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PECTINESTERASE ACTIVITY IN SOME FRUITS

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Pectinesterase (PE) activity in brine extracts from cherries, plums, currants, gooseberries and strawberries was investigated. The activity of the enzyme as well as the optimal parameters of this activity were found to differ considerably depending on the kind of fruit. Pectinesterase was most active in cherries and least active in strawberries and red currants.

Alcoholic fermentation of fruit musts and mashes causes degradation of pectic substances. The degree of this degradation depends mainly on the activity of endogenous pectynolytic enzymes occurring in the fruits and musts. One of these enzymes is pectinesterase (pectin pectylhydrolase EC 3.1.1.11) responsible for the release of metoxyl groups from pectins and creating conditions for the formation of methanol (MeOH). Pectinesterase may also lead to the appearance of poorly esterified pectins which can combine with calcium ions (Ca^{++}) and cause opacity of juices and wines [17]. Completely methylated pectin cannot be hydrolysed by polygalacturonase and its activity depends on the demethylating activity of PE [13]. According to Jakob et al. [5] this enzyme may also act on the volatile esters of juices and musts thereby altering their aroma: Accordingly, technological processes must ensure maximum hydrolysis of pectins with a simultaneous minimization of PE activity.

Pectinesterase is not a uniform enzyme, displaying different physical and chemical properties depending on its origin [13, 18]. Some fruits have been found to contain multiple PE forms [9, 10]. Optimum pH for PE of higher plants is usually in the range 5.5-9.5 [16, 18, 20], while optimum temperature varies from 30 to 50°C [10, 12, 13, 18]. Thermal sensitivity of PE also varies depending on its origin, with some forms being inactivated at 60°C and others at temperatures in excess of 80°C [2, 3, 11]. Pectinesterases of various origin were investigated by Zetelaki-Horwath [22, 23] (PE from *Aspergillus niger*), Dongowski and Bock [4] (PE from Asp. niger, oranges, tomatoes and white cabbage), Awad [1] persimmon, Jen and Robinson [6] (paprika), Pozsar-Hajnol and Polacsek-Racz [13] (tomatoes), Polacsek-Racz and Pozsar-Hajnol [12] (peaches, apples, carrots) and McFeeters et al. [11] (cucumbers), among others. All these studies

revealed a diversity of PE and of optimal parameters of its activity which may be due to the kinds and variety of fruit as well as to other factors. The precise determination of these parameters is needed for a better understanding of the changes induced by PE and for selecting raw material processing technologies minimizing MeOH production.

The aim of this research was the determination of the activity of PE from some fruits and of the optimal parameters of this enzyme's activity (pH and temperature).

MATERIAL AND METHODS

Following kinds and varieties of fruits were used:

Faworytka strawberries (*Fragaria grandiflora*), Zielony Butelkowy gooseberries (*Grossularia reclinata Mill.*), Goliat black currants (*Ribes L.Eucoreosma*), Red Cross red currants (*Ribes L. Ribesia*), Łutówka cherries (*Cerasus austera Roem.*) and Węgierka Zwykła plums (*Prunus L.*).The fruits were picked in 1987 on a plantation near Wieliczka in southern Poland. Fully ripe fruits were stored for 12—24 h at +7°C before analyses. The characteristic of the analysed fruits, conforming to Polish norms [15], is given in Table 1.

Table 1. Physico-chemical characteristic of the investigated fruits

Kind of fruit	Extract % weight	Total sugar as invert sugar, g/dm^3	Total acidity as apple acid, g/dm^3	pH
Strawberry	7.8	49.8	9.5	3.72
Gooseberry	9.5	65.2	16.3	3.45
Cherrys	13.7	112.4	11.8	3.41
Plums	16.3	124.8	11.4	3.90
Black currants	16.4	123.3	27.5	3.03
Red currants	12.6	84.9	21.9	2.85

ENZYME EXTRACTION

PE was extracted from fruit tissue with a variety of solutions and methods [1, 3, 11-13]. In our preliminary experiments we used the following solutions to extract PE: distilled H_2O, 5% water solution of NaCl (brine), 0.15 M Sörensen phosphate buffer (pH = 7.5) with 5% NaCl (buffer + brine), 5% water solution of NaCl with 1% Triton X-100 (brine + Triton), 1.6 M NaCl with 1% Triton, and 1.6 M NaCl with 5% Triton (Table 2). We found that PE was extracted best by 1.6 M NaCl with 1% Triton X-100, and by 5% NaCl with 1% Triton X-100. In the main body of experiments we used the former solution (1.6 M NaCl + 1% Triton).

