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Analytical Methods

Dabsyl derivatisation as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography

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ABSTRACT

The commonly applied HPLC method to determine biogenic amines in dry fermented meat after dansylation was compared with an alternative dabsylation procedure. The use of dabsyl chloride at 70 °C resulted in a 25-min reduction of the derivatisation time, in comparison with the dansylation at 40 °C. Furthermore, the use of irritating ammonia to remove the excess of dansyl chloride can be avoided. Introduction of the SPE cleaning procedure on the C18 cartridge resulted in a reliable and sensitive method of biogenic amines determination in a complex protein–fat matrix, which is typical of dry fermented sausages.

The biogenic amines tryptamine (TRYP), phenethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), serotonin (SER), tyramine (TYR), and the natural polyamines spermidine (SPD) and spermine (SPM), were separated by means of gradient HPLC, using the two coupled C18 Chromolith reversed-phase columns.

1. Introduction

Biogenic amines (BAs) are basic nitrogenous compounds, formed mainly by microbial decarboxylation of the free amino acids in a foodstuff (Karovičová & Kohajdová, 2005). Fermented foods can certainly be considered as a reservoir for accumulation of BAs. This is because the fermentation process gives the predominant microbial flora an opportunity to decarboxylate the increasing amount of free amino acids (Lorenzo, Martínez, Franco, & Carballo, 2007).

In the dry fermented sausages, the main biogenic amines are tyramine (TYR), putrescine (PUT), cadaverine (CAD), spermine (SPM) and spermidine (SPD), and to a lesser extent, phenethylamine (PHE), histamine (HIS), tryptamine (TRYP), and serotonin (SER) (Ruiz-Capillas & Jiménez-Colmonero, 2004). Since high amounts of these organic substances can induce toxicological risks and health problems, monitoring of their levels is very important. Hence, several analytical procedures for the determination of BAs in food samples have already been elaborated.

One considerable difficulty is the isolation of BAs from a complex matrix of the food sample. Solid samples are most frequently extracted with acidic solvents which also act as deproteinisation agent during the liquid solid extraction (LSE). While hydrochloric acid is used for the extraction of cheese samples, it is not recommended for the extraction of meat and fish, because of the possible occurrence of turbidity (Innocente, Biasutti, Padovese, & Moret, 2007). In the meat and fish analysis, trichloroacetic acid (TCA) (Ferreira et al., 2006; Masson, Johansson, & Montel, 1999) or, more frequently, perchloric acid ($HClO_4$) (Dadáková, Křížek, & Pelikánová, 2009; Eerola, Hinkanen, Lindfors, & Hirvi, 1993; Latorre-Moratalla et al., 2008) is used. Most research dealing with solid food samples limits the sample clean-up to LSE, although the resulting extracts still contain several interfering compounds. To remove these compounds and concentrate the sample, solid phase extraction (SPE) can either be used after the LSE procedure, or directly as the matrix solid phase dispersion (MSPD) (Calbiani et al., 2005). Strong cationic-exchange cartridges (SCX) or silica adsorbents are mainly used for the extraction of aliphatic and biogenic amines. However, the difference in the pK_a-values of amines makes it difficult to simultaneously elute the target analytes from the adsorbent

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and the use of ion-pair reagents is often required (Molins-Legua & Campins-Falcó, 2005). Another possibility to enhance the coelution of the target compounds is the SPE sample purification on a C18 cartridge after derivatisation (Soufleros, Bouloumpasi, Zoutou, & Loukou, 2007).

Amongst the available analytical techniques, HPLC is by far the most frequently used to separate and quantify BAs. Since most BAs present in the food samples neither show an adequate absorption, nor exhibit significant fluorescence, derivatisation has to be performed in order to increase the sensitivity needed for a subsequent UV, VIS or fluorescence detection (Önal, 2007). The use of an HPLC system equipped with an UV detector requires derivatisation with a chromophoric reagent such as dansyl-chloride (Dns-Cl) (Dadáková et al., 2009; Saarinen, 2002), or dabsyl chloride (Dbs-Cl) (Krause, Bockhardt, Neckermann, Henle, & Klostermeyer, 1995; Romero, Bagur, Sánchez-Viñas, & Gázquez, 2003). Although dansyl chloride is the most widely used derivatisation reagent in the analysis of BAs by means of RP-HPLC–UV, this method demonstrates certain drawbacks, when applied to the determination of BAs in food samples. One drawback is the long derivatisation time, and another one is that the alkaline ammonia has to be used to remove the interfering by-products. In this study, the dansylation procedure (Eerola et al., 1993) is compared with the closely related, yet faster dabsylation procedure. Although dabsylation is less frequently applied in meat analysis, its advantage over the predominantly used dansyl chloride reagent is that dabsyl derivatives show absorbance in the range of 436–460 nm. In that way, interferences from UV-absorbing biological compounds present in the meat extracts are mostly avoided (Aboul-Enein, 2003). For dabsylation of biogenic amines in several foodstuffs, small variations amongst the methods can be found (Castillo & Castells, 2001; Krause et al., 1995; Romero, Bagur, Sánchez-Viñas, & Gázquez, 2000). Therefore this study is carried out to determine the critical parameters affecting the derivatisation yields of the aforementioned BAs. An ultimate goal is to develop a reliable and robust method for the detection and quantification of several biogenic amines in the meat products.

