EXAMINATION OF THE BIOGEN AMINE CONTENT OF HUNGARIAN WINES

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Summary

The present paper reports on the results obtained in the determination of the total biogen amine, histamine and tiramine content of Hungarian wines.

The alkalized wine sample is lyophilized, then the components to be determined are extracted from the solid residue with absolute n-butanol. The condensation products of histamine + ortophthalaldehyde and of the tyramine + α-nitroso-β-naphtol can be determined by fluorescence spectroscopy. The total of biogen amine is measured with ninhydrine reaction by spectrophotometry.

The results show that the histamine, tiramine and biogen amine content of Hungarian wines are within the limits accepted on international level.

Introduction

The increasingly stricter requirements towards the quality of wines for export-necessitate the wide knowledge of the constituents of wines. Hence the concentration of materials having physiological effect present in wines in micro amounts determined. Last years research was focussed mainly on the biogen amine content of wines. Within this, special attention was paid to the histamine and tyramine concentration.

In Hungary research work in this direction started only a few years ago and at first the biogen amine content of the Hungarian champagnes was studied.

Our present paper reports on all the research work done and results achieved concerning the biogen amine, histamine and tyramine content of Hungarian wines.

The research was planned on the basis of the following considerations:

— to elaborate a procedure suitable for the separation of the constituents to be determined;

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— to select an appropriate detection method;
— optimization of measuring conditions;
— to examine the biogen amine, histamine and tyramine concentration in wines characteristic of Hungarian wine districts throughout several years.

Biogen amine content of wines

Substances containing nitrogen, e.g. biogen amines play an important role in the quality of wines.

The biogen amines are produced by the decarboxylation of amino acids. As co-factor, pyridoxal phosphate takes part in the raction in which the amine compound is formed through an intermediary of Schiff base type.

As an example, Figs 1 and 2 represent the simplified reaction scheme of histamine and tiramine formation.

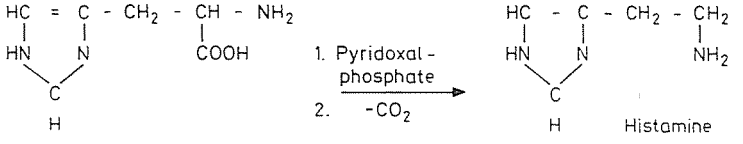

Fig. 1. Simplified reaction scheme of histamine formation

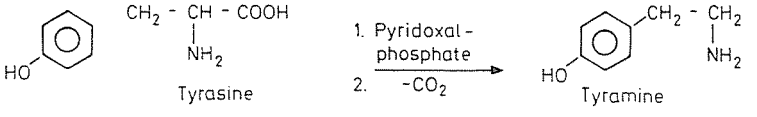

Fig. 2. Simplified reaction scheme of tyramine formation

Histamine has various physiological effects. In a certain small amount it can be used as pharmaceutical in the medicine (to cure vascular diseases, to help the secretion of gastric juice, to cure allergic diseases), however, in a higher concentration it is poisonous. It has a phlogistic effect, but plays an important role also in the onset of a shock. Histamine can be regarded as cause of the mackerel intoxication whose classical symptoms are nausea, head-ache, urticaria, etc.

Histamine occurs, first of all, in fermentation products, e.g. in cheeses and wines in greater quantities [3]. The average histamine content present in foods does not reach the toxic level, it is not damaging for the health. At one meal one has to consume a histamine amount of between 70–700 mgs for the clinical symptoms of histamine intoxication to appear [1].

Since 1945 it has been known that histamine in the form of biogen amine is present in the majority of wines. Red wines contain, in general, a higher quantity compared with white ones.

The determination of histamine present in wines is important not only because it is poisonous in itself, but also since it is able to react with the pharmaceutical inhibitor of the monoamino-oxidase which is especially dangerous in the presence of alcohol, as chronic intoxication may set on.

