ELSEVIER

Journal of Chromatography A, 838 (1999) 191–195

JOURNAL OF CHROMATOGRAPHY A

Short communication

Isotachophoretic determination of 3-methylhistidine

František Kvasnička

Department of Food Preservation and Meat Technology, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic

Abstract

A capillary isotachophoretic (cITP) method to determine the concentration of 3-methylhistidine (3-MeHis) in meat and meat products is described. A clear separation of the 3-MeHis from histidine, 1-methylhistidine and other components of acidic sample hydrolyzate was achieved within 20 min. Method characteristics (linearity, accuracy, precision and detection limit) were determined. Low laboriousness, sufficient sensitivity and low running cost are the important attributes of cITP method. The developed method was successfully applied to analyses of real samples and used for the determination of lean meat content in meat and meat products.

Keywords: Isotachophoresis; Meat; Food analysis; Methylhistidines; Histidine; Amino acids; Creatinine

1. Introduction

Lean meat content is the best quality parameter of meat products. Using this parameter, meat products may be divided very easily into different quality classes (and prices) which are understandable for consumers in every (European) country. There are some typical components (for structural formula see Fig. 1) of meat proteins, e.g., 3-methylhistidine (3-MeHis; [*S*]-1-methylimidazole-4-alanine; τ-methyl-L-histidine), N_{ϵ}-methyllysine and creatinine [1,2]. Especially 3-MeHis is a normal constituent of the myofibrillar protein actine and myosine. Levels of protein-bound 3-MeHis in different species of meat are constant[1] [3,4]. Hitherto no 3-MeHis residues are determined in other foodstuffs, rich in proteins as milk, eggs, soya etc. That is why the determination of 3-MeHis in meat product can be used as an index of lean meat content. For the determination of 3-MeHis mostly high-performance liquid chromatography (HPLC) methods with fluorimetric [3,5,6,7] detection after derivatization prior to analysis or direct UV detection at 210 nm (only model mixture of His and 3-MeHis) [8] are used.

A capillary isotachophoretic (cITP) method for the determination of 3-MeHis is presented as an alternative to the above mentioned technique. cITP is a simple, quick, sufficiently sensitive and inexpensive method and therefore well suited for routine analysis.

2. Experimental

2.1. Chemicals

Standards of 3-MeHis[2], 1-methylhistidine[3] (1-MeHis; [*S*]-1-methylimidazole-5-alanine; π-methyl-L-histidine), morpholinethanesulfonic acid (MES)

[1] 5 mg/g of non-collagen nitrogen [3] or 750 μg/g meat protein [4].

[2] This structure corresponds to IUPAC 1-methyl-histidine.

[3] This structure corresponds to IUPAC 3-methyl-histidine.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00122-3

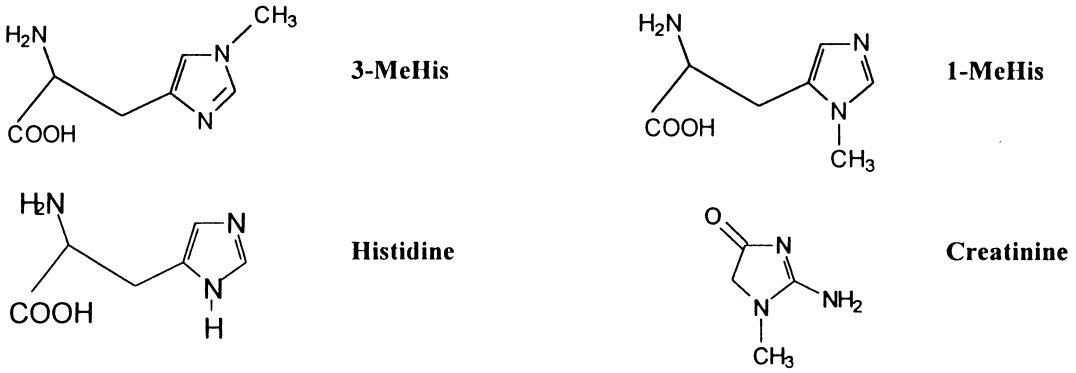

Fig. 1. Structural formulas of typical components of meat proteins.

and ε-aminocaproic acid (EACA) were purchased from Sigma–Aldrich (Czech Republic), L-histidine (His) from Reanal (Budapest, Hungary), creatinine (Creat) from Jenssen (Ghent, Belgium), analytical-reagent grade ammonium hydroxide, sulphuric acid, barium hydroxide octahydrate and acetic acid (HAc) were purchased from Lachema (Brno, Czech Republic).

