A screening method for the simultaneous determination of putrescine, cadaverine, histamine, spermidine and spermine in fish by means of high pressure liquid chromatography of their 5-dimethylaminonaphthalene-1-sulphonyl derivatives

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Eine Screening-Methode für die gleichzeitige Bestimmung von Putrescin, Cadaverin, Histamin, Spermidin und Spermin im Fisch mittels Hochdruck-Flüssigchromatographie ihrer 5-Dimethylaminonaphthalin-Sulfonyl-Derivate

Zusammenfassung. Eine einfache und schnelle Methode für die Extraktion, Derivatisierung und Bestimmung von Putrescin, Cadaverin, Histamin, Spermidin und Spermin als 5-Dimethylaminonaphthalin-1-sulfonyl-Derivate mit Hilfe der Hochdruck-Flüssigchromatographie (HPLC).

Die Amine werden mit einer wäßrigen Trichloressigsäure-Lösung aus dem Fisch extrahiert und mit Hilfe von 5-Dimethylaminonaphthalin-1-sulfonyl (Dansylchloride) derivatisiert. Die Reaktionsmischung wird auf eine RP-8-Säule injiziert und durch Gradientelution getrennt. Die beschriebene Methode bringt einen beträchtlichen Zeitgewinn und eine Verringerung der Analysenkosten.

Summary. A simple and rapid method is described for the extraction, derivatization and subsequent determination by means of high pressure liquid chromatography of putrescine, cadaverine, histamine, spermidine and spermine as their 5-dimethylaminonaphthalene-1-sulphonyl derivatives.

The amines are extracted from the fish material by an aqueous trichloroacetic acid solution and derivatized by means of 5-dimethylaminonaphthalene-1-sulphonylchloride (dansyl chloride). The reaction mixture is then injected on a RP-8 column using gradient elution to separate the amine derivatives. Use of the method results in a considerable saving with respect to both time and costs.

1 Introduction

According to Mietz and Karmas [1], the most common, fastest and generally most accepted evaluation of the decomposition of fish has been based on the organoleptic analysis of the material. The subjectivity of the method and the difficulty of training people to precisely detect any decomposition remains a problem.

The determination of volatile acids [2] and later of acetic acid, succinic acid, ethanol, volatile bases and amines, trimethylamine [3], changes in the lipid content and fatty acid spectrum [4, 5], the extent of hydrogen sulphide liberation [6], volatile reducing agents [7] and of nucleotide breakdown products [8] were all attempts to evaluate more objectively the degree of decomposition.

Non-volatile acids, such as amino acids [9, 10], hypoxanthine [11], indole and skatole [12] and especially histamine and histidine [13, 14], have received much attention and in some cases have been correlated with the degree of decomposition.

Putrescine, cadaverine, histamine, spermine and spermidine have been identified in decomposed fish and pork [15, 16]. In order to evaluate the degree of decomposition of tuna fish, a chemical quality index was defined by Mietz and Karmas [1]:

$$\text{quality index} = \frac{(\text{mg/kg histamine} + \text{mg/kg putrescine} + \text{mg/kg cadaverine})}{(1 + \text{mg/kg spermidine} + \text{mg/kg spermine})}.$$

This quality index was correlated with the degree of decomposition of the fish material, as evaluated by organoleptically skilled analysts and can be used to classify the quality of fish material as acceptable, borderline or unacceptable [17]. A method has been described for the determination of putrescine, cadaverine, histamine, spermidine and spermine [18].

In this method, the amines are extracted with a 5% aqueous trichloroacetic acid solution and, upon alkalinization with sodium hydroxide, extracted three times with 5 ml *n*-butanol/chloroform (1+1). An equal amount of *n*-heptane is added to the organic phase, which is then extracted three times with 1 ml of 0.2 *N*-HCl. The acidic extract is brought to dryness.

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To the residue, a saturated sodium bicarbonate solution is added together with a solution of dansyl chloride in acetone and allowed to react overnight. The dansyl derivatives are then extracted with three 5 ml portions of diethyl ether and the organic phase brought to dryness. The residue is then dissolved in 1 ml acetonitrile and 10 µl of solution injected onto a RP-8 column. Separation of the amine derivatives is achieved using an elution gradient consisting of a combination of methanol, acetonitrile and 0.02 *N*-HAc.

