A C T A A L I M E N T A R I A P O L O N I C A
Vol. VIII (XXXII), No. 1-2 1982

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IMPROVEMENT OF POLISH TOBACCO BY THE POLYSACCHARIDE--HYDROLYZING ENZYMES TREATMENT

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Key words: tobaco, hemicelulose, celulose, pectinolytic enzymes, hydrolysis of polysaccharide

The result of studies on improving Polish tobacco by the enzymatic treatment were presented. The polysaccharide-hydrolyzing enzymes hemicellulase, cellulase and commercial pectinolytic preparation Pektopol, were applied.

Polish low grade tobacco, mainly dark, and Machorka type are characterized by the unfavourable chemical composition and poor flavouring properties. The necessity exists for improvement of the discussed tobacco by suitable processing.

Among many improvement methods some foreign patents appeared in literature, concerning the enzymatic treatment of tobacco, especially of tobacco stems, with the purpose to obtain a raw material for production of sheet tobacco [1, 2, 3, 4, 6, 7, 8, 11, 13].

The studies were carried out on the possibilities for applying a suitable enzymatic treatment for improvement of Polish lowgrade tobacco by the lowering of the level of chemical components unfavourably affecting the flavour of tobacco, and namely of proteins [10] and polysaccharides.

The present studies cover the acting of polysaccharide-hydrolyzing enzymes upon the tobacco in order to improve its organoleptic properties.

EXPERIMENTAL

MATERIALS

The experimental material consisted of low-grade tobacco Mocny Skroniowski, Machorka, and commercial cigarette blend „Klubowe" with a big content of dark tobacco.

They were treated by the following polysaccharide-hydrolyzing enzymes:
— Cellulase from *Aspergillus niger* — produced by Fluka A. G., Chem. Fa.
— Fungal Hemicellulase, manufactured by Koch-Light Lab. Ltd.
— Polish pectolytic preparations: Pektopol P and its thermostable variety Pektopol PT.

The Pektopol preparations obtained from *Aspergillus* mould are characterized by a wide range of activity. They contain hydrolyses such as cellulase, hemicellulase and pectinase. Besides, they reveal weak proteolytic and amylolytic activity.

METHODS

In the conducted studies aimed at enzymatic improvement, tobacco was treated by soaking or spraying with the water solution of enzyme or enzymatic preparation.

Numerous trials of improving tobacco with the use of various doses of enzymes in variable conditions of their effect such as time and temperature were conducted.

After the enzymatic process and after eventual drainage of water solution of the enzyme, tobacco was dried at about 50°C to humidity 12-14% in order to inactivate the enzyme.

The results of the conducted processes of improvement were studied on the basis of analysis of chemical composition, physical and organoleptic properties of tobacco.

The influence of enzymatic treatment on the content of the major constituents, the most important ones from viewpoint of tobacco's quality was examined, i.e. of water-soluble carbohydrates, water-soluble reducing substances, pentosanes, cellulose and pectic substances including water-soluble pectins, pectic acid and protopectins.

Water-soluble carbohydrates and water-soluble substances reducing Fehling's solution were determined by Bertrand's method. Pentosanes were determined by Jenke's method [9], cellulose — by Seifert's method [12]; pectins were determined acc. to Jacin [5].

THE RESULTS OF THE STUDIES AND DISCUSSION

In the first stage of the studies on the effect of polysaccharide-hydrolyzing enzymes, the trials were conducted with the aim of improving Machorka tobacco, with purified, crystallic enzymes: cellulase and hemicellulase.

Tobacco was sprayed with water solution of the enzyme in such

quantity as to introduce 1% of the enzyme, by weight, to the mass of tobacco and simultaneously to moisten the tobacco to a defined humidity.

The following conditions of treatment were applied:

— dose of enzymes 1% of tobacco, by weight,
— humidity of tobacco 30%,
— temeprature 20°C
— time of the process 10 hours.

As the enzymes of this type contain usually the system of polysaccharide-hydrolyzing enzymes, the influences of the discussed enzymes upon the carbohydrate components of tobacco were examined, and namely: — cellulose, pentosanes, pectins and water-soluble carbohydrates, and water solubles, reducing Fehlin's solution.

The results of the improvement of tobacco Machorka with the use of hemicellulase and cellulase are presented in Table 1.

