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CHANGES IN THE POLYPHENOLIC COMPOUNDS OF APPLE PULP

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Key words: polyphenolic compounds, apple must.

The content of polyphenolic compounds and the colour of apple must obtained from the apple pulp being stored before pressing was studied. Changes in the polyphenolic compounds were intensive in the first minutes after apple comminution. Especially susceptible to the reaction were procyanidins and flavanols. The changes in polyphenolic compounds were accompanied by the must browning process being more intensive in case of turbid than clear must.

INTRODUCTION

Polyphenols are commonly found in raw material of plant origin. Apples, being a part of such compounds, contain among other things: catechins, procyanidins and chlorogenic acid, i.e. components which are very susceptible to enzymatic oxidation [2, 6, 7, 9]. Such a reaction takes place after milling apples in the presence of oxygen. Colourless polyphenols are oxidized to quinones which are characterized by high reactivity. They become condensed between one another and yield coloured products with other apple components [8, 11].

In apple must production, after milling the raw material, apple pulp is getting brown due to the reactions taking place in the pulp. The changes in polyphenolic compounds have an adverse effect on the quality and nutritive value of must.

The intensity of enzymatic oxidation within various groups of polyphenols is different and does not affect the must colour in the same degree. The authors of the present study tested kinetics of the changes within polyphenols in apple pulp. At the same time, they determined the colour of must samples, both clear and turbid, obtained from the pulp

and calculated linear correlation coefficients between the parameters of must colour and polyphenols.

MATERIALS AND METHODS

The material used in the studies was made up of apples of the Golden Delicious variety. The apples were comminuted by hand on a plastic grate. A "0" sample was obtained after mixing the pulp with 1.7% NaCl (10% water solution) in the course of apple grating. To the other samples was added NaCl after pulp storage for 3, 5, 15, 30, 60, 120 and 240 minutes at 20°C. The time measurement started after grating a half of 1 kg apple portions. Two kg samples of pulp were prepared for pressing. The must pressed out of the samples was called turbid, whereas clear must was that centrifuged for 15 minutes on a laboratory centrifuge (type 310) of 16 000 rpm (19 200 g).

Polyphenols in the must were determined quantitatively and qualitatively and the colour of must was described. The following components were determined: total polyphenols with Folin-Ciocalteu'a reagent [1], flavonoids and non-flavonoids according to Karmling and Singlenton [1], procyanidins according to Pompei and Peri [12], flavanols with vanilin reagent [1]. The content of total polyphenols, flavonoids and non-flavonoids was converted into gallic acid, flavanols into catechin, while procyanidins into cyanidin. The degree of polymerization of procyanidins was determined as described by Rangana and Parpia [13].

The determinations of quantitative changes within polyphenols were made by the method two-dimensional TLC, with cellulose MN-300 Merck. Formic acid — water (2:98) was the first solvent and n-amyl alcohol — acetic acid — water (10:6:5) the second [4]. Extracts for chromatography were prepared according to Sniegieryev and Zhranko [16]. Identification of polyphenols was made on the basis of observations of chromatograms in ultraviolet light and after spraying with different solvents [10, 14, 15, 17].

The measurements of colour were made by means of a colorimeter "MOMCOLOR D" in a C.I.E. tristimulus system. The colour of turbid musts was measured in reflected light, whereas the colour of clear musts in transmitted light. On the basis of the measured values: X_1, X_2, Y and Z, the dominant wavelenght (λ_d) and excitation purity Pe were calculated [5].

RESULTS AND DISCUSSION

Quantitative changes of polyphenols resulting from the reactions taking place in the pulp are shown in Fig. 1.

The data presented in Fig. 1 indicate that a large reduction in the content of polyphenols took place during the initial stage of enzymatic oxidation in the apple pulp. The degree and intensity of these changes depended on the type of polyphenolic compounds. The content of total polyphenols, being statistically significant, decreased in the samples examined during 60 min. and reached 62%. Leaving the pulp for 240 min

