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The purification of a commercial pectinlyase from *Aspergillus niger*

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The purpose of this study was to examine the purification of an enzymic commercial preparation containing pectinlyase by means of a simple, inexpensive and quick method which can be adopted for industrial applications. The following adsorbents were tested: activated carbon, graphite, γ-alumina, titania and bentonite. Given the good results obtained with bentonite, a purification procedure was optimized with this support. The enzymic preparation diluted at a 1 : 2 ratio was twice put into contact with 2% bentonite at pH 4·0 and at a temperature of 25°C for 3 h. After precipitation of pectins at pH 3·2, the enzyme solution was brought to pH 6·0 and then concentrated by means of ultrafiltration through a membrane with a cut-off of 50 000. Almost total recovery of enzymic activity was obtained, with a purification of 4·5 and a reduction in the contents of brown pigments of about 90%.

INTRODUCTION

Pectic enzymes are generally used by the fruit-juice processing industry for degrading the pectins in a wide variety of fruits such as apples, pears, berries, cranberries, grapes and tropical fruits, so as to obtain settling of solids, clarification, reduction in viscosity, increase in filtering velocity and in juice extraction yield. Juice depectinization can be achieved either by employing two enzymes in series, i.e. pectinesterase (PE, EC 3.1.1.11) and endo polygalacturonase (PG, EC 3.2.1.15), which de-esterify and depolymerize the pectin molecule, respectively (Rombouts & Pilnik, 1980), or by using pectinlyase (PL, EC 4.2.2.10), an endo-enzyme capable of directly depolymerizing highly esterified pectins by breaking down the glucosidic bond via a β elimination mechanism (Albersheim, 1968).

The use of PL is preferable in fruit-juice processing as it permits the use of only a single enzyme and avoids the reduction of fruit-juice stability due to the coagulation of pectin partially de-esterified with endogenous Ca^{+2} (Alana *et al.*, 1989).

No specific commercial PL preparation is available for industrial applications. Y23 (Seishin Pharm.), a product derived from *Aspergillus japonicus*, is the only preparation available, as PL on the market, but it can only be used for laboratory because of difficulty in obtaining it and its high cost. Of the various commercial preparations employed in the beverage industry, the best proved to be cytolase PCL5 (Genecor), a product derived from *Aspergillus niger*, which, however, is characterized by a high rate of impurities, including extraneous proteins, pectins and melanoidins. In the present study, an attempt has been made to purify the PL of PCL5 by a quick and simple method, the principal step being adsorption, easily applicable on a large scale by the food processing industry.

The following adsorbents were used: activated carbon, graphite, bentonite, γ-alumina and titania.

Only a few studies have been conducted on PL purification (Bush & Codner, 1970; Ishii & Yokotsuka, 1972, 1975; Lim *et al.*, 1983; Parini *et al.*, 1988; Alana *et al.*1989; Zhou *et al.*, 1989; Bugbee, 1990; Zhou *et al.*, 1990; Alana *et al.*, 1991; Okai & Gierschner, 1991; Polizeli *et al.*, 1991), and only a few of these concern PL derived from *Asperillus niger*.

Even though the use of adsorbents is one of the main methods for enzyme and protein purification (Zittle, 1953; Green & Wase, 1986; Bailey & Ojama, 1990), it does not yet appear that these have been employed for PL purification. As a matter of fact, the method offers several advantages such as mechanical resistance, which is an important factor in the design and construction of adsorption columns, thermal resistance, which permits thermal sterilization to maintain sterility in biochemical separation processes and stability with respect to organic solvents as well as with respect to considerable variations in pH and also to microbiological degradation.

Unfortunately, protein adsorption on inorganic

supports is usually an irreversible process. Another drawback is that the desorbed protein may turn out to be conformationally altered with respect to the native protein (Soderquist & Walton, 1980). The use of adsorbents, therefore, has a greater possibility of being successful in the removal of undesired extraneous proteins.

Finally, other drawbacks which may be encountered include the difficulty of obtaining reproducible results (Green & Wase, 1986) and the risk of blockage of chromatographic columns due to the slow rate of the process.

MATERIALS AND METHODS

The enzyme preparations employed included: Pectinol 80 SB, Cytolase M219, M104 and PCL5 (batch 44) and Pectinol 80 SB, all supplied by Genecor; Pectinex 3L supplied by Novo; Biopectinases L63 and L100 supplied by Biocon; Pectinol D5L, Rohapect D5S and PC supplied by Rohm; Ultrazym SE 604 and 100 supplied by Ciba Geigy; Rapidase C80 supplied by Gistbrocades; and Pectolyase Y23 supplied by Seishin Pharm.