The determination of tyramine is also necessary, as together with histamine it has significant effect upon the vascular system and especially upon the blood pressure. Its toxic effect manifests itself when patients treated with drugs containing monoamino-oxidase inhibitor are given tyramine-containing food or drink–whose tyramine content in itself would not be damaging. In these cases tyramine does not decompose to the physiologically uneffective 4-hydroxy-phenyl acetic acid, the tyramine gets into the blood circulation yielding hypertension. Cheese and wine were found to contain tyramine in relatively high amount.

As the amount of the total biogen amine may have a role in the taste of wines, their determination is of high importance.

Researches have been carried on so far only in the field of identification, except the determination of some amines being present in higher quantities, like e.g. histamine and tyramine.

Due to the spreading of thin layer-, as well as high pressure-liquid chromatography, the identification of biogen amines became possible. The following amines could be determined in wines: tyramine, putrescine, cadaverine, histamine, benzylamine, ethyl-amine, isopropyl-amine, isobutyl-amine, hexyl-amine. Out of these amines, however, some occur only in very low concentrations, while there are some which are not present in every wines.

According to literature data, the determination of histamine has been done so far by the following methods: thin layer chromatography, gel electrophoresis, high pressure liquid chromatography, separation technique with ion exchange and various biochemical and physicochemical processes.

The tyramine content was detected by gas-chromatographic, thin layer-chromatographic and paper-chromatographic methods. A comprehensive table including the histamine content of wines examined so far, was published by Tejodor and Marine. According to this, authors of several papers found concentration values between 0–21 mg/l.

Preparatory operations

The foods are, generally, very complicated multicomponent systems, and so are the wines. This is why the component to be determined must be separated from the other constituents disturbing the measurement.

In our case, the analysis of the total biogen amine content is disturbed by the presence of amino acids, while with the tyramine analysis, first of all, histidine and spermidine can interfere. The histidine content of wines is quite considerable. In the detection of tyramine tyrosine may appear as an interferent. The colouring agents of wines can also modify the results.

The first part of this paper, therefore, treats separation methods. In our previous experiments liquid-liquid extraction and ion-exchange technique have been applied, and the histamine content of wines was measured. The results obtained were published in the periodical "Borgazdaság" [6].

Later on, separation technique was looked for which is able to separate the amino acids from the amines (thereby the direct determination of the total biogen amine content became possible) as well as the colouring agents of wines.

These days lyophilization (freeze drying) has widely spread in food industry. The essence of this technique is that the substance is dehydrated in high vacuum and at low temperature and an anhydrous material is left behind as end-product.

This method was used in the course of our experiments. According to our considerations, after lyophilization of the wine, the amines will be extracted with solvents of appropriate selectivity.

The amino acids are readly soluble in hydrous media, however, if absolute organic solvent is applied, exclusively the amines dissolve.

From among the organic substances monovalent alcohols of various carbon atom number were used. On the basis of our experiments absolute n-butanol proved to be the most selective solvent.

The following separation technique has been employed:

— to the wine sample of 8 cm^3 0.8 cm^3 of 1 mol/l sodium hydroxide was added,
— this solution was then loyphilized according to a previously set program for 48 hours,
— afterwards 8 cm^3 of absolute n-butanol were added in two portions. To promote dissolution, the sample was stirred with glass rod.
— The solid parts were separated by centrifugation, with a speed of 1500 r.p.m. lasting for half an hour. Such a high performance was needed to separate also the floating tiny particles. In this way a clear solution was obtained.
— Since the absolute n-butanol mixed with water only in a limited degree, and the reagents applied later on are all aqueous solutions, the extract has been completed to a volume of 10 cm^3 with absolute methanol. Thus, the system did not separate to two phases in the following steps.

Possibilities of detection

To the detection of the histamine content spectrofluorometric method was used. Histamine does not show fluorescence in itself, however, its condensation product obtained with ortho-ophthalic-aldehyde shows sensitive fluorescence [5].

The fluorescent complex is as follows: (Fig. 3).

