DETERMINATION OF AMINO ACID CONTENT IN ROMANIAN SALAMI BY ION-EXCHANGE CHROMATOGRAPHY

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Abstract: *Free amino acid content might influence the levels of biogenic amines in salamis, but is not necessary for a salami with high levels of free amino acids to have high levels of biogenic amines. Biogenic amines are aliphatic, alicyclic or heterocyclic organic bases with biological activity present in meat, cheese, fish and wine products. They are formed by amination and transamination of aldehydes and ketones or by decarboxilation of aminoacids. Free amino acids composition has been evaluated to serve as quality and tipicality of several salami varieties. Eight types of Romanian manufactured salamis (Banatean, Ardelenesc, Sinaia, Sibiu, Vanatoresc, Ghiudem, Babic, Plai) were investigated. The analysis of amino acids was carried out on an automatic amino acid analyzer (Amino Acid Analyzer AAA400, Ingos Ltd., Czech Republic) equipped with an Ostion LG ANB ion-exchange column (36x0.37 cm). Free amino acids were separated by stepwise gradient elution using five Li^+ buffer- systems. Colorimetric detection was accomplished at 570 nm and 440 nm after post-column derivatisation with ninhydrin reagent. For the identification of the peaks observed in the test samples a comparison of retention times between standards and the actual test samples was carried out. All the extractions and HPLC analysis were made in triplicate. The LC ion-exchange post-column derivatisation method used has proved to be suitable for amino acid determination in salamis. The main free amino acids were glutamine, alanine, glutamic acid and gamma amino-butyric acid. The Babic salami sample presented the highest level of amino acids, 240 mg/g, the Banatean salami sample revealed the lowest level of amino acids, 115 mg/g. The aim of the work was to asses the amino acid levels in Romanian salami. The absolute amounts of free amino acids vary to a large extent according to salami type and age but relative amounts (of individual free amino acids) reveal important differences in several salami varieties*

Key words: *Ion-exchange chromatography, amino acids, salami*

INTRODUCTION

Free amino acid content might influence the levels of biogenic amines in salamis, but is not necessary for a salami with high levels of free amino acids to have high levels of biogenic amines. Biogenic amines are aliphatic, alicyclic or heterocyclic organic bases with biological activity present in meat, cheese, fish and wine products. They are formed by amination and transamination of aldehydes and ketones or by decarboxilation of aminoacids.[1]

Degradation of proteins manifests itself in accumulation of free amino acids which can be converted into corresponding biogenic amines during salami ripening [2]. Salami were observed to contain ample pyridoxal phosphate which is important for amino acid decarboxylase activity [3].

The liberation of amino acids in salami ripening was recognized very early [4] and their contribution to salami flavour has been discussed [5]. Free amino acids composition has been evaluated to serve as quality and tipicality of several salami varieties [6].The absolute amounts of free amino acids vary to a large extent according to salami type and age but relative amounts (of individual free amino acids) reveal important differences in several salami varieties [7].

Chromatographic methods are generally used for the determination of amino acids ,

e.g. overpressured –layer chromatography , high performance liquid chromatography , chromatography on an amino acids analyzer, gas chromatography , capillary electrophoresis [1,5,8,9,11].

MATERIAL AND METHODS

2.1 Sampling

The salami samples were purchased in May 2009 from supermarkets in Cluj-Napoca. Eight types of Romanian manufactured salamis (Banatean, Ardelenesc, Sinaia, Sibiu, Vanatoresc, Ghiudem, Babic, Plai) were investigated.

2.2 Sample preparation for biogenic amines, free and total amino acid analisys

Samples (3 g) were extracted with 10 ml cold 10% trichloroacetic acid for 1 hour with gentle agitation on a shaker at room temperature (Laboshake LS 500i, C. Gerhardt GmbH & Co. KG, Germany). The extracts were then centrifuged (Heraeus Labofuge 400R, Thermo Fischer Scientific Inc., Germany) at 5,000 rpm for 10 min. The supernatants were filtered through a 0.2 µm pore membrane filter (Sartorius AG, Germany) and stored at -20 °C.

2.3 The experimental conditions

The analysis of biogenic amines was carried out on an automatic amino acid analyzer (Amino Acid Analyzer AAA400, Ingos Ltd., Czech Republic) equipped with an Ostion LG ANB ion-exchange column (6x3.7 cm). The were separated by stepwise gradient elution using a Na^+/K^+-citric buffer system (Ingos Ltd., Czech Republic). Colorimetric detection was accomplished at 570 nm after post-column derivatisation with ninhydrin reagent [6]. Chromatographic conditions for the determination free amino acids are shown in table 1.

Table 1.

Chromatographic parameters

Length of column	36 cm
Column diameter	0.37 cm
Column temperature	60^0C
Eluent flow rate	0.30 mL min^{-1}
Reagent flow rate	0.20 mL min^{-1}
Reactor temperature	121^0C
Sample volume	100 µl
Detector wavelength	570nm and 440 nm
Time of analysis	92 min

For the identification of the peaks observed in the test samples a comparison of retention times between standards and the actual test samples was carried out, a chromatogram for separation of standards is shown in Figure1.

