A C T A A L I M E N T A R I A P O L O N I C A
Vol. V (XXIX), No. 4 1979

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CONTENTS OF FREE AMINOACIDS AND AMIDES IN SUGAR BEET AND SUGAR INDUSTRY SEMIPRODUCTS

Key words: aminoacids content, amids content, sugar beet, sugar halfproducts, liquid chromatography

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The content of aminoacids and acid amides was determined by the liquid chromatography method in sugar beet and in diffusion juices, after the main liming, and in the thin and thick juices and molasses.

To evaluate the quality of sugar beet and the semiproducts used in the sugar industry one must determine the content of aminoacids and amides, i.e. the non-sugars which are particularly harmful for the correct course of the technological process. The content of free aminoacids and amides in the sugar beets, sugar juices and molasses is determined by the liquid chromatography method [1, 2, 3, 5]. No current data concerning the content of this group of compounds in the sugar raw materials and semiproducts are available in the Polish sugar industry.

The purpose of this study was develop, choose and adapt to our possibilities the liquid chromatography methods designed for the determination of the harmful nitrogen non-sugars occurring in sugar beets and in the diffusion juices including those after main liming and the thin and thick juices, as well as in molasses.

MATERIALS AND METHODS

Sugar beet samples including 50 roots each were taken from an industrial plantation run by one of the sugar factory, in October 1976 and 1977. The pulp obtained from these root samples was used for chromatographic determinations. Additionally beet slices and the corresponding factory-made diffusion juices (after main liming, thin, thick) and molasses

were taken in 1977 from the same sugar mill. Appropriate sugar juices were also obtained under laboratory conditions from the beets cultivated in an experimental field belonging to the same sugar factory. The content of sugar in the pulp and in juices was determined by the polarimetric method.

The contents of aminoacids and amides in the extraxt obtained from the beet pulp were determined by digestion, using a buffer solution with lithium citrate and norleucine as the internal standard [4].

DETERMINATION OF AMINOACIDS IN SUGAR JUICES FROM VARIOUS PRODUCTION STAGES

40 g samples of the diffusion juice, the juice after main liming and thin juice, as well as 15 g samples of thick juice and 10 g samples of molasses were weighed with an accuracy of ± 0.01 g and put into the 100 cm^3 measuring flasks. Then 40 cm^3 of the buffer solution with 0,1 N lithium citrate of pH 1.5 were added to each flask. The content was mixed mechanically for 20 minutes and then some buffer solution was added to fill the flasks to the mark, and mixed once again.

Exactly 50 cm^3 of liquid samples obtained in this way were measured, 10 cm^3 of 20% trichloracetic acid were added to each sample and the precipitate was centrifuged at a speed of 4500 rpm. Then, 5 cm^3 samples of clear supernatants were measured and placed in two 10 cm^3 measuring flasks. One flask was filled to the mark with the buffer solution containing 0.25 N lithium citrate of 2.24 pH, the other with the buffer solution containing 0.2 N sodium citrate of pH 2.22.

AMINOACID ANALYSIS CONDITIONS

The previously prepared samples were put automatically in 1 cm^3 doses into a column a dimension 50×0.8 cm and 15×0.8 cm (in case of sugar beet) and 48×0.8 and 13×0.8 (in case of juices). Automatic "Jeolco" liquid chromatograph type JLC-6AH equipped with two columns and two developing systems, the buffer solution with sodium citrate, were used, A standard solution of aminoacids containing 0.1 μM of each aminoacid per 1 cm^3 (except proline whose content was 0.2 μM and glutamine — 0.4 μM) was introduced, together with the samples, to an automatic analyser.

The aminoacids separation conditions, including asparagine and glutamine, were as follows:

— stationary phase, sulphonated spherical resin, type LCR-2,
— moving phase, buffer solution with sodium citrate of pH 5.29 ± 0.1,
— eluent flow rate: 15 cm^3/min.,
— ninhydrine reagent flow rate: 0.56 cm^3/min.,
— column temperature and pressure: 65°C, 10-12 kg/cm^2,

— flow chamber: 2 mm,
— recorder tape speed: 120 mm/h.
For neutral and dicarboxylic aminoacids and their amides:
— stationary phase: sulphonated spherical resin, type LCR-2,
— moving phase, buffer solution with lithium citrate,
— eluent flow rate: 0.84 cm^3/min.,
— ninhydrine reagent flow rate: 0.56 cm^3/min.,
— variations of temperature and pressure — 36°C to 60°C during 65 min., 20-22 kg/cm^2.