— to 4 cm^3 of histamine solution (later on of the prepared wine sample) 0.8 cm^3 1 mol/1 NaOH solution (pH 11–12) was added;
— following this 0.2 cm^3 of 1% OPT solution was added by pipette and then the system was mixed well. The OPT solution was prepared by dissolving 0.1 g OPT in 10 cm^3 metanol;
— after 4 minutes of reaction time 0.4 cm^3 of 3 mol/1 hydrochloric acid (pH 2) was added. The obtained complex was stable for some 15–20 minutes;
— as next step, excitation and emission spectra were recorded.

The effect of amines as well as of chloride and sulphate ions upon the stability of the complex was also studied. Chloride ions stabilize the systems, therefore re-acidification was performed with hydrochloric acid. Thus the complex obtained was stable for 30–40 minutes.

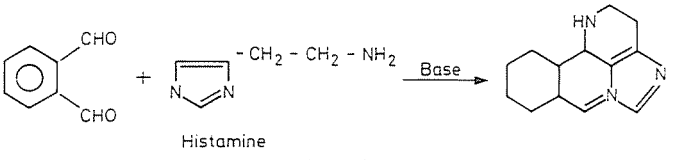

Fig. 3. Reaction between histamine and orthophthalaldehyde

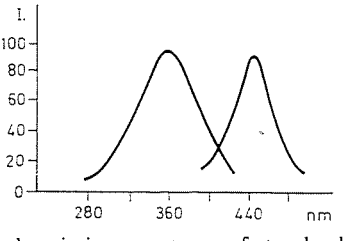

Fig. 4. Excitation and emission spectrum of standard histamine solution
λ_{ex}: 360 nm λ_{em}: :450 nm

The excitation and emission spectra recorded with standard histamine solution are shown in Fig. 4. The excitation and emission spectra recorded with wine extract are shown in Fig. 5.

One can observe that the spectrum of the standard solution agrees with literature data, its excitation maximum is at a wavelength of 360 nm, and the

emission maximum is at 450 nm. However, the spectrum of wine extract shows a difference, its excitation maximum being at a wavelength of 340 nm and the emission maximum at 420 nm.

In order to decide whether the shift in wavelength is caused by the presence of another fluorescent compound or not, various amounts of standard histamine solutions have been added to the wine extract and the spectra were recorded under various parameters (Fig. 6).

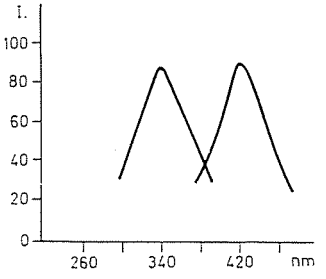

Fig. 5. Excitation and emission spectrum of wine extract
λ_{ex}: 340 nm λ_{em}: 420 nm

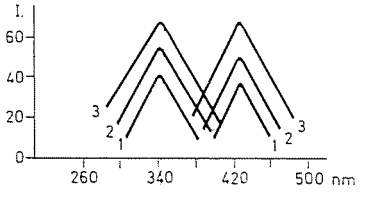

Fig. 6. Excitation and emission spectra of wine extract recorded after standard histamine solution addition under various parameters
1. λ_{ex}: 320 nm λ_{em}: 400 nm
2. λ_{ex}: 340 nm λ_{em}: 420 nm
3. λ_{ex}: 360 nm λ_{em}: 450 nm

It can be seen that the position of the peaks did not change, only the intensities varied what refers to the fact that the system does not contain fluorescent compounds other than histamine. To clear up the shifting wavelength, further studies have to be carried out. It is noteworthy, however, that Spanish authors obtained similar results when examining Spanish wines [1].

This fact excludes, however, the application of a calibration curve, therefore the method of standard addition was used with the examination of samples.

The tyramine content was also determined by spectrofluorometry. The para-substituted phenols when reacted in dilute nitric acid with α-nitroso-β-

naphthol yield a characteristic yellow fluorescent compound, and so does the tyramine.

The combining reaction results in the following compound (Fig.7).

The combining reaction is as follows:

— 1 cm^3 of 0.1 % alcoholic α-nitroso-β-naphtol solution and 1 cm^3 of 1 mol/1 HNO_3 solution (containing 2.5 ml of 2 % $NaNO_2$ solution in 100 ml) are added to 5 cm^3 of the extract;
— the mixture was homogenized and kept at a temperature of 60 ± 5 °C for 1 hour;
— after the reaction time had elapsed, the sample was cooled to room temperature and then the reagent excess was extracted with 10 cm^3 1,2- dichlorethane.

