ANALYSIS OF ALDEHYDES IN DRY SAUSAGE

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Background

Formations of aliphatic nonbranched hydrocarbons, particularly aldehydes formed from lipid oxidation or lipolysis are important flavour compounds in fermented meat products (*Leistner, 1995*). They offer both positive and negative properties. Certain aldehydes contribute off-flavours while others are considered to be essential components of the characteristic flavour of the product (*Hamilton, 1994*).

For the measurements of aldehydes the thiobarbituric acid test (TBA) is one of the oldest and most commonly used methods especially in meat products (*Rossell, 1994*). Despite its wide application this method has been criticised, e.g. because TBA can also react with other than lipid oxidation compounds (*Prior & Löliger, 1994*). Other spectrophotometric methods used to determine aldehydes are the anisidine value (AV), the reaction with *N,N*-dimethyl-*p*-phenylenediamine (DPPD) and reaction with 2,4-dinitrophenyl hydrazine (2,4-DNPH) (*Rossell, 1994; Reindl & Stan, 1982*). Chromatographic methods are applied to produce information on individual compounds formed during oxidation process. Gas chromatography is much applied for its high resolution and the possibility of coupling a mass spectrometer for compound identification. Aldehydes as flavour compounds are analysed mainly by headspace techniques (*Prior & Löliger, 1994*).

Hydrazones formed after reaction with 2,4-DNPH can also be analysed by liquid chromatography. This is due to their stable and non-volatile character. Sample pre-treatments before derivatisation as well as purification of formed hydrazones are necessary. Reindl and Stan (*Reindl & Stan, 1982*) reported a method to isolate aldehydes by distillation into a trap filled with 2,4-DNPH and clean up of the formed hydrazones with hexane. This study introduces a solid phase extraction technique both to the sample extraction and to the clean-up of derivatives prior reversed phase LC-analysis.

Objectives

This study is part of EU-project FAIR-CT96-3227: *Control of bioflavour and safety in Northern and Mediterranean fermented meat products (FMP)*. The objective of this EU-project is the improvement of quality and safety of fermented meat products by studying raw materials and fermented meat products (FMP) in order to better characterise raw materials in respect to metabolic potential and FMP in respect to the present of undesirable flavour compounds and desirable flavour compounds as related to the type of product (Northern or Mediterranean). The objective of this present study is according of sub-task 1.2.b of the EU-project to characterise of FMP with respect to undesirable compounds. The present study focused on testing the applicability of the proposed method to quantitatively determine free extracted aldehydes from fermented sausages and analysing the aldehyde concentrations in 4 different sausage types by the method.

Materials and Methods

The validation of the method was assayed with different types of dry sausages. Dry sausage samples in Figure 2 are FAIR-CT96-3227-project samples (market products) according to sub-task 1.2.b. Samples were Norwegian type (N), Belgian Northern type (BN), Belgian Southern type (BS) and Italian type (I). Measured aldehydes were butanal, hexanal, heptanal, trans-2-octenal, octanal, trans-2-nonenal, 2,4-decadienal, nonanal and decanal. A 5g portion of thawed and just before analysis homogenised sausage sample was weighed into screw-cap tube and homogenised with ultra-turrax in 10 ml cold 99% ethanol twice. Sample extract was purified with C_2 column. The formation of derivatives was proceeded by adding 10 ml of 2 mg/ml 2,4-DNPH reagent. The formed hydrazones were purified with silica SPE-columns. After loading the sample, column was washed with 2ml n-hexane. Aldehydes were eluted from column with 50% acetonitrile. Samples were analysed by reverse-phase high performance liquid chromatograph (HPLC). Solvents were purified water as A and acetonitrile as B. Gradients began at 60 % and ended at 90 % acetonitrile in 10 min. Chromatographic conditions were: flow rate 1,0 ml/min; column temperature 40°C; injection volume 50 µl; wavelength 360 nm with 500 nm as reference.

Results and discussion

Linearity of the method was tested by forming derivatives of standard solutions at different concentrations. The linear quantitation area showed to be in the concentration range 0.02-4.8 µg/ml, where the correlation coefficients of linear regression curves for each analyte were > 0.99. The linearity of butanal, hexanal, heptanal and octanal was, however, good up to 20 µg/ml with the same correlation coefficient. The recovery was analysed with spiked dry sausage. The values were depended on aldehyde compound: a recovery of butanal was in the range of 70-90%, hexanal 40-60%, heptanal 40-50 % and others were lower. Limit of detection for separate aldehydes were found to be in the range of 5-37 ng/ml, when injecting standard solution. Limits of determinations calculated for sample matrix were 4-30 µg/kg. The precision of the method was assayed by six replicate extractions. Precision values calculated as coefficient of variations were 4-15 % for butanal, hexanal and heptanal, and higher for other determined aldehydes. Examples on chromatograms of aldehyde standards and a sausage sample are shown in Figure 1.

The purpose of this study was to develop and test solid phase extraction in sample pre-treatment instead of distillation procedure (*Reindl and Stan, 1982*). The method proved to be specific and easy to perform in two days, and therefore it is applicable for the determination of lower aldehydes i.e. butanal, hexanal and heptanal in meat and meat products (Figure 1).

The results of aldehyde contents in 4 different sausage types are shown in Figure 2. There were great differences between concentrations detected in Mediterranean (high concentration) and Northern (low concentration) type of sausages. The analysis of

aldehydes in the project samples is a part of co-operation study in the EU-project and the results shown in Figure 2 will be connected and evaluated against other measured parameters and processing conditions in another summary report. The suggested reason for the different aldehyde contents were different maturing times and conditions in sausage production.

