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# Determination of Central Nervous and Organ Tissue in Meat Products through GC-MS Analysis of Marker Fatty Acids from Sphingolipids and Phospholipids

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## Introduction

To protect consumers of meat products from material potentially infected by bovine spongiform encephalopathy (BSE), Swiss legislation (1) prohibits the use of specified bovine risk material for the production of meat products for human consumption, principally tissue of the central nervous system (CNS), i.e. brain and spinal cord. These risk materials have to be removed during slaughtering.

Various methods were developed for the enforcement of this regulation. *Lücker et al.* described two approaches, one based on the determination of cholesterol, the other on neuron-specific enolase (2–4). The detection limit obtained through the immunochemical determination of neuron-specific enolase was specified as 0.01 % CNS tissue in a meat product. Cholesterol only enabled the detection of 0.5 % CNS tissue, but this method has the advantage of not being hampered by intensive heat treatment of the product. As also certain other tissues have a high cholesterol content, this latter method was only recognized as screening procedure (5).

*Niederer and Bollhalder* (6) determined CNS tissue in meat products and cosmetics through the analysis of brain-specific fatty acids. Using the ratios of the cis/trans isomers of nervonic acid (C24:1), they were able to distinguish between CNS tissue from cow, sheep and pig. In spiked sausages, species identification of brain was possible down to a content of 0.05 %, and CNS material was detectable down to 0.01 %. Two sample preparation procedures were used:

1. The sphingolipids and other fatty-acid-containing polar components were isolated from the lipids of low polarity, transesterified with hydrochloric acid, and the resulting fatty acid methyl esters (FAME) analyzed by GC-MS.
2. Raw extracts were transesterified; the FAMEs of interest were isolated by reversed phase HPLC and analyzed by GC-MS.

*Barcarolo et al.* (7) refined the determination of nervonic acid and its isomers by an on-line LC-GC-FID method enabling the accurate determination at concentrations of 0.1–10 mg/kg of meat product. A similar method was described by *Hellgren* for the analysis of bioactive sphingolipids in meat and fish products (8). Intact sphingolipids were determined in plant material by HPLC-MS/MS by *Humpf* and *Seefelder* (9).

The work described in this paper is based on a method derived from that of *Niederer* and *Bollhalder*, following their first strategy of initially isolating the fatty acids bonded to polar moieties, then formation of methyl esters, and GC-MS analysis. Among some 60 samples of meat products tested for CNS material, one seemed positive. Interrogation of the producer revealed, however, that the product (a “mortadella” sausage) contained no CNS material. The fatty acids originated from pork stomach, which was not on the list of the ingredients. As the detection of organ tissue is another subject of interest, the method was re-optimized to include a broader range of fatty acids. Two fractions of lipids were analyzed in order to separately determine the fatty acids from different polar lipids, enriching the data material serving as finger print.

According Swiss legislation, most organs may be used to prepare meat products (except of, e.g., eyes). They must be listed in the ingredients, with the exception of heart and tongue, which belong to muscle meat (10). The European Union introduces similar legislation in 2003.

## Experimental

Meat products as well as test samples of meat for sausages and organs were homogenized. 2 g of the homogenate was mixed with 10 ml acetone and kept in an oven at 60°C for 1 h in order to open the cell structures. 40 ml of water was added and the sample extracted with 20 ml, then 10 ml of methyl tert. butyl ether (MTBE).

10 ml of the combined MTBE extract was diluted with 10 ml of hexane and transferred onto a cartridge containing 500 mg silica gel (J.T. Baker 7086-03), previously rinsed with about 5 ml MTBE/hexane 1+1. The triglycerides and other materials of low polarity were removed from the cartridge with two 10 ml portions of MTBE/hexane 1+1. Then fraction A was eluted with 10 ml of methanol/MTBE 1+3 and fraction B with 10 ml methanol.

Fraction A was evaporated to dryness in a stream of nitrogen and re-dissolved in 10 ml methanol. Then the fatty acids bonded in sphingo- and phospholipids (esters and amides) were converted to FAMEs adding 1 ml of concentrated hydrochloric acid and heating in an oven at 75°C for 3 h. After addition of 5 ml of water, the



FAMES were extracted with 4 ml hexane containing the internal standard (1 µg/ml methylhexacosanoate, FAME-26). Fraction B was treated in the same way, but without the initial solvent evaporation step.

1 ml of the FAME solution was evaporated to dryness in a stream of nitrogen and silylated by addition of 30 µl N,O-bis(trimethylsilyl)trifluoroacetamide/1% trimethylchlorosilane and 30 µl pyridine. The mixture was warmed on a heating plate thermostated at 60°C, diluted with 1 ml of hexane, and injected into GC.

GC analysis involved 15 µl injections either by the on-column or by concurrent solvent recondensation (CSR) large volume (LV) splitless injection (11). A GC 8000 equipped with on-column and vaporizing injector, a vapor exit, and an autosampler AS800 with a 50 µl syringe was connected to a mass spectrometer MD800, both from ThermoFinnigan (Milano, Italy).

For CSR-LV splitless injection, a 3 m×0.32 mm i.d. precolumn was used, for on-column injection a 0.5 m×0.53 mm i.d. precolumn, both coated with a thin film (some 3 nm) of OV-1701-OH (prepared in the laboratory according to ref. (12)). The precolumns were connected by a press-fit connector to a 30 m×0.25 mm i.d. separation column, coated in the laboratory with an 0.15 µm film of PS-255 (a methylpolysiloxane, Fluka, Buchs, Switzerland). Helium was the carrier gas at 40 kPa inlet pressure. The oven temperature was programmed from 60°C (2 min) at 20°/min to 230°C and at 4°/min to 320°C (2 min).