The remaining conditions were as for tryptophane and alkaline aminoacids. Proline was determined at the wavelength of 440 nm, the remaining aminoacids — at 570 nm.

RESULTS AND DISCUSSION

Table 1 gives the average determined contents of individual aminoacids and amides (from four samples) in sugar beets coming from the 1976 and 1977 campaigns. The beets were taken from industrial planta-

Table 1. Average contents (from 4 samples) of aminoacids and amides in sugar beets from the 1976 and 1977 campaigns

Aminoacids and amides	Sugar beets from 1976		Sugar beets from 1977	
	mmol/100g d.m.	share in %	mmol/100g d.m.	share in %
Aspartic acid	1.232	4.92	0.400	4.18
Treonine	0.420	1.68	0.257	2.69
Serine	0.759	3.03	0.482	5.04
Asparagine	1.171	4.68	0.465	4.86
Glutamic acid	1.156	4.62	0.501	5.24
Glutamine	16.533	66.03	5.460	57.10
Proline	traces	traces	0.043	0.45
Glycine	0.266	1.06	0.126	1.32
Alanine	0.933	3.73	0.357	3.73
Alpha-aminobutyric acid	0.152	0.61	0.066	0.69
Valine	0.343	1.37	0.199	2.08
Cystine	0.062	0.25	traces	traces
Methionine	0.189	0.75	0.062	0.65
Isoleucine	0.569	2.27	0.324	3.39
Leucine	0.596	2.38	0.364	3.81
Tyrosine	traces	traces	traces	traces
Phenylalanine	0.083	0.33	0.059	0.62
Tryptophane	0.166	0.66	0.103	1.08
Lysine	0.150	0.60	0.112	1.17
Histidine	0.094	0.37	0.082	0.86
Arginine	0.163	0.65	0.099	1.03
Total	25.037	100	9.561	100

tions belonging to the same sugar factory. From the results of tests and of the analysis of the vegetation conditions it can be said that climatic conditions have a great effect on the content of aminoacids in the beets. In the sample taking region the year 1976 was relatively dry and 1977 — relatively wet. In 1976 the content of aminoacids and amides determined in the beets was approximately three times higher than in the 1977. The content of glutamine — the nitrogen non-sugar most harmful for the production process — was also higher. At elevated temperature and in an alkaline medium glutamine becomes cyclised into pirolidonecarboxylic acid with the liberation of amonia.

This is the cause of the undesired decrease of the alkalinity of juices, especially at the concentration station. The excessive amounts of soluble calcium salts produced in the juice affect actively the increase in the amount of molasses and result in a decreased output of sugar from the beets.

From a comparison of results from the two successive campaigns it can be said that in spite of distinct differences between the amount of

Table 2. The contents of aminoacids and amides in sugar beets and sugar juices obtained from these beets under laboratory conditions, in mmol/100 g d.m.

Symbol	Beets	Diffusion juice	Juice after main liming	Thin juice	Thick juice
Asp	0.327	0.343	0.384	0.441	0.377
Thr	0.191	0.214	0.161	0.192	0.171
Ser	0.307	0.360	0.342	0.389	0.311
Asp-NH_2	0.281	0.277	0.199	0.141	0.093
Glu	0.304	0.339	0.451	0.597	0.486
Glu	3.311	3.921	1.521	1.262	0.392
Pro	0.016	0.030	0.019	0.054	0.031
Gly	0.108	0.154	0.103	0.145	0.103
Ala	0.270	0.297	0.287	0.329	0.270
AABA	0.049	0.061	0.055	0.089	0.036
Val	0.141	0.169	0.129	0.142	0.098
Cys	traces	traces	traces	traces	traces
Met	0	0.032	0.034	0.069	0.036
Ile	0.214	0.248	0.221	0.267	0.202
Leu	0.269	0.288	0.229	0.296	0.197
Tyr	0	0	0.021	0.089	0.048
Phe	0.048	0.075	0.066	0.086	0.037
Trp	traces	traces	0.051	0.092	0.054
Lys	0.106	0.128	0.077	0.111	0.072
His	0.049	0.077	0.032	0.080	0.024
Arg	0.104	0.128	0.079	0.118	0.066
Total	6.095	7.139	4.461	4.989	3.104
Glutamine %	100	118.42	45.94	38.11	2.43