Amino acid standards were used to prepare standard curves which in turn were used to determine the concentration of the sought components in the test samples. All the extractions and HPLC analysis were made in triplicate. Results were expressed as mean ± standard deviation (m±SD).

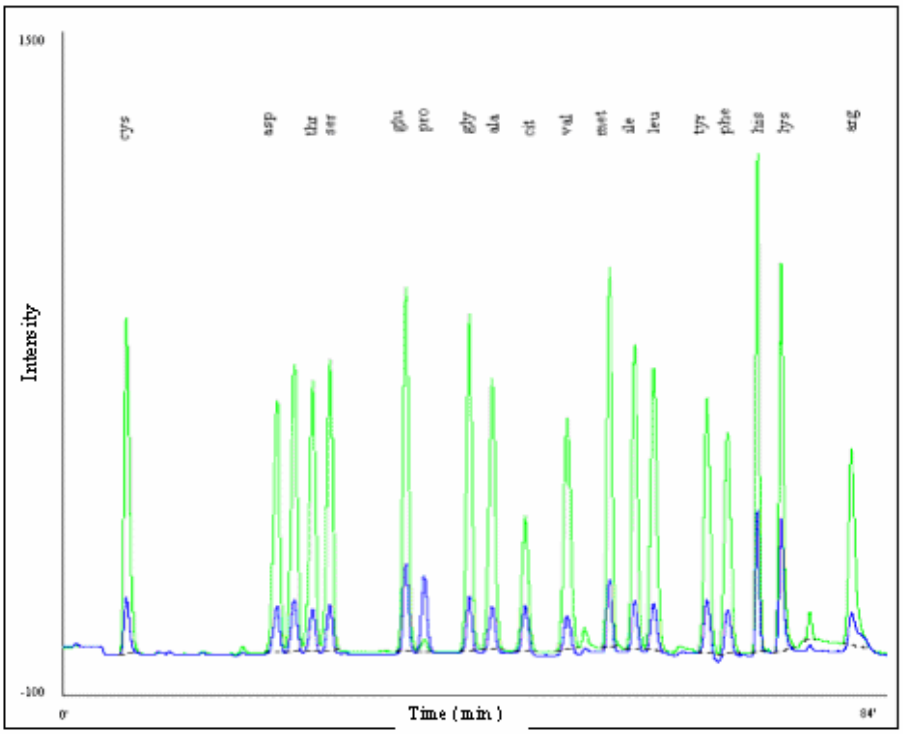

Figure 1. The separation of amino acid standards.

RESULTS AND DISCUSIONS

3.1 Free amino acid content

Free amino acid content values registered from 2.06 mg/g in Vanatoresc salami to 11.49 mg/g in Sinaia salami. The major free amino acids in sample were: glutamine (from 0.006 mg /g in Sibiu salami to 3.12 mg/g in Sinaia salami), alanine (from 0.102 mg/g in Vanatores salami to 1.33 mg/g in Babic salami), glutamic acid (from 0.174 mg/g in Sibiu salami to 1.25 mg/g in Babic salami) and gamma amino-butyric acid (from 0.007 mg/g in Vanatoresc to 1.10mg/g in Babic salami).

In the salami samples analyzed, metionine, ornitine, histidine, arginine and proline were found in low levels.

When taking into consideration the Babic salami sample we found high levels of free amino acids, the Sinaia salami sample presented high levels of free amino acids also. Free amino acid content influences the levels of biogenic amines in salamis, but is not necessary for a salami with high levels of free amino acids to have high levels of biogenic amines. These findings are consistent with data presented by other authors [12,13,14,15].

3.2 Total amino acid content

Total amino acid content in Romanian salamis was also investigated in this study. The Babic salami sample presented the highest level of amino acids, 240 mg/g, the Banatean salami sample revealed the lowest level of amino acids, 115 mg/g.

Total amino acid content of the samples was studied and results are presented in Fig.2

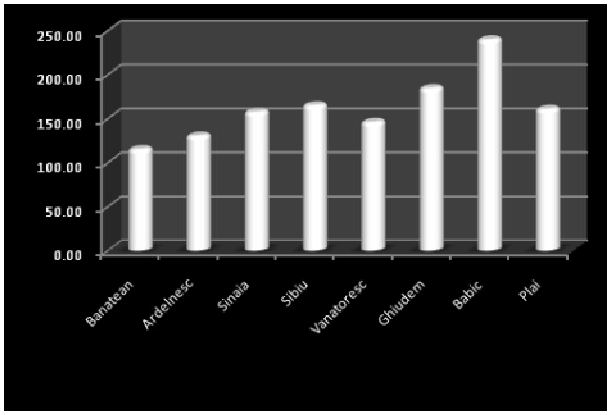

Fig 2. Total amino acids found in samples

Amino acids found in Romania salami are shown in Table 2.

