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INTERRELATION BETWEEN THE EFFECT OF ENZYMATIC CLARIFICATION OF APPLE JUICES AND THE AMOUNT AND QUALITY OF POLYPHENOLS. PART II. CHANGES OF POLYPHENOLS DURING THE PRODUCTION OF APPLE JUICE AND THEIR EFFECT ON PECTINOLYSIS

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The inhibitory effect of polyphenols of apple juices on the activity of pectinolytic enzymes depends on their content in the juices and also on their forms of occurrence. Oxidized polyphenols as well as reduced forms of these compounds are clearly inhibitory. Effective methods of counteracting the adverse effect of polyphenols on pectinolytic enzymes may be PVP additions to the juices, brief aeration of the juices, and additions of antioxidants.

In the first part of this research [15] it was found that nearly all of the studied polyphenols inhibit the activity of polygalacturonases and pectinesterase to a greater or lesser extent, with the degree of inhibition depending mainly on the amount and form of the polyphenols. The degree of oxidation of these compounds is of primary importance here [13]. Other authors [2, 14, 16, 21, 28] have found that the inhibitory effect of polyphenols increases with the increasing degree of their oxidation. This is especially true of leucoanthocyanidins and catechins which are compounds particularly susceptible to oxidation.

One method of safeguarding polyphenols against oxidation, and hence of counteracting increases of their inhibitory effect, is the addition of antioxidants such as l-ascorbic acid or SO₂ to pulps or juices [6, 18, 19, 26]. Juices containing SO₂ were found to contain about 50% more polyphenols than control samples [18]. This phenomenon makes possible another method of avoiding the inhibitory effect of polyphenols on pectinolytic enzymes consisting in intense oxydation (15-30 min) of the pulp or juice prior to pectinolysis, and subsequent removal of the oxidized and polymerized polyphenols by leaving them in the pulp or by filtration. This procedure has already been applied in practice (e.g. [3, 9, 22]).

Yet another way of inactivating polyphenols in apple juices consists in adding c. 0.04% doses of porous polymers of the polyvinyl polypyrrolidone (PVPP) or polyvinyl pyrrolidone (PVP) type. These compounds are capable of adsorbing polyphenols in quantities depending on their chemical structure. According to literature reports [4, 7, 9, 12, 23, 29], PVPP or PVP remove about 60% of polyphenols, considerably reducing their adverse effect on pectinolytic enzymes, with the remaining polyphenols improving the sensory properties of the juices.

The aims of the second part of our investigations, concerning the effect of apple juice polyphenols on the enzymatic decomposition of pectins, were:

— the determination of the effect of industrially applicable technological processing on quantitative changes in the polyphenols fraction of apple juices, and hence on the course of pectinolysis, and

— the determination of the effect of peculiarities of different apple varieties, and of the content of pectinic substrate on the course of enzymatic pectinolysis.

MATERIAL AND METHODS

Juices were produced from four apple varieties (Landsberska, Boiken, Mc Intosh, Idared) which are characterized chemically in Table 1. The order in which the apple varieties were processed corresponded to the order in which they ripened; in all cases the ripeness was assessed according to industrial standards.

Table 1. Basic chemical composition and activity of oxidizing enzymes in the studied apple varieties

Apple variety	Extract (%)	Titratable acidity (%)	pH	Total polyphenols (mg/kg)	Total pectins (%)	Activity of	
						o-diphenol oxidase (mg purpgal) 100 g	peroxidase (mg purpgal) 100 g
Landsberska	10.2	0.9	3.38	3000	1.00	3.42	1.78
Boiken	10.0	0.9	3.36	2800	0.72	15.40	2.56
Mc Intosh	13.0	0.7	3.65	2400	0.98	38.30	7.73
Idared I	13.5	0.7	3.70	3300	0.94	4.80	3.60
Idared II	13.0	0.6	3.55	3400	0.70	10.32	8.08

The juices from all the apple varieties were obtained on laboratory scale according to the following technological modifications:

- 1) control-comminution, juice separation, filtration, pouring into jars, preservation;
- 2) addition of SO₂ to the juice, filtration, pouring into jars, preservation;
- 3) addition of PVP to the juice, filtration, pouring into jars, preservation;

- 4) aeration of the juice (30 min), filtration, pouring into jars, preservation;
- 5) aeration of the juice (60 min), filtration, pouring into jars, preservation.