As this method is too laborious and time-consuming for the routine screening of these amines, research was undertaken to develop a less time-consuming method.

2 Materials and methods

2.1 Reagents

The amines were purchased from Laboratoria Flandria, Ghent, Belgium (histamine hydrochloride) and from Fluka AG, Buchs SG, Switzerland (cadaverine dihydrochloride, putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride).

Trichloroacetic acid, diethyl ether and buffer pH 9 were obtained from Merck. Phosphate buffer pH 9 (Titrisol, Merck) was used undiluted.

A 5% solution of trichloroacetic acid in water was prepared (solution A). A stock solution (B) was prepared by dissolving approximately 0.17 g of each amine in 100 ml solution A. Consequently, a tenfold dilution of stock solution B was prepared (solution C).

5-Dimethylaminonaphthalene-1-sulphonylchloride (Dns-Cl) was obtained from Janssen Chimica, Beerse, Belgium. The dansyl reagent (50 mg dansyl chloride in 10 ml acetone, reagent grade, Merck) was prepared daily.

The methanol and the water used in the elution gradient were of HPLC grade (Burdick and Jackson High Purity Solvents).

2.2 Apparatus

A Spectra-Physics SP8000B high-pressure liquid chromatograph was used in combination with a Spectra-Physics SP8400 UV-detector set at 254 nm. Separations were achieved using a 250 × 3 mm Hibar pre-packed column, Lichrosorb RP-8, 10 µm, Merck. The column oven temperature was set at 40 °C.

2.3 Sample preparation

To 10 g of the homogenized fish sample, 30 ml of the 5% trichloroacetic acid solution (temperature 89°–90 °C) was added and the mixture was stirred vigorously by means of a glass rod for 2 min.

The suspension was filtered through a filter paper (Faltenfilter, Ederol, FRG) and 1 ml of a tenfold dilution of the filtrate, in 5% trichloroacetic acid, was transferred to a test tube, to which subsequently 1 drop 4 *N*-NaOH, 1 ml buffer pH 9 and 2 ml dansyl reagent were added. The test tube was closed, stirred for 30 s, wrapped in aluminium foil and placed in a thermostated oven at 55 °C for 1 h. The reaction mixture was then cooled in the refrigerator prior to HPLC analysis.

2.4 Analytical procedure

The standard addition method was used to quantitate the levels of the different amines in the sample.

To two aliquots of 10 g of the same homogenized fish sample, 1 ml and 2 ml of solution C were added; subsequently solution A was added so as to obtain 30 ml of the extraction reagent. To a third aliquot of the same extract (blank sample), 30 ml solution A was pipetted.

This procedure was carried out to make sure that the added amines had reacted completely with the dansyl chloride. Depending on the nature of the sample, it was necessary to dilute the extract with solution A so as to obtain an excess of dansyl chloride in order to completely convert the amines in to their dansyl derivatives. The method described above was used to determine the amounts of these amines in sardines, tuna, salmon, shrimp, herring and cod.

2.5 HPLC conditions

After equilibration of the column with a methanol/water mixture (70/30) at a rate of 77 ml/h, a 20-µl aliquot of the sample extract was injected on to the column via a sample loop. From the moment of injection, the methanol content gradually increased to 80% over a period of 7 min after which a steeper increase in the methanol content, up to 100% over a period of 4.5 min, was programmed. The eluant was then held for 7.5 min and subsequently lowered in 0.1 min to the original methanol/water mixture (70/30) which was kept constant for 10 min in order to re-equilibrate the column prior to the next injection.

3 Results

The time necessary for the extraction of the amines is illustrated in Fig. 1. For all the amines, an extraction time of 2 min seemed sufficient in order to reach a plateau; during this time there was no significant difference in the observed peak height. Figure 2 B shows the separation of the dansyl derivatives of putrescine, cadaverine, histamine, spermidine and spermine added in a concentration of 10 mg/kg to a fish sample. Figure 2 A shows the chromatogram of the same extract without the added amines. As can be seen, the re-