2. Experimental

2.1. Preparation of the amine standard solutions

The standards (either in the form of the free bases, or the respective hydrochlorides), i.e. SPM, SPD, CAD, PUT, PHE, TYR, SER, HIS and TRYP were purchased from Sigma Aldrich (Bornem, Belgium). The mixed stock solution, containing 1000 $\mu g \cdot mL^{-1}$ of each individual amine, was prepared by dissolving adequate amounts of the amines in a mixture of methanol and 1 M HCl (1:1, v/v) (both chemicals purchased from VWR International, Leuven, Belgium). A separate internal standard (IS) stock solutions, containing 1000 $\mu g \cdot mL^{-1}$ 1,7-diaminoheptane (Sigma Aldrich), was made in an analogous way. These solutions were stored for periods of up to one month at the temperature −28 °C. Seven working solutions, at the concentrations of 0.08, 0.40, 0.80, 2.00, 4.00, 6.00, 8.00 $\mu g \cdot mL^{-1}$ of each individual amine, respectively, were obtained from the stock solution by an appropriate dilution with 0.4 M $HClO_4$ (VWR International). These amine mixtures were stored at 4 °C for one week. The IS stock solution was diluted with 0.4 M $HClO_4$ to the concentration of 8 $\mu g \cdot mL^{-1}$, in order to obtain an IS working solution.

2.2. Liquid–solid extraction of the meat sample (LSE)

The 2-g aliquot of a finely cut dry fermented meat sample was weighed and then spiked with 200 μL of IS working solution. The 10-mL aliquot of 0.4 M $HClO_4$ was added and the entity was homogenised with an Ultra-Turrax T18 homogeniser (IKA, Staufen, Germany). The extracts were stored at 4 °C to crystallise the fat. The meat sample was centrifuged (Heraeus Labofuge 200, Fisher Scientific, Tournai, Belgium) for 10 min at 1000 g and the upper fat layer was removed. Subsequently, the extraction was repeated for the second time. The filtered supernatants were combined and the resulting volume was made up to 25 mL with 0.4 M $HClO_4$.

2.3. Derivatisation

2.3.1. Dansylation

The 2-mL aliquot of the amine mixture, or sample extract was transferred to the 10-mL test tube, and the pH was adjusted to 9.5–10.0 by adding 400 μL 2 M NaOH and 600 μL buffer solution (0.95 M $NaHCO_3$). The dansyl chloride solution was freshly prepared each day by ultrasonic dissolution of 10 mg Dns-Cl (1-dimethylamino-naphtalene-5-sulfonyl chloride, Sigma Aldrich) per 1 mL acetonitrile (Fisher Scientific). The 4-mL portion of dansyl chloride solution was added to the test tube and then thoroughly vortexed. Following the procedure elaborated by Eerola et al. (1993), derivatisation was carried out for 45 min at 40 °C. After this incubation period, the excess of dansyl chloride was removed by adding a portion of concentrated ammonia (VWR International) and incubating the test tube at room temperature in the dark. In order to obtain a chromatogram without an interference of Dns-Cl, three different volumes (i.e. 0, 100 and 200 μL) of ammonia and two different incubation times (i.e. 30 and 60 min) were tested.

2.3.2. Dabsylation

An analogous volume of the extract as for dansylation (i.e. 2 mL) was used for the derivatisation with dabsyl chloride (4-dimethylaminoazobenzene-4′-sulfonyl chloride, Sigma Aldrich). To study the optimal pH value for dabsylation, portions of 2 M NaOH varying from 150 to 500 μL were added to cover the pH range from 7.4 to 10.6. Buffering of the samples was done by adding 600 μL of 0.95 M $NaHCO_3$. The 4-mL portion of the dabsyl chloride solution (5 mg Dbs-Cl per 1 mL acetonitrile) was added to the sample. Incubation was performed at a temperature of 70 and 80 °C for 20 min, in order to establish a better dabsylation yield. Ultimately, the reaction was stopped by cooling the test tubes in an ice bath for 30 min.

2.4. Sample purification and concentration

2.4.1. Liquid–liquid extraction (LLE)

Three times, a 2-mL portion of diethyl ether (Fisher Scientific) was added to the derivatised sample and shaken for 1 min. Each time, after separation of the two phases, the upper organic layer was transferred to the second test tube. Ultimately, the three organic fractions were combined and evaporated to dryness at 30 °C under a steady stream of nitrogen.

2.4.2. Solid-phase extraction (SPE)

To obtain good recovery of the biogenic amines, the experiment was set up to determine the best possible conditions of carrying out the SPE procedure. Therefore the different eluted fractions were collected separately and each one was analysed for the target compounds. In the first experiment, SPE purification of the sample was directly applied after the perchloric acid extraction. The Gracepure C18 cartridge (1000 $mg \cdot 6\ mL^{-1}$) (Grace Davison Discovery Sciences, Lokeren, Belgium) was activated with 6 mL acetonitrile and equilibrated with 6 mL ultra pure water. The extract was loaded on top of the cartridge and washed twice with two 4.5-mL portions of ultra pure water. Elution of the biogenic amines was forced with the three 4.5-mL portions of acetonitrile. In the