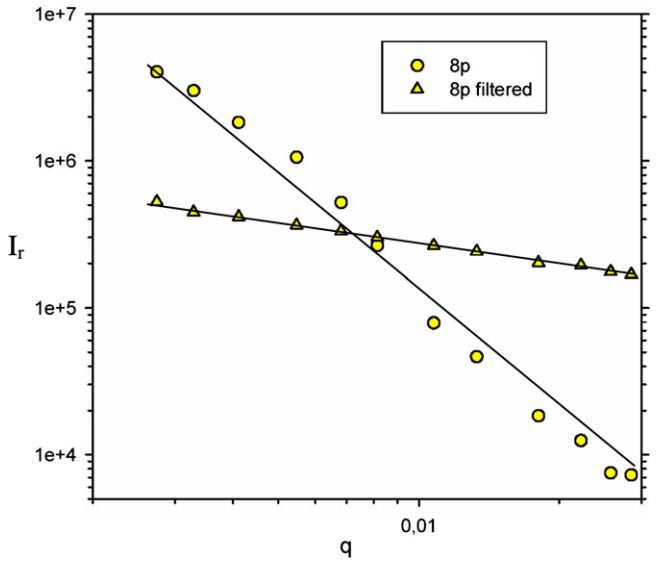

Fig. 4. Angle dependency of the experimental scattered intensity I_r of filtered and unfiltered hydrolysates.

The more the angle dependence, the bigger the particles predominant in the scattering will be. However, since the scattering of big particles is much greater than the small particles, a small proportion of large particles can have a predominant effect on the scattering intensity, even in very low concentration. Upon filtration a small proportion of large particles was removed with 0.45 μm filter.

The MT68 permeate was measured as well but showed almost no scattering, indicating very small particles of the size of amino acids or dipeptides.

In conclusion, the solution contains one distribution of particle sizes in the retentate (around 34 nm) with only a small proportion of "big" particles (greater than 0.45 μm). Comparison between HPLC and light scattering experiments would require relating the diffusion coefficient to the molecular weight through some adequate model (for instance the Mark–Houwink–Sakadura equation). Getting information from light scattering on size distribution and not only on mean radius through additional measurements would also be interesting.

Results were disappointing in as much as no information on the size of particles in the permeate was obtained, and therefore, giving no insight on the retention rate of the molecules. This is due to the nature of the solution (a complex mixture of salts and peptides) and to the small size of the molecules in the permeate.

3.3. Nanofiltration

3.3.1. Total recycling mode

In order to concentrate a mixture of peptides obtained by enzymatic hydrolysis or to concentrate the peptide fractions of low MW, membranes with low MWCO must be used (Afonso & Borquez, 2002; Garem, 1995; Martin-orue, Bouhallab, & Garem, 1998).

A NF membrane of MWCO 300 Da ($L_p = 2.97$ l/h/m^2/bar) was selected to carry out the concentration of the peptide mixture. It was initially tested in total recycling mode of the retentate and the permeate, in the same way as the procedures described previously (Sections 1 and 2. The results obtained are presented in Table 4.

Steady fluxes are satisfactory for a NF membrane, and become particularly interesting at 40 °C (157 l/h/m^2 at 35 bars).

The rate of decrease in COD is high, which is interesting in so far as the NF permeate is intended to be rejected without any additional treatment.

Table 4
Performances (steady fluxes, nitrogen retention rate, COD retention rate) of the NF membrane 300 Da MT04 in total recycling mode for various pressures

Pressure (bars)	Flux (l/h/m^2) at 15 °C	$RR_{nitrogen}$ (%)	RR_{COD} (%)
25	$J_{2h} = 40$	98	–
30	$J_{2h} = 47$	92	–
35	$J_{2h} = 58$ (157 at 40 °C)	100	97.8