aminoacids and amides in sugar beets, the percentage shares of individual aminoacids do not vary to a greater extent. Among the identified aminoacids and amides, glutamine had the highest share. In the determination methods used, no gamma-butyric acid, usually determined with other aminoacids, was found to be present [1, 3]. In Table 2, the results of determination of aminoacids and amides contained in sugar beets and (after main liming, thin and thick), are specified. In the diffusion juices the same number of aminoacids and amides was found as in the beets, whereas their quantity was some 18% higher. This phenomenon may be explained by the higher degree of extraction of the stations where juices is being extracted from these beets, as well as by the possibility of peptide decomposition. The differences between the total amount of aminoacids and amides in the juices after main liming and in the thin and thick juices are undoubtedly caused by conversion of glutamine to piolidonecarboxylic acid and, also, by a partial adsorption of aminoacids on the precipitat. The juice after the main liming was filtered prior to

Table 3. Contents of aminoacids and amides in sugar beets and juices obtained from these beets under industrial conditions, in mmol/ 100 g d.m.

Symbol	Beets	Diffusion juice	Juice after main liming	Thin juice	Thick juice
Asp	0.421	0.430	0.411	0.518	0.497
Thr	0.466	0.472	0.322	0.401	0.380
Ser	0.640	0.648	0.318	0.390	0.378
Asp-NH_2	0.701	0.711	0.346	0.305	0.271
Glu	0.813	0.819	1.072	1.267	1.252
Glu-NH_2	7.011	7.798	3.820	3.194	0.994
Pro	0.047	0.047	0.043	0.079	0.058
Gly	0.191	0.190	0.194	0.231	0.225
Ala	0.468	0.481	0.351	0.406	0.394
AABA	0.063	0.072	0.059	0.060	0.056
Val	0.237	0.230	0.164	0.202	0.198
Cys	traces	traces	traces	traces	traces
Met	0.093	0.087	0.075	0.069	0.070
Ile	0.416	0.430	0.299	0.341	0.334
Leu	0.405	0.416	0.306	0.327	0.320
Tyr	traces	traces	0.031	0.156	0.151
Phe	0.050	0.083	0.070	0.060	0.062
Trp	0.103	0.089	0.042	0.088	0.075
Lys	0.071	0.100	0.104	0.119	0.106
His	0.104	0.103	0.056	0.078	0.031
Arg	0.098	0.101	0.067	0.109	0.078
Total	12.398	13.307	8.15	8.40	5.93
Glutamine %	100	111.22	54.48	45.56	14.18

chromatographic analysis, whereas in the production process this operation is not carried out. Only juices after saturation I and II, and thick juice are filtered.

The conversion of glutamine takes place during the entire juice purification and condensation cycle, this is indicated by the rather small growth of the content of glutamic acid. In the juice after main liming the content of glutamine compared to its content in the beets is reduced by 54.1%, in thin juice — by the 61.9% and in thick juice — by 88.2%.

Results of the determination of aminoacids and amides in beets and industrial juices are given in Table 3. In this case the decomposition of glutamine at the main liming station is weaker (45.5%), more of it (8.9%) is adsorbed on the precipitate after saturation I and II, and less is decomposed (31.4%) at the condensation station.

Table 4 gives the determined contents of aminoacids and amides in molasses. Molasses 1, 2 and 3 were taken from the same sugar factory as the beet samples. From the analytical data it results that all the aminoacids and acid amides present in sugar beets in a 25% amount pass qualitatively to the molasses.

Table 4. Contents of aminoacids and amides in molasses from 1977 campaign, in mmol/100 g d.m.