The adsorbents employed included: activated carbon, bentonite, titania (BDH, England), graphite (Lonza) and γ-alumina with granulometry of 125–250 μm (Snam Progetti).

Apple pectin (esterification grade of 72–75%, Roth) was used after further esterification as a substrate for determining pectinlyase activity.

All other reagents were RP grade and were supplied by Carlo Erba (Italy) except for bovine serum albumin BSA (Pentex, Miles) and Coomassie Brilliant Blue G-250 (Serva).

Pectin esterification

Apple pectin (150 g) was first washed at room temperature with 0·5 M HCl in 60% ethanol, then in 60 and 96% ethanol, and finally with anhydrous methanol so as to eliminate impurities such as acids, sugars, polyphenols and other polar components. The pectin was then esterified with 2 litres of 1 M H_2SO_4 in anhydrous methanol at 2–3°C and stirred from time to time over a period of 4 weeks. The esterified pectin thus obtained was filtered and washed with 60 and 96% ethanol and ethyl ether, and allowed to dry at about 45°C overnight (Kohn *et al.*, 1983). Pectin esterification grade was equal to 97% (Kertesz, 1951), and a molecular weight of about 26 500 was determined by measuring viscosity according to Owens *et al.* (1946).

Determination of pectinlyase activity

In a 10 ml tube containing 2·0 ml of PL diluted in 0·1 M citric-phosphate (C-P) buffer and thermostated at 25°C, 2·0 ml of 1·0% esterified pectin diluted in the same buffer were added under stirring. After 1 min, the reaction was stopped with 2·0 ml of 0·5 M H_2SO_4. The order of the reagents was inverted for the blank mixture by adding first the acid and then the enzyme to the pectin solution. The reaction mixture was read against the blank at 235 nm. The increase in absorbance observed was due to the double conjugate bond of the $\Delta^{4:5}$ unsaturated uronide formed during the reaction.

The molar extinction coefficient at 235 nm, used for calculating enzyme activity, was 5550 $M^{-1}cm^{-1}$ (Albersheim, 1968).

Protein determination

Protein determination was done according to Bradford (1976). Two volumes of 0·06% Coomassie Brilliant Blue in 3% perchloric acid were mixed with one volume of 0·2 M acetate buffer at pH 5·65 and with one volume of protein solution. For the blank, one volume of the same buffer in which the enzyme had been diluted was employed in lieu of the protein solution. The blue complex formed in the sample was read against the blank at 592 nm. Bovine serum albumin was used as a reference.

Adsorption as a function of the pH

50 mg of the various supports, including activated carbon, graphite, bentonite, γ-alumina and titania, were equilibrated in a 10-ml test tube with 2·5 ml of 0·1 M C-P buffer at pH values ranging from 2·8 to 7·0 for 20 min at room temperature. Bentonite, in particular, was dispersed by means of a small spatula and contemporaneously sonicated. Ten trials were carried out for each support. PL (PCL5) (2·5 ml) was then added as such or after dilution. As the enzyme solution has a high buffer capacity at a pH of about 4·2, the pH value was adjusted by means of 2 M HCl or NaOH in order to bring it to pre-established values. The test tubes were then stirred for about 12 h at room temperature and then centrifuged at 2600 *g* for 20 min.

Finally, the enzyme activity and the total protein of the supernatants were determined and the colour of the supernatants was measured at 420 nm.

Adsorption as a function of concentration

Various quantities of the support from 50 mg to a maximum of 250 mg were equilibrated as described above with 2·5 ml of 0·1 M C-P buffer at optimum purification pH. They were then placed in contact with 2·5 ml of PL at the same pH value for 12 h.

Adsorption as a function of time

300 ml of diluted enzyme in 1 : 2 and 1 : 4 ratios were brought into contact with bentonite at 1 and 2%, respectively, following the procedure described above. The solution was stirred at room temperature. About 2·0 ml of the solution at a time were subsequently taken and centrifuged at 2600 *g* for 10 min and then analysed.