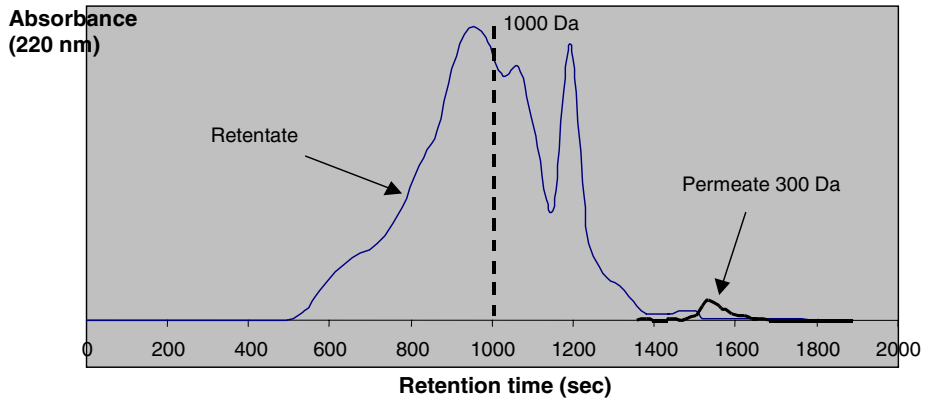

Fig. 5. HPLC profiles of the retentate (4.8 g/l) and the permeate obtained by the NF membrane MT04 300 Da (at 35 bars).

With regard to the retention of peptides, analyses by HPLC confirm the results of Table 4. It is observed on the chromatogram of Fig. 5 that the retention of the solutes of size higher than 1000 Da is total. However, it is difficult to draw a conclusion about the retention of the solutes of size lower than 1000 Da (Pontalier, Ismail, & Ghoul, 1997).

Thus, the membrane MT04 seems well-suited for the concentration of peptides. In addition, it can be easily cleaned (nearly 85% of recovery of the initial flowrate after simple rinsing with water), which shows this membrane is not sensitive to fouling. This characteristic is important because the membrane has to preserve its performances in spite of the high peptide concentrations that will be encountered during effective concentration.

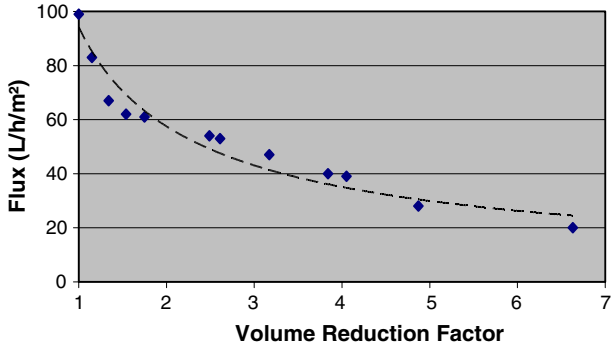

Fig. 6. Evolution of flux (35 bars, 40 °C) according to the VRF (membrane MT04).

3.3.2. Concentration mode

A volume $V_i = 3.9$ l of solution with an initial peptide content $C_i = 45.3$ g/l has been concentrated with the NF membrane MT04 of MWCO 300 Da previously tested in total recycling mode.

The operating conditions are a velocity of 2.5 m/s, a pressure of 35 bars and a temperature of 40 °C. The RR of peptides is almost always constant and high (97–98%) and the fluxes have been measured at various volume reduction factors (VRF) (Fig. 6).

From an initial volume $V_i = 3.9$ l of solution, $V_f = 0.55$ l of concentrate ($C_f = 296.6$ g peptide/l) and $V_p = 3.1$ l of permeate ($C_p = 2.93$ g/l) were recovered.

The global mass balance of peptides over the whole duration of the concentration can be expressed as:

$$V_i \cdot C_i = V_f \cdot C_f + V_p \cdot C_p.$$

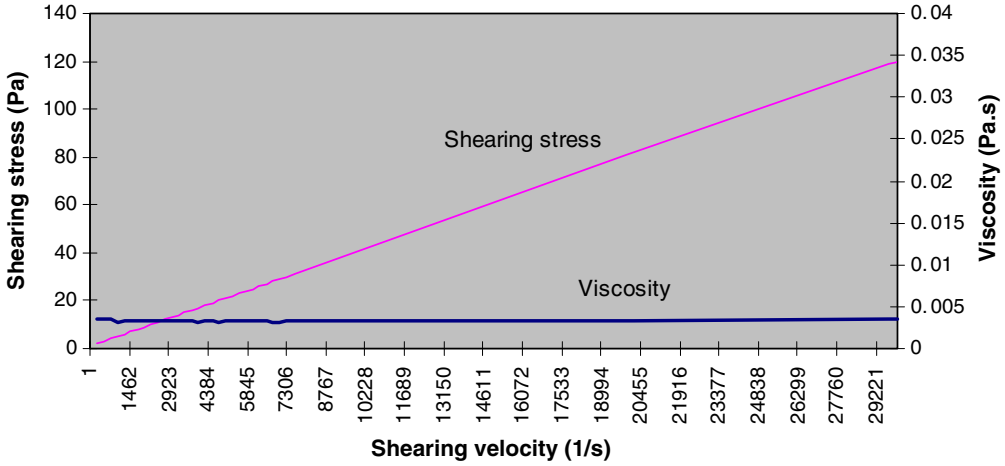

Fig. 7. Evolution of the shearing stress and viscosity according to the shearing velocity at 40 °C (solution at 300 g/l).

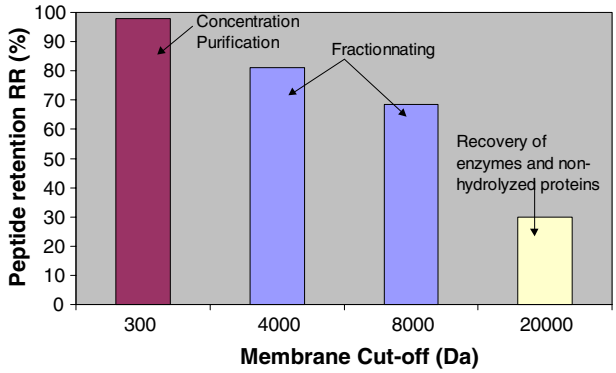

Fig. 8. Peptide retention of three UF membranes and one NF membrane.

This assessment being checked (with a margin of 2.6%), the volume reduction factor (VRF), the mass concentration factor of peptides (MCF) and the percentage of peptides lost in permeates (PL) can be calculated:

$$\mathrm{VRF} = V_i/V_f = 7$$

$$\mathrm{MCF} = V_i \cdot C_i/V_f \cdot C_f = 6.54$$

$$\mathrm{PL} = (V_p \cdot C_p/V_i \cdot C_i) \times 100 = 5\%.$$

These values are very encouraging because the final peptide contents of the concentrate is almost 300 g/l with low peptide losses in the course of concentration. These results could still undoubtedly be improved because the viscosity of the concentrate was measured to only 3×10^{-3} Pa s (at 40 °C), which will make it possible to push the concentration further, more especially as the fluid has a Newtonian behaviour (Fig. 7).

4. Conclusions

Performances of three UF membranes and one NF membrane were tested in order to determine their use in a process integrating an enzymatic reactor and some membrane filtration steps for the treatment of blue whiting peptide solutions. According to their performances in terms of peptide retention, these membranes can be used for various purposes (Fig. 8).

The UF membrane of high MWCO (20 kDa) is well-suited for the separation of peptides and non-hydrolyzed proteins. Now, the regeneration conditions of this membrane must be improved.

On the other hand, the UF membranes of intermediate MWCO (4 and 8 kDa) seem promising for the fractionation of peptides, since their flux and their retention are good.

By the end, the MWCO 300 Da NF membrane allows peptides to be concentrated in good conditions of both fluxes and recovery rate. Nevertheless, it could be interesting to test an NF or reverse osmosis membrane with a lower MWCO in order to retain the totality of the molecules. The risk is then to concentrate salts and also to need an additional desalination step (Cros, Bourseau, Vandanjon, Quemeneur, & Jaouen, 2003).

Future work will consist in testing the biological activities of each fraction. An improvement of the anti-oxydative, anti-radical or anti-tumoral properties of the fractions containing peptides of low molecular weight will be evaluated. If the results are encouraging, the process of concentration and fractionation will be optimized from a technical-economic point of view.

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