Table 2. The influence of solvent upon the extraction of PE

Kind of fruit	The solution used for extraction						Standard deviation for 6	Variation coefficient V (%) for 6	Number of extractions
	water	brine*)	bufor**) + brine	brine + 1% Triton	1,6 M NaCl + 1% Triton	1,6 M NaCl + 5% Triton			
	PE activity in extract***)								
1	2	3	4	5	6	7	8	9	10
Strawberry	—	100	115	130	122	138	3.80	3.52	6
Gooseberry	3	100	107	160	162	164	2.20	1.36	6
Cherrys	30	100	109	146	158	158	1.45	0.95	6
Plums	18	100	98	135	137	131	1.82	1.41	6
Black currants	25	100	125	142	166	160	3.35	2.08	5
Red currants	2	100	133	138	140	143	3.73	2.66	6

*) 5% NaCl solution

**) buffer + brine in ratio 1:1

***) expressed as percentage of the brine extract

Extraction was performed by adding 70 cm^3 of cold (5°C) extraction solution to 70-g samples of chilled comminuted fruits. The samples thus prepared were homogenized for 30 s in an MPW-302 mixer with cutting blades, and then stored for 1 h in a refrigerator (5°C). Next, the suspension was centrifuged off (2000 g for 5 min), the suspension filtered through a double gauze and immediately analysed.

ACTIVITY DETERMINATION

PE activity was determined by the modified Kertesz method [7, 13]. The substrate in which activity and optimum pH and temperature were determined was a 0.5% solution of pectin A (Kǿbenhavns Pektinfabrik, Denmark) in 0.1 M NaCl. The degree of pectin esterification, determined by double titration [8, 14] was 65.5%. The pH of the pectin solution and enzyme extract was adjusted precisely to 7.5. 5 cm^3 of the extract were added to 50 cm^3 of the substrate and the mixture was titrated with 0.025 M NaOH (gooseberries, strawberries, red currants, and plums) or with 0.05 M NaOH (cherries and black currants) at a rate enabling the maintaining of pH at a constant level (7.5). The reaction was carried out in a thermostat (30°C) for 30 min, and the amount of lye that was used was recorded at 1-min intervals. PE activity was calculated on the basis of the amount of used-up base, assuming that 1 cm^3 of 0.01 M NaOH is equivalent to 320.34 μg MeOH. The PE unit was defined as the number of micrograms of MeOH released by the enzyme from 1 g of fruits during 1 min at 30°C.

In determining optimum pH and temperature of PE activity we used the same amounts of fruit extract and substrate as in activity determinations. To find optimum temperature, the pH of the substrate and extract was adjusted to the optimal value, and determinations were made at specific temperatures ranging from 30 to 80°C.

RESULTS AND DISCUSSION

The PE activity in fruit tissue extracts depended very closely on the kind of solvent used in extraction (Table 2). The experiments confirmed the advisability of selecting the most suitable solvent to extract PE from the various fruits. The 1.6 M NaCl was found to have no adverse effect on PE activity in the studied extracts. An inhibitory effect of brine solutions (in excess of 7.5%) in enzyme extraction was found by Polacsek-Racz and Pozsar-Hajnol [12], but in our experiments we used a different mixture (brine + Triton) and a different method of extraction.