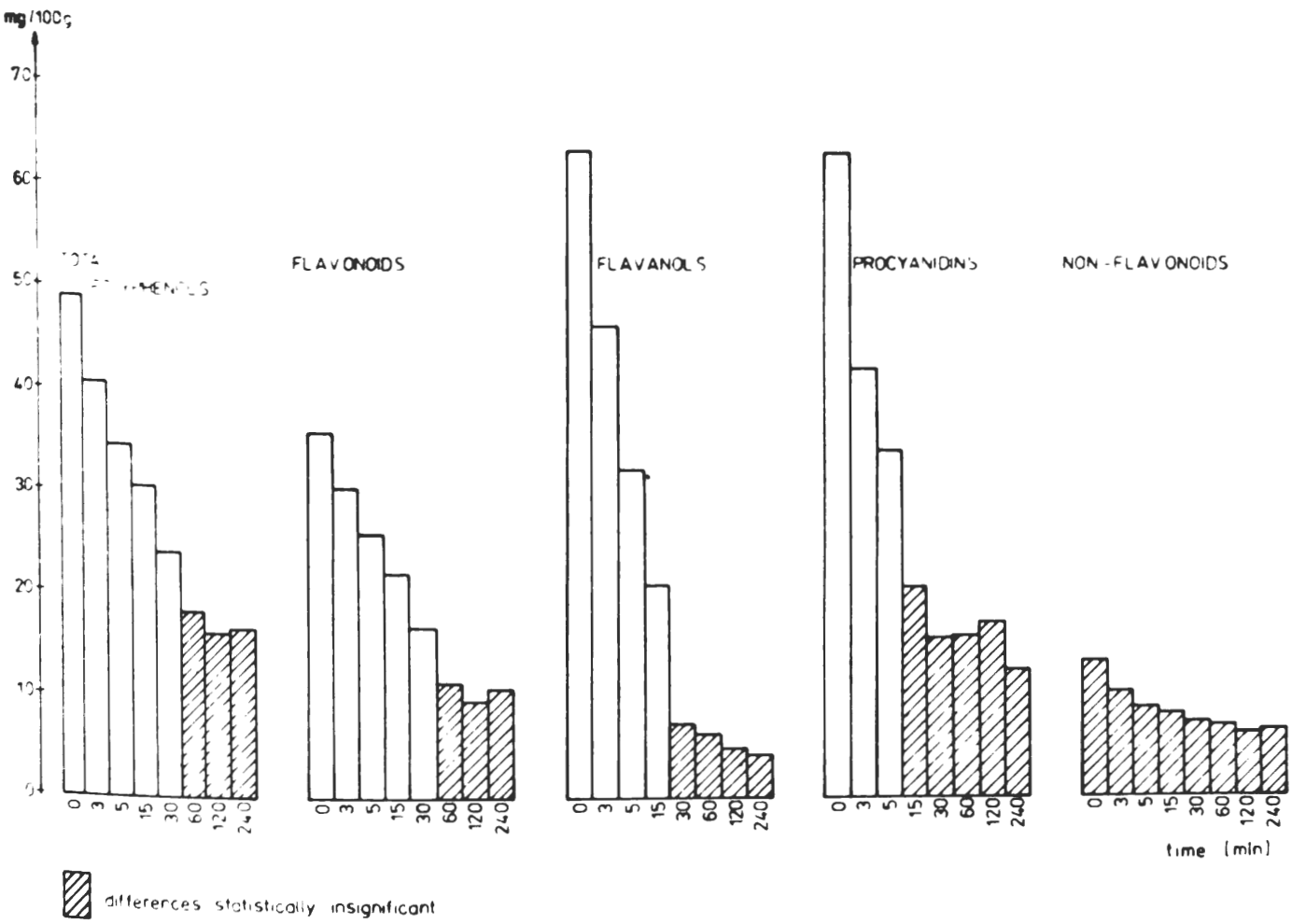

Fig. 1. Effect of apple pulp storage time on the content of polyphenolic compounds in the must

did not affect significantly the changes within the compounds. The content of flavonoids which constitute the main part of polyphenols was decreasing significantly in the pulp samples left for 60 min. After that time their content decreased by 68% and further changes were not significant. Higher intensity and degree of changes were revealed by a group of flavanols belonging to flavonoids. Significant changes of these compounds occured after 30 min. and amounted to 88%. Longer storage of the pulp i.e. up to 210 min. did not affect flavanols significantly, their content decreased only by 5%.

Procyanidins underwent statistically significant changes in the shortest period of time, i.e. 15 min., in comparison with the groups of compounds presented in this paper. Their content decreased by 68%.