On-column injection was performed at a rate of 3 µl/s, resulting in concurrent solvent evaporation. The vapor exit was closed 10 s after starting injection. CSR-LV-splitless injection involved a 105×5 mm i.d. injector liner, with a constriction at the bottom and a 1 cm plug of deactivated glass wool above the constriction. The inlet of the precolumn was installed at the orifice of the constriction, i.e. just below the glass wool. The sample was injected at maximum speed (about 100 µl/s), inserting the needle into the injector by merely 15 mm (band formation (13)). The injector was thermostated at 330°C; the splitless period lasted 3 min, during which the septum purge was also closed (auto pressure pulse (11)).

Quantitative determination involved MS in the electron impact (EI)-selected ion monitoring (SIM) mode, using the following masses (fatty acid:number of double bonds, m/z): 26:0 (internal standard), 87; 22:0 OH, 383; 23:0 OH, 397; 24:0, 382; 24:1, 348; 24:0 OH, 455; 24:1 OH cis/trans, 409; 25:0 OH, 425; 25:1 OH cis/trans, 423; 26:1 OH, 437; 26:0 OH, 439.

As no standards were available for a majority of the FAMES analyzed, quantitative data was based on uncorrected peak areas obtained by MS-SIM normalized on the internal standard FAME-26:0. No absolute concentrations were needed, because the detection of CNS or organ tissue was based on the comparison of the FAME concentrations with reference materials. Peak areas of the FAMES of interest were divided by the area of FAME-26:0 and multiplied by 1000 in order to obtain suitable formats.

## Results

### Confirmation of the method

The steps of the method are summarized in figure 1. They were checked through the following experiments:

1. Extraction of the sphingo- and phospholipids from the homogenated sample was checked for brain tissue as well as for an experimental sausage prepared from typical sausage meat and 1 % brain tissue (produced by the Ausbildungszentrum für die Schweizer Fleischwirtschaft, Spiez, Switzerland in behalf of the Swiss Federal Office of Public Health, SFOPH). In the third extract, at most 3 % of the fatty acids of interest were found, indicating that a double extraction is sufficient.
2. Transfer of the extract onto the SPE cartridge with MTBE resulted in some 10 % breakthrough of FAMES from brain tissue (experiment with sausage homogenated with 10 % brain; MTBE collected after passage of the SPE re-analyzed passing it through another cartridge). Transfer with hexane (turbid solution) resulted in apparently low capacity and substantial initial breakthrough, suggesting that the components of interest were not dissolved and passed the SPE cartridge adsorbed to particles. With MTBE/hexane 1+1, breakthrough remained below 4 % for all components of interest, i.e. this solvent mixture dissolved the lipids to be analyzed and was sufficiently weak an eluent to prevent elution from the silica gel.