Each of the five technological modifications had three technological variants: A — with no enzymatic processing of the juice, B — with the application of the Pektopol PT pectinolytic preparation, C — with the Pectinol DHT preparation. Moreover, in the second year of studies, the juice from Idared apples was supplemented with 0.2% apple pectin preparation in order to study the effect of polyphenols on pectinolysis in a medium with increased substrate content. It was also possible to investigate changes in pectins during their enzymatic degradation.

The apples were washed, comminuted, and the juice separated in a juice extractor. Before pouring into jars with twist-off caps, the juice was filtered through a filter paper, thermally deaerated, pasteurized at 85°C for 20 min, and then cooled to 30°C.

The juices were processed enzymatically with the Pektopol PT and Pectinol DHT preparations applied in doses recommended by the producers (0.5 and 0.05 g/dm³, respectively). In both cases the parameters of pectinolysis were identical: 45-50°C and 90 min.

Sulphur dioxide (50 mg/dm³) was added directly to the juice taken from the juice extractor, in the form of a 4% water solution. The polyvinyl pyrrolidone K-30/PVP dose in the juice was 0.04%, and aeration was done mechanically with a magnetic mixer.

The following determinations were made in the juices:

- extract by the refractometric method,
- titratable acidity (as apple acid) by the potentiometric method,
- dynamic viscosity with a Höppler viscosimeter,
- total polyphenols (as tannic acid) by the Folin-Denis colorimetric method using the Folin-Ciocalteu reagent,
- leucoanthocyanidins (as cyanidin chloride) by the colorimetric method according to Swain and Hillis [22],
- catechins (as dl-catechin) by the colorimetric method using the vanilin reagent according to Swain and Hillis [24],
- methanol content by the colorimetric method with chromotropic acid according to Ishii and Yokotsuka [8],
- total pectins by their precipitation with ethanol by the method of Ishii and Yokotsuka [8], and then colorimetrically by the carbazole method modified by Bartholomae [1].

In the Idared apple juice supplemented with the apple pectin preparation there were two additional determinations:

- degree of pectins esterification by the titration method,
- mean molecular masses of the pectins by the viscosimetric method.

The obtained results were interpreted statistically by two- and three-directional variance analysis.

RESULTS AND DISCUSSION

It is evident from the obtained extract contents and titratable acidity that the main factor responsible for differences between juices is apple variety. Juices from Landsberska and Boiken varieties had 10.0% extract, while in Mc Intosh and Idared varieties the figure stood at 13.0-13.5%. The acidity of juices from the former two varieties amounted to 9.0 g of acids per dm^3 , while that of juices from the latter two varieties was 6.5-6.7 g of acids per dm^3 . Similar relationships were observed in the case of the juices' dynamic viscosity. Comparing polyphenols contents in apples (Table 1) and in juices produced from the (Table 2), it can be seen that the total polyphenols content in apples is three times that in juices. The drop of polyphenols content in the juices is due to their aeration in the juice extractor.

Modifications in the technology of producing apple juices led to significant changes in total contents of polyphenols, catechins, and leucoanthocyanidins in the juices. The contents of these compounds in juices from apples of the different varieties are given in Table 2.

The differences in total polyphenols content in the juices, due to the technology of juice production, were fairly substantial (four- to eight-fold). The highest polyphenols content was in samples 1 (control) and 2 (SO_2 addition), and the lowest in sample 5 (aeration of the juice for 60 min).

Another factor affecting the total content of polyphenols in juices is apple variety. The Idared variety was found to contain the largest amount of these compounds (on average 1150 mg per dm^3), next came the Landsberska variety (about 1000 mg/ dm^3), the Boiken variety (880 mg/ dm^3), and last was the Mc Intosh variety (800 mg polyphenols per dm^3). The significance of both these factors, i.e. technological modifications and apple variety, in affecting polyphenols content in juices is also indicated by results of variance analysis.

