EFFECTS OF PECTINASE IMMOBILIZATION DURING HOLLOW FIBER ULTRAFILTRATION OF APPLE JUICE

MARÍA E. CARRIN, LILIANA CECI and JORGE E. LOZANO[1]

PLAPIQUI (UNS-CONICET)
12 de Octubre 1842
(8000) Bahía Blanca — Argentina

Accepted for Publication May 16, 2000

ABSTRACT

The immobilization of commercial pectinases by physical adsorption on hollow fiber ultrafiltration (HFUF) membranes was studied in view of its possible application in fruit juice clarification. The optimal pH during immobilization and washing was investigated, as well as the effects of enzyme and pectin concentrations and retentate flow on the operation. Results indicated that permeate flux was not initially increased when pectin solution or apple juice was ultrafiltred through pectinase immobilized on HFUF membranes. However, enzyme immobilization greatly extended the membrane operation, by keeping permeate flux constant during prolonged periods at a reasonable yield.

INTRODUCTION

The objectives of clarified apple juice concentration are mainly to reduce costs and to increase shelf-life. Before concentration, pressed juice must be clarified. A conventional clarification process includes hydrolysis of pectin and starch with specific enzymes, flocculation of turbidity with clarifying agents (bentonite, gelatin and/or silica-sol) and filtration through plate and frame or vacuum Oliver-type filters, in order to eliminate insoluble solids and destroy pectic substances. This process involves several separate operations, which are hard work and time consuming.

The application of ultrafiltration (UF) as an alternative to conventional processes for clarification of apple juice was demonstrated by Heatherbell *et al.* (1977). However, the acceptance of UF in the fruit processing industry is not yet completed, because there are problems with the operation and fouling of membranes. During UF two fluid streams are generated: the ultrafiltred solids

[1] Corresponding author. PLAPIQUI (UNS-CONICET), Camino La Carrindanga Km 7, C.C. 717, 8000 BAHÍA Blanca, Argentina.

Journal of Food Process Engineering 23 (2000) 281-298.

free juice (permeate), and the retentate with variable content of insoluble solids which, in the case of apple juice, are mainly remains of cellular walls and pectin. Permeate flux (J_p) results from the difference between a convective flux from the bulk of the juice to the membrane and a counter diffusive flux or outflow by which solute is transferred back into the bulk of the fluid (Cheryan 1986). As a result of this mechanism, pectin and other large solutes are brought to the membrane surface by convective transport during the HFUF of apple juice. Consolidation of this gel layer on the HFUF membrane has a drastic effect on the performance of the operation.

Commercial pectic enzymes, or "pectinases", are used in apple juice manufacturing to depectinize pressed juices in order to remove turbidity and prevent cloud-forming (Grampp 1976). The available commercial pectinase preparations used in apple processing generally contain a mixture of pectinesterase (PE), polygalacturonase (PG) and pectinlyase (PL) enzymes (Dietrich *et al.* 1991). Endo-Polygalacturonase and Pectinlyase among others, have been immobilized on different organic and inorganic supports, with uneven results (Pifferi and Prezious 1987; Spagna *et al.* 1995). Enzyme immobilization by physical adsorption is a simple and well established technique (Gekas 1986; Szaniawski 1996). However, immobilized pectinase enzymes are not currently available commercially. In view of the high molecular weight and viscosity of pectin, the use of immobilized pectinase in most fruit processing applications may be rather limited (Kulp 1975). Despite the different types of supports and reactor configurations proposed for a continuous performance of enzymatic reaction, immobilization of enzymes on micro, or ultrafiltration membranes, appear as interesting alternatives for treating cloudy fruit juices (Alkorta 1995). The use of pectinase immobilized on ultrafiltration membranes is expected to hydrolyze the pectin to lower molecular weight species (mainly anhydrogalacturonic acid, AGA) at the membrane-permeate interface, resulting in an increase of the permeate flux or at least an extension of the membrane operation without cleaning. Glutaraldehyde has been used as enzyme molecules cross-linking and spacing agent (Atallah and Hultin 1977; Synowiecki *et al.* 1982).

The objective of the present work is to determine the best conditions for commercial pectinase immobilization on polysulfone hollow fiber; the capability of physically immobilized enzyme to hydrolyze the gel layer; and the influence of the enzyme layer on the resistance to permeate flux. Alternatively, the influence of glutaraldehyde on the immobilization process will be also verified.

MATERIAL AND METHODS

The pectin samples used were: (A) commercial HMP citrus pectin obtained from Braspectin (Gelfix, Brazil), with anhydrogalacturonic acid content (AGA)

62.0% and esterification degree (ED) 54.2%; and (B) Genu pectin type B from Hercules Co. (150 grade USA-SAG type B rapid set). Pectin dispersions were prepared as suggested by the IFT Committee (1959) Method 5-54. Pectin concentration (C_p) was varied in the range 1.2 to 10 mg/L. Malic acid (4g/L) and sucrose were added to 12°Brix soluble solids, simulating a clarified apple juice at different pH values. Galacturonic acid was quantitatively determined by the colorimetric phenyl-phenol method (Blumenkrantz and Asboe-Hansen 1973). Standard error (S) for determination of galacturonic acid on permeate flux was 2.85 mg/L.

Enzyme (Röhapect D5S) was diluted in 0.1M acetate buffer at pH = 4.6 to 5.6 and 0.1M phosphate buffer at pH = 7. Free enzyme maximum activity was at pH = 4.6 and 50C and the pectinase was inactivated at pH = 7.0 (Ceci and Lozano 1998). Apple juice was extracted from Granny Smith apples by following the experimental procedure described in a previous work (Toribio and Lozano 1979).