Table 1. Carbohydrate components in Machorka tobacco before and after enzymatic hydrolysis with hemicellulase and cellulase

Components	Contents in tobacco, % dry matter		
	before	after the enzymatic treatment with	
		cellulase	hemicellulase
Cellulose	17.7	13.50	12.10
Pentosanes	5.50	3.85	3.80
Carbohydrates soluble in water	1.04	2.23	2.50
Water solubles reducing Fehling's solution	4.30	5.60	5.80

As it can be seen from the table, application of both hemicellulase and cellulase in the improvement of tobacco causes more than double increase in the amount of water-soluble carbohydrates and increase in the content of reducing substances. These favourable changes take place due to the decrease of quantity of cellulase by 23.7-31.6% and of pentosanes by about 30%.

The enzyme hemicellulase giving, as seen in Table 1, better results in case of treatment of tobacco Machorka, was applied in the improvement of tobacco Mocny Skroniowski.

The following treatment conditions were applied:

— spraying the enzyme water solution on the tobacco,
— dose of enzyme: 1% of enzyme by weight,
— humidity of tobacco 30%,

— temperature 20°C,
— time of process 10 hours.

The results are presented in Table 2.

Table 2. Results of improving of Mocny Skroniowski tobacco with hemicellulase

Components	Contents in tobacco, % dry matter	
	before	after enzymatic treatment
Cellulose	13.10	9.05
Pentosanes	5.10	4.30
Carbohydrates soluble in water	7.30	9.00
Water solubles reducing Fehling's solution	11.19	13.07

As it is illustrated in Table 2, in tobacco Mocny Skroniowski the action of hemicellulase causes hydrolysis of cellulose and pentosanes, respectively by 31 and 15.7%. Hydrolysis of these polysaccharides is a source of monosaccharides, the amount of which increases in tobacco Mocny Skroniowski by 20%.

The studies upon the improving tobacco with the use of Polish Pektopol preparations with a lower degree of purification were conducted, as well.

Many trials were conducted with spraying of water solution of the preparation on tobacco in such quantity as to introduce 1% of the preparation, by weight, to the mass of tobacco and simultaneously to moisten tobacco to a defined humidity, and then the process was conducted in variable conditions of time and temperature.

The following conditions of the enzymatic process were applied:
— humidity of tobacco: 30 and 50%
— temperature 20-22°C and 53-55°C
— time of treatment: 4, 10 and 20 hours.

The results of analysis of the carbohydrate components — cellulose, pentosanes, pectic substances, water-soluble carbohydrates, water-soluble substances reducing Fehgling's solution, in the initial tobacco Machorka and in that one treated by Pektopol preparation, are presented in Table 3.

As it results from Table 3, the effect of preparation Pektopol on tobacco machorka causes significant lowering in amount of cellulose, reaching to 21%. The best results were obtained at humidity of tobacco equal to 50%; rise in temperature increases the effect. The required treatment time at humidity of 50% at 53-55°C is 4 hours. The similar though slightly worse results were obtained at 20-22°C no earlier than after 20 hours.

The enzymatic hydrolysis of pectic substances, affected by Pektopol preparation, as it results from Table 3, manifested, first of all, by the loss

Table 3. Results of improving Machorka tobacco with Pektopol P

Components	Contents in tobacco before enzymatic treatment (% dry matter)	Contents in tobacco (% dry matter) after enzymatic treatment in conditions											
		temperature 20-22°C						temperature 53-55°C					
		humidity of tobacco 30%, time (hour)			humidity of tobacco 50%, time (hour)			humidity of tobacco 30%, time (hour)			humidity of tobacco 50%, time (hour)		
		4	10	20	4	10	20	4	10	20	4	10	20
Cellulose	17.70	15.60	15.50	15.50	14.50	14.50	14.40	16.30	16.20	16.00	14.10	14.00	14.00
Pentosanes	5.50	5.23	4.95	4.81	4.81	4.40	3.64	4.81	4.40	3.85	3.44	3.16	3.04
Carbohydrates soluble in water													
— before inversion	0.27	0.50	0.60	0.80	0.65	0.75	0.85	0.40	0.42	0.42	0.70	0.80	0.90
— after inversion	1.04	1.25	1.55	1.80	1.40	1.80	1.90	1.10	1.28	1.30	1.75	1.90	2.05
Water solubles reducing Fehlings solution													
— befor inversion	0.97	1.50	1.80	1.84	1.55	2.20	2.30	1.52	1.80	1.80	1.60	2.40	2.50
— after inversion	4.30	5.05	5.50	6.00	5.32	6.00	6.20	4.95	5.50	5.40	5.80	6.30	6.50
Pectins	9.95	8.40	8.20	8.10	8.00	7.90	7.90	8.10	7.50	7.30	7.50	5.90	5.70
— pectin soluble in water	1.35	1.70	1.50	1.45	1.70	1.60	1.70	1.80	1.60	1.70	1.90	1.80	1.60
— pectic acids	3.70	3.50	3.60	3.50	3.40	3.50	3.40	3.50	3.20	3.00	3.10	2.00	2.20
— protopectin	4.90	3.20	3.10	3.15	2.90	2.80	2.80	2.80	2.70	2.60	2.50	2.10	1.90

in protopectins and increase in the amount of water-soluble pectins leads to very distinct decrease of general quantity of pectins.