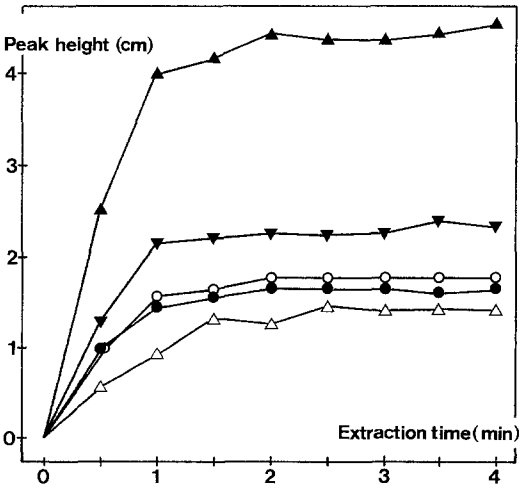

Fig. 1. Relation ship between peak height (cm) for cadaverine (▲), putrescine (●), histamine (▼), spermidine (○), spermine (△), and extraction time (in min)

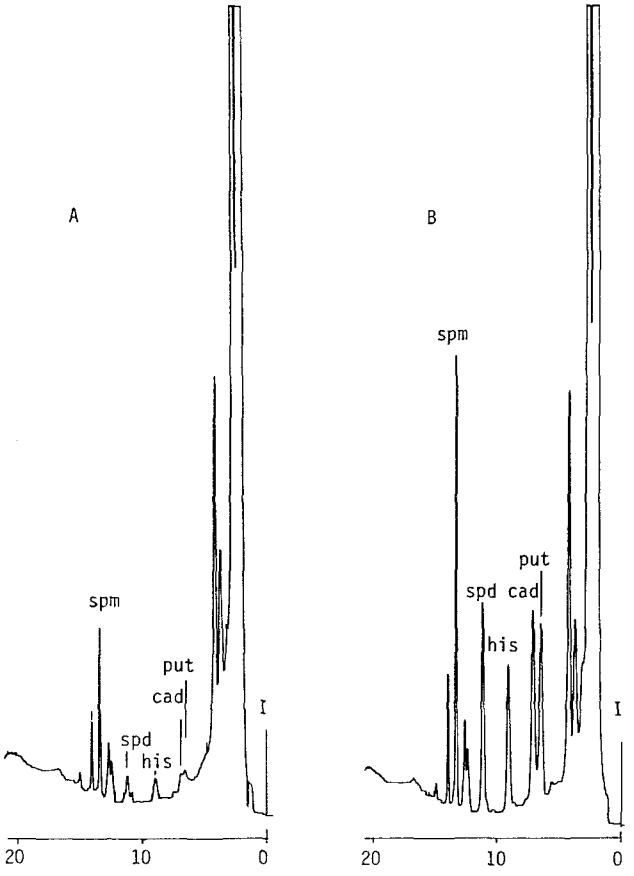

Fig. 2 A, B. High-pressure liquid chromatography of an extract of a fish sample (**A**) and the chromatogram of the same fish sample extract to which 10 mg/kg of each amine is added (**B**). – *spm* spermine; *put* putrescine; *cad* cadaverine; *spd* spermidine; *his* histamine

tention times increased with the number of dansyl residues on the molecules and the carbon chain length of the amines.

Increasing the steepness of the water-methanol gradient yielded lower retention times for tris-Dns-spermidine and tetra-Dns-spermine. However, no separation between bis-Dns-putrescine and bis-Dns-cadaverine resulted. The proposed elution gradient is the best compromise with regard to the separation of these dansyl derivatives.

The reaction time of 60 min at 55 °C is sufficient for the conversion of the amines into their dansyl derivatives (Fig. 3). Doubling the reaction time to 120 min did not increase the amount of derivatives in the reaction mixture, as established by HPLC analysis. The reproducibility of the method is evidenced from the coefficients of variation, as shown in Table 1.