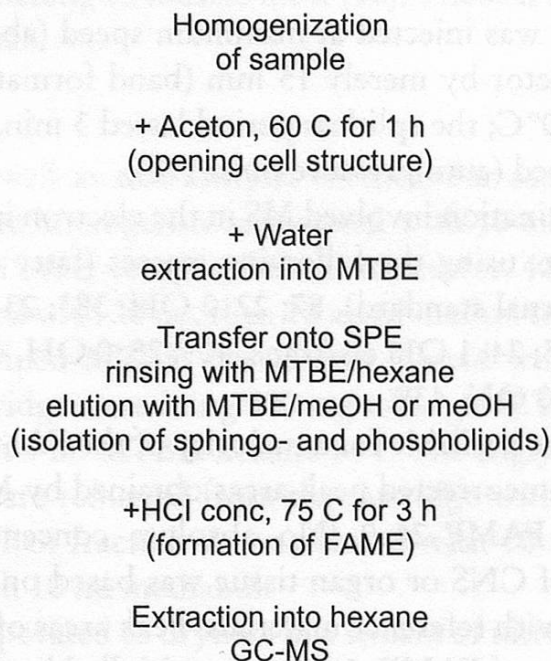


Figure 1 Steps of the analytical procedure

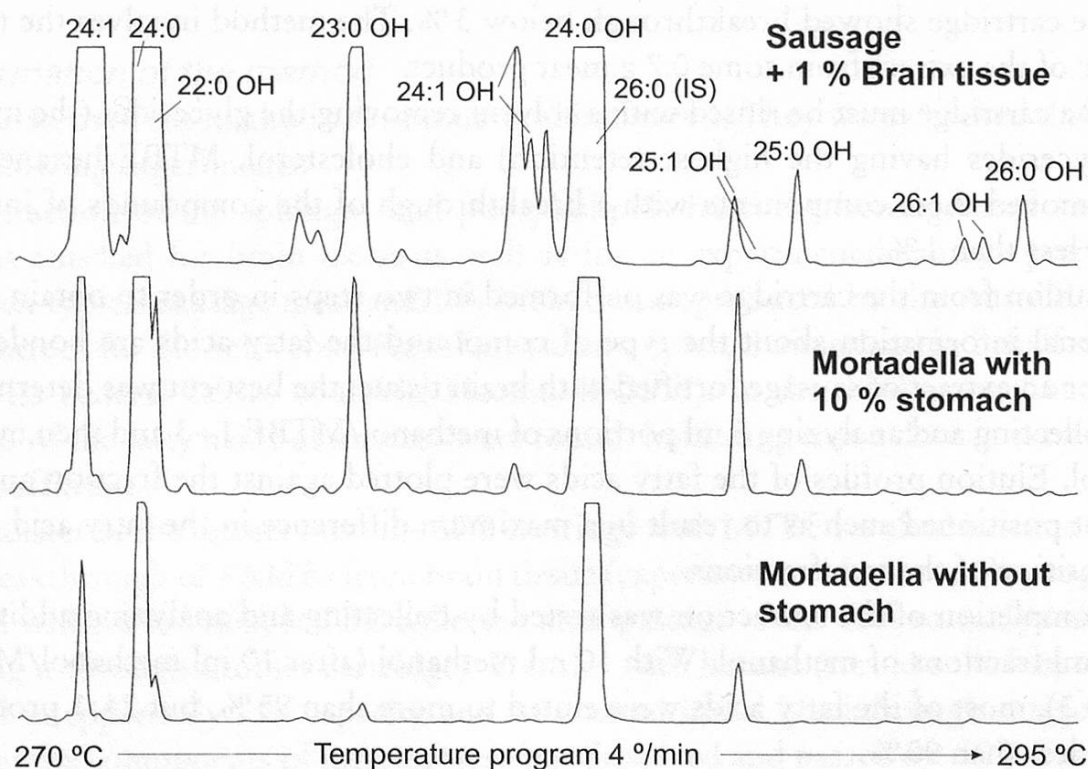


3. Capacity of the SPE cartridge: loading the extract from 2 g spiked sausage onto the cartridge showed breakthrough below 3 %. The method involves the transfer of the extract from some 0.7 g meat product.
4. The cartridge must be rinsed with a solvent removing the glycerides (the mono-glycerides having the highest retention) and cholesterol. MTBE/hexane 1+1 removed these components with a breakthrough of the compounds of interest of less than 1 %.
5. Elution from the cartridge was performed in two steps in order to obtain additional information about the type of compound the fatty acids are bonded to. For an extract of sausage fortified with brain tissue, the best cut was determined collecting and analyzing 5 ml portions of methanol/MTBE 1+3 and then methanol. Elution profiles of the fatty acids were plotted against the fraction and the cut positioned such as to result in a maximum difference in the fatty acid composition of the two fractions.
6. Completion of the extraction was tested by collecting and analyzing additional 5 ml fractions of methanol. With 10 ml methanol (after 10 ml methanol/MTBE 1+3), most of the fatty acids were eluted to more than 95 %, but 24:1 probably to less than 90 %.
7. Transformation to FAME: to control the formation of FAME from the phospho- and sphingolipids, the SPE eluate from a sausage fortified with 1 % brain tissue was heated with hydrochloric acid for a varied duration. After 2 h, the FAME reached at least 90 % and after 3 h more than 97 % of the amounts obtained after reaction overnight.
8. Extraction of the FAME from the reaction medium diluted with water: a second extract with 2 ml hexane yielded 10–15 % of the FAMEs of interest. The procedure was left with a single extraction step, but using 4 ml hexane.
9. There was no significant difference between the results obtained with on-column and splitless injection. The precolumn had to be replaced after some 40 analyses with on-column injection.

Repeatability of quantitative results was largely determined by the performance of the GC-MS analysis. When disregarding small components and some first results in a series of analyses, relative standard deviations of data obtained over several weeks remained below 15 %. Linearity was tested for two types of experimental sausages (with and without liver) spiked with 0, 0.1, 0.5, and 1 % of pork brain.

### **Chromatograms**

The bottom GC-MS-TIC chromatogram in figure 2 shows the silylated FAMEs of interest from a “mortadella” (an Italian sausage) assumed to be free of organ and CNS tissue. The center chromatogram is from a similar mortadella containing some 10 % pork stomach (according to a traditional recipe, as confirmed by the producer). The top chromatogram was obtained from an experimental sausage prepared with 1 % brain tissue (pork).



**Figure 2 GC-MS-TIC chromatograms of two mortadellas, one with and the other without pork stomach, as well as of a sausage spiked with 1% brain tissue during its preparation**