The best results, expressed in decrease of amount of pectins were obtained at 55°C and humidity of tobacco 50%. Under these conditions, the decrease in amount of pectins during 4, 10 and 20 hours is, respectively: 24.6; 40.7 and 42.7%. The most economical time for hydrolysis of pectins, under these conditions, is 10 hours.

Depending on the conditions of treatment, the changes in pentosanes' content have place (Table 3); the highest degree of hydrolysis is obtained during the longer time, i.e. 20 hours; at 55°C and humidity of tobacco = = 50%, good results are obtained already after 4 hours.

As it results from Table 3, under the effect of Pektopol a significant increase in quantity of water-soluble carbohydrates can be observed: the higher the humidity of tobacco and temperature and the longer the treatment time, the higher the said increase. The highest increments in quantity of water-soluble saccharides are obtained at about 55°C and humidity of tobacco 50% during 20 h but results obtained at a shorter time are often not very much lower. As it can be seen from the table, the increments in amount of watersoluble carbohydrates are not so high as it could result from the hydrolysis of cellulose. Breakdown of cellulose into the above molecular fragments probably takes place. As a result of treatment of tobacco Machorka with the use of Pektopol preparation, the high rise in quantity of substances reducing Fehling's solution, was observed. This increase is propertional to the increase of water-soluble carbohydrates and watersoluble pectins.

The best results for the examined chemical components are obtained at 55°C, humidity of tobacco = 55% during 10 hours but the results obtained at lower moisture and temperature are also satisfactory for the technological process.

The presented above studies concerned use of Pektopol in a dose of 1% of tobacco, by weight. It was also interesting to examine the higher doses of this preparation. Results of the experiments with increased doses of Pektopol are presented in Table 4. From the industrial viewpoint, the trials were conducted at about 20°C and humidity of tobacco 30% during optimal time 10 hours. As it results from Table 4, the increase in dose of preparation Pektopol, to 2% of tobacco, by weight, enhances favourable changes in chemical composition of tobacco Machorka. During the treatment of tobacco Machorka with Pektopol, the favourable changes in physical properties of the raw material were also observed. After the treatment tobacco was more flexible.

The experiments were also carried out with the aim to improve the commercial cigarette blend Klubowe, using Pektopol PT in the following conditions:

— dose of the preparation: 1 and 2% of tobacco, by weight,

Table 4. Effect of concentration of Pektopol P on carbohydrate components of Machorka tobacco

Components	Contents in tobacco, % dry matter		
	before	after the treatment in conditions:	
		1% of enzyme	2% of enzyme
Cellulose	17.70	15.50	10.68
Carbohydrates soluble in water	1.04	1.55	1.78
Water solubles reducing Fehling's solution	4.30	5.50	7.10
Pectins	9.95	8.20	7.50
— pectin soluble in water	1.35	1.50	1.69
— pectic acids	3.70	3.60	3.28
— protopectin	4.90	3.10	2.53

— humidity of tobacco 30%,
— optimal temperature of Pektopol's action: 53-55°C,
— time of enzymes' effect: 4 and 20 hours.

The results of the analysis of carbohydrate components of cigarette blend Klubowe before and after treatment with Pektopol PT are presented in Table 5. The treatment of commercial cut tobacco Klubowe with Pektopol, similarly as in the case of tobacco Machorka, causes lowering in the amount of cellulose and pectins, by 31 and 26%, respectively. Hydrolysis

Table 5. Contents of carbohydrate components in cigarette filler „Klubowe" before and after enzymatic treatment with Pektopol PT

Components	Contents in tobacco, % dry matter			
	before	after the enzymatic treatment in conditions:		
		4 hours		20 hours
		1% of enzyme	2% of enzyme	1% of enzyme
Cellulose	13.24	12.90	10.50	9.12
Carbohydrates soluble in water	4.29	4.92	5.10	5.71
Water solubles reducing Fehling's solution	8.19	9.25	10.99	12.83
Pectins	7.88	7.57	6.44	5.86
— pectin soluble in water	1.45	1.24	0.89	0.85
— pectic acids	3.35	3.37	3.63	3.28
— protopectin	3.08	2.96	1.92	1.73

of the discussed polysaccharides is a source of monosaccharides and oligosaccharides. The comparative organoleptic evaluation revealed a distinct improvement of flavour properties of cigarettes produced from the improved cut tobacco Klubowe.