2.2. Isotachophoresis

The electrophoretic analyser used was a EA 100 (Labeco-Villa, Spišská Nová Ves, Slovak Republic) with column coupling. The separation were performed in a PTFE pre-separation capillary (90 mm× 0.8 mm I.D.) which was coupled with a PTFE analytical capillary (90 mm×0.3 mm I.D.). Zones were detected by conductivity and UV detection, respectively. The isotachopherograms were evaluated by a personal computer software package supplied with the analyser. Cationic analysis of 3-MeHis was performed with a leading electrolyte comprising of 5 m*M* NH_4OH+10 m*M* MES and a terminating electrolyte of 10 m*M* EACA+5 m*M* HAc. The driving current applied to the pre-separation capillary was 100 μA and to the analytical capillary 20 μA which was decreased to 5 μA during detection. Each analysis required 20 min.

2.3. Calibration

The external standard technique was used. A standard of 3-MeHis was injected (in duplicate) from the 100 μmol/l stock solution at five-levels (50 to 4000 pmol) by the use of a 10-μl Hamilton syringe.

2.4. Sample treatment

Samples of lean meat and ham were obtained fresh from a local butcher. In case of direct hydrolysis a 5-g homogenised sample was weighed into a 50-ml Erlenmeyer flask and 30 ml of 30% H_2SO_4 was added. The flask was placed into an oven (110°C) for 12 h.

Hydrolysis of sample after extraction of soluble peptides was as follows: 5-g of homogenised sample was weighed into a 100-ml glass centrifugation tube, 30 ml of 80% ethanol was added, the mixture was sonicated for 15 min and after the extraction centrifuged. Clear supernatant was discarded and extraction procedure repeated (twice). A 30-ml volume of 30% H_2SO_4 was added and tube placed into an oven (conditions as above).

Hydrolyzate was quantitatively transferred into a 50-ml volumetric flask and made up to volume with distilled water. A 5-ml aliquot (after filtration through paper) was transferred into a 50-ml volumetric flask and neutralised with a hot solution of 1 *M* $Ba(OH)_2$ to pH 5–7 (using indicator paper). The flask was then cooled to laboratory temperature and made up to volume with demineralised water. Filtrate (through paper) was used for cITP analysis. Sample was injected using a 10-μl Hamilton syringe or internal valve with a fixed volume 35 μl.