The catechins content in control juices (sample 1A) ranged widely from 80 mg/ dm^3 in the Boiken variety to 280 mg/ dm^3 in the Idared apple juice. Aside from apple variety, a significant factor affecting the differences in catechins content in the juices was the method of juice production. The changes in this content were of the same character as in the case of total polyphenols but the differences themselves were much greater: juices from Mc Intosh apples produced according to method 2 (SO_2 addition) contained about 600 mg of catechins in every dm^3 , while in aerated juice (sample 5) this content was about 120 times lower, amounting to about 5 mg/ dm^3 .

The leucoanthocyanidins content in the juices ranged from 350 mg/ dm^3 (Boiken) to 750 mg/ dm^3 (Idared). Similarly as in the case of total polyphenols and catechins, here too the SO_2 addition caused an apparent increase of the content of leucoanthocyanidins, ranging from 20 to 100% depending on apple variety. A PVP addition to the juices reduced the content of these compounds by 10-90%, and aeration of the juices — by about 50%.

The technological modifications of juice production aimed at varying the

Table 2. Contents of polyphenol compounds in apple juices

Technological variant	Apple variety												
	Landsberska			Boiken			Mc Intosh			Idared			
	Total poly-phenols (mg/dm ³)	catechins (mg/dm ³)	leucoanthocyanidins (mg/dm ³)	total poly-phenols (mg/dm ³)	catechins (mg/dm ³)	leucoanthocyanidins (mg/dm ³)	total poly-phenols (mg/dm ³)	catechins (mg/dm ³)	leucoanthocyanidins (mg/dm ³)	total poly-phenols (mg/dm ³)	catechins (mg/dm ³)	leucoanthocyanidins (mg/dm ³)	
1	A	1000	170	380	950	80	360	850	240	500	1200	280	800
	B	1000	240	450	800	70	340	800	200	450	1100	250	700
	C	1000	180	380	900	80	350	750	170	400	1150	230	750
2	A	1600	420	880	1550	170	800	1450	550	850	1600	450	980
	B	1700	370	850	1500	150	750	1500	580	900	1550	430	870
	C	1500	360	870	1600	160	800	1550	620	950	1550	430	870
3	A	800	140	440	800	40	300	700	150	350	600	50	400
	B	750	110	300	750	30	280	750	180	400	560	40	370
	C	750	110	340	750	40	300	700	140	320	600	70	380
4	A	300	30	180	250	20	180	200	20	170	750	100	480
	B	400	30	200	250	20	170	200	25	165	700	80	450
	C	300	30	200	300	25	170	150	10	130	650	60	430
5	A	200	30	140	200	15	150	150	10	135	400	30	280
	B	200	30	130	200	15	140	100	5	80	380	30	260
	C	200	30	140	150	20	150	100	5	85	350	30	240

polyphenols content in the juices, exploited the most important line of transformations of these compounds, namely that based on oxidation. An addition of 50 mg of sulfur dioxide per dm³ of juice inhibited these transformations, and the greatest increase of polyphenols content due to SO₂ was observed in juices originally displaying the highest activity of the oxidizing enzymes (Mc Intosh). In Landsberska and Idared juices these changes were small, this being a result of low activities of oxidizing enzymes (Table 1).

Similar relationships were observed in aerated juices, since the greatest losses of polyphenols were found in juices produced from apples with the most active oxidation system (Mc Intosh), with the lowest losses due to oxidation being observed in juices from apples with the lowest activities of diphenol oxidases (Idared and Landsberska).

As can be seen from Table 2, the compounds most labile in this respect are catechins. This observation is compatible with literature reports on their reactivity [20]. It is known that some of the polyphenols in unoxidized form inhibit polygalacturonases and pectinesterase, and so by preventing their oxidation one also increases their inhibitory effect. Other polyphenols, however, become inhibitors only after oxidation, and in this case aeration, a process accelerating oxidation, increases their inhibitory potential [14].