CONCLUSIONS

1. The polysaccharide-hydrolyzing enzymes, used in the experiments: fungal hemicellulase, cellulase from *Aspergillus niger* and pectolytic preparation Pektopol — cause partial hydrolysis of cellulose and pectosanes, leading to the increase of watersoluble saccharides in tobacco.

2. The effect of hemicellulase in the applied conditions of treatment of Machorka and Mocny Skroniowski, causes lowering of cellulose's level by about 31%, of pentosane by 15.7 and 30.9%, respectively, and rise in water-soluble saccharides by 20 and 14%, resp.

3. The action of cellulase on tobacco in the applied treatment conditions causes decrease in quantity of cellulose by more than 20%, of pentosanes by about 30% and rise in amount of water-soluble carbohydrates by more than 100%.

4. The best results of improving tobacco with the use of Pektopol are as follows: 1% of enzyme for the mass of raw tobacco, temperature about 50°C, humidity of tobacco 50%, time 20 h. The optimal time — from the technological viewpoint — is 10 h. The effect of Pektopol preparation causes lowering in quantity of cellulose content by about 20%, of pentosanes by about 40%, increments of water-soluble saccharides by almost 100% and qualitative changes of pectins and the decrease of their total amount by more than 40%.

5. Polish raw tobacco improved by the enzymatic method, as shown by the organoleptic evaluation, is characterized by more favourable flavouring and technological properties.

LITERATURE

1. Bavley A. et al: Pat U.S.A. 3 240 214, 1966.
2. Bernard I.: Pat. Australia 420 623, 1972.
3. De la Burde R. L.: Pat U.S.A. 3256 888, 1966.
4. De la Burde R. L.; Crayton H.: Pat U.S.A. 3 256 889, 1966.
5. Jačin H. et al: J. Agr. Fd. Chem., 1967, **15**, (6), 1057.
6. Denig W.: Pat. Germany 252 121, 1912.
7. Kopsch R., Raban J.: Pat. Germany 2 045 181, 1972.
8. Mitchell T. G.: Pat. U.S.A. 3 974 838, 1976.
9. Modrzejewski K.: Metody badań w przemyśle celulozowo-papierniczym, script of Technical University, Łódź 1961.

10. Podlejski J., Olejniczak W.: Zeszyty Naukowe Politechniki Łódzkiej, Chemia Spożywcza 1980, **35**, 353.
11. Rosen W. E.: Pat. U.S.A. 3 889 689, 1975.
12. Seifert K.: Das Papier 1956. **10**, 301.
13. Silberman H.: Pat. U.S.A. 3 513 857, 1970.

Manuscript received: February, 1980
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USZLACHETNIANIE KRAJOWYCH TYTONI ENZYMAMI HYDROLIZUJĄCYMI POLISACHARYDY

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Streszczenie

Przeprowadzono badania nad uszlachetnianiem krajowych tytoni metodą enzymatyczną. Do tego celu zastosowano enzymy hydrolizujące polisacharydy hemicelulazę grzybową, celulazę z *Aspergillus niger* oraz krajowy preparat pektolityczny Pektopol. Wykonano próby laboratoryjne uszlachetniania tytoniu różnymi dawkami enzymów w zmiennych warunkach ich działania takich jak: czas i temperatura. W przeprowadzonych badaniach ustalono dawkę enzymu, warunki, efektywność działania oraz sposób prowadzenia procesu obróbki enzymatycznej.

Badania potwierdziły skuteczność stosowanych enzymów w rozkładzie polisacharydów tytoniu. Powodują one częściową hydrolizę celulozy i pentozanów prowadzącą do zwiększenia w tytoniu ilości cukrów rozpuszczalnych w wodzie. Ponadto hydroliza pektyn za pomocą preparatu Pektopol powoduje zmniejszenie ogólnej ilości pektyn, jak również ich zmiany jakościowe. Badania wykazały przydatność enzymów hydrolizujących polisacharydy do modyfikacji składu chemicznego tytoniu, a tym samym poprawy jego właściwości degustacyjnych i technologicznych.