The excitation and emission spectra of the standard solution and wine extract have been recorded (Fig. 8). It has been stated that the two spectra well agree with those found in the literature. The excitation maximum was at a wavelength of 465 nm, while the emission maximum at 565 nm.

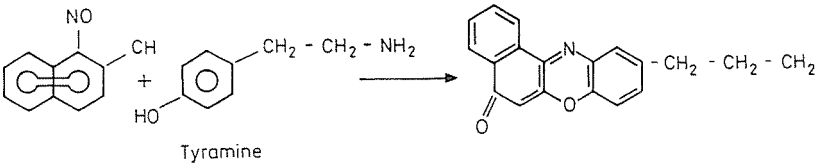

Fig. 7. Reaction between tyramine and α-nitroso-β-naphthol

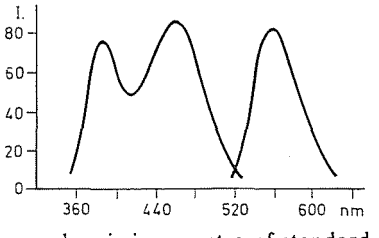

Fig. 8. Excitation and emission spectra of standard tyramine solution
λ_{ex}: 465 nm λ_{em}: 565 nm

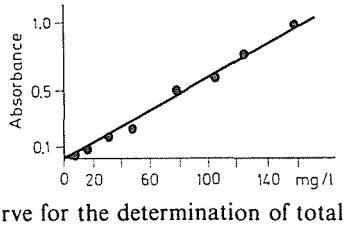

Fig. 9. Calibration curve for the determination of total biogen amine content

The calibration curve was taken and used in the serial measurements.

The total biogen amine content of the Hungarian wines has not been determined so far. Our earlier investigations concerned the champagnes.

The determination of biogen amine content of wines was performed as follows:

— 1 cm^3 of ninhydrine reagent (ninhydrine reagent: 12.6 g of sodium acetate 3 H_2O + 2.5 cm^3 glacial acetic acid + 2.0 g ninhydrine dissolved in 25 cm^3 water and 75 cm^3 ethanol) is added,

— the system was heated on a hot water bath for 30 minutes, then completed with ethanol to a final volume of 10.0 cm^3,

— the absorbance was measured at a wavelength of 570 nm by spectrophotometer. The calibration curve was taken with standard histamine solutions (Fig. 9).

Results and discussion

The total biogen amine, histamine and tyramine concentration of pure once-tinned wines of 1981 and 1982 vintage have been examined with the method developed by us (Tables 1, 2, 3).

Table 1

Results for 1981 vintage wines obtained in December 1981

Wine district	Sort	Total biogen amine content mg/l	Histamine mg/l	Tyramine mg/l	L-malic acid mg/l	L-lactic acid mg/l
Badacsony	Ezerfürtü	15.82	0.195	0.880	3.310	0.220
	Szürkebarát	25.81	0.910	0.880	0.950	2.170
	Jubileum	20.30	0.610	0.620	2.840	1.350
	Rajnai Rizling	41.00	0.753	0.570	4.900	0.209
Alföld	Jubileum	55.40	3.100	0.760	3.150	0.880
	Olaszrizling	28.10	1.085	0.900	3.010	0.610
	Zenit	40.20	1.010	0.760	3.930	0.320
	Kékfrankos	18.90	0.850	0.680	3.680	0.950
Villány—Siklós	Olaszrizling	20.60	0.210	0.620	3.210	0.610
	Tramini	55.60	0.280	1.380	0.810	2.860
	Cabernet	40.80	0.610	0.790	0.570	2.690
	Kékfrankos	18.90	0.690	0.690	0.660	4.640
	Oportó	25.00	0.950	0.790	0.570	2.150
Eger	Leányka	30.50	1.500	0.480	3.900	2.500
	Zenit	32.80	0.810	0.290	2.130	0.480
	Kékfrankos	50.60	1.650	0.770	3.260	0.260
	Merlot	43.40	0.790	0.500	3.870	1.830
	Zweigelt	20.30	2.100	0.460	2.360	0.930