Table 3. Methanol and pectins content in apple juices

Technological variant	Apple variety								
	Landsberska		Boiken		Mc Intosh		Idared		
	methanol (mg/dm ³)	total pectins (mg/dm ³)	methanol (mg/dm ³)	total pectins (mg/dm ³)	methanol (mg/dm ³)	total pectins (mg/dm ³)	methanol (mg/dm ³)	total pectins (mg/dm ³)	
1	A	7.8	197	3.3	70	8.6	144	8.8	150
	B	12.6	102	6.0	54	14.4	78	25.2	120
	C	9.4	146	5.6	60	12.2	92	14.6	130
2	A	11.0	168	6.0	120	8.0	136	10.4	180
	B	12.2	124	9.6	80	12.0	102	20.2	160
	C	12.0	164	8.4	100	11.4	114	18.4	160
3	A	7.6	164	4.2	84	7.4	126	7.8	170
	B	9.6	77	7.6	52	16.8	64	14.4	100
	C	8.4	111	6.0	63	15.0	88	12.2	120
4	A	7.6	162	4.0	80	8.2	134	10.0	185
	B	14.4	70	5.8	48	15.2	72	19.6	120
	C	12.0	70	5.2	60	14.8	84	14.2	150
5	A	10.0	127	8.2	100	8.2	140	10.4	180
	B	14.4	64	10.8	82	16.0	88	16.8	140
	C	12.4	111	9.2	90	14.8	96	13.2	150

The polyphenols being substrates for diphenol oxidases that were reported in apple juice include p-coumaric, caffeic, and chlorogenic acids, and also catechins and leucoanthocyanidins [5, 11, 15, 21, 24]. Flavonol glucosides and phloretin derivatives are immune to these enzymes [10].

As shown in the first part of this research, the strongest inhibitors of pectinolytic enzymes are catechin, flavonols-quercetin, kempherol, rutin, phenylpropenic acids (o-, m-, and p-coumaric), and tannic acid [15]. Given this, it may be suspected that the seemingly advantageous process of juices aeration does not lead to the expected improvement of the action of pectinolytic enzymes, this being due to the fact that the polyphenols, active inhibitors in reduced form, are not substrates of phenol oxidases and hence remain unchanged, while the inhibitory force of catechin-type compounds increases greatly [16, 28].

The 0.04% PVP addition to juices reduces the total polyphenols content by 5 (Mc Intosh) to 40% (Idared). The greatest losses were in the catechins fraction, ranging from 25% in Mc Intosh juices to 80% in Idared juices (Table 2).

The apple variety factor and the related differences in the activity of oxidizing enzymes have in this case a reverse effect. The least losses of the determined polyphenols fractions occurred in juices from Mc Intosh apples having the most active oxidases system, while the highest losses of polyphenols content were observed in juices from Idared apples which are characterized by the lowest activity of oxidizing enzymes. This may indicate that PVP more readily adsorbs compounds of low molecular mass, a fact confirmed by other authors [4, 7, 12]. Judging by the effect of PVP on enzymatic decomposition of pectins, it seems that an addition of this compound, particularly to juices with a weak system of oxidizing enzymes or with these enzymes protected against oxidation (addition of antioxidants), may effectively prevent the adverse effect of polyphenols on pectinolytic enzymes.

The observed changes in the amounts of methanol released in the juices indicate pectins deesterification, while differences in the amounts of pectins are indicators of their degradation. The contents of methanol and pectins in the studied apple juices are given in Table 3.

The amount of methanol released in juices depends on the joint effect of three factors: apple variety, the method of juice production, and the pectinolytic preparation used in pectinolysis. When the Pektopol PT preparation was added to the juice, the increase of methanol content ranged from 70 to about 190%, while in the case of the Pectinol DHT preparation this increase was much lower, ranging from 20 to 75%. As regards apple variety and the related differences in polyphenols content in juices, the largest amounts of methanol were released in juices from Idared apples having the highest content of polyphenols. The reverse dependence was also observed. This kind of relationship is also confirmed by the effect of technological processes modifying the polyphenols content in juices on pectins deesterification. In aerated juice samples in which polyphenols were oxidized and polymerized, the increases of methanol were small, while in juices with SO₂ in which the seemingly increased polyphenols content was due to the

reduced form of these compounds, the increments of released methanol were greater. This may be evidence of stronger inhibition of pectinesterase activity by oxidized polyphenols.