Table 2.

Amino acid content of salami samples (mg/g)

Sample / Amino acid	Banatean	Ardelnesc	Sinaia	Sibiu	Vanatoresc	Ghiudem	Babic	Plai
				Mean±SD				
asp	12.46 ± 0.55	14.66 ± 0.51	16.55 ± 0.62	18.56 ± 0.82	15.46 ± 0.71	19.95 ± 0.82	25.50 ± 1.02	15.11 ± 0.72
OHpro	0.55 ± 0.002	0.62 ± 0.02	0.51 ± 0.01	0.27 ± 0.01	1.34 ±0.08	0.50 ± 0.02	1.33 ± 0.09	2.27 ± 0. 11
thr	6.10 ± 0.22	6.60 ± 0.18	8.00 ± 0.30	8.65 ± 0.37	6.41 ± 0.30	9.61 ± 0.48	11.67 ± 0.62	7.86 ± 0.37
ser	6.18 ± 0.23	7.09 ± 0.20	8.29 ± 0.31	8.43 ± 0.36	8.38 ± 0.46	10.19 ± 0.52	13.41 ± 0.74	7.92 ± 0.38
glu	19.15 ± 0.73	21.68 ± 0.62	25.01 ± 0.86	24.54 ± 0.93	23.29 ± 0.88	27.87 ± 1.12	32.76 ± 1.42	22.05 ± 0.96
pro	7.53 ± 0.35	8.91 ± 0.28	9.67 ± 0.36	9.02 ± 0.41	11.27 ± 0.59	11.28 ± 0.58	15.43 ± 0.77	11.48 ± 0.43
gly	7.07 ± 0.32	8.09 ± 0.27	10.47 ± 0.38	10.49 ± 0.45	12.99 ± 0.61	12.28 ± 0.62	17.97 ± 0.86	14.73 ± 0.65
ala	7.70 ± 0.36	8.90 ± 0.28	11.02 ± 0.42	11.46 ± 0.52	9.28 ± 0.54	12.34 ± 0.62	16.53 ± 0.82	11.43 ± 0.43
val	3.79 ± 0.19	3.61 ± 0.11	5.61 ± 0.21	5.58 ± 0.24	4.43 ± 0.22	6.28 ± 0.33	7.40 ± 0.39	5.29 ± 0.23
cys	0.32 ±0.001	0.36 ± 0.01	0.41 ± 0.01	0.47 ± 0.02	0.56 ± 0.02	0.69 ± 0.02	0.87 ± 0.06	0.45 ± 0.02
met	2.41 ± 0.10	2.62 ± 0.06	3.42 ± 0.08	3.73 ± 0.13	1.96 ± 0.09	3.06 ± 0.17	4.89 ± 0.19	3.50 ± 0.18
ile	3.49 ±0.15	3.87 ± 0.12	4.81 ± 0. 11	5.07 ± 0.19	3.85 ± 0.19	5.88 ± 0.26	7.49 ± 0.39	4.88 ± 0.21
leu	9.55 ± 0.48	10.78 ± 0.36	13.06 ± 0.54	14.31 ± 0.63	11.15 ± 0.58	15.88 ± 0.76	20.52 ± 0.96	12.89 ± 0.55
tyr	3.91 ± 0.20	4.55 ± 0.13	5.50 ± 0.22	5.43 ± 0.25	4.91 ± 0.27	6.72 ± 0.35	8.85 ± 0.54	5.40 ± 0.22
phe	4.82 ± 0.22	5.51 ± 0.18	6.61 ± 0.27	6.78 ± 0.32	6.31 ± 0.30	8.40 ± 0.39	11.02 ± 0.62	6.48 ± 0.29
lys	9.36 ± 0.46	10.54 ± 0.35	12.77 ± 0.41	14.56 ± 0.66	10.12 ± 0.51	14.93 ± 0.74	19.31 ± 0.96	12.75 ± 0.55
his	3.41 ± 0.18	3.85 ± 0.12	5.61 ± 0.22	6.83 ± 0.26	3.43 ± 0.19	5.55 ± 0.25	7.11 ± 0.38	4.96 ± 0.22
arg	7.56 ± 0.35	8.46 ± 0.27	10.35 ± 0.38	11.13 ± 0.52	11.00 ± 0.59	13.27 ± 0.42	17.96 ± 0.86	11.57 ± 0.43
Total	115.38 ± 5.093	130.7 ± 4.07	157.66 ± 5.71	165.3 ± 7.09	146.16 ± 7.13	184.68 ± 8.47	240.01 ± 11.69	161.03 ± 6.9

All determination were carried out in triplicate and mean value ± standard deviation (SD) reported.

CONCLUSIONS

The aim of the work was to assess the amino acid levels in Romanian salami. The absolute amounts of free amino acids vary to a large extent according to salami type and age but relative amounts (of individual free amino acids) reveal important differences in several salami varieties.

The HPLC ion-exchange post-column derivatisation method used has proved to be suitable for amino acid determination in salamis.

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