The activity of PE from the studied fruits is characterized in Table 3. The greatest PE activity was found in cherries and black currants (5.97 and 4.96 U g, respectively) while the lowest figures were in strawberries and red currants (0.47 and 0.75 U g). The high PE activity in sweet cherries has already been described by Schmid [19]. Our earlier studies of demethylation of pectins during fermentation of fruit musts [20] also revealed a relatively high activity of PE in

Table 3. Characteristic PE activity in the investigated fruits

Kind of fruit	PE activity (U g^{-1}) in 30°C, pH — 7.5	PE activity (U^{-1}) in temp. optimum, pH — 7.5
Strawberry	0.47	1.40 (60°C)
Gooseberry	3.57	15.95 (70°C)
Cherrys	5.97	22.10 (60°C)
Plums	2.68	4.50 (60°C)
Black currants	4.96	12.48 (50°C)
Red currants	0.75	4.70 (60°C)

cherry, plum and black currant musts. In all the fruits except plums the activity of the enzyme measured at optimal temperature was about four times greater than at 30°C. It is noteworthy that PE activity in fruits may depend not only on the fruit kinds but also on fruit variety, climatic and soil conditions, ripeness, and crop year. In 1987 the vegetation cycle and harvests of fruits were much delayed.

OPTIMAL pH

The results of determinations of optimal pH are presened in Fig. 1a-f. In strawberries and plums this pH was 7.5, in gooseberries and black currants — 8.0, and in cherries and red currants — $\geqslant$ 9.0. The activity of PE in cherries (Fig. 1d) and red currants (Fig. 1e) was increasing up to the maximum pH value that was investigated (9.0) and it is likely that the optimal pH in these fruits is higher. The curves for gooseberries and currants (Fig. 1b, c, e) are characteristic, indicating untypical changes of PE activity in the pH range 7.0-8.0. These results indicate that there may exist two PE forms here. Jen and Robinson [6] demonstrated the presence of serveral forms of polygalacturonase and PE in sweet paprika, with a second pH optimum for the investigated enzymes appearing in the stage of full ripeness of the fruit. Versteeg et al. [21] demonstrated the existence of two PE isoenzymes in orange juice. In order to confirm our results, activity measurements ought to be performed for a purified enzyme isolated from fruit tissue. In all the investigated fruits PE was found to be inactive at pH ranging from 4 to 5-6. As is known, however, PE of fruit origin released MeOH in juices and musts, while the pH of the substrate is usually below 4.0. The likely explanation is that PE activity at pH ranging from 4 to 6 is low and was not determined in the presence of the highly methylated substrate. Pozsar-Hajnol and Polacsek-Racz [13] demonstrated that PE activity may be determined with highly methylated pectin as substrate only when pH exceeds 5.5.

OPTIMAL TEMPERATURE

The changes of PE activity in the studied fruits depending on temperature are illustrated in Fig. 2a-f. The extracts from strawberry, cherry, plum, and red-currant tissue were most active at 60°C, while the PE of gooseberries attained