The different relationships observed in Mc Intosh apple juices may be caused by the high activity of their phenol oxidases. Some role is also played by the degree of pectins esterification and by other factors inhibiting the activity of pectinesterase, this being also true of the other apple varieties. Hence, it is difficult to draw final conclusions from the accumulated data, and further studies are required.

The greatest amounts of pectins in juices produced from the various apple varieties occurred in the Idared variety which also contained the largest amount of polyphenol compounds. Thus, their degradation in this apple variety was least intense. SO₂ additions did not alter pectins content to any great extent, while drops in polyphenols contents in juices treated with PVP (sample 3) or subjected to 30-min aeration led to considerable degradation of these substances. The longer (60 min) aeration of juices caused the relatively lowest decrease of pectins content. This proves that the activity of pectinolytic enzymes is weakened by unoxidized polyphenols. Similar results were obtained by Pisarnicki and Gołyszewa [18] who aerated apple juices for 24 h and observed that the rate of pectins degradation decreases with the increase of the degree of polyphenols oxidation. They also demonstrated that SO₂ added to the juices reduces the adverse effect of this phenomenon on pectinolysis.

In order to determine the effect of polyphenols on pectinolysis in juices with increased pectins content, the process was studied in juices supplemented with 0.2% apple pectin (Table 4).

The data in Table 4 indicate that the increase of polyphenols content in juices (sample 2) caused small increases of methanol content and very small losses of pectins, while the decrease of polyphenols content (samples 3 and 4) caused a fairly substantial increase of methanol content as well as a significant drop of pectins content.

To get a better idea about the changes of pectinic substances during enzymatic degradation, the degrees of their esterification and mean molecular masses were determined (Table 4). Worth pointing out is that the decrease of pectins esterification level, which is also a measure of pectinesterase activity, was in this case small and correlated with the increase of methanol content in the juices. Given the intentionally increased content of pectins in the apple juices, even the smallest changes of pectins esterification degree were accompanied by fairly substantial releases of methanol.

The changes of molecular masses of pectins, being the result of the action of polygalacturonases, depend largely on the quantitative proportions between exo- and endo- forms of these enzymes. The Pectinol DHT preparation used in the experiments was found to have a predominantly endo-PG effect, while Pektopol PT displayed greater activity of the exo- type.

As regards the effect of the various technological modifications on enzymatic

Table 4. Characteristic of Idared apple juices supplemented with 0.2% of apple pectin preparation

Technological variant	Total polyphenols (mg/dm ³)	Catechins (mg/dm ³)	Leucoanthocyanidins (mg/dm ³)	Methanol (mg/dm ³)	Total pectins (mg/dm ³)	Degree of pectins esterification (%)	Mean molecular mass of pectins (M·10 ³)	
1	A	1200	240	580	43.6	1790	70.4	209.2
	B	1000	200	470	80.0	1100	64.0	95.5
	C	800	220	430	44.4	1700	70.2	72.3
2	A	1550	420	840	44.4	1890	70.6	200.8
	B	1600	480	980	68.4	1500	68.8	155.8
	C	1500	370	780	52.4	1670	70.3	68.0
3	A	750	100	520	46.0	1820	70.8	201.2
	B	600	60	350	82.8	1050	62.3	70.9
	C	700	80	470	64.8	1350	67.1	59.0
4	A	780	60	430	42.0	1940	72.9	209.4
	B	600	40	320	76.0	1010	64.2	145.1
	C	550	30	320	61.2	1290	66.4	71.5
5	A	440	30	320	48.4	1870	72.8	200.4
	B	410	30	240	72.0	910	66.3	110.8
	C	430	30	280	60.0	1230	68.4	70.0

degradation of pectins, the PVP addition (sample 3) was found to improve the process most, whereas the worst results were obtained by adding SO₂ to the sample (number 2).

Generally speaking, when the juice contains greater amounts of pectins, the inhibitory effect of polyphenols on pectinolytic enzymes may be weaker, with the reduced forms of polyphenols playing the greater role here. It thus seems that a good method of preventing the adverse effect of polyphenols on enzymatic pectinolysis is the addition of PVP. A positive effect may also be achieved by brief aeration of the juices, but this method is dangerous in that it may lead to excessive oxidation changes in the polyphenols fraction in juices with a high activity of oxidizing enzymes, thereby leading to results opposite to those desired.