Table 2

Results for 1981 vintage wines obtained in December 1982

Wine district	Sort	Total biogen amine content mg/l	Histamine mg/l	Tyramine mg/l	L-malic acid mg/l	L-lactic acid mg/l
Badacsony	Ezerfürtü	25.82	0.200	1.110	2.126	1.153
	Szürkebarát	39.37	1.500	1.220	0.662	2.890
	Jubileum	26.25	0.810	0.650	2.835	1.345
	Rajnai rizling	38.93	1.200	0.750	3.969	0.737
Alföld	Jubileum	76.57	5.430	1.130	1.020	2.470
	Olaszrizling	38.07	1.800	1.550	1.610	2.880
	Kékfrankos	25.37	1.110	1.400	1.500	2.930
	Zenit	52.50	1.740	1.380	1.210	2.400
Villány—Siklós	Olaszrizling	27.57	0.390	0.715	1.210	2.010
	Tramini	64.32	0.318	1.760	0.656	3.580
	Cabernet	50.70	0.780	0.900	0.318	3.880
	Kékfrankos	24.07	0.950	0.740	0.520	4.930
	Oportó	30.18	1.290	0.880	0.378	4.180
Eger	Leányka	38.50	2.580	0.610	3.780	2.979
	Zenit	40.25	1.659		0.741	2.851
	Kékfrankos	69.57	2.780	1.770	0.567	3.395
	Merlot	57.50	1.710	1.800	1.937	4.579
	Zweigelt	26.25	3.600	1.320	0.520	2.562

Table 3

Results for 1982 vintage wines obtained in December 1982

Wine district	Sort	Total biogen amine content mg/l	Histamine mg/l	Tyramine mg/l	L-malic acid mg/l	L-lactic acid mg/l
Badacsony	Ezerfürtü	20.16	0.320	0.810	3.010	0.350
	Szürkebarát	23.40	0.710	0.580	1.980	1.550
	Jubileum	28.20	0.730	0.610	4.300	0.400
	Rajnai rizling	30.80	1.320	0.800	1.150	2.300
Alföld	Jubileum	31.50	1.250	0.680	2.780	2.050
	Olaszrizling	31.85	1.850	0.920	1.420	3.940
	Zenit	28.00	2.400	0.680	2.830	2.020
	Kékfrankos	44.63	2.500	0.990	0.950	3.810
Villány—Siklós	Olaszrizling	25.26	0.190	0.810	0.987	3.018
	Tramini	33.60	0.336	0.980	1.218	2.470
	Cabernet	37.62	0.580	0.750	1.324	2.682
	Kékfrankos	44.63	0.780	1.150	1.182	2.017
Eger	Leányka	28.35	1.950	0.570	4.180	1.480
	Zenit	16.63	1.140	0.510	2.880	2.370
	Kékfrankos	52.74	1.000	0.810	3.750	0.810
	Merlot	37.10	0.990	0.650	1.890	2.880
	Zweigelt	14.88	1.110	0.390	0.470	2.180

It can be stated that regarding the above compounds no significant difference exists among the wines of various sorts, vintages and geographical origin. The concentration values obtained agree with those accepted in international standard.

If the measured data are considered in function of the age of wine then one can observe that the histamine, tyramine and total biogen amine concentration increases with the age. This seems to prove the fact that in the course of the biological decomposition of malic acid, the lactic acid bacteria form amines from amino acids, as a "by-product" of the acid decomposition. Accordingly, there is no wine which contains, e.g. both malic acid and histamine in a concentration of 0 mg/l.

According to general observation, the seasoned red wines contain histamine in higher amount compared to the white ones of similar age. The reason for this is that the biological decomposition of malic acid occurs more frequently in red wines than in white ones.

In summary the conclusion can be drawn that the method developed is suitable for the selective separation of biogen amines and for the determination of histamine, tyramine and total biogen amine concentration.

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