Determination of Androstenone in Pig Fat Using Supercritical Fluid Extraction and Gas Chromatography–Mass Spectrometry

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A method for the analysis of androstenone (5α-androst-16-en-3-one) in boar fat samples is described. Sample workup is based on supercritical fluid extraction (SFE), with final determination using gas chromatography–mass spectroscopy (GC–MS) in selected ion monitoring (SIM) mode. Careful tuning of the supercritical fluid density and extraction temperature enables fairly selective extraction of the target analyte. Further selectivity can be obtained by choosing alumina as the support on which the fat is loaded, as indicated by full-scan chromatograms. The extraction can be speeded up by effective spreading of the sample on the support and by using the lowest possible amount of sample with acceptable precision and accuracy in the quantitation. The detection limit for androstenone was 0.05 μg/g of fat using optimized conditions. No significant differences in accuracy were found when the SFE/GC–MS method was compared with two other methods (based on radioimmunoassay and liquid chromatography) designed for the analysis of androstenone in pig fat.

Keywords: *Supercritical fluid extraction; boar taint; male pigs; androstenone*

INTRODUCTION

The use of male pigs in meat production is advantageous in terms of carcass leanness, production costs, and animal welfare. The main disadvantage is the so-called boar taint, which has been shown to be associated with, among other components, androstenone (5α-androst-16-en-3-one) (Patterson, 1968). Various techniques have been used in methods designed for the determination of androstenone, e.g., radioimmunoassay (RIA) (Andresen, 1976; Claus, 1974), microtiter plate immunoassay (Claus et al., 1988), gas chromatography–mass spectroscopy (GC–MS) (García-Regueiro and Diaz, 1989; Schilt et al., 1989), and liquid chromatography (Hansen-Møller, 1994). Selectivity problems may sometimes be encountered in methods using immunotechniques. Cross-reactions were reported for 5α-androst-16-en-3α-ol (8%), 5α-androst-16-en-3β-ol (12%), and 4,16-androstadien-3-one (80%) in the antiserum raised against androstenone (Claus et al., 1988). According to the authors, these reactions did not cause any severe problems. However, it was found by others (García-Regueiro and Diaz, 1989) that boar back fat could contain 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol, at concentrations of ca. 0.49 and 1.8 μg/g, respectively. If low concentrations of androstenone need to be determined, these findings indicate that cross-reactions may lead to less accurate androstenone determinations using methods based on immunochemistry. A properly designed method based on chromatography can be made very selective but usually requires several workup steps prior to the final determination. These are often performed manually, making them time- and labor-consuming.

Supercritical fluid extraction (SFE) is nowadays gaining interest as an alternative sample workup technique. Liquid-like solvating capabilities combined with almost gas-like transporting properties enable fast and efficient extractions of target analytes. The merits of the technique have been thoroughly reviewed by several authors (Chester et al., 1992; King and Hopper, 1992; Riekkola and Manninen, 1993; Camel et al., 1993; Janda et al., 1993). Carbon dioxide has almost exclusively been used as the extraction fluid, due to its inertness and nontoxic properties. The solvent power is easily tuned by varying the density and temperature. If required, the polarity of the fluid can be changed by adding polar modifiers. These favorable properties of supercritical fluids have resulted in a large number of applications in various fields. In analysis and characterization of food products, SFE has been used for selective extractions of pesticides (Murphy and Richter, 1991; France and King, 1991; Hopper and King 1991), cholesterol (Ong et al., 1990; Engelhardt et al., 1991; King et al., 1993), fatty acids (Artz and Sauer, 1992; Heikes, 1993), hydroperoxides (Sugiyama et al., 1990), nitrosamines (Maxwell et al., 1993), and sulfonamides (Cross et al., 1993). Some preliminary results of carbon dioxide extraction of androstenone in fat have been reported recently (Zabolotsky et al., 1993). The method was, however, not optimized and was performed only on spiked samples. The high solubility of fat in supercritical carbon dioxide

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(King et al., 1989, 1993; Lembke and Engelhardt, 1993) makes it difficult to extract target analytes from a bulk fat matrix. Hence, careful optimization is necessary to avoid coextraction of fat.

In this paper we present a selective and fast method for the determination of androstenone in pig fat. Our method is based on extraction with supercritical carbon dioxide and a final analysis using GC–MS. To our knowledge, this is the first optimized supercritical fluid extraction of androstenone in boar fat samples.

EXPERIMENTAL PROCEDURES

Equipment. Extractions were carried out on a Hewlett-Packard 7680T (Wilmington, DE) controlled by a Windows-based software (Hewlett-Packard). The analytes were trapped on a solid