CONCLUSIONS

1. Apples of the Landsberska and Idared varieties contain more polyphenols than the Boiken and Mc Intosh varieties.

2. The inhibitory effect of apple juice polyphenols on the activity of pectinolytic enzymes depends on their content in the juices, the degree of their oxidation, and on the quantity and quality of the pectic substrate. The activity of pectinolytic enzymes is also important here.

3. The adverse effect of polyphenols on pectinolytic enzymes may be effectively counteracted by a PVP addition to the juices or their brief aeration, and also by additions of antioxidants. The selection of the most suitable method depends largely on the amount of pectins in the juices.

4. Further studies are planned in view of the lack of univocal results concerning the effect of polyphenols on pectinesterase contained in the enzymatic preparations, and the effect of various amounts of pectins on the inhibition of pectinolytic enzymes.

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WSPÓLZALEŻNOŚĆ POMIĘDZY EFEKTEM ENZYMATYCZNEGO KLAROWANIA SOKÓW JABŁKOWYCH A ILOŚCIĄ I JAKOŚCIĄ POLIFENOLI W NICH ZAWARTYCH. CZ. II ZMIANY POLIFENOLI W CZASIE OTRZYMYWANIA SOKU JABŁKOWEGO I ICH WPŁYW NA PROCES PEKTYNOLIZY

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Streszczenie

Przeprowadzone badania dotyczyły wpływu różnic odmianowych jabłek (Landsberska, Boiken, Mc Intosh, Idared) oraz zawartości substratu pektynowego (dodatek preparatu pektynowego) na przebieg enzymatycznej pektynolizy, prowadzonej z udziałem preparatów pektynolitycznych Pektopol PT i Pectinol DHT. Określano również wpływ dodatku przeciwutleniacza (SO_2), PVP, procesu depektynizacji oraz napowietrzania soków na zawartość związków polifenolowych. W zależności od zastosowanego sposobu otrzymywania, soki jabłkowe zawierały zróżnicowane ilości polifenoli, a mianowicie: katechin ogółem od 5 do 620 mg/dm^3 , leukoantocyjanidyn ogółem od 80 do 980 mg/dm^3 i polifenoli ogółem od 150 do 1700 mg/dm^3 (tab. 2). Dodatek SO_2 do soków w ilości 50 mg/dm^3 powodował zahamowanie przemian oksydacyjnych polifenoli i stąd pozorny wzrost ich zawartości, przy czym w zależności od poziomu aktywności enzymów utleniających (tab. 1) obserwowany „wzrost” zawartości polifenoli był zróżnicowany (w sokach z jabłek odmiany Mc Intosh — największy, a Landsberska i Idared — najmniejszy). Dodatek PVP do soków w ilości 0,04% przyczyniał się do spadku zawartości polifenoli, przy czym największe straty występowały we frakcji katechin (od 25% Mc Intosh, do 80% — Idared). Efektem napowietrzania soków były straty w zawartości polifenoli, zwłaszcza katechin, przy czym w sokach z jabłek o najaktywniejszym systemie oksydacyjnym (Mc Intosh) były największe. Przebieg procesu pektynolizy soków pod wpływem preparatów Pektopol PT i Pectinol DHT oceniano na podstawie spadku lepkości oraz zawartości pektyn ogółem i metanolu (tab. 3), a w sokach wzbogaconych dodatkiem preparatu pektyny jabłkowej na podstawie stopnia zestyfikowania oraz średniej masy cząsteczkowej pektyn (tab. 4).

Stwierdzone zróżnicowanie w ilościach uwalnianego metanolu oraz stopnia degradacji pektyn (tab. 3 i 4) pozwala przypuszczać, że inhibicyjne właściwości mają zarówno utlenione jak i zredukowane formy polifenoli, przy czym stopień powodowanej przez nie inhibicji zależy od ilości zawartych w sokach pektyn. Stąd też dobór skutecznej metody zabezpieczającej enzymy pektynolityczne przed niekorzystnym wpływem polifenoli należy uzależniać także od poziomu zawartości pektyn w sokach.