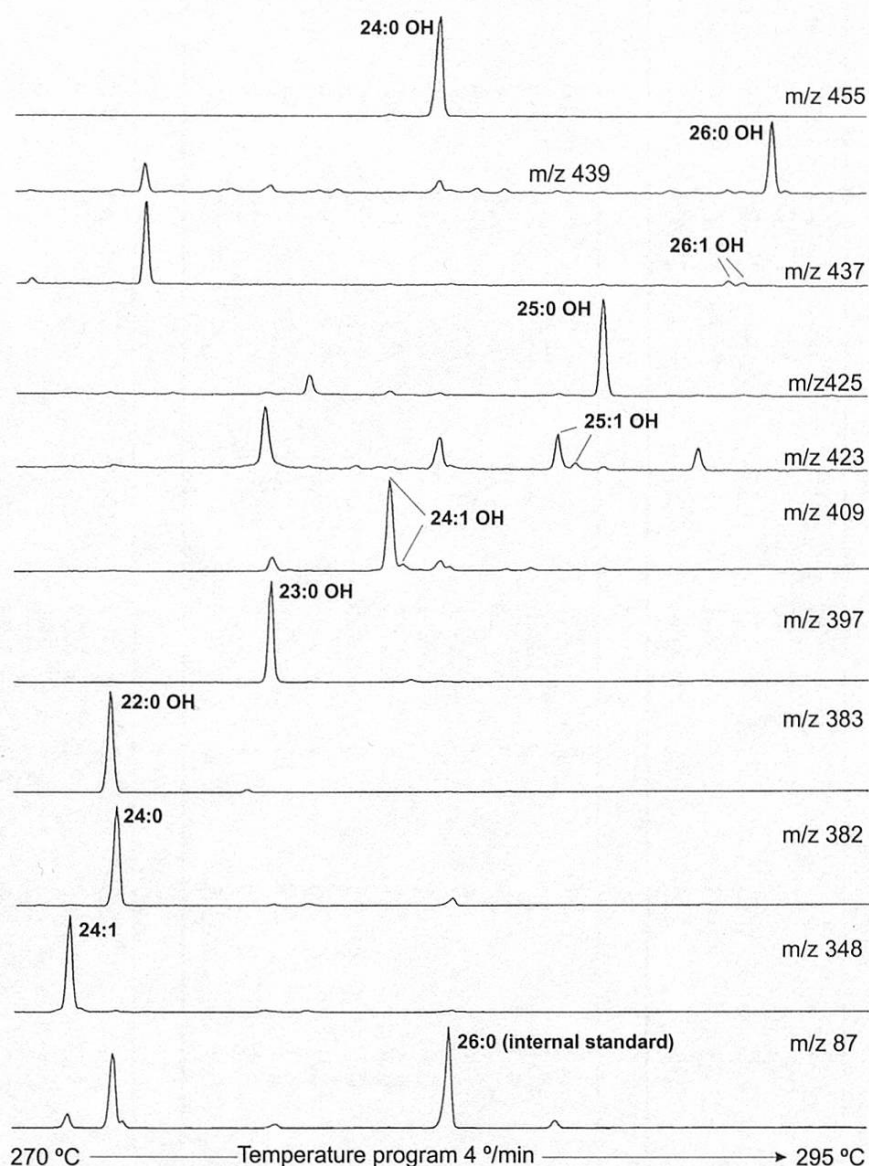
The analysis was focused on the  $C_{22}$ – $C_{26}$  fatty acids, since the  $C_{16}$ – $C_{20}$  acids did not show interesting selectivity for CNS or organ tissue. Most of the relevant fatty acids are hydroxylated. Some of them also contain a double bond and co-exist as cis and trans isomers. The chromatograms readily show the potential of the method for the determination of small amounts of CNS or organ tissue, but also that separation would need substantial improvement to be complete and sufficiently reliable without selective detection.

Figure 3 shows the GC-MS chromatograms of the relevant silylated FAMEs as detected by the most selective ions (EI-SIM). Mostly the fragment resulting from loss of 59 amu (methylated carboxyl group) was used, as typical for beta-hydroxy FAMEs. For 24:0 OH,  $M^+-15$  was preferred in order to achieve reliable separation from 26:0, the internal standard. 26:0 was monitored at  $m/z$  87, since 24:0 OH forms a negligibly small signal at this mass.

### **Calibration of FAME contents**

Numerous samples of brain and organ tissue obtained from the slaughter-house of Zürich were analyzed to calibrate the fatty acid contents in comparison with meat samples typically used for the production of sausages and other meat products.





**Figure 3 GC-MS/SIM chromatograms of the silylated FAMES analyzed: extract from pork brain**

Values shown in table 1 and 2 are averages of usually some five samples. They represent SIM peak areas normalized on the internal standard without application of response factors, i.e. they do not indicate absolute concentrations. Table 1 lists the data from fraction A (eluted from the SPE cartridge with methanol/MTBE 1+3), table 2 those from fraction B (methanol).

In fraction A, the fatty acids analyzed are present at massively higher concentrations in the CNS material than in organ tissue and meat (up to a factor of 10000). In fraction B, values for the organ tissue are in the same range as those for CNS material and easily 100 times higher than in meat, suggesting that minor additions of the following organ tissue can clearly be distinguished from meat: liver, spleen, kidney, and stomach.



Table 1

**Normalized peak areas of fatty acids in lipids of fraction A from the SPE column: known CNS and organ tissue c, cis; t, trans**