Conclusions

The present study focused on testing the applicability of the proposed method to quantitatively determine free extracted aldehydes from fermented sausages and analysing the aldehyde concentrations in 4 different sausage types (Northern and Mediterranean type) by the method. There were great differences between concentrations detected in Mediterranean (high concentration) and Northern (low concentration) type of sausages.

Literature

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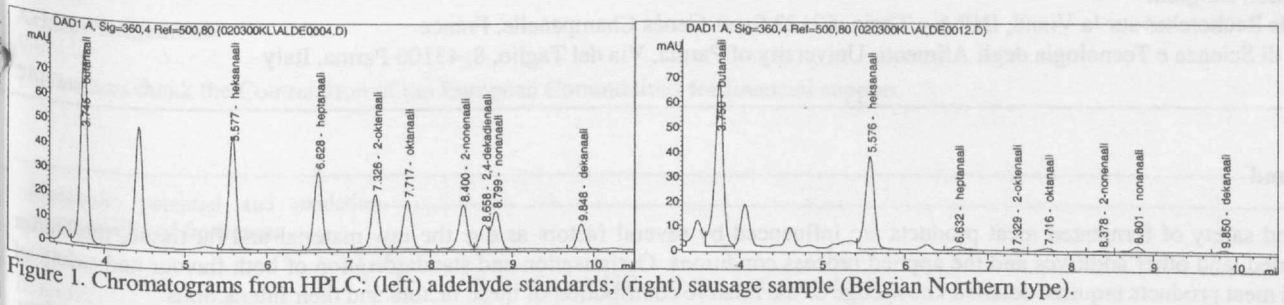

Figure 1. Chromatograms from HPLC: (left) aldehyde standards; (right) sausage sample (Belgian Northern type).

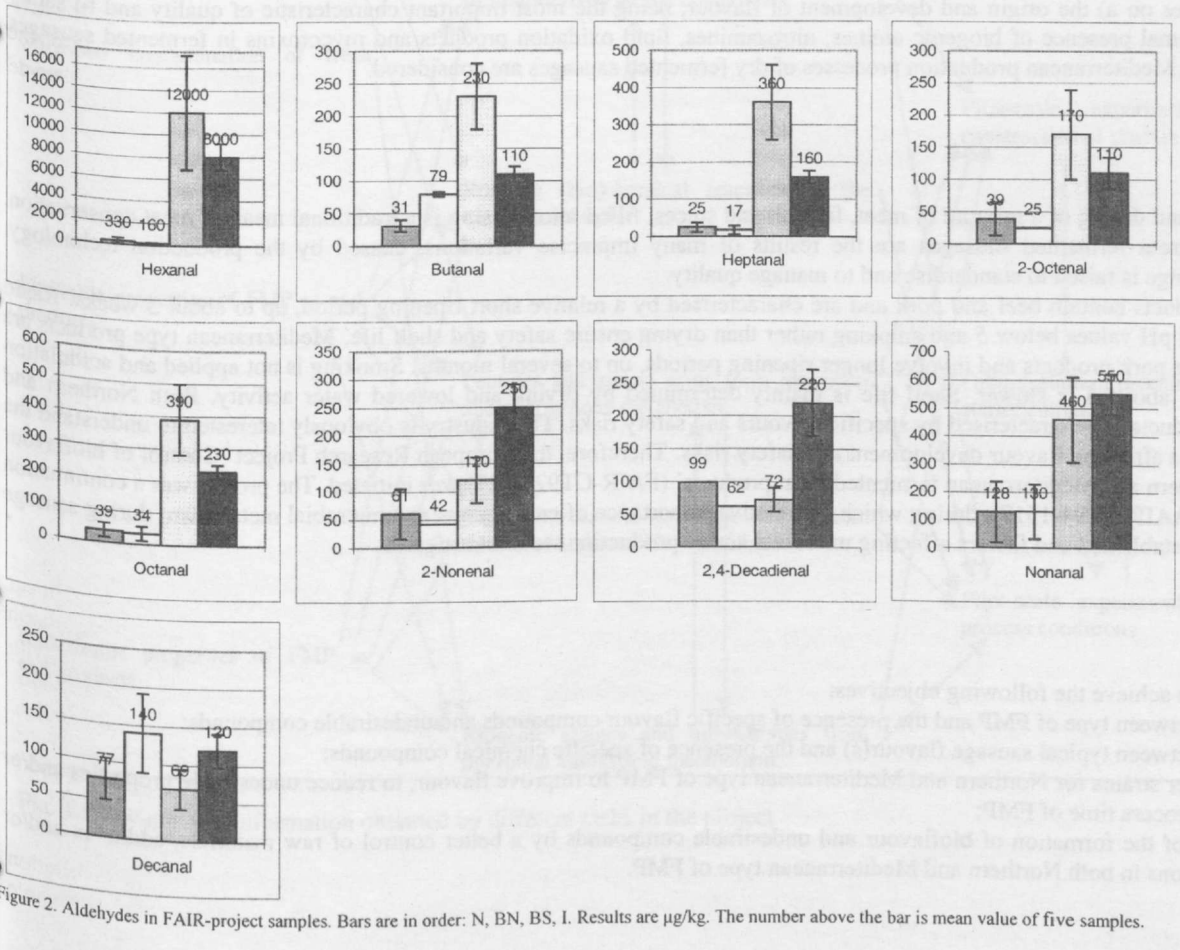

Figure 2. Aldehydes in FAIR-project samples. Bars are in order: N, BN, BS, I. Results are µg/kg. The number above the bar is mean value of five samples.