Ultrafiltration

A 50 ml sample of PL diluted in a 1 : 2 ratio was brought into contact with bentonite under optimum conditions as established experimentally in previous trials. One volume of enzyme solution was then diluted in one volume of water, brought to a pH of approximately 3·2 by adding 2 M HCl and placed in a refrigerator at 3°C overnight in order to eliminate pectins by precipitation. The solution was subsequently ultrafiltrated at pH 4·0 and 6·0 through membranes with cut-off values of 10 000 (PM 10, Amicon), 30 000 (YM 30, Amicon) and 50 000 (XM 50, Amicon). Finally, the residue was washed twice with 0·1 M C-P buffer at the same pH.

RESULTS AND DISCUSSION

Analysis of commercial enzymic preparations

Table 1 shows that, amongst the enzymic liquid preparations, cytolase PCL5 (PCL5), and amongst the solid ones, pectolyase Y23 (Y23) exhibit the greatest activities and specific activities.

PCL5 was chosen for the present study since Y23 is not an enzymic preparation normally used in the food processing industry, as the product is not easily available on the market and has a high cost. Moreover, the optimum pH of PCL5's PL, which is equal to 5·8 as against Y23's optimum pH of 7·0, makes the former product particularly interesting for its possible applications in food processing. Table 2 shows the characteristics of these two products.

It should, however, be noted that PCL5 presents a drawback due to its high level of impurities represented by the presence of extraneous proteins and pectins, as well as by the high concentration of brown pigments

Table 1. Characteristics of enzymic preparations containing pectinylase (PL)

Liquid products	Activity (μmol(min ml)	Protein (mg/ml)	Specific activity (unit/mg)
Cytolase M219	14·3	20·8	0·69
Cytolase M104	44·0	35·4	1·24
Cytolase PCL5	86·0	17·2	5·00
Pectinol 80 SB	11·0	9·33	1·18
Pectinol D5L	22·5	12·2	1·84
Pectinex 3XL	29·6	8·25	3·59
Biopectinase L63	27·7	13·2	2·10
Biopectinase L100	29·9	21·3	1·40
Solid products	**Activity (μmol(min mg))**	**Protein (mg/mg)**	**Specific activity (unit/mg)**
Pectoylase Y23	1·987	0·128	15·52
Rapidase C80	0·041	0·101	0·41
Ultrazym 100	0·066	0·058	1·14
Ultrazym SE 604	0·059	0·168	0·35
Rohapect D5S	0·026	0·055	0·47
Rohament PC	0·009	0·091	0·10

Table 2. Physical and chemical characteristics of enzymic preparations Y23 and PCL5

Enzymic preparation	Origin	Optimum pH[a]	Isoelectric pH	MW
Y23[b]	*Aspergillus japonicus*	7·0	7·7	32 000
PCL5[c]	*Aspergillus niger*	5·8	3·6	38 000

[a] Apple pectin esterified to 97% was used as substrate.
[b] Ishii *et al.*, 1975.
[c] Okai *et al.*, 1991.

such as melanoidins, brown nitrogenous polymers and copolymers resulting from the Maillard browning reaction (O'Brien & Morrissey, 1989). In fact, PCL5 which has been diluted 250 times exhibits an absorbance of about 0·35 UA at 420 nm.

The following parameters were considered during the adsorption trials of the enzymic preparations, namely recovery of enzymic activity (recovery %), degree of purification (purification) and percent decrease of absorbance at 420 nm (D_{420}%). These parameters were all referred to the supernatant after adsorption.

Recovery % (Rec) = (final activity/initial activity) × 100

Purification (Pur)

= (initial specific activity/final specific activity)

D_{420}%

= ((initial $Abs_{420\ nm}$-final $Abs_{420\ nm}$)/initial $Abs_{420\ nm}$)× 100

where $Abs_{420\ nm}$ = absorbance at 420 nm.

Activity as a function of pH

Table 3 shows the relationship between the parameters of adsorption of the enzyme, pH value and the dilution of the enzyme (PCL5) employed on the various supports tested.

An increase in activity of the supernatant after adsorption was noticed in some of the trials, with recovery rates of over 100%. This result may be due to the adsorption of enzymic inhibitors such as brown pigments.

At higher dilutions, the level of activity was seen to depend to a greater extent upon the type of support being used, and a greater correlation with the pH value was also observed. In other words, the effects of the nature of surface support would seem to be more pronounced at low surface coverage; this observation was reported also by Norde *et al.* (1986).