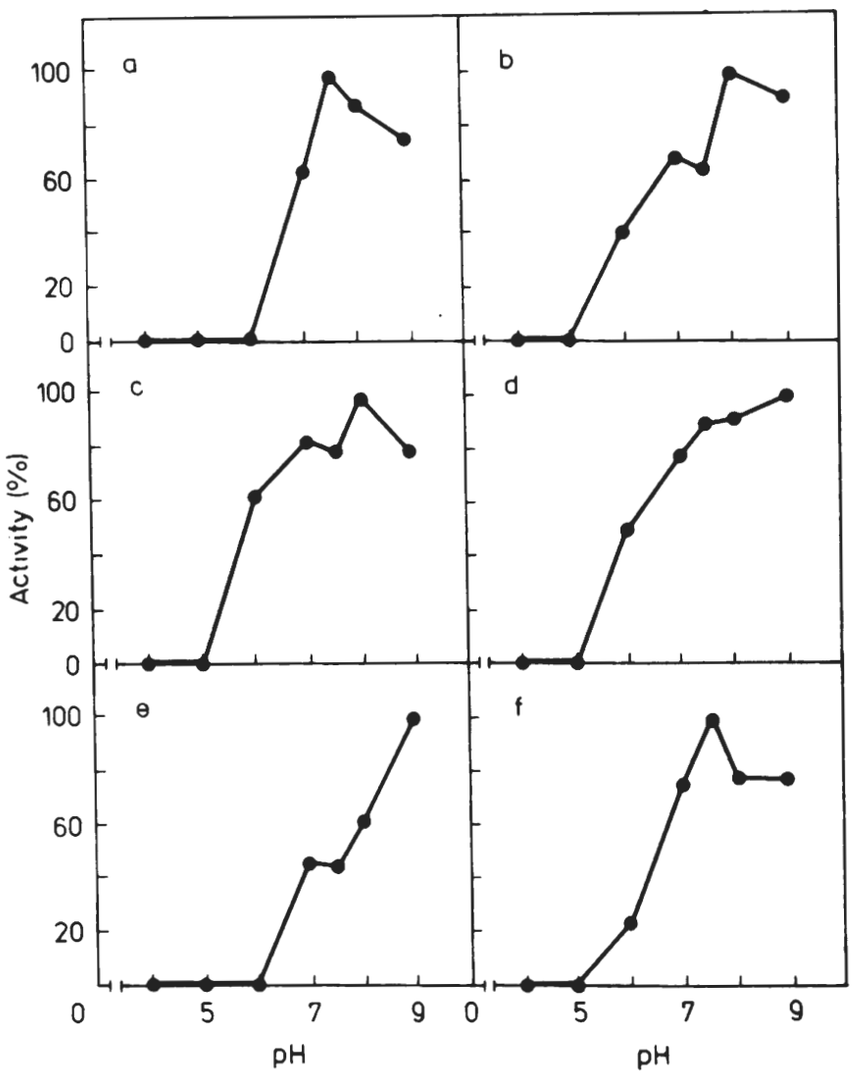

Fig. 1. Effect of pH on PE activity; a — strawberry, b — gooseberry, c — black currant, d — cherry, e — red currant, f — plum

maximum activity at 70°C. The lowest optimal temperature was in the case of black currants (50°C). At 30°C the activity of PE from plums and black currants was 40-60% lower than at optimal temperature. The activity of PE from the remaining fruits at 30°C ranged from 10 to 30% of the maximum value. At 80°C the demethylation of the substrate with fruit PE extracts was severely inhibited. The greatest activity at this high temperature was demonstrated by PE from strawberries, plums and cherries (20% of maximum activity) and the least activity — by the enzyme from gooseberries and currants (about 1% of peak activity).

EFFECT OF TEMPERATURE ON PE ACTIVITY

Fig. 3 presents the changes of PE activity due to temperature during demethylation of a 0.5% pectin solution. Pectin demethylation with extracts of PE from the studied fruits in temperatures from 30 to 80°C varied considerably. Temperatures of 30-60°C applied for 30 min failed to significantly affect the activity of PE from red currants (Fig. 3e) and plums (Fig. 3f). Temperature exerted a particularly singificant effect in extracts from black currants, cherries, and gooseberries. Temperatures of 40-50°C (black currants and cherries) and 50-60°C