Out of the polyphenols examined non-flavonoids were affected by the changes to a smaller extent. After 240 min storage of the pulp, 51% of

non-flavonoids was left, the largest changes occured during the first 5 min and amounted to 35%.

The decreasing intensity of the changes within the groups of polyphenols in the course of extended time of pulp storage, resulted probably not only from the depletion of the substrates but also from enzyme inhibiting factors — condensation products of polyphenols.

As can be seen in Fig. 2. the degree of polymerization of procyanidins within the range of 0-15 minutes of the pulp storage did not change and amounted to 1. After a longer period of time, when the must was pressed out of the pulp, this degree increased. The highest value of 4.284 was achieved after 120 minutes.

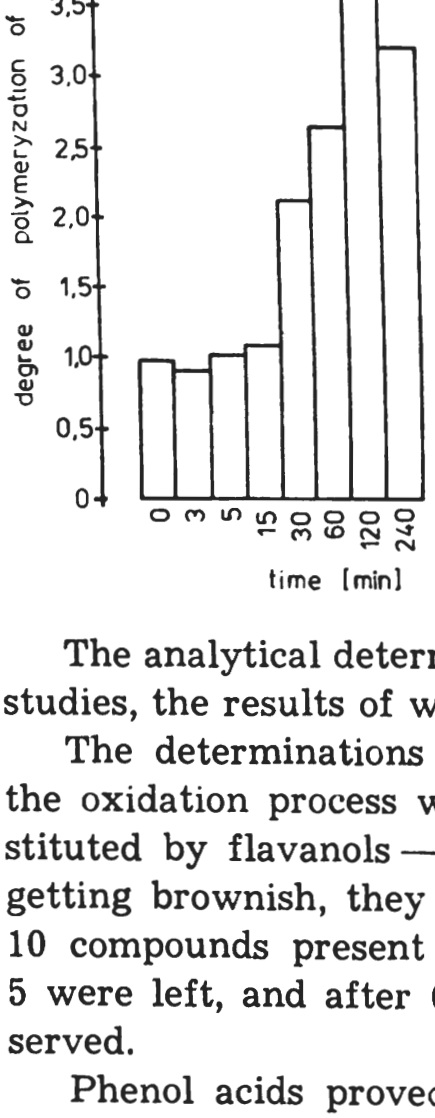

Fig. 2. Effect of the apple pulp storage time on the degree of polymerization of procyanidins

The analytical determinations have been supported by chromatographic studies, the results of which are presented in Table 1.

The determinations show that in the must from the pulp in which the oxidation process was inhibited, the most numerous group was constituted by flavanols — catechins and procyanidins. When the pulp was getting brownish, they underwent the most remarkable changes. Out of 10 compounds present at the begining of the test, after 15 min. only 5 were left, and after 60 min. only a slight amount of catechin was observed.

Phenol acids proved to be more resistant to enzymatic oxidation in the apple pulp. In the sample not subjected to oxidation, 5 compounds were detected, and after 240 min pulp storage, slight qualities of 3 compounds were detected. It is interesting to note the presence of chlorogenic acid in the juice pressed out after 240 min pulp storage. As we know, this compound is oxidized readily by polyphenoloxidase. This was most

Table 1. Effect of the apple pulp storage time on chromatographic determinations of polyphenolic compounds in the apple must

Storage time min	Number and concentration of polyphenols on the chromatograms																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	+++	+	+	+	+	++	+++	+	+	+	+	+++	+	++	+	+	+	+++
3	+++	+	+	+	+	+	++	+	+	+	+	++	+	+		+	+	++
5	+++	+	+	+	+	+	++	+	+	+	+	++	+	+		+		++
15	++	+	+	+	+	+	+		+	+	+	+		+				++
30	++	+		+	+		+		+	+	+			+				+
60	+	+		+			sl		+	+	+							
120	+	sl		+			sl		+	+	+							
240	sl	sl		sl					sl	sl								

Explanations: number of compounds on the chromatograms: 1 — chlorogenic acid, 2-5-phenol acids, 6-8-catechins, 9-11-flavanol glycosides, 12-18-procyanidins, concentration of compounds: +++ large, ++ medium, + small, sl — slight

likely due to the reaction of quinone reduction in chlorogenic acid up to the phenol by procyanidins. According to Lea [7] procyanidins are not oxidized by polyphenoloxidase, but by quinones of chlorogenic acid only.