|                     |        | Fraction A: normalized peak areas |       |         |         |         |       |         |         |      |       |      |      |
|---------------------|--------|-----------------------------------|-------|---------|---------|---------|-------|---------|---------|------|-------|------|------|
|                     |        | 22OH                              | 23OH  | 24:1OHc | 24:1OHt | 25:1OHc | 25:OH | 26:1OHc | 26:1OHt | 26OH | 24OH  | 24:1 | 24:0 |
| <i>CNS material</i> |        |                                   |       |         |         |         |       |         |         |      |       |      |      |
| Spinal cord         | cow    | 14000                             | 8000  | 3200    | 380     | 140     | 7500  | 350     | 180     | 1600 | 25000 | 4000 | 3500 |
|                     | pig    | 20000                             | 2200  | 1400    | 800     | 30      | 550   | 90      | 170     | 310  | 12300 | 1900 | 2500 |
| Brain               | calf   | 28298                             | 6531  | 3870    | 730     | 175     | 172   | 323     | 374     | 1364 | 29184 | 7428 | 8188 |
|                     | pig    | 47337                             | 8320  | 5236    | 2778    | 163     | 2178  | 261     | 708     | 962  | 31460 | 4116 | 4094 |
|                     | cow    | 59762                             | 10846 | 5580    | 3133    | 225     | 2580  | 294     | 875     | 1168 | 29289 | 5563 | 8195 |
| <i>Organ tissue</i> |        |                                   |       |         |         |         |       |         |         |      |       |      |      |
| Eye                 | pig    | 8.0                               | 45    | 0.7     | 0.25    | 0.04    | 1.5   | 0.1     | 0.1     | 0.4  | 6.0   | 0.6  | 1.2  |
| Heart               | pig    | 46                                | 4.8   | 1.8     | 0.04    | <0.05   | 0.5   | <0.05   | <0.05   | 0.5  | 5.8   | 49   | 62   |
| Liver               | calf   | 194                               | 198   | 8.9     | 0.00    | <0.05   | 7.3   | <0.05   | <0.05   | 1.3  | 106   | 34   | 171  |
|                     | cow    | 250                               | 400   | 25      | 1.5     | 3.0     | 20    | 0.1     | <0.05   | 9.0  | 200   | 8.0  | 80   |
|                     | pig    | 60                                | 8.0   | 6.0     | 0.1     | 0.1     | 0.9   | 0.1     | <0.05   | 0.3  | 10    | 35   | 75   |
| Lung                | pig    | 3.7                               | 0.1   | 0.3     | 0.0     | 0.1     | 0.3   | <0.05   | <0.05   | 0.3  | 1.0   | 3.7  | 5.7  |
| Spleen              | calf   | 140                               | 14    | 4.3     | 0.6     | <0.05   | 3.0   | <0.05   | <0.05   | 1.1  | 20    | 280  | 160  |
| Kidney              | cow    | 700                               | 280   | 2.3     | 0.9     | 0.6     | 3.1   | <0.05   | <0.05   | 0.8  | 80    | 9    | 40   |
|                     | pig    | 250                               | 120   | 19      | 2.2     | 0.7     | 11    | 0.1     | <0.05   | 1.0  | 97    | 35   | 50   |
| Diaphragm           | pig    | 35                                | 6.6   | 1.3     | 0.3     | <0.05   | 1.1   | <0.05   | <0.05   | 0.6  | 20    | 9    | 20   |
| Stomach             | pig    | 350                               | 310   | 30      | 0.7     | 2.8     | 50    | 0.5     | 0.2     | 3.8  | 340   | 30   | 80   |
| <i>Sausage meat</i> |        |                                   |       |         |         |         |       |         |         |      |       |      |      |
|                     | calf   | 40                                | 9.0   | 2.0     | 0.6     | 0.3     | 2.0   | 0.1     | <0.05   | 1.0  | 35    | 9.0  | 24   |
|                     | cow    | 20                                | 6.0   | 1.0     | 1.0     | 0.2     | 1.0   | <0.05   | <0.05   | 1.0  | 15    | 6.0  | 10   |
|                     | pig    | 40                                | 7.0   | 2.0     | 0.5     | 0.2     | 1.0   | 0.1     | 0.1     | 1.0  | 20    | 8.0  | 15   |
| Rind                | pig    | 8                                 | 1.5   | 0.2     | 0.10    | 0.10    | 0.3   | <0.05   | <0.05   | 0.2  | 2.0   | 4.0  | 4.0  |
| Bacon               | pig    | 20                                | 8.0   | 2.0     | 0.3     | 0.10    | 2.0   | 0.3     | <0.05   | 0.5  | 8.0   | 8.0  | 5.0  |
|                     | turkey | 11                                | 2.3   | 0.5     | 0.0     | 0.08    | 0.8   | <0.05   | <0.05   | 0.3  | 8.6   | 2.3  | 5.4  |
|                     | hen    | 45                                | 16    | 2.0     | 0.3     | 0.07    | 5.5   | <0.05   | <0.05   | 1.1  | 31    | 11   | 34   |

Table 2

**Fatty acids in lipids of fraction B (methanol) in the same reference materials as in table 1**

|                     |        | Fraction B: normalized peak areas |      |         |       |       |       |
|---------------------|--------|-----------------------------------|------|---------|-------|-------|-------|
|                     |        | 22OH                              | 23OH | 24:1OHc | 25:OH | 26OH  | 24OH  |
| <i>CNS material</i> |        |                                   |      |         |       |       |       |
| Spinal cord         | cow    | 490                               | 120  | 25      | 40    | 20    | 130   |
|                     | pig    | 240                               | 26   | 9.0     | 7.4   | 6.6   | 55    |
| Brain               | calf   | 233                               | 23   | 11      | <0.1  | 2.3   | 39    |
|                     | pig    | 91                                | 18   | 12      | 4.3   | 1.7   | 36    |
|                     | cow    | 3818                              | 73   | <0.1    | <0.1  | 4.1   | 7.0   |
| <i>Organ tissue</i> |        |                                   |      |         |       |       |       |
| Eye                 | pig    | 0.4                               | 0.1  | <0.03   | <0.03 | <0.03 | 0.1   |
| Heart               | pig    | 180                               | 3.6  | 0.4     |       |       | 1.9   |
| Liver               | calf   | 1250                              | 150  | 4.0     | 0.5   | 0.8   | 65    |
|                     | cow    | 120                               | 100  | 3.0     | 3.0   | 1.5   | 25    |
|                     | pig    | 230                               | 4    | 0.8     | <0.03 | <0.03 | 2     |
| Lung                | pig    | 1.4                               | 0.2  | 0.08    | 0.1   | 0.2   | 0.3   |
| Spleen              | calf   | 90                                | 2.3  |         |       |       | 0.5   |
| Kidney              | cow    | 30                                | 2.6  |         |       |       | 1.0   |
|                     | pig    | 155                               | 30   | 1.9     | 3.5   | 0.3   | 30    |
| Diaphragm           | pig    | 15                                | 0.4  | <0.03   | 0.07  | 0     | 0.5   |
| Stomach             | pig    | 220                               | 150  | 18      | 16    | 0.8   | 90    |
| <i>Sausage meat</i> |        |                                   |      |         |       |       |       |
|                     | calf   | 30                                | 1    | <0.03   | <0.03 | <0.03 | 0.2   |
|                     | cow    | 20                                | 0.6  | <0.03   | <0.03 | <0.03 | 0.2   |
|                     | pig    | 20                                | 0.7  | 0.1     | <0.03 | <0.03 | 0.4   |
| Rind                | pig    | 6                                 | 0.1  | <0.03   | <0.03 | <0.03 | 0.1   |
| Bacon               | pig    | 10                                | 0.5  | <0.03   | <0.03 | <0.03 | 0.3   |
|                     | turkey | 15                                | 0.5  | <0.03   | <0.03 | <0.03 | <0.03 |
|                     | hen    | 50                                | 2.4  |         |       |       | 0.3   |