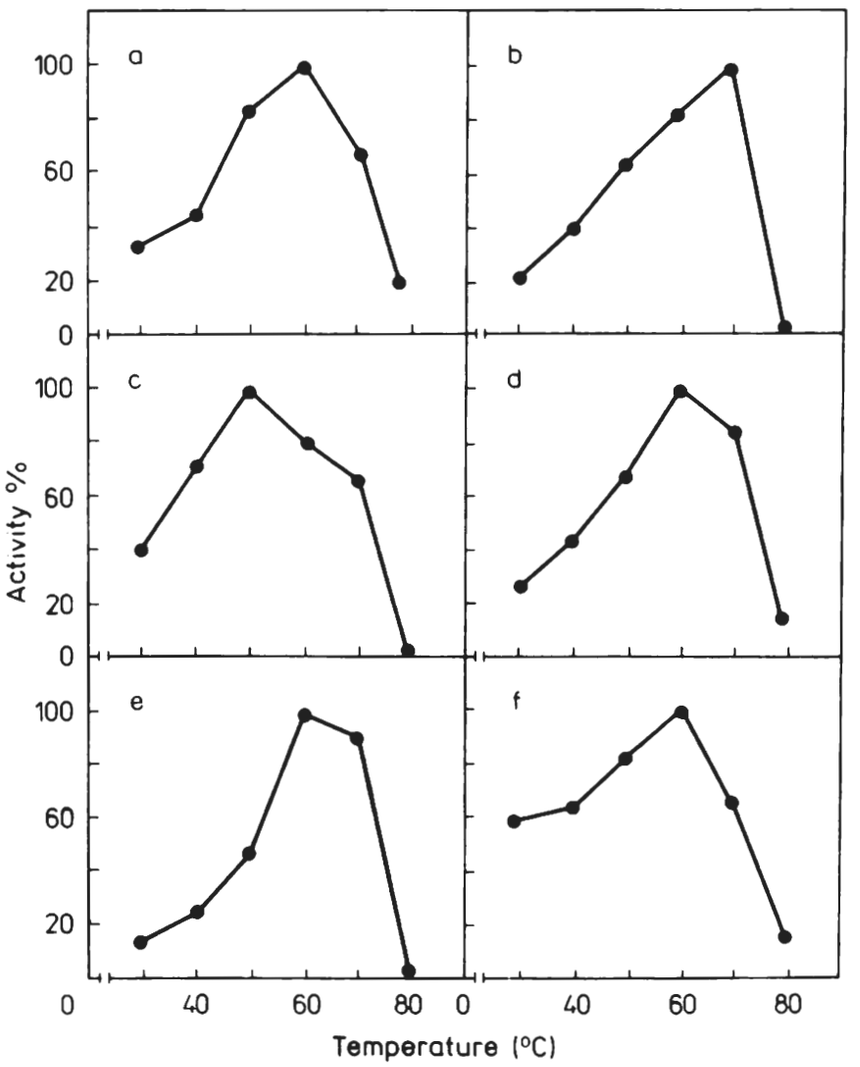

Fig. 2. Effect of temperature on PE activity; explanations — see Fig.1

(gooseberries) reduced the demethylation rate to its minimum level after 15-25 min. At 80°C demethylation was almost completely arrested after a mere 1-5 min.

Various thermal stabilities of what were probably different forms of PE was observed by Brady [3] in experiments with bananas, and by Versteeg et al. [21] in orange juice. Schmid [19] demonstrated that cherry must heated to 70°C loses its PE activity in a matter of several minutes. In this research we did not determine the temperature of inactivation of PE from the studied fruits. The inactivation temperature may be different from that causing the lowest pectin demethylation rate.

EFFECT OF SUBSTRATE CONCENTRATION ON PE ACTIVITY

Figs 4 and 5 illustrate the effect of substrate concentration on the activity of plum PE. The activity of this enzyme peaked at the Michaelis constant $Km = 6.25 \times 10^{-1}$ g/dm^3. When the enzyme was fully saturated by the substrate, the reaction rate (Vmax) was 3.32 μg/g/min. These figures are only a rough estimate of the enzyme-substrate affinity since we performed our experiments with fruit tissue extracts and not with isolated and purified enzymatic protein.