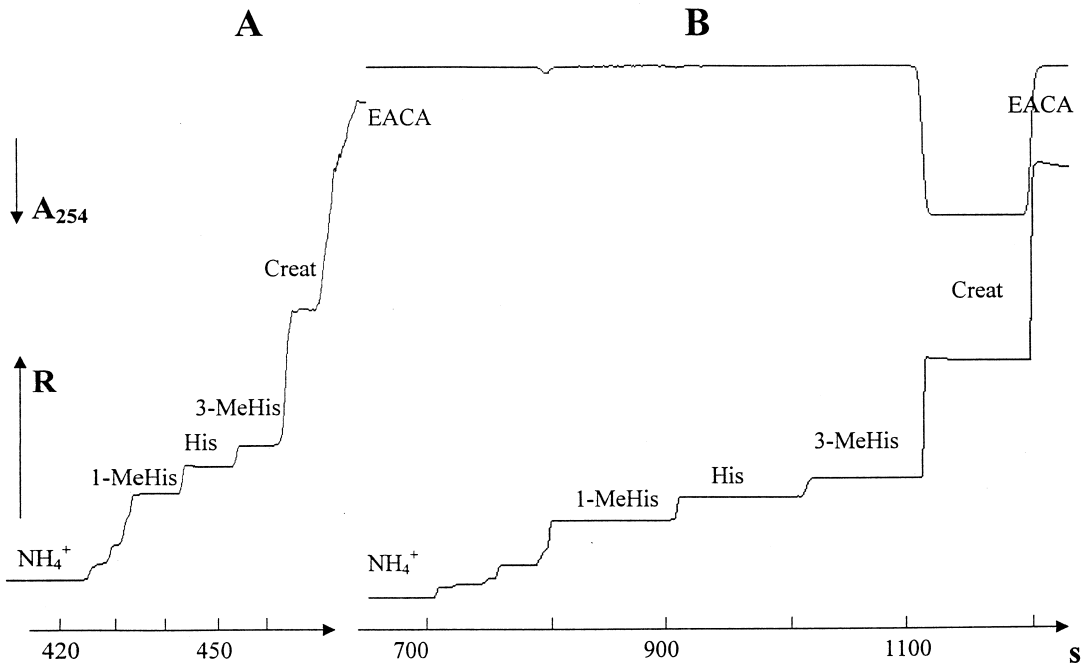

Fig. 2. Isotachopherogram of model mixture (injected 3.5 nmol each); A=preseparation capillary; B=analytical capillary; R=signal from conductimeter; A_{254}=signal from UV detector.

3. Results and discussion

Desired resolution (see Fig. 2) of 3-MeHis from His and 1-MeHis (and other sample constituents) was achieved by separation based on differences of ionic mobilities according to p*K* values, i.e., pH of leading electrolyte was selected near the centre of p*K* values of histidine derivatives (6.0).

The calibration data are given in Table 1. The 3-MeHis content (see Table 3) was calculated by means of the calibration equation (see Table 1). The method characteristics, i.e., linearity, precision, accuracy (recovery) and detection limit are summarised in Table 2. Results clearly show that the method is suitable for the intended purpose.

Table 1
Calibration data – qualitative and quantitative parameters

Species	RSH[a]	Regression model SL[b]=*a*·pmol+*b*		
		a	*b*	*r*
NH_4	0.00	–	–	–
1-MeHis	0.16	–	–	–
Histidine	0.22	–	–	–
3-MeHis	0.25	0.1518	−1.0260	0.999933
Creatinine	0.50	–	–	–
EACA	1.00	–	–	–

[a] Relative step height (–).
[b] Step length (μC).

Table 2
Method characteristics for 3-MeHis

Characteristic	Value
Precision[a] (RSD, n=6)	1.5%
Accuracy (recovery)[b]	95.0±3%
Linearity	1–100 μmol/l
Detection limit[c]	0.2 μmol/g of dry matter

[a] Repeated injection of the same sample extract (pork leg B, see Table 3).
[b] Three different real samples (pork, beef and chicken meat) spiked at concentration 5 μmol/g dry matter.
[c] The shortest detectable step length is 0.5 s; at 5 μA it corresponds to 2.5 μC and from the calibration equation this charge equals to 17 pmol of 3-MeHis; LOD of HPLC with fluorimetric detection is 1.6 pmol of 3-MeHis [7].