Immobilization Steps

Hollow fiber was treated with NaOH 0.1 N by reverse flux for 2 h, and soaked with distilled water for 1 h in reverse flux and ½ h in normal flux. Enzyme solution at different concentrations (C_e = 1 to 60 g/L) and pH values (pH_e = 3.6 to 5.6) was then pumped through the fiber (normal flux) for 2 h, to assure immobilization phenomena occurred. Finally, the hollow fiber with active immobilized pectinase, or immobilized pectinase membrane (IPM), was rinsed with buffer solution at the required pH (pH_w) for 10 min. Szaniawski (1996), working with lower enzyme concentrations, found that most of the pectinase was immobilized by adsorption during the first few minutes. Enzymes were immobilized under two operational conditions: Reynolds number (Re) = 3528 (Retentate flow, Q = 0.1 L/min; transmembrane pressure, ΔP_{TM} = 0.25 $kg_f cm^{-2}$); and Re = 3411 (Q = 0.04 L/min; ΔP_{TM} = 0.085 $kg_f cm^{-2}$). For some experiments, up to 0.4% glutaraldehyde was added to the enzyme solution, previous to immobilization.

Pilot Plant Ultrafiltration Unit

A pilot scale UF unit (Amicon model DC50P) with a single hollow fiber cartridge (0.45 m^2) was used. The unit had a 40 L storage tank, sanitary stainless steel pump and pipes, a flow reversing valve, pressure control and streams flow meters for permeate and retentate fluxes. The main driving force of UF is the transmembrane pressure (ΔP_{TM}), which in the case of HFUF can be defined as (Constenla and Lozano 1996):

$$\Delta P_{TM} = (P_i + P_o) / 2 - P_{ext} \quad (1)$$

where P_i = pressure at the inlet of the fiber, P_O = outlet pressure, and P_{ext} = pressure on the permeate side. The unit was operated in batch mode and process parameters were juice volume (V_o) = 27 L; temperature (T) = 50C; Q = 9.4 L/min; ΔP_{TM} = 0.6 kg_f cm^{-2}. The HF cartridge was cleaned after each experiment by rinsing with water, NaOH solution and final rinsing with water. No systematic increase in the resistance of the HF cartridge to water flow after cleaning was observed, indicating the membrane cleaning procedure was effective in removing both the foulant and the immobilized enzyme. Permeate was continuously returned to reservoir. Permeate samples (2 mL) were collected at selected intervals.

Lab Unit

A lab HFUF unit with a single hollow fiber (cutoff 50,000; 46 cm length; 0.11 cm diameter) including a peristaltic pump, differential manometers, flowmeter and constant temperature recirculating bath operated in batch mode was used (Fig. 1). Retentate initial volume (V_o) per assay was 0.5 L. Temperature was kept constant at 50C.

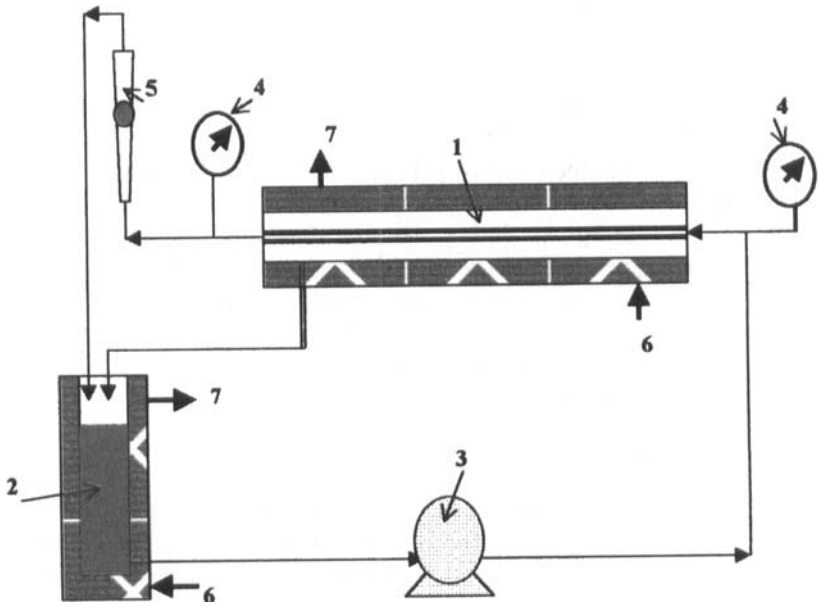

FIG. 1. SINGLE FIBER UF SYSTEM: (1) POLYSULFONE HOLLOW FIBER; (2) PECTIN OR ENZYME SOLUTION RESERVOIR; (3) PERISTALTIC PUMP; (4) MANOMETERS; (5) FLOWMETER; (6) THERMOSTATIZED WATER IN; (7) THERMOSTATIZED WATER OUT.

Three types of experiments were performed: (1) pectin solution ultrafiltration (UF) through a clean hollow fiber, or control fiber; (2) pectin solution UF through a hollow fiber with active immobilized pectinase (IPM); and (3) UF of pectin solution through inactive immobilized pectinase. Results are given as a reference permeate flux (J_r):

$$J_r = J^o - J_p \tag{2}$$

where J^o is the initial flux of water permeating through a clean fiber; and J_p is the permeate flux. A new clean hollow fiber was used in each experiment. Standard error (S) for permeate flux determination was 0.01 L/min cm^2.

Scanning Electron Microscopy

Assayed fibers were cut in rings at determined distances from the inlet, fixed with OsO_4 and ruthenium red, then dehydrated and embedded with Spurr resin. Fiber "rings" were finally cut with a LKB microtome and observed through a TEM JEOL 100 CXL microscope.

RESULTS AND DISCUSSION

TEM Studies

Figure 2 shows a TEM micrograph of a clean fiber (a), after enzyme immobilization (b), and after pectin filtration (c) through the immobilized enzyme. As Fig. 2b shows, a primary adsorption layer of enzyme on the membrane skin was produced after immobilization, which originates an additional resistance to permeate flux. Finally in Fig. 2c it can be observed that during HFUF of pectin solution a gel layer was effectively developed on the previously immobilized enzyme.