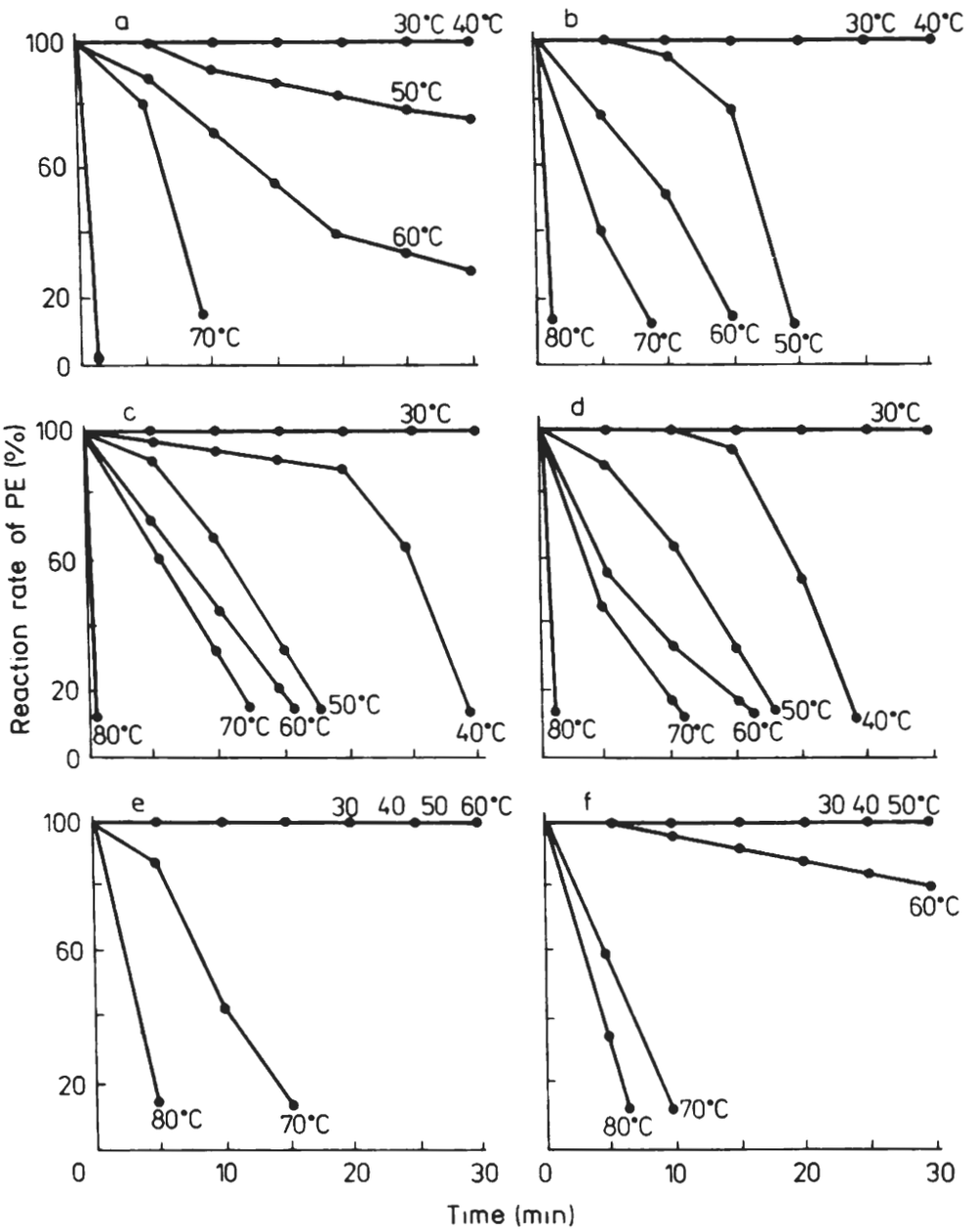

Fig. 3. Effect of time and temperature on the reaction rate of PE; explanations — see Fig.1