Table 3 lists the mean values for the sausage meat samples of calf, cow and pig from tables 1 and 2 as well as results from 42 meat products from the market for which it was assumed that they contained neither CNS nor organ tissue. The average values for the meat products well agreed with the averages for the sausage meat samples, confirming the absence of additions. The maximum values for the meat products in the third line were used as threshold for the detection of admixed organ or CNS materials.

### *Pork stomach in mortadella*

Among the about 120 meat products analyzed, none contained CNS material. One contained soybean components, which increased the concentration of some of the relevant fatty acids. 12 samples of mortadella contained pork stomach.

Table 3

**Mean values for the samples of sausage meat from tables 1 and 2 and of 42 meat products assumed to contain meat only, as well as the maximum values for the meat products used as threshold for suspecting the addition of organ or CNS material**

| <i>Fraction A</i>   | 22OH | 23OH | 24:1OHc | 24:1OHt | 25:1OHc | 25:OH | 26:1OHc | 26:1OHt | 26OH | 24OH | 24:1 | 24:0 |
|---------------------|------|------|---------|---------|---------|-------|---------|---------|------|------|------|------|
| Mean sausage meat   | 23   | 6    | 1.3     | 0.4     | 0.16    | 1.2   | 0.08    | 0.02    | 0.7  | 15   | 6    | 10   |
| Meat products: mean | 33   | 6    | 1       | 0.2     | 0.2     | 2     | 0.1     | 0.1     | 1    | 19   | 6    | 12   |
| maximum             | 90   | 17   | 4       | 0.9     | 0.4     | 7     | 0.2     | 0.2     | 2    | 52   | 13   | 29   |
| <i>Fraction B</i>   |      |      |         |         |         |       |         |         |      |      |      |      |
| Mean sausage meat   | 17   | 0.6  | 0.02    |         |         | <0.1  |         |         | <0.1 | 0.20 |      |      |
| Meat products: mean | 15   | 0.7  | 0.0     |         |         | 0.1   |         |         | 0.1  | 0.4  |      |      |
| maximum             | 30   | 1.8  | 0.2     |         |         | 0.5   |         |         | 0.3  | 1.8  |      |      |



Table 4

**Mortadella with and without pork stomach. At bottom: values used as threshold and for stomach**

| Sample                               | Fraction A |      |         |         |       |      | Fraction B |      |      |         |       |      |
|--------------------------------------|------------|------|---------|---------|-------|------|------------|------|------|---------|-------|------|
|                                      | 22OH       | 23OH | 24:1OHc | 25:1OHc | 25:OH | 24OH | 24:0       | 22OH | 23OH | 24:1OHc | 25:OH | 24OH |
| <i>Mortadella containing stomach</i> |            |      |         |         |       |      |            |      |      |         |       |      |
| 1                                    | 268        | 120  | 12.4    | 0.6     | 21.2  | 158  | 82         | 121  | 24   | 2.5     | 2.7   | 17   |
| 2                                    | 85         | 60   | 5.8     | 0.5     | 10.7  | 72   | 22         | 59   | 36   | 4.2     | 5.1   | 24   |
| 3                                    | 74         | 46   | 5.3     | 0.4     | 8.1   | 56   | 19         | 32   | 18   | 2.1     | 2.4   | 11   |
| 4                                    | 74         | 35   | 4.0     | 0.5     | 8.3   | 68   | 16         | 19   | 11   | 1.0     | 2.5   | 13   |
| 5                                    | 136        | 52   | 7.0     | 0.6     | 8.    | 90   | 23         | 28   | 13   | 1.4     | 1.8   | 8.0  |
| 7                                    | 90         | 29   | 4.0     | 0.2     | 6.3   | 58   | 28         | 23   | 7.5  | 0.8     | 1.4   | 5.3  |
| 8                                    | 76         | 25   | 3.8     | 0.3     | 5.2   | 50   | 20         | 21   | 6.8  | 0.8     | 1.5   | 6.0  |
| 9                                    | 69         | 25   | 3.6     | 0.2     | 4.9   | 46   | 18         | 20   | 7.5  | 0.9     | 1.3   | 5.3  |
| 10                                   | 98         | 35   | 5.4     | 0.3     | 7.1   | 70   | 24         | 22   | 11   | 1.4     | 1.9   | 8.6  |
| 11                                   | 90         | 64   | 7.2     | 0.8     | 13.2  | 79   | 25         | 37   | 22   | 3.0     | 3.0   | 14   |
| 12                                   | 89         | 30   | 4.4     | 0.3     | 5.7   | 58   | 22         | 19   | 8.3  | 0.9     | 1.4   | 5.6  |
| <i>Mortadella without stomach</i>    |            |      |         |         |       |      |            |      |      |         |       |      |
| 13                                   | 50         | 6.0  | 1.6     | 0.17    | 0.1   | 20   | 18         | 11.5 | 0.3  | <0.05   | 0.14  | 0.7  |
| 14                                   | 33         | 4.3  | 1.1     | 0.11    | 0.9   | 15   | 12         | 13.4 | 0.6  | 0.05    | 0.18  | 0.7  |
| 15                                   | 25         | 6.7  | 1.4     | 0.13    | 2.4   | 12   | 11         | 10.9 | 0.7  | 0.18    | 0.11  | 0.4  |
| 16                                   | 23         | 5.7  | 1.1     | 0.14    | 1.9   | 15   | 10         | 11.0 | 0.7  | 0.08    | 0.08  | 0.3  |
| 17                                   | 36         | 7.1  | 1.6     | 0.18    | 2.3   | 23   | 16         | 9.7  | 0.7  | 0.13    | 0.11  | 0.4  |
| 18                                   | 46         | 5.6  | 1.5     | 0.14    | 1.1   | 22   | 13         | 9.3  | 0.6  | <0.05   | 0.16  | 0.7  |
| <i>Comparison</i>                    |            |      |         |         |       |      |            |      |      |         |       |      |
| Threshold                            | 90         | 17   | 4       | 0.4     | 7     | 52   | 29         | 30   | 1.8  | 0.2     | 0.5   | 1.8  |
| Stomach                              | 350        | 310  | 30      | 3       | 50    | 340  | 80         | 220  | 150  | 18      | 16    | 90   |