The linearity of the detector response to increasing amounts of amine added to the blank sample is illustrated by the coefficients of correlation, which are for putrescine $r=0.9998$, for cadaverine $r=0.9991$, for

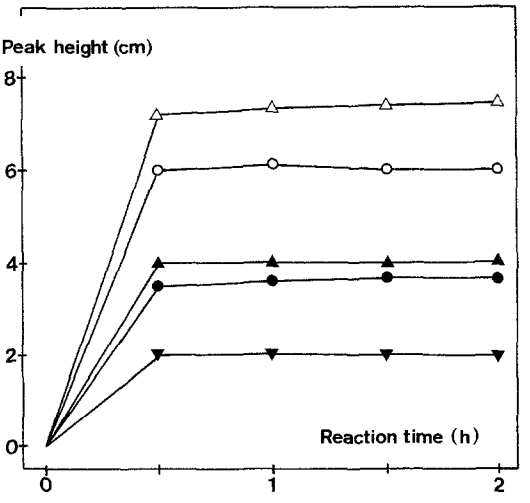

Fig. 3. Relationship between peak height (cm) of each extracted and derivatized amine and the reaction time (h)

Table 1. Reproducibility ($n=5$) of the determination of the amines using the method described

Amine	Coefficient of variation
Putrescine	5.50%
Cadaverine	7.82%
Histamine	3.93%
Spermidine	5.32%
Spermine	4.09%

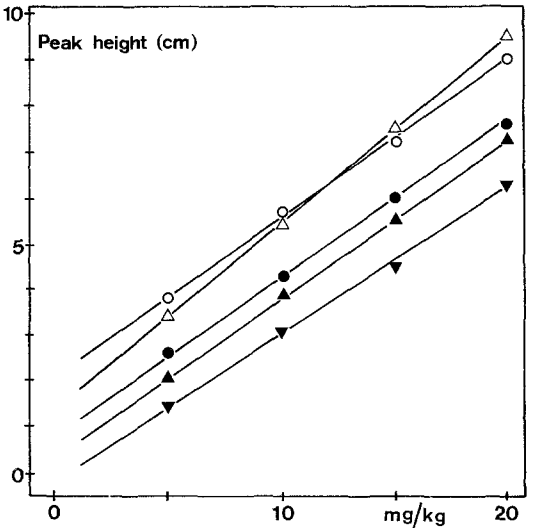

Fig. 4. Linearity between peak height (cm) of each extracted and derivatized amine and the amounts (mg/kg) added to the fish sample

histamine $r=0.9995$, for spermidine $r=0.9989$, and for spermine $r=0.9994$ (Fig. 4).

4 Discussion

The proposed method allows for easy quantification of the dansyl derivatives of putrescine, cadaverine,

Table 2. Advantages of the method

	Earlier methods	Our method
Sample preparation	Appr. 8 h	Appr. ½ h
HPLC eluent	Acetonitrile (expensive)	Water and methanol
HPLC separation	30 min	20 min

histamine, spermidine and spermine in contrast to more laborious methods [16]. The advantages of the method are given in Table 2. By employing our method, the analysis time and costs per sample are significantly reduced. Although the equipment used in the present work was not automated, the technique allows for full automation.

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References

1. Mietz JL, Karmas E (1977) J Food Sci 42:155–158
2. Hillig F, Clark EP (1938) J Ass Offic Anal Chem 21:688–698
3. Hillig F, Shelton LR, Loughrey JH, Eisner J (1985) J Ass Offic Anal Chem 41:763–766
4. Wood G, Hintz L (1971) J Ass Offic Anal Chem 54:1019–1023
5. Wood G, Hintz L, Salwin H (1969) J Ass Offic Anal Chem 52:904–910
6. Deckert W (1961) Chem Ztg 85:224–227
7. Farber L, Lerke PA (1958) Food Technol 12:677–761
8. Jones NR, Murray J, Livingston EL, Murray CK (1964) J Sci Food Agric 15:763–767
9. Shewan JM, Jones NR (1957) J Sci Food Agric 8:491–494
10. Migauchi DT, Malins DC (1957) Commer Fish Rev 19:26–29
11. Spinelli J, Eklund M, Migauchi D (1964) J Food Sci 29:710–714
12. Reay GA, Shewan JM (1949) Adv Food Res 2:342–346
13. Sagar OS, Horwitz W (1957) J Assoc Off Anal Chem 40:892–898
14. Edmunds NJ, Eitenmiller RR (1975) J Food Sci 40:516–520
15. Tabor H, Tabor CW (1964) Pharmacol Rev 16:245–250
16. Lakritz L, Spinelli AM, Wasserman AE (1975) J Agric Food Chem 23:344–348
17. Karmas E (1981) Lebensm Wiss Technol 14:273–278
18. Spinelli AM, Lakritz L, Wasserman AE (1974) J Agric Food Chem 22:1026–1030

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