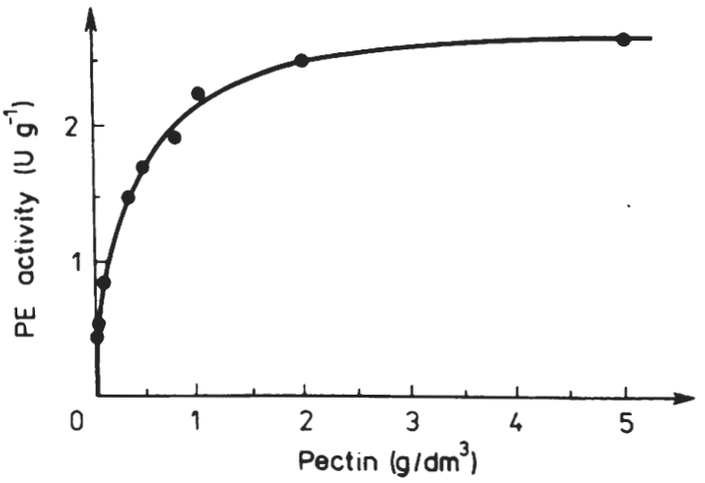

Fig. 4. Effect of substrate concentration on the activity of PE from plums (30°C, pH 7.5)

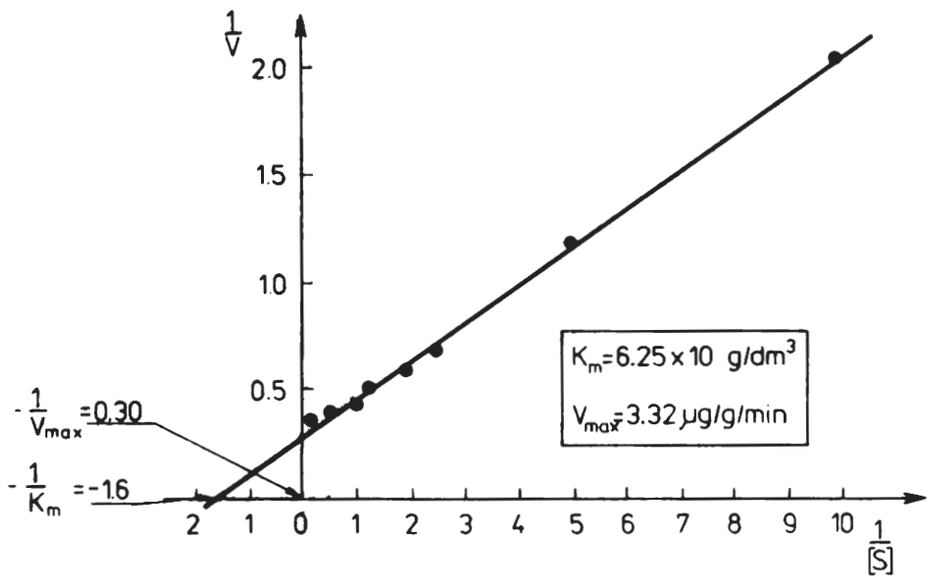

Fig. 5. Graphical representation according to Lineweaver-Burk of the relation between PE activity from plums and substrate concentration

In general, our experiments show that the PE activity in the investigated fruits varies, and that optimal parameters of enzyme activity depend on the origin of the enzyme (kind of fruit). Accordingly, the various fruits should be processed differently in order to limit MeOH liberation.

CONCLUSIONS

1. The investigated fruits differ as to pectinesterase activity. The highest activity of this enzyme was in cherries and black currants (5-6 U/g) and the lowest — in strawberries and red currants (0.5-0.75 U/g).

2. Maximum activity of pectinesterase from the studied fruits occurs in the pH range of 7.5-9.0, and optimal temperature is 50-70°C.

3. During pectins demethylation with pectinesterase from the investigated fruits at 80°C, the rate of this reaction drops to its minimal value after 1-5 min. During 30 min of activity of the enzyme from plums and red currants at 30, 40, 50 and 60°C, the rate of pectins demethylation is close to maximum.

LITERATURE

1. Awad M.: J. Food Sci., 1985, 50, 1643.
2. Awad M., Young R. E.: J. Amer. Soc. Hort. Sci., 1980, 105 (5), 638.
3. Brady C. J.: Aust. J. Plant. Physiol., 1976 (3), 163.
4. Dongowski G., Bock W.: Die Nahrung 1984, 28 (5), 507.
5. Jakob M., Hippler R., Lüthi H. R.: Lebensm. Wiss. Technol., 1973 (6), 138.
6. Jen J. J., Robinson M. L.: J. Food Sci., 1984, 49, 1085.
7. Kertesz Z. J.: Pectic enzymes. In: Colowick S. P., Kaplan N. V.: Methods in Enzymology, Acad. Press, New York, vol. I, 1955, 158.
8. Kujawski M., Tuszyński T.: Die Nahrung 1987, 31 (3), 233.
9. Markovic O.: Collect. Czech. Chem. Commun., 1974, 39 (3), 908.
10. Markovic O., Heinrichova K., Lenkey B.: Collect. Czech. Commun., 1975, 40 (3), 769.