Table 5

Percent pork stomach in the mortadella as calculated from data in table 4

| Sample | % Stomach in the mortadella |      |         |         |       |      |      |      |      |         |       |      | Mean (%) |
|--------|-----------------------------|------|---------|---------|-------|------|------|------|------|---------|-------|------|----------|
|        | 22OH                        | 23OH | 24:1OHc | 25:1OHc | 25:OH | 24OH | 24:0 | 22OH | 23OH | 24:1OHc | 25:OH | 24OH |          |
| 1      | 56                          | 37   | 37      | 18      | 40    | 41   | 79   | 50   | 15   | 13      | 16    | 18   | 34       |
| 2      | 14                          | 17   | 15      | 14      | 18    | 16   | 10   | 22   | 24   | 23      | 31    | 26   | 19       |
| 3      | 11                          | 13   | 13      | 9       | 13    | 11   | 7    | 9    | 11   | 11      | 14    | 11   | 11       |
| 4      | 11                          | 9    | 9       | 12      | 14    | 15   | 3    | 4    | 7    | 5       | 15    | 14   | 10       |
| 5      | 29                          | 15   | 19      | 16      | 14    | 21   | 12   | 8    | 8    | 7       | 10    | 8    | 14       |
| 7      | 16                          | 7    | 9       | 3       | 10    | 12   | 18   | 6    | 5    | 4       | 8     | 5    | 8        |
| 8      | 12                          | 6    | 8       | 4       | 8     | 9    | 8    | 4    | 4    | 4       | 8     | 6    | 7        |
| 9      | 10                          | 6    | 7       | 2       | 7     | 8    | 6    | 4    | 5    | 4       | 7     | 5    | 6        |
| 10     | 18                          | 9    | 13      | 7       | 11    | 15   | 14   | 5    | 7    | 7       | 11    | 9    | 11       |
| 11     | 16                          | 19   | 19      | 22      | 24    | 18   | 15   | 12   | 14   | 16      | 18    | 15   | 17       |
| 12     | 15                          | 8    | 10      | 5       | 8     | 12   | 10   | 4    | 5    | 4       | 8     | 6    | 8        |

Traditionally mortadella is produced with 10–25 % pork stomach. In Switzerland, stomach must be listed in the ingredients, but the producers obviously hesitate to write this information on a label. Some producers eliminated the stomach from the recipe, others simply did not declare it. The distinction of mortadella samples with or without stomach was based on whether several fatty acids exceeded the threshold values repeated at the bottom of table 4. For the samples listed as “without stomach”, none of the values reached the threshold, while for those in the upper part of the table, listed as “containing stomach”, many exceeded it. This distinction turned out to be correct, either confirmed by the declaration or by inquiries of the producer.

The amount of stomach tissue added to the sausage was estimated by subtraction of the medium values of the given acid in a mortadella without stomach and division by the value for the stomach (table 5). The resulting percentages were averaged in the column at the right. The mean percentage of stomach varied between 6 and 34 %. For about a third of these samples, this estimation could be confirmed by information from the producers.

## Conclusion

The analysis of the  $C_{22}$ – $C_{26}$  fatty acids from phospho- and sphingolipids in meat products enables the sensitive detection not only of CNS material, but also of organs, such as liver, stomach, kidney, and spleen. The detection of organ tissue is primarily of interest to check the declaration of ingredients of meat products. For the detection and possibly specification of organs it is useful to subdivide the lipids of which the fatty acids are analyzed, since this enriches the data material. The detection limit for stomach is around 3 %. No attempt was made to determine animal species, but according to *Niederer* and *Bollhalder* (6) this should be possible through the cis/trans ratio of the 24:1 fatty acids.

No meat product contained CNS material, and no non-declared organ tissue was detected except pork stomach in 6 samples of mortadella. The common suspicion that sausages and other meat products frequently contain dubious by-products of slaughtering could not be confirmed.