Flux Determinations

Figure 3 and 4 show the evolution of reference permeate flux with time, under the different procedures assayed. The J_r values for a membrane without enzyme (WE) are in general lower than after pectinase immobilization by adsorption (that means higher permeate flux, closer to water permeation rate). It was also verified that in general, J_r proved more stable (reaching a plateau) when IPM was used. The influence of enzyme content on J_r, at different C_p values and constant Q was plotted in Fig. 3. Results showed that only at the lower pectin concentration and elevated C_e, an improvement on permeate flux was obtained. On the other hand, increasing C_p, enzyme immobilization had a

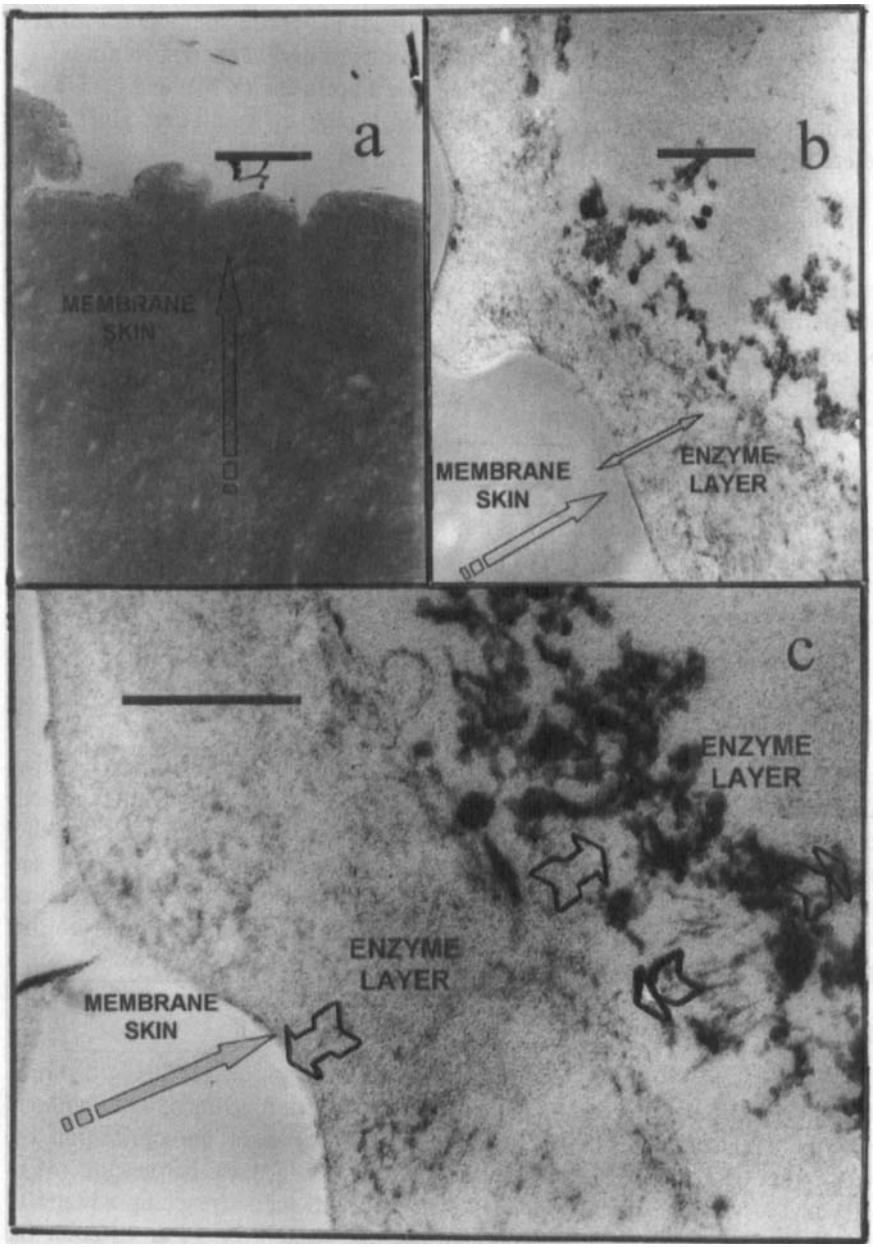

FIG. 2. TEM MICROGRAPHY OF (a) A CLEAN HFUF MEMBRANE; (b) AFTER PHYSICAL IMMOBILIZATION OF A COMMERCIAL PECTINASE; AND (c) AFTER THE ULTRAFILTRATION OF A PECTIN SOLUTION. BAR = 0.625 μm.

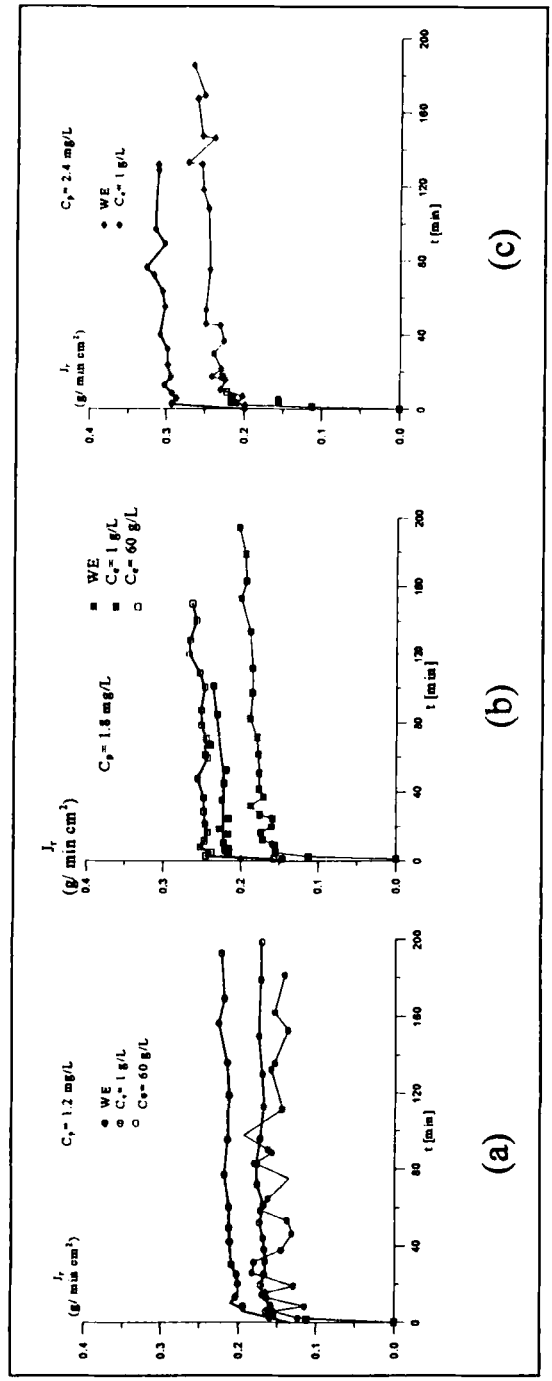

FIG. 3. REFERENCE PERMEATE FLUX (J_r) VS TIME, WITHOUT (WE) AND WITH TWO LEVEL OF IMMOBILIZED ENZYME CONCENTRATION

Pectin concentration: (a) C_p = 1.2 mg/L. (b) C_p = 1.8 mg/L; and (c) C_p = 2.4 mg/L; Q = 0.1 L/min.

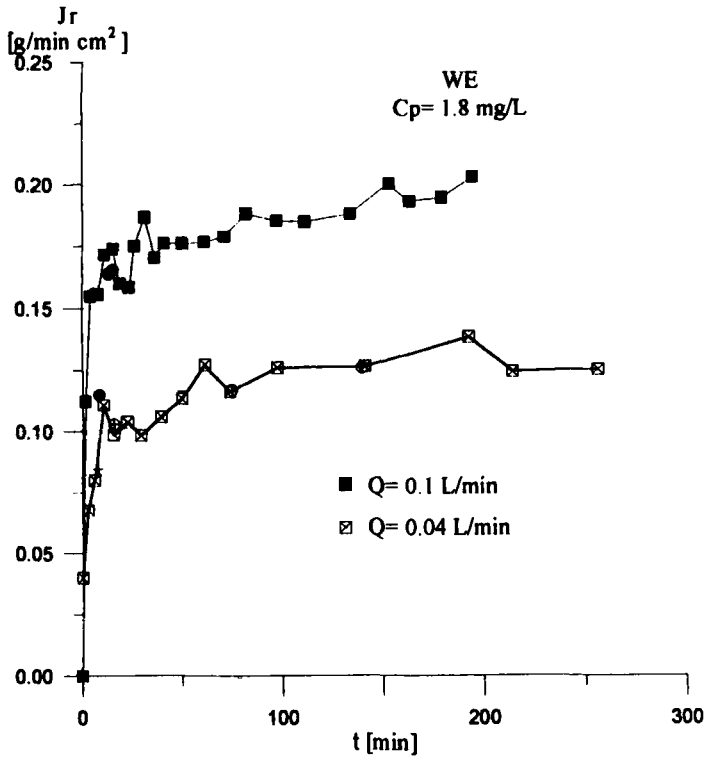

FIG. 4. J_r AS A FUNCTION OF TIME, WITH RETENTATE FLOW (Q) AS A PARAMETER

negative effect on J_p. When Q is reduced to laminar conditions (Fig. 4) permeate flux through WE improves more than 60% (J_r is closer to J^o). The same behavior was observed when IPM was assayed. This behavior was attributable to a lower drag force, increasing both the rate of formation of the gel layer and the residence time of pectin molecules on the immobilized pectinase.

Figure 5 shows a very interesting result associated with the influence of pH: when it is elevated to pH=7, the enzyme was inactivated, the pectin gel layer was not reduced and J_r increased. Moreover, pectinase remained adsorbed on the membrane, increasing the resistance to permeate flux.

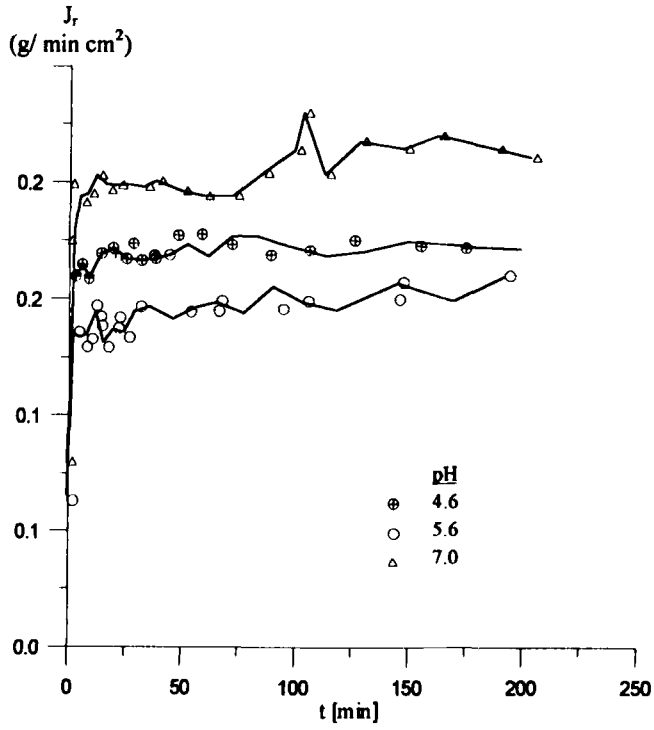

FIG. 5. INFLUENCE OF THE pH OF IMMOBILIZATION AND WASHING ON J_r

Resistance Determination

An approach to predict the flux of permeate is the resistance-in-series model (Constenla and Lozano 1996). Theoretically, this model could predict J_p throughout the range of ΔP_{TM}. When enzymes are immobilized on the membrane, permeate flux can be approximated as:

$$J_p = \frac{\Delta P_{TM}}{R_i + R_g + R_e + R_h} \tag{3}$$

where R_i = intrinsic resistance of the membrane, which is a property of the mechanical and chemical structure of the membrane material; R_g = gel resistance, which depends upon the extent of polarization, and the physical

properties of both the gel layer and the permeate which must pass across it; R_e = enzyme resistance; and R_h = effect of the layer of hydrolyzed pectin on total resistance. The intrinsic resistance of membrane was considered constant. Resistance due to the enzyme layer was also considered stable during the experimental run. On this basis, the following relationship for J_r and the different flux resistance was proposed and fitted with the SYSTAT nonlinear multiparametric program:

$$J_r = J^\circ - J_p = J^\circ - \frac{\Delta PTM}{R_i + \beta t^\delta + \phi t^\varphi + R_e} \qquad (4)$$

where $\beta\, t^\delta = R_g$; $\phi\, t^\varphi = R_h$. R_i values can be easily determined experimentally using water as permeant through a clean membrane. This method is valid also to determine R_e, but using an IPM. R_g parameters were obtained by fitting experimental values resulting from the ultrafiltration of a pectin solution through a clean membrane. Finally, R_h parameters, ϕ and φ, were calculated with Eq. (4) for different retentate flow, type of pectin, pectin and enzyme concentration, and immobilization and washing pH, after 300 min of ultrafiltration of pectin solutions. The more negative R_h values the more effective was the pectin hydrolysis. R_e and R_h values are listed in Tables 1 and 2. Some interesting conclusions arise from these values:

(1) Independently of C_e value, the resistance attributable to the enzyme layer R_e remains practically constant (Table 1 and Fig. 6).
(2) On the other hand, the resistance which characterized the layer of hydrolyzed pectin (R_h) drastically increased with pectin concentration greater than $C_p \cong 1.5$ mg/L, depending on enzyme concentration (Fig. 6). This last behavior indicated that for the most concentrated pectin solutions ($C_p > 1.8$ mg/L) immobilized enzymes were unable to prevent the growing of the gel layer, resulting in an increase of the total resistance to flux.

As Fig. 7 shows, Eq. (4) fits very well the experimental variation of J_r with time.

Determination of Hydrolyzed Pectin

Figure 8 shows the amount of hydrolyzed pectin which flows through the UF membrane as galacturonic acid (C_a), which is the main product after pectin hydrolysis. Results indicated that C_a rapidly increased with C_e, having a maximum at about $C_e = 5$ g/L. The use of glutaraldehyde as a crosslinking agent did not report any improvement on the permeate flux (results are not

shown). Moreover, when a relatively concentrated glutaraldehyde solution was used, the result was a remarkable enzyme inactivation. This behavior was attributable to the reduction of the enzyme activated sites by the crosslinking agent.

TABLE 1.
VARIATION OF R_e AND R_h WITH TYPE OF PECTIN, RETENTATE FLOW, PECTIN AND ENZYME CONCENTRATION, AFTER 300 MIN ULTRAFILTRATION. (pH_e = pH_w = 4.6)

Pectin	Q (L/min)	C_p (mg/L)	C_e (g/L)	$R_e^{(1)}$ (kg_f min/g)	$R_h^{(2)}$ (kg_f min/g)
A	0.1	1.2	60	0.9047	-2.0888
A	0.1	1.5	60	1.7458	-2.0084
A	0.1	1.8	1	2.1852	0.2742
A	0.1	1.8	60	1.8258	4.0843
A	0.1	2.4	1	1.5712	41.5655
A	0.1	1.2	1	2.034	1.7303
A	0.04	1.2	1	1.6325	0.6194
A	0.04	1.8	1	2.0937	5.0822
B	0.04	1.2	1	1.3957	-0.4157
B	0.04	1.8	1	1.4007	0.3856
B	0.04	1.8	0.5	1.6300	8.3212
B	0.04	1.8	10	1.9060	4.9423
B	0.04	10.0	1	1.3209	0.2724
B	0.04	10.0	60	2.8107	4.0053

(1)Experimentally determined.
(2)Calculated with Eq. (4)

TABLE 2.
VARIATION OF R_e AND R_h WITH pH_e AND pH_w, AFTER 300 MIN ULTRAFILTRATION OF A PECTIN TYPE B SOLUTION (Q = 0.04 L/min)

C_p (mg/L)	C_e (g/L)	pH_e	pH_w	$R_e^{(1)}$ (kg_f min/g)	$R_h^{(2)}$ (kg_f min/g)
1.8	1	4.0	4.0	1.4134	0.8652
1.8	1	4.6	4.6	1.4007	0.3856
1.8	1	4.6	6.0	1.2001	-3.0301
1.8	1	5.0	5.0	1.8369	-0.7735
10	1	4.6	4.6	1.3209	0.2724
10	1	4.6	6.0	1.3581	-2.8970
10	60	4.6	4.6	2.8107	4.0053
10	60	4.6	6.0	2.3979	3.5072

(1)Experimentally determined.
(2)Calculated with Eq.(4)

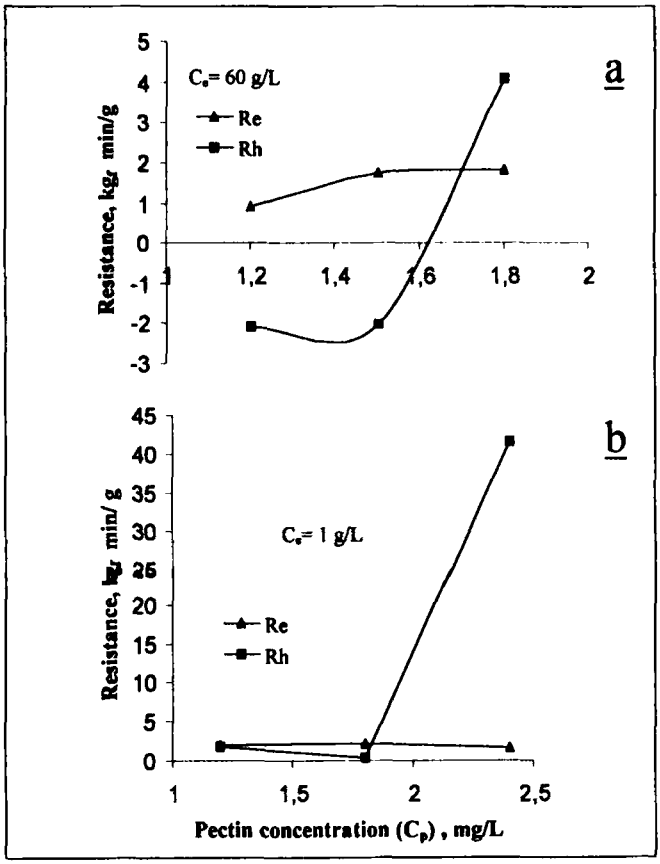

FIG. 6. VARIATION OF ENZYME LAYER RESISTANCE (R_e) AND HYDROLYZED PECTIN LAYER (R_h) AS A FUNCTION OF PECTIN CONCENTRATION (C_p)
(a): C_e = 60 g/L; (b): C_e = 1 g/L; Q = 0.1 L/min

Pilot Plant Ultrafiltration Results

Physical immobilization of Rohapect D5S commercial enzyme, buffered to pH = 4.6, was made by ultrafiltration of the enzyme solution (1 or 5 g/L) for 2 h at very low ΔP_{TM}. Figure 9 shows the variation of galacturonic acid concentration (C_a) on the permeate flux as a function of time, with and without immobilized pectinase on a hollow fiber cartridge. Results indicated that UF of pectin solution through a hollow fiber membrane after immobilization and

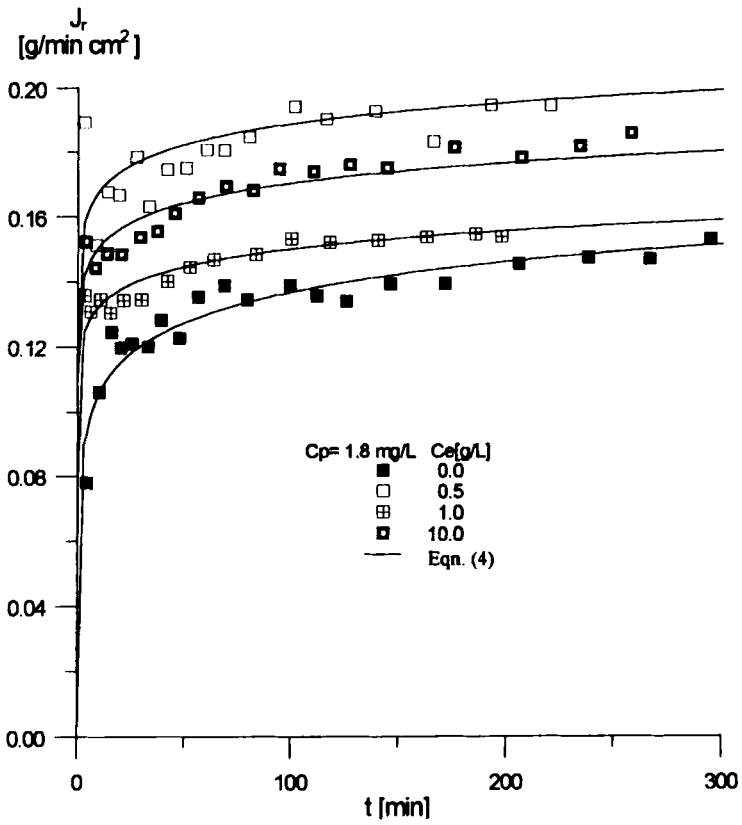

FIG. 7. EXPERIMENTAL VARIATION OF J_r WITH TIME (Q = 0.04 L/MIN)
Full line represents Eq. (4)

washing of enzymes at the optimal pH = 4.6 results in a continuous increase of C_a, attributable to an active enzymatic surface. On the other hand, washing immobilized enzymes with a neutral (pH_w = 7) solution resulted in an important inactivation of the pectinase, manifested as a constant low-level permeation of galacturonic acid. Finally, Fig. 10 shows the variation of Q_p with time, in the case of apple juice ultrafiltration, with and without pectic enzyme immobilization treatment. While during UF without immobilized enzymes the permeate flux decreases continuously, three periods were observed after pectinase immobilization: (1) an initial period, characterized by a rapid decrease in Q_p; (2) an intermediate period, where Q_p reached a plateau; and (3) a final period, where

Q_p approached a linear steady increase with time. Explanation for this behavior should be looked for by studying the complex diffusion-reaction process of pectin degradation and passage through enzyme film and pectin gel. A work about this topic is in progress. An enzyme may be identified as active toward gel layer if its direct addition into an operating UF system causes an increase in permeate flux. While it is true in the case of free enzymes, the mechanism is not so simple when pectinase is immobilized on a UF membrane.

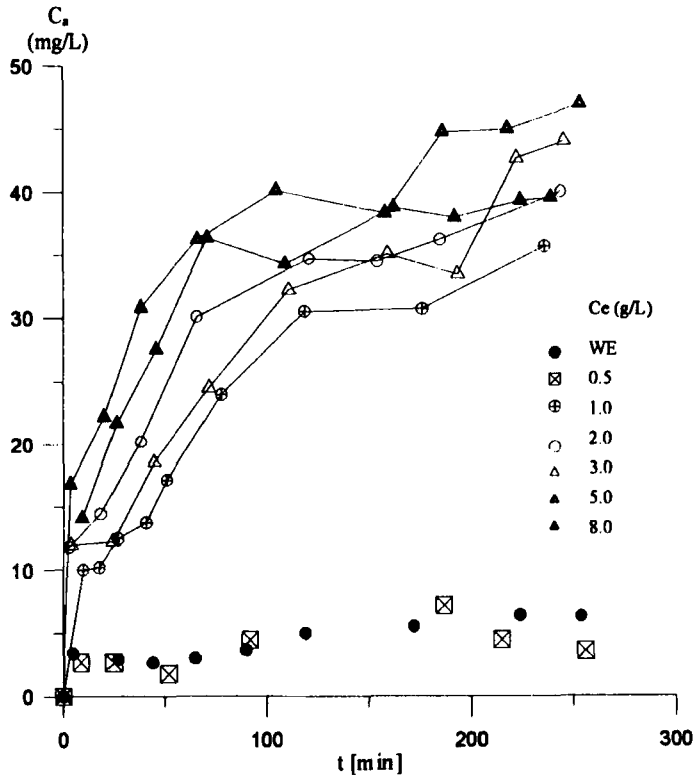

FIG. 8. HYDROLYZED PECTIN AS GALACTURONIC ACID CONCENTRATION (C_a) IN PERMEATE FLUX AS A FUNCTION OF ENZYME CONCENTRATION
(C_p = 80 mg/L; $pH_e = pH_w = pH_p$ = 4.6)

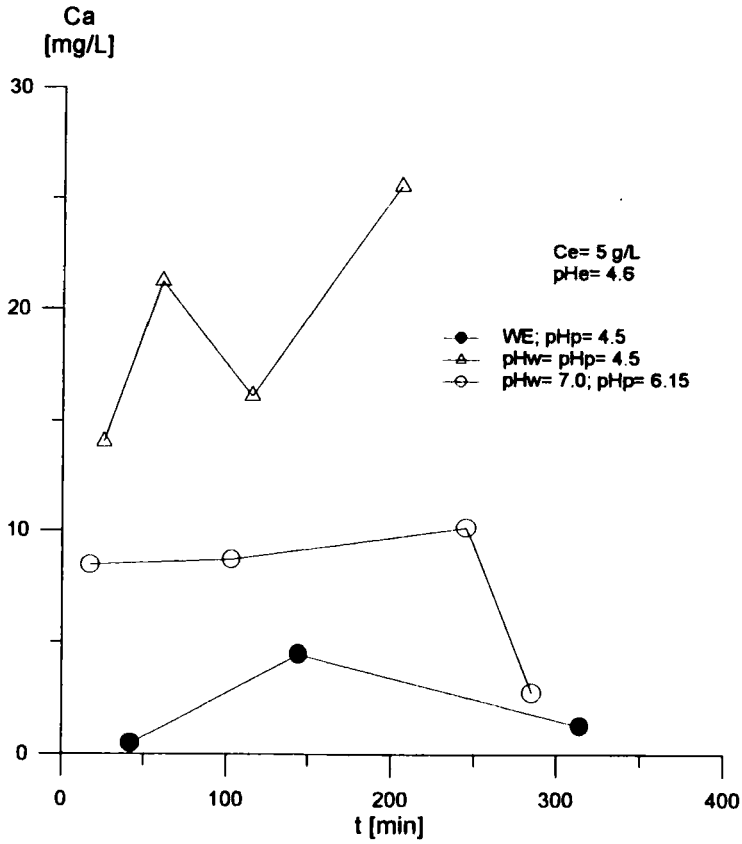

FIG. 9. CHANGES OF THE GALACTURONIC ACID CONTENT (C_a) IN THE PERMEATE FLUX, DURING HFUF OF A PECTIN SOLUTION IN A PILOT PLANT EQUIPMENT, UNDER DIFFERENT IMMOBILIZATION CONDITIONS

CONCLUSIONS

As a conclusion, although physical immobilization of commercial pectinase on HFUF membranes (IPM) practically does not increase the permeate flux, it operates some control on the gel layer formed during ultrafiltration of apple juice. The pectinase layer partially hydrolyzes the pectin molecules continuously deposited during ultrafiltration, therefore retarding the rate of formation of the gel layer on the membrane and around the pores. This effect allows Q_p to be kept at sufficiently high values to reduce the membrane cleaning steps, extending the period of operation within a reasonable yield. It should be emphasized that

immobilization by physical adsorption of commercial pectic enzymes results in quite a simple process, which requires little care and labor. Moreover, pectinase immobilized by this method can easily be removed by conventional membrane washing.

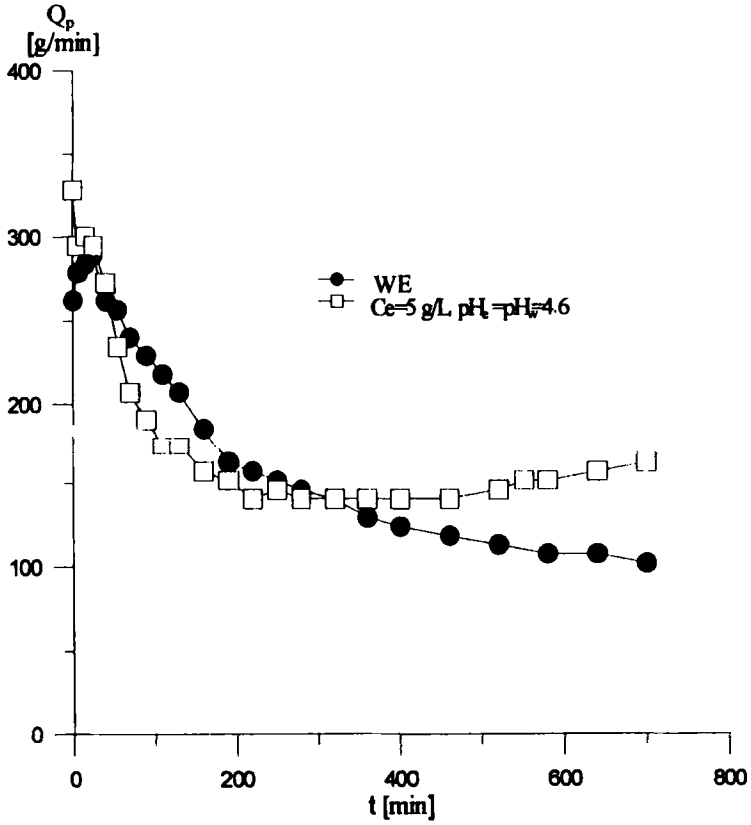

FIG. 10. REFERENCE PERMEATE FLUX DURING APPLE JUICE ULTRAFILTRATION THROUGH MEMBRANES WITH AND WITHOUT PECTINASE IMMOBILIZATION, IN A PILOT PLANT EQUIPMENT

NOMENCLATURE

C_a:	Galacturonic acid concentration.	[mg/L]
C_e:	Enzyme concentration.	[g/L]
C_p:	Pectin concentration	[mg/L]

DI:	Internal diameter HFUF	[cm]
IPM	Immobilized pectinase membrane	
J^o:	Water permeate flux in a clean membrane	[g/min cm^2]
J_p:	Permeate flux	[g/min cm^2]
J_r:	Reference flux ($J^o - J_p$)	[g/min cm^2]
pH_e:	pH of the enzyme solution.	
pH_w:	pH of solution used for removing excess enzyme on fiber.	
pH_p:	pH of pectin solution	
Q:	Retentate flow	[L/min]
Q_p:	Permeate flow (Pilot plant UF system)	[L/min]
R:	Flux resistance	[kg_f min/g]
Re:	Reynolds number	
t:	Time	[min]
V_o:	Initial volume	[L]
ΔP_{TM}:	Transmembrane pressure	[kg_f/cm^2]

REFERENCES

ALKORTA, I., GARBISU, C., LLAMA, M.J. and SERRA, J.L. 1995. Viscosity decrease of pectin and fruit juices catalyzed by pectin lyase from *Penicillium Italicum* in batch and continuous-flow membrane reactors. Biotechnol. Techniques *9*, 95-100.

ATALLAH, M. and HULTIN, H. 1977. Preparation of soluble conjugates of glucose oxidase and catalase by cross-linking with glutaraldehyde. J. Food Science *42*, 7-10.

BLUMENKRANTZ, N. and ASBOE-HANSEN, G. 1973. New method for quantitative determination of uronic acids. Analytical Biochem. *54*, 484-489.

CECI, L. and LOZANO, J. 1998. Determination of enzymatic activities of commercial pectinases for the clarification of apple juice. Food Chem. *61*, 237-241.

CHERYAN, M. 1986. *Ultrafiltration Handbook*. Technomic Publishers, Lancaster, PA.

CONSTENLA, D.T. and LOZANO, J.E. 1996. Predicting stationary permeate flux in the ultrafiltration of apple juice. Lebensmittel-Wissenschaft und-Technologie *29*, 587-592.

DIETRICH, H., PATZ, C., SCHÖPPLAIN, F. and WILL, F. 1991. Problems in evaluation and standardization of enzyme preparations. Fruit Processing *1*, 131-134.

GEKAS, V.C. 1986. Artificial membranes as carriers for the immobilization of biocatalysts. Enzyme Microb. Technol. *8*, 450–459.

GRAMPP, E.A. 1976. New process for hot clarification of apple juice for apple juice concentrate. Fluss. Obst *43*, 382–388.

HEATHERBELL, D.A., SHORT, J.L. and STAUEBI, P. 1977. Apple juice clarification by ultrafiltration. Confructa *22*, 157–169.

IFT. 1959. Pectin Standardization. Final Report of the IFT Committee. Food Technol. *13*, 496–500.

KULP, K. 1975. Carbohydrases. In *Enzymes in Food Processing*. Second Edition. (G. Reed, ed.) Academic Press, New York.

PIFFERI, P.G. and PREZIUSO, M. 1987. Immobilization of endo-polygalacturonase on γ-alumina for the treatment of fruit juices. Lebensm. Wiss. U Technol. *20*, 137–142.

SPAGNA, G., PIFFERI, P.G. and GILIOLI, E. 1995. Immobilization of pectinlyase from *aspergillus niger* for application in food technology. Enzyme and Microbial Technol. *17*, 729–738.

SYNOWIECKI, J., SIKORSKI, Z. and NACZK, M. 1982. Immobilization of amylases on krill chitin. Food Chemistry *8*, 239–246.

SZANIAWSKI, A. 1996. Effects of pectin concentration and crossflow velocity on permeability in the microfiltration of dilute pectin solutions by macroporous titania membranes containing immobilized pectinase. Biotechnol. Prog. *12*, 403–405.

TORIBIO, J.L. and LOZANO, J.E. 1984. Non-enzymatic browning in apple juice concentrate during storage. J. Food Science *49*, 889–892.