Three compounds, identified as flavanol glucosides were not susceptible

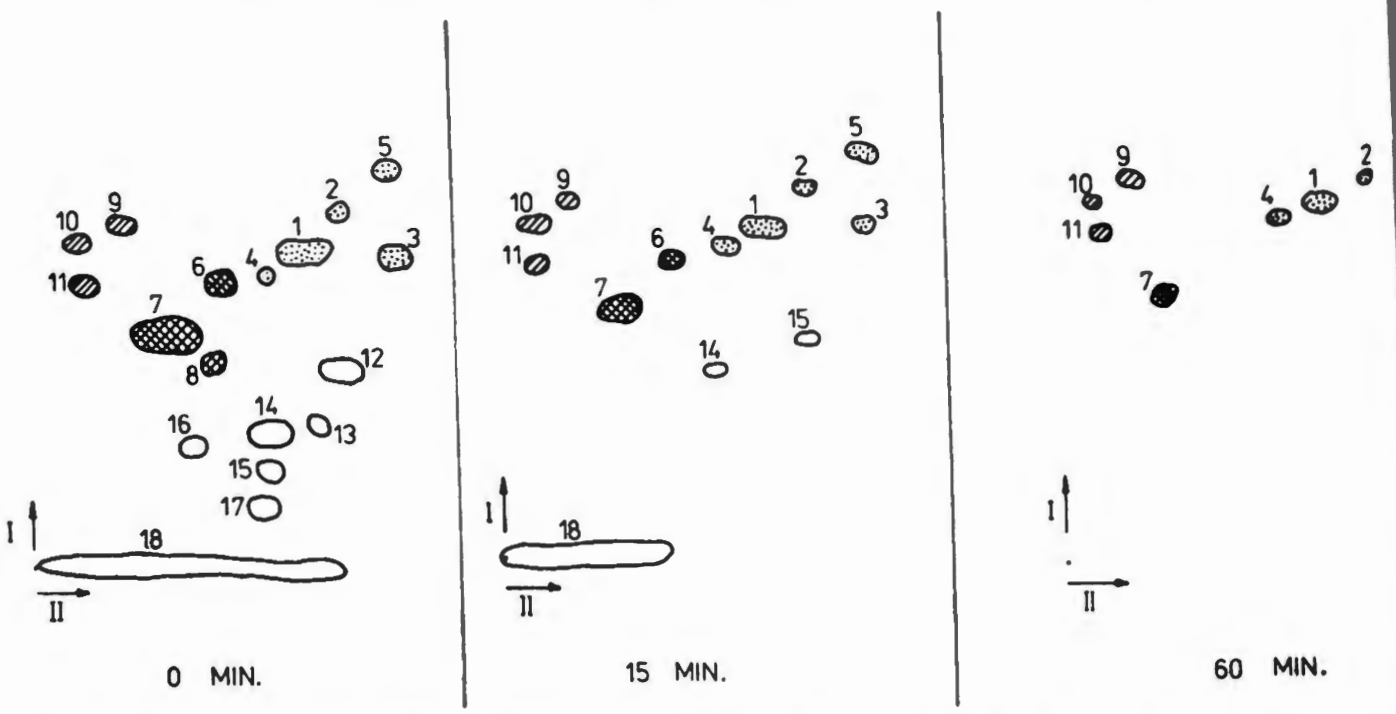

Fig. 3. Chromatograms of polyphenolic compounds extracted from apple must, pressed out from the pulp after 0, 15 and 60 min.

to the changes in pulp, two of them were still present after 240 min of storage. According to Van Buren et al. [2] flavanol glucosides are not a substrate for polyphenoloxidase.

The results of chromatographic studies confirmed the analytical determinations. Procyanidins and catechins proved to be the group of polyphenols contained in apples most susceptible to enzymatic changes.

Together with the determinations of polyphenols, the colour of clear and turbid musts obtained from the pulps exposed to the activity of tissue enzymes was measured. The results of measurements are shown in Fig. 4 and 5.