## Summary

$C_{22}$ – $C_{26}$  fatty acids from sphingo- and phospholipids were used for the determination of CNS material (BSE) and organ tissue (declaration) in meat products. The lipids of interest were isolated from the bulk lipids using a silica gel cartridge, the fatty acids converted to methyl esters, silylated, and analyzed by GC-MS. Two fractions of lipids were analyzed separately, as this enhanced the selectivity of the method. No CNS material was detected in about 120 samples. Only in six of some 60 samples non-declared organ tissue was found – all mortadella, which is traditionally prepared with pork stomach (declaration required in Switzerland). The detec-



tion limit for stomach is around 3 %. Other organ tissues shown to be detectable by the method are liver, kidney, and spleen.

## Zusammenfassung

C<sub>22</sub>–C<sub>26</sub> Fettsäuren aus Sphingo- und Phospholipiden wurden zum Nachweis von ZNS-Material (Hirn, Rückenmark; BSE-Problematik) und Innereien (Deklaration) in Fleischprodukten verwendet. Diese Lipide wurden mittels einer Kieselgel-Kartusche aus den übrigen Lipiden isoliert, die Fettsäuren sauer zu Methylestern umgewandelt, silyliert und mit GC-MS analysiert. Zwei Lipidfraktionen wurden getrennt analysiert, weil damit die Selektivität der Methode erhöht werden konnte. In ca. 120 Proben von Fleischprodukten wurde kein ZNS-Material nachgewiesen. Unter ca. 60 Proben befand sich nur ein Produkt (6 Proben) mit nicht deklarierten Innereien: Mortadella, der traditionell mit Schweinemagen hergestellt wird (Deklaration in der Schweiz vorgeschrieben). Schweinemagen war ab einem Zusatz von ca. 3 % nachweisbar. Folgende andere Innereien erwiesen sich als nachweisbar: Leber, Milz und Niere.

## Résumé

La fraction C<sub>22</sub>–C<sub>26</sub> d'acides gras provenant de sphingo- et phospholipides a été utilisée pour déterminer la présence de cervelle, de moelle épinière (problème ESB) ainsi que de tissu d'organes (soumis à déclaration) dans les produits carnés. Les sphingo- et phospholipides ont été isolés des lipides principaux à l'aide d'une cartouche de silice, dérivatisés en esters méthylique, silylés et finalement analysés par GC-MS. Deux fractions ont été analysées séparément afin d'augmenter la sélectivité de la méthode. Aucun matériel de tissu nerveux central n'a été décelé dans la centaine d'échantillons de produits carnés analysée. Six produits seulement sur quelques 60 échantillons renfermaient du tissu d'organes non déclaré: des saucisses mortadelle, traditionnellement fabriquées avec l'estomac de porc (déclaration obligatoire en Suisse). Le tissu d'estomac est détectable à partir d'une teneur d'environ 3 %. Le foie, la rate et le rein sont d'autres organes dont la présence peut être décelée par cette méthode.

## Key words

Fatty acids from sphingo- and phospholipids, Hydroxy fatty acids, Determination of CNS material, Organ tissue, Sausages

## References

- 1 Tierseuchenverordnung SR 916.401, Bern, 1995.
- 2 Lücker, E., Eigenbrodt, E., Wenisch, S., Failing, K., Leiser, R. and Bülte, M.: Development of an integrated procedure for the detection of central nervous tissue in meat products using cholesterol and neuron-specific enolase as markers. *J. Food Protection* **62**, 268–276 (1999).
- 3 Lücker, E., Eigenbrodt, E., Wenisch, S., Leiser, R. and Bülte, M.: Identification of central nervous system tissue in retail meat products. *J. Food Protection* **63**, 258–263 (2000).

- 4 Lückner, E., Horlacher, S., Eigenbrodt, E. und Bülte, M.: Verfahren zum Nachweis von im Hinblick auf BSE unerwünschten Zutat in Fleischerzeugnissen. *Fleischwirtschaft* 5, 74–77 (2000).
- 5 Horlacher, S., Lückner, E. und Bülte, M.: Nachweis von Risikomaterial in Fleischerzeugnissen. *Lebensmittelchemie* 55, 121–122 (2001).
- 6 Niederer, M. and Bollhalder, R.: Identification of species specific central nervous tissue by GC-MS – a possible method for supervision of meat products and cosmetics. *Mitt. Lebensm. Hyg.* 92, 133–144 (2001).
- 7 Barcarolo, R., Bau, A., Moreno, J.B., Dimitrova, B. and Anklam, E.: Method development for the analysis of nervonic acid in meat-derived food using on-line LC-GC technique. 25<sup>th</sup> Int. Symp. on Capillary Chromatography, Riva del Garda 2002.
- 8 Hellgren, L.I.: Occurrence of bioactive sphingolipids in meat and fish products. *Eur. J. Lipid Sci. Technol.* 103, 661–667 (2001).
- 9 Humpff, H.-U. und Seefelder, W.: Identifizierung und Charakterisierung von komplexer Sphingolipiden in pflanzlichen Lebensmitteln. *Lebensmittelchemie* 55, 141 (2000).
- 10 Swiss «Lebensmittelverordnung» Art. 124, Bundeskanzlei Berne, 2000.
- 11 Magni, P. and Porzano, Th.: Large sample volume splitless injection. Proceedings 25<sup>th</sup> Int. Symp. on Capillary Chromatography, Riva del Garda 2002.
- 12 van der Hoff, G.R., van Zoonen, P. and Grob, K.: Deactivation of precolumns for capillary GC by a thin layer of OV-1701. *J. High Resol. Chromatogr.* 17, 37–42 (1994).
- 13 Grob, K. and Biedermann, M.: The two options for sample evaporation in hot GC injectors: thermospray and band formation. Optimization of conditions and injector design. *Anal. Chem.* 74, 10–16 (2002).

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