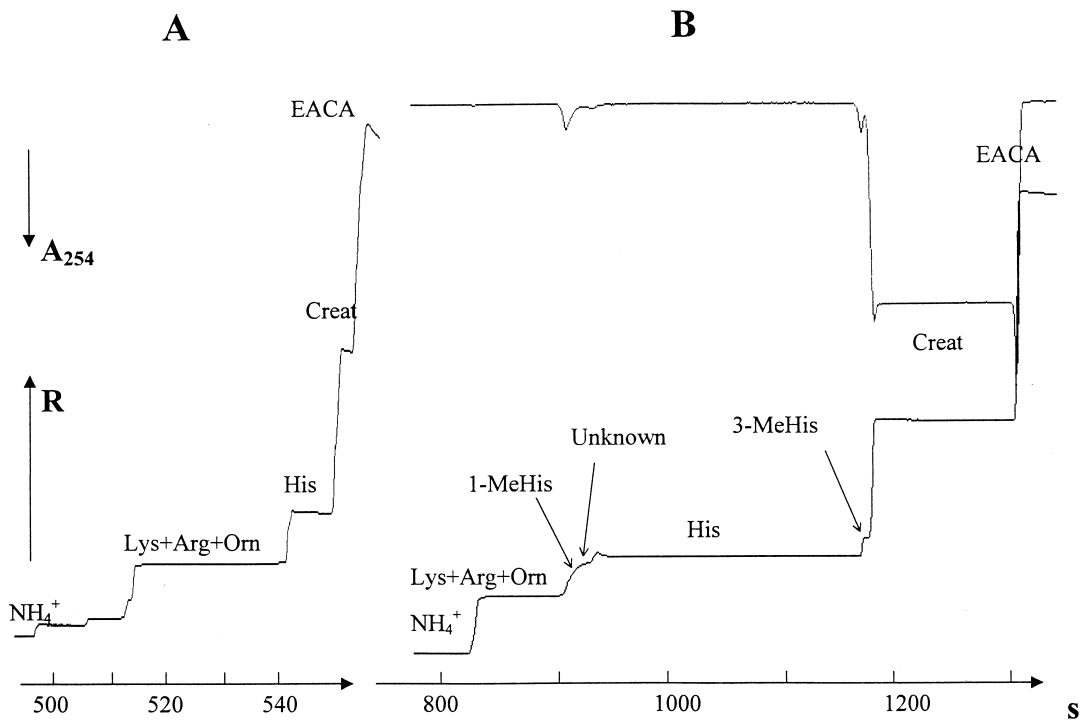

Fig. 3. Isotachopherogram of pork meat after acidic hydrolysis with 30% sulphuric acid (without extraction by 80% ethanol prior to hydrolysis); A=preseparation capillary; B=analytical capillary; R=signal from conductimeter; A_{254}=signal from UV detector; step denoted as 3-MeHis corresponds to 350 pmol.

Fig. 3 shows an isotachopherogram of a sample of pork meat hydrolyzate. It is clear that no interfering compound with 3-MeHis was found in this sample (and in the other analysed samples). The evidence that the step denoted as 3-MeHis in Fig. 3 corresponds to the 3-MeHis content was made by the standard addition method (see Table 2). Besides 3-MeHis other meat protein components such as 1-MeHis, His and creatinine can be determined in one run.

In contrast to literature data, results summarised in Table 3 show that 3-MeHis content varies for different kinds of meat (Table 3, column A). The differences may be caused by presence of soluble protein fraction or dipeptide balenine and anserine [1,7] which are usually removed by extraction prior to hydrolysis [1,5]. After extraction with 80% ethanol the differences in 3-MeHis content decreased (Table 3, column B). We have found reasonably high levels (comparable to 3-MeHis ones) of 1-MeHis in meat samples. Up to now there are no literature references to 1-MeHis content in meat. We have also

Table 3
Results of sample analyses

Sample	Content of 3-MeHis (μmol/g DS)		3-MeHis/1-MeHis	
	A[a]	B[a]	A	B
Pork leg	14.0	6.5	1.77	0.96
Beef meat – hind quarter	7.9	5.9	0.36	0.58
Chicken leg	7.1	5.9	0.08	0.72

[a] A=Direct hydrolysis of sample; B: hydrolysis after extraction with of 80% ethanol prior to hydrolysis.

found that the ratio of 3-MeHis/1-MeHis varies for different kinds of meat and potentially can be used for meat origin recognition (see Table 3).

The developed cITP method is being used in the project ''Direct method for lean meat content determination'' which is now running in our department.

4. Conclusions

The presented results provide evidence that the developed isotachophoretic method of the 3-MeHis determination in meat and meat products is reliable and reproducible. Other meat protein constituents such as creatinine and 1-MeHis can be determined in one run together with 3-MeHis. cITP can easily be an alternative method to HPLC. Low laboriousness (neutralisation of acidic hydrolyzate and filtration only), sufficient sensitivity (0.2 μmol 3-MeHis/g dry matter) and low running cost (at least 10-times lower than HPLC) are the important attributes of the cITP method.

References

[1] W.R.D. Rangeley, R.A. Lawrie, J. Food. Technol. 11 (1976) 143.
[2] H.F. De Brabander, K. De Wasch, P. Batjoens, G. Temmermen, in: Proceedings of Euro Food Chem IX, 1997, p. 17.
[3] D. Jones, D. Shorley, Ch. Hitchcock, J. Sci. Food Agric. 33 (1982) 677.
[4] W. Arneth, Proc. BAFF 36 (1997) 197.
[5] W. Arneth, Proc. BAFF 35 (1996) 192.
[6] S. Min, Y. Yisheng, Y. Lu, J. Chromatogr. 581 (1992) 272.
[7] M. Dunnet, R.C. Harris, J. Chromatogr. B 688 (1997) 47.
[8] Histidine and 3-methylhistidine, http://www.hplc.com/shodex/english/dc010906.htm, accessed April 1998.