The dominant wavelenght (λ_d) is characterized by the hue of colour, excitation purity (Pe) — chroma and Y — lightness. Values λ_d and Pe, in case of apple must, increase during the browning process and Y-decrease [3]. As can be seen in Fig. 4 and 5, for clear must, the browning process developed for 15 min when the pulp was left before pressing. Parameters for the must colour after that time revealed the highest values λ_d = 572 nm, Pe = 0.235 and the smallest Y = 67.80%. When the apple must was left for a longer period of time that 15 min, the parameters of colour for clear must were improved. Lea [7] measured the chroma of the apples must by tristimulus colorimetry. He found that the colour rises for a short period time and then declines.

In the case of turbid must, during the time of the browning process,

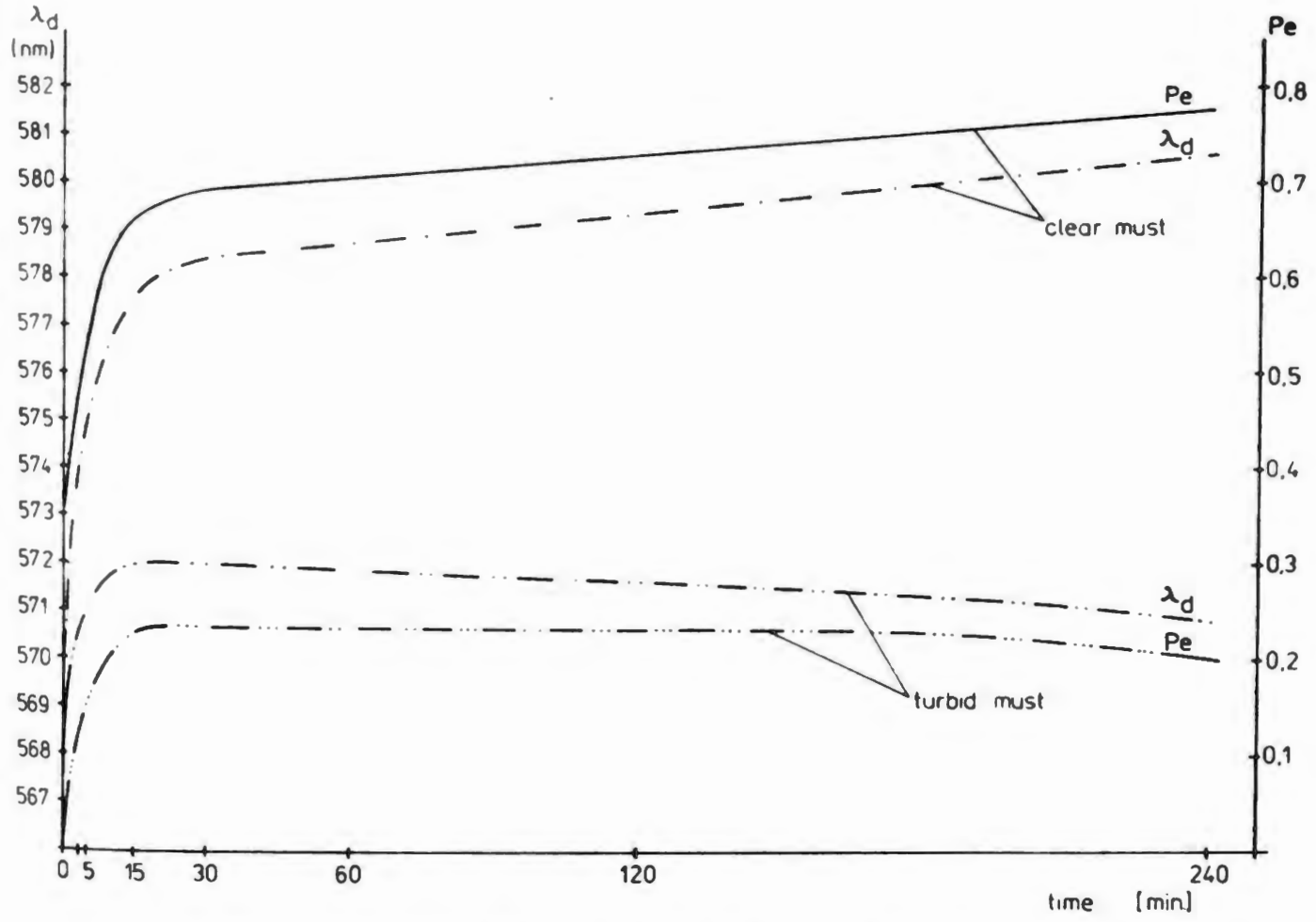

Fig. 4. Effect of apple pulp storage on the colour of clear and turbid must

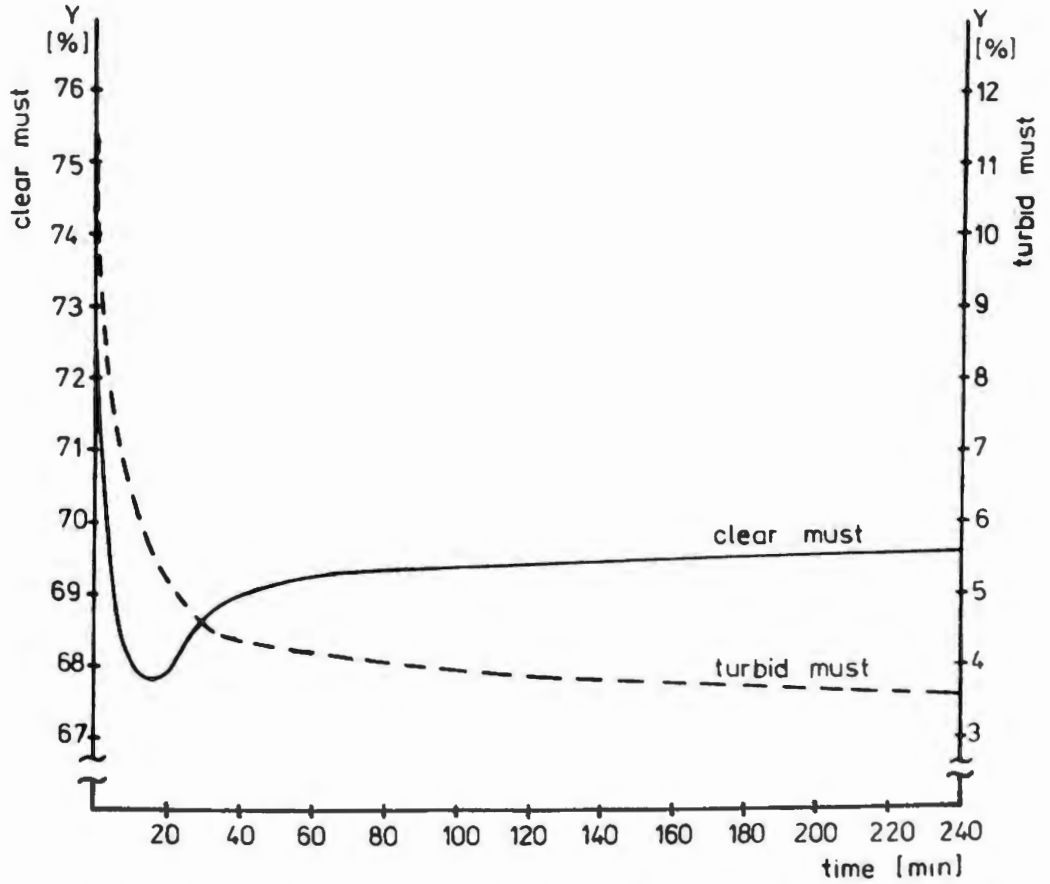

Fig. 5. Effect of apple pulp storage on the lightness of clear and turbid must

the colour of the must had worse parameters in comparison with the clear must. The dominant wavelenght increased to $\Delta\lambda_d = 8.6$ nm, purity to $\Delta Pe = 0.335$ and lightness decreased to $\Delta Y = 5.69\%$. For clear must, the values were $\Delta\lambda_d = 3.0$ nm, purity $\Delta Pe = 0.155$ and lightness $\Delta Y = 4.57\%$, respectively. Time extension for the pulp left for more than 15 min before pressing, resulted in a deterioration of the colour of turbid must.

Instrumental measurements were in agreement with visual evaluation. Clear must of "0" sample was white-green. When the pulp was left for a longer period of time, i.e. 15 min, the colour was gradually changing to dark yellow and in the samples from 30-240 minutes the colour was changing into slightly lighter one. Turbid must in "0" sample was green-yellow, after 3 min of storage pulp — yellow, after 5 min dark yellow, after 15 min — yellow-brown, after 30 min — brown-yellow, after 60 and 120 min — brown, and after 240 min — dark brown. The differences between the samples from 0 to 15 min of the pulp storage were remarkably larger than between the samples from 30-240 min.