11. McFeeters R. F., Fleming H. P., Thompson R. L.: J. Food Sci., 1985, 50, 201.
12. Polacsek-Racz M., Pozsar-Hajnal K.: Acta Alimentaria 1976, **5** (3), 189.
13. Pozsar-Hajnal K., Polacsek-Racz M.: Acta Alimentaria 1975, **4** (3), 271.
14. Collective work: Food Chemical Codex, National Academy of Sci., Washington D. C. 1972.
15. Polska Norma: PN-66/A-79120.
16. Rexova-Benkova L., Markovic O.: Adv. Carbohydr. Chem. Biochem., 1976, 33, 323.
17. Rombouts F. M., Pilnik W.: Flüss. Obst 1971, 38(3), 93.
18. Sapożnikowa E. V.: Pektinowyje Wieśćestwa Płodow, Izd. Nauka, Moskwa 1965.
19. Schmid P.: Z. Lebensmitt. Untersuch. und Forschung 1975, 168(1), 23.
20. Tuszyński T.: Materiały XVII Sesji Naukowej KTiCHŻ PAN, Łódź 1986, II, 445.
21. Versteeg G., Rombouts F. M., Spaansen G. H., Pilnik W.: J. Food Sci., 1980, 45, 969.
22. Zetelaki-Horvath K.: Acta Alimentaria 1981, 10(4), 371.
23. Zetelaki-Horvath K.: Acta Alimentaria 1983, 12 (1), 11.

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CHARAKTERYSTYKA AKTYWNOŚCI PEKTYNOESTERAZY W NIEKTÓRYCH OWOCACH

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Streszczenie

W doświadczeniach stosowano owoce truskawek, agrestu, czarnych i czerwonych porzeczek oraz wiśni i śliwek (tab. 1), w których oznaczano aktywność pektynoesterazy (PE) oraz określano optymalne parametry jej działania. Do ekstrakcji PE z tkankami owoców stosowano różne roztwory, z których najlepszy był 1,6 MNaCl + 1% Tritonu X-100 i ten użyto w zasadniczych doświadczeniach (tab. 2). Aktywność PE oznaczano miareczkową metodą Kertesza, a substratem był 0,5% roztwór pektyny w 0,1 M NaCl.

Stwierdzono, że badane owoce charakteryzują się różnymi aktywnościami PE (tab. 3). Największą aktywność enzymu miały wiśnie (5,97 J/g) i czarne porzeczki (4,96 J/g) a najmniejszą truskawki (0,47 J/g) i czerwone porzeczki (0,75 J/g). Wyznaczono optymalne parametry (pH i temperatura) działania PE badanych owoców, które mieszczą się w następujących granicach: pH od 7,5 do $\geqslant$ 9,0 i temperatura 50-70°C (rys. 1 i 2). Otrzymane wyniki (rys. 1b, c i e) wskazują na możliwość istnienia w agreście i porzeczkach dwóch form PE.

Wykazano, że działanie temperatury w zakresie 30-60°C w ciągu 30 min nie ma istotnego wpływu na szybkość reakcji demetylacji pektyn pod wpływem PE z czerwonej porzeczki i śliwki (rys. 3). PE śliwek wykazywały maksymalną aktywność dla stałej Michaelisa $Km = 6{,}25 \times 10^{-1}$ g/dm^3, a w warunkach pełnego wysycenia enzymu przez substrat szybkość reakcji (Vmax) była równa 3,32 µg/g/min (rys. 4 i 5).