Symbol	Molasses 1	Molasses 2	Molasses 3	Molasses 4
Asp	1.138	1.311	1.101	1.431
Thr	0.392	0.434	0.529	0.983
Ser	1.003	1.112	1.062	1.884
Asp-NH_2	0.064	0.072	0.018	0.032
Glu	1.720	1.749	1.639	1.958
Glu-NH_2	0.091	0.099	0.045	0.084
Pro	0.874	0.704	0.912	1.112
Gly	1.884	1.664	1.826	2.500
Ala	2.140	3.008	2.949	3.529
AABA	0.135	0.156	0.177	0.639
Val	1.586	2.756	2.724	3.016
Cys	1.194	1.378	1.773	1.340
Met	0.147	0.123	0.178	0.261
Ile	1.320	1.611	1.509	2.259
Leu	1.204	1.722	1.628	2.209
Tyr	0.716	0.156	0.127	1.450
Phe	0.190	0.315	0.356	0.317
Trp	0.379	0.206	0.518	0.761
Lys	0.120	0.159	0.193	0.252
His	0.092	0.102	0.095	0.115
Arg	0.101	0.186	0.185	0.236
Total	16.49	19.02	19.54	26.35

CONCLUSIONS

1. Nineteen aminoacids and 2 acid amides have been identified in sugar beets, sugar juices and molasses.

2. The sugar beets examined were characterised by high contents of glutamine, a nitrogen onu-sugar which is harmful for the correct course of the sugar production process.

3. The content of aminoacids and amides in sugar beets depends on the climatic conditions under which the beets were grown.

4. Approximately 50% glutamine determined in sugar beets are decomposed at the main liming station, further decomposition takes place at the juice condensation station.

5. Some 25% of aminoacids and acid amides pass from the beets to the molasses.

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Manuscript received: June, 1978.

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ZAWARTOŚĆ WOLNYCH AMINOKWASÓW I AMIDÓW KWASOWYCH W BURAKACH CUKROWYCH I PÓŁPRODUKTACH CUKROWNICZYCH

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Streszczenie

Oznaczono metodą chromatografii cieczowej zawartość aminokwasów i amidów kwasowych w korzeniach buraków cukrowych oraz w pochodzących z tych korzeni sokach: dyfuzyjnym, po głównym nawapnieniu, rzadkim, gęstym i melasie.

Z rezultatów przeprowadzonych badań i analizy warunków wegetacji roślin wynika, że istotny wpływ na ilość aminokwasów i amidów kwasowych zawartych w burakach cukrowych wywierają czynniki klimatyczne (tab. 1). W roku stosun-

kowo suchym związków tych było około trzykrotnie więcej niż w roku mokrym.

Porównując wyniki otrzymane podczas dwóch kolejnych kampanii przerobu buraków cukrowych stwierdzono, że mimo wystąpienia wyraźnych różnic w ilości aminokwasów i amidów kwasowych zawartych w korzeniach buraków cukrowych (mmole/100 g s.s.) ich udziały procentowe ulegają niewielkim wahaniom.

Jakościowo, wszystkie zidentyfikowane w burakach cukrowych aminokwasy i amidy kwasowe przechodzą przez poszczególne etapy procesu technologicznego fabrykacji cukru i gromadzą się w melasie (tab. 1, 2, 3). Spośród zidentyfikowanych w burakach cukrowych aminokwasów i amidów kwasowych, w największych ilościach wystąpiła glutamina, która jest szczególnie szkodliwym dla procesu technologicznego związkiem chemicznym. Udział glutaminy wynosił prawie 60% w stosunku do sumy aminokwasów i amidów kwasowych, oznaczonych ilościowo w burakach cukrowych.

Niewielkie, ilościowe przyrosty kwasu glutaminowego w półproduktach wskazują, że przemiana glutaminy zachodzi w całym cyklu oczyszczania i zagęszczania sóków cukrowniczych.

Zawartość glutaminy jest mniejsza: w soku po nawapnieniu głównym o 54,1%, w soku rzadkim o 61,9% i w soku gęstym o 88,2%, w stosunku do zawartości oznaczonej w burakach cukrowych (tab. 2).

Do melasu przechodzą jakościowo wszystkie aminokwasy i amidy kwasowe w ilości stanowiącej około 25% sumy tychże związków oznaczonych w korzeniach buraków cukrowych (tab. 4).