Such colour changes were probably due to changes within polyphenols. During the first 15 min of oxidation of colourless polyphenols to coloured quinones, the process of must browning developed. Time extension for the reaction in the pulp resulted in a development of condensation and polymerization processes and consequently to a deeper brownish colour, but only in case of turbid must. Polymerized compounds are less soluble and therefore they were probably removed from clear must during centrifuging. On the other hand, in turbid must, these molecules remained suspended with colloidal substances and therefore the must brown.

Table 2 shoes the results of calculations of linear correlation coef-

Table 2. Linear correlation coefficients between the changes of polyphenols in the storage pulp and the parameters of colour apple musts

Compounds / Parameters		Total polyphenols	Flavanols	Procyanidins	Non-flavonoids	Flavanoids	Degree of polymerization of procyanidins
Clear must							
wavelength	λd	-0.662	-0.725	-0.802	-0.754	-0.608	0.284*)
purity	Pe	-0.841	-0.916	-0.930	-0.847	-0.803	0.483
lightness	Y	0.482	0.510	0.565	0.490	0.458	−0.222*)
Turbid must							
wavelength	λd	-0.937	-0.962	-0.945	-0.909	-0.904	0.663
purity	Pe	-0.895	-0.935	-0.940	-0.840	-0.870	0.595
lightness	Y	0.922	0.952	0.951	0.898	0.888	−0.619

Explanation: *) — lack of significant linear correlation coeficient

ficients between the content of polyphenols and the parameters of colour for clear and turbid musts obtained from the pulp left for various periods of time before pressing.

Comparison of these coefficients for the samples of clear and turbid musts show that changes within polyphenols are more correlated with the parameters of turbid musts than those of clear musts. The difference is most likely due to the removal of polymerized highmolecular polyphenols during centrifuging the samples with clear musts. All groups of polyphenols examined had statistically significant correlation coefficients with colour parameters. The highest values were revealed by flavanols and procyanidins. Thus, these compounds affected the colour of apple must remarkably.

CONCLUSION

1. Reactions causing changes in the polyphenolic compounds occur in the apple pulp, the rate of these changes is large in the first minutes after milling the apples and it decreases after a longer period of pulp storage.

2. The rate and quantity of the changes within polyphenols depend on their kind. For example, statistically significant changes developed when the pulp was left for the following periods of time: procyanidins — 15 min, flavanols — 30 min, total polyphenols and flavanoids — 60 min.

3. Changes within polyphenols in pulp are accompanied by a must browning process which is more intensive in case of turbid than of clear must.

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ZMIANY ZWIĄZKÓW POLIFENOLOWYCH W MIAZDZE JABŁKOWEJ

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Streszczenie

W pracy badano dynamikę zmian zawartości związków polifenolowych i barwy moszczy pozyskiwanych z miazgi jabłkowej przetrzymywanej 0, 3, 5, 15, 30, 60, 120 i 240 min przed tłoczeniem. Stwierdzono, że przemiany związków polifenolowych zachodzą intensywnie w pierwszych minutach po rozdrobnieniu jabłek. Statystycznie istotne zmiany procyjanidyn nastąpiły w czasie 15 min pozostawienia miazgi przed tłoczeniem, flawanoli w czasie 30 min, flawonoidów i polifenoli w ciągu 60 min.

W pomiarach barwy moszczy mętnych stwierdzono, że w miarę wydłużania czasu przetrzymywania miazgi przed tłoczeniem wzrasta ich zbrunatnienie. Natomiast dla moszczy klarownych, brunatnienie barwy rosło do 15 min przetrzymywania miazgi, a następnie od 30 do 240 min następowało pojaśnienie barwy.

W otrzymywaniu moszczy mętnych w celu zachowania jasnej barwy należy zapobiegać procesom utleniania w chwili rozdrabniania. Natomiast dla moszczy klarownych jasną barwę uzyskuje się również przez przedłużanie czasu przetrzymywania miazgi przed tłoczeniem, ale otrzymuje się produkty ubogie w związki polifenolowe.