Activated carbon and graphite, at pH 2·8 and at a dilution ratio of 1 : 4, gave good results, with a recovery rate of over 100% and a degree of purification of 1·46. The effect of pH becomes more evident at higher dilution (1 : 8); PL remains in the supernatant due to a preferential adsorption of extraneous proteins. Activated carbon is generally acidic, featuring various functional groups on the surface such as carbonyl, carboxyl, phenol, lactone, quinone and ether groups, as well as a very large inner surface due to a high number of pores with diameters normally larger than 1–2 nm. This latter feature, in particular, accounts for activated

Table 3. PL (PCL5) adsorption on different supports

Adsorbents	pH	Dilution 1 : 2			Dilution 1 : 4			Dilution 1 : 8		
		Rec %	Pur	D_{420}%	Rec %	Pur	D_{420}%	Rec %	Pur	D_{420}%
Activated carbon	2·8	128	1·31	19	122	1·46	55	95	0·90	70
	4·0	124	1·25	15	115	1·22	46	79	0·67	55
	6·5	100	1·21	10	86	1·10	23	54	0·60	33
Graphite	2·8	121	1·40	15	122	1·46	46	77	0·91	59
	4·0	110	1·10	8	109	1·23	26	67	0·60	34
	6·5	100	1·00	6	90	1·04	17	60	0·59	18
Bentonite	2·8	104	1·29	44	110	2·10	67	23	1·98	79
	4·0	112	1·20	34	115	1·90	63	78	1·54	67
	6·5	97	1·06	18	76	1·00	25	49	0·91	16
γ-Alumina	2·8	84	0·82	—	95	1·05	54	48	0·41	63
	4·0	91	0·92	—	110	1·35	42	55	0·49	49
	6·5	88	0·84	—	84	1·00	25	27	0·28	24
Titania	2·8	110	1·10	10	98	0·98	41	76	0·75	43
	4·0	100	1·10	—	89	0·89	35	88	0·85	32
	6·5	100	1·00	8	94	1·10	—	88	0·75	8

Ten trials were carried out for each support. Rec 3%, Pur 4·5% (95% of confidence interval).

carbon's remarkable adsorption properties, which it displays also with respect to polar macromolecules. Given these characteristics, a lowering of the pH value may be expected to facilitate the adsorption of PCL5 proteins and melanoidins on such supports, especially due to increased electrostatic interactions between the protonated amino groups of the proteins and the partially dissociated carboxyl groups of the support. Moreover the lowering of pH values favours the preferential adsorption of proteins having a greater molecular weight and higher pI values; the enzyme with a higher pI value has a higher net positive charge and exhibits stronger electrostatic interactions with the support. In fact, PL has a low pI value (3·6) (Okai & Gierschner, 1991), which is inferior to that of the other enzymes present in PCL5, such as β-glucosidase (βG, pI 3·9), pectinesterase (PE, pI 4·2) and polygalacturonase (PG, pI 5·2). Moreover, PL's molecular weight is relatively low (38 000), below, for example, that of βG (100 000).

With regard to adsorption of melanoidins, the trend is as predicted, with an increase in D_{420}% as pH values decrease. Such results are in line with those of the behaviour reported in the literature for several enzymes (Bailey & Cho, 1983; Cho & Bailey, 1979).

Compared to activated carbon, graphite is practically devoid of any pores, featuring a well-ordered hexagonal and rhombohedral crystalline structure. Moreover, graphite also features less reactive groups at the surface. Given these differences, it can be concluded that graphite is more hydrophobic and usually less reactive with respect to polar compounds than activated carbon.

Table 3 shows, in fact, that there is a slightly lower adsorption of melanoidins on graphite as compared to activated carbon (D_{420}%). Furthermore, significantly different recovery rates are observed only at lower concentrations (1 : 8) (Norde *et al.*, 1986) with a lower recovery rate for graphite with a trend less dependent upon the pH value. These findings are in line with those of other authors according to whom the adsorption of proteins increases (Gupta *et al.*, 1983), while the effects of pH are less marked (Norde *et al.*, 1986) as the hydrophobicity of the surface increases.

As can be seen in Table 3, bentonite is the support which generally gives the highest results. Bentonite is mainly made up of montmorillonite, the elementary unit layers of which comprise three sheets, i.e. two of silica tetrahedrons and a central one of alumina octahedrons (Kirk-Othmer, 1964). Because of this structure, water and other polar molecules may penetrate into the unit layers, thus tending to swell the bentonite.

The partial substitution of Al^{3+} with Mg^{2+} and of Si^{4+} with Al^{3+} leads to a deficiency in the charge balanced by adsorbed cations. The cations of montmorillonite can be exchanged with organic cations or may form coordination complexes with organic anions. Although essentially a cation exchanger, bentonite has an anion-exchange capacity which, albeit moderate, may take place along the rupture lines of the sheets and the hydroxy groups of the surface. Bentonite, therefore, is also capable of adsorbing the anions present in the buffer such as phosphate anions.

As with the other supports, the greatest differences in activity were noted for the enzyme dilution of 1 : 8 (Table 3), with a marked adsorption at lower pH values (Fig. 1), probably due to the fact that the enzymes become positively charged and intermolecular protein interactions diminish. For pH values ranging from 4·0 to 5·5, a central minimum adsorption area (Fig. 1) is encountered where PL bentonite cationic interaction is reduced even though other bonds persist, including Van der Waals and hydrogen bonds. Moreover, strong interactions, especially of an electrostatic nature, between the proteins, may occur given the different pI values (ranging from 3·6 to 5·2) of the PL and of the other enzymes present, including βG, PE and PG, and these interactions probably prevail over those with the surface of the exchanger. When the pH of the solution is greater than 5·5, i.e. higher than the pI of the aforesaid enzymes, high adsorption is noted,

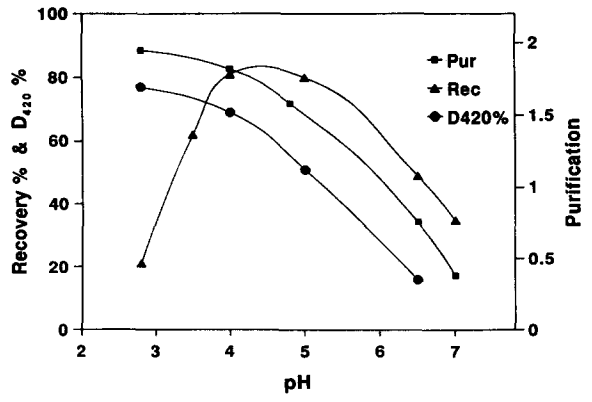


Fig. 1. Adsorption of PL (PCL5) on bentonite (1%), as a function of pH (dilution ratio 1 : 8, at 25°C for 12 h).

albeit less marked than in the acid zone. This finding may be partially due to an anion exchange mechanism of bentonite or to the formation of direct hydrogen bonds with protein via water molecules or the phosphate ion of the buffer adsorbed onto the surface; however, the most important adsorption mechanism is probably represented by the formation of metal–protein complexes due to anionic carboxyl oxygen and to the neutral amine nitrogen of the anionic form of PL, directly coordinated to the hexahydrated metal cation of the montmorillonite, as already reported in the literature (Gupta *et al.*, 1983).

Purification was seen to increase as the pH value decreased (Fig. 1). Such increase in purification is closely related to a greater selectivity of the adsorbent with respect to extraneous proteins in relation to the higher MW and to the higher pI of the protein itself. This can be accounted for, as mentioned above, by the high content of β-glucosidase with a molecular weight of about 100 000 and a pI of about 3·9 (Shoseyov *et al.*, 1988). Colour reduction, D_{420}%, which is ascribable to the adsorption of brown pigments, was found to be greater at a pH value of 3·0 where PL and extraneous protein adsorption is greater. It may therefore be inferred that these substances are strongly associated with the protein.

Therefore, as can be seen in Fig. 1, optimum adsorption conditions are attained at a pH value of about 4·0, as not only is the recovery rate optimal but purification and D_{420}% are also good. At lower pH values, in fact, recovery is too low, while at pH values greater than 5·0 a sharp drop in the values of all the aforementioned parameters was noted. For this reason, all subsequent trials were conducted at pH values of 4·0.

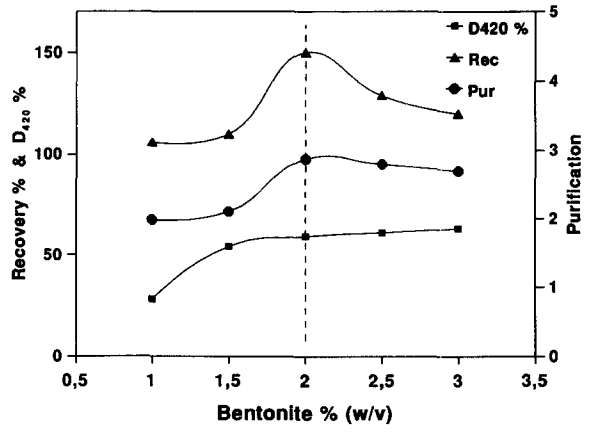
Adsorption trials were carried out for γ-alumina, also at pH 5·0; results (not shown in Table 3) were more or less identical to those obtained at pH 4·0. The recovery and purification values for γ-alumina are therefore lower than those obtained with the other adsorbents, with a maximum at pH values between 4·0 and 5·0 and a sharp drop in both these parameters at pH values greater than 5·0.

Both acid and basic sites were found to be present on the surface of γ-alumina (Goodboy & Fleming, 1984). At pH values below 4·0 a slightly greater adsorption rate can be noted as the enzymes are positively charged and can thus interact with the basic sites of the support. At pH values from 4·0 to 5·0, the neutral amino acid groups of the enzymes present can react directly via hydrogen bonds with surface hydroxyl groups of γ-alumina. At pH values greater than 5·0 the recovery rate is lower and therefore adsorption is greater; this is due to aluminium atoms which act as adsorptive electrophilic centres in attracting donor groups such as –OH and $-NH_2$ (Green & Wase, 1986).

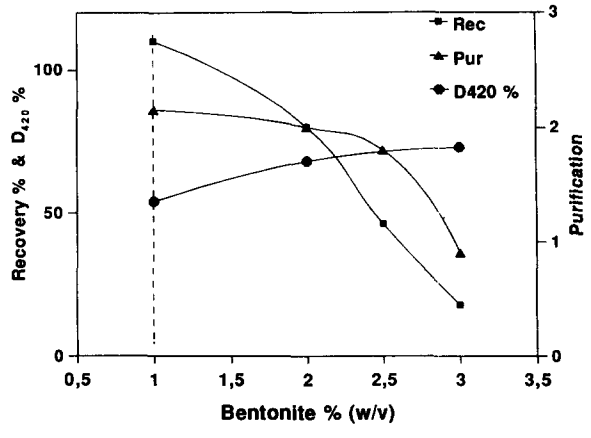
The values of the parameters considered, for TiO_2 were found to be negligible (Table 3).

Adsorption as a function of bentonite concentration and time

As can be seen in Fig. 2(a), at a dilution ratio of PCL5 of 1 : 2 the maximum recovery rate and purification as well as a high D_{420}% were obtained with a bentonite concentration of 2%, whereas at a dilution ratio of 1 : 4 (Fig. 2(b)), the best results were obtained with an adsorbent concentration of 1%. Optimum results were obtained, therefore, for both dilution ratios considered, using 25 ml of PCL5 for each gram of bentonite.



(a)



(b)

Fig. 2. PL(PCL5) adsorption as a function of bentonite concentration (w/v) and of dilution ratio (a) 1 : 2, (b) 1 : 4, at pH 4·0, 25°C for 12 h.

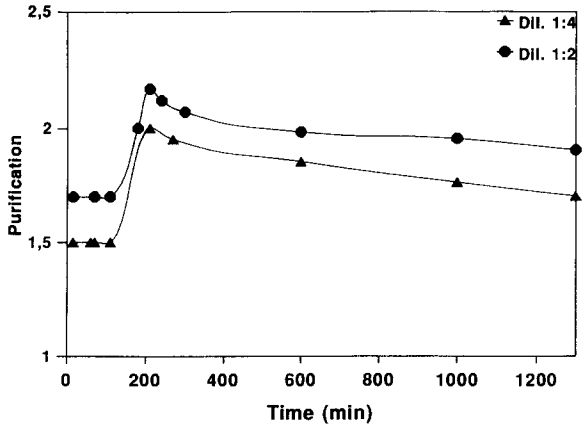


Fig. 3. PL (PCL5) purification as a function of contact time, at dilutions 1:2 (2% bentonite) and dilution 1:4 (1% bentonite) at pH 4·0, 25°C for 12 h.

As the effect of time on adsorption is amply reported in the literature (Blade & Boulton, 1988), it was decided here to examine its effect on purification. As can be seen in Fig. 3, after adsorption on bentonite, an increase in purification as a function of contact time can be seen to take place in successive stages, the former of which after a time equal to or less than 10 min and the latter at about 3 h, after which the degree of purification can be seen to gradually diminish.

The total adsorption process is dynamic and may be divided into five steps (Wahlgreen & Arnebrant, 1991): (1) transport to the surface, (2) adsorption, (3) structural rearrangement, (4) desorption or exchange, and (5) transport away from the surface. The adsorption rate during the first stage (Fig. 3) is probably controlled by steps 1 and 2.

The increase in purification after the first stage can be attributed to conformational changes (step 3) or to exchange with other species of the proteins in solution (step 4), so that low MW proteins are replaced by high MW proteins; the latter mechanism is similar to the exchange process observed to occur between blood proteins at solid surfaces, i.e. the so-called Vroman effect (Horbett & Brash, 1986). This process is all the more likely to occur because bentonite continues to swell during adsorption, thus increasing the separation between unit layers and permitting increasingly greater organic macromolecular cations to penetrate between them.

Summing up, then, the optimum conditions for the adsorption of PCL5 onto bentonite were found to be as follows: enzyme dilution ratio 1:2, support concentration 2%, adsorption pH 4·0 and contact time 3 h at room temperature. The above-mentioned method has been further improved by adding to the procedure a second adsorption with bentonite, under the same conditions as before (Table 4).

Table 4. PL (PCL5) purification at optimal conditions

Step	pH	Parameters		
		Recovery %	Purification	D_{420}%
1° Adsorption	4·0	100	2·5	50
2° Adsorption	4·0	100	4·1	55
Precipitation	3·2	98	4·3	60
UF (XM 50)	6·0	90	4·5	93

Table 5. PL (PCL5) ultrafiltration after adsorption and precipitation

Membrane	PH	Recovery %		Purification		D_{420}%	
		Residue	Filtrate	Residue	Filtrate	Residue	Filtrate
PM 10	4·0	25·2	0·30	—	—	—	—
YM 30	4·0	61·3	38·7	—	—	23	77
XM 50	4·0	81·9	10·5	0·9	1·2	17	83
XM 50	6·0	90·1	3·2	1·1	—	67	33

Ultrafiltration (UF)

Table 5 shows the effects of UF on pectinlyase activity after treatment of PCL5 with bentonite under optimum conditions and after precipitation of pectins in an acid environment.

A loss of about 75% of the total catalytic activity was observed after filtering through a polysulphone PM10 membrane. Given that activity is practically nil in the ultrafiltrate, it may safely be assumed that such a drop in activity is brought about by the deactivation of the enzyme due to the conformational changes occurring in the proteins following on the interaction with the membrane during filtration. Such conformational changes can take place according to different protein adsorption models as described in the literature (Lundstrom & Elwing, 1990; Wahlgreen & Arnebrant 1991).

On the other hand, use of the YM30 membrane made out of cellulose acetate does not lead to any deactivation of the enzyme. In fact, only about 60% of the enzyme was recovered as the MW of the enzyme was very close to membrane cut-off, while the brown pigments were seen to be distributed between the cake and the filtrate.

Despite the high cut-off of the XM50 membrane, about 82% of enzyme activity at pH 4·0 is recovered in the cake. This can be accounted for by the membrane's polarity, which is half-way between that of the PM and of YM membranes. This leads to the membrane interacting with PL without deactivating it. In fact, as already mentioned, this process may be favoured by the electrostatic interactions which take place between the proteins of the PCL5 at this pH value. The increase of pH leads to repulsions with both the other proteins in the solution and with the melanoidins which, at a pH value of 6·0, are partially salified. This in turn leads to an increase in recovery and, above all, a considerable variation in the colour of the filtrate, which goes from 17 to 67% without, however, any associated increase in the degree of purification.

CONCLUSIONS

Amongst the adsorbents tested for the purification of the PL contained in the PCL5 preparation, i.e. acti-

vated carbon, graphite, bentonite, γ-alumina and titania, the best results were obtained by the adsorption of extraneous proteins on bentonite.

These results included almost the total recovery of enzymic activity, a final purification of 4·1, a drop in brown pigments of about 55% as measured at 420 nm (D_{420}% and a reduction in pectin content.

Subsequent treatment of PCL5 by precipitation and ultrafiltration was found to reduce the recovery rate by about 90%, while the degree of purification was seen to increase slightly (4·5) and a further reduction in D_{420} by about 93% was observed.

The procedure employed can be very easily carried out whether for laboratory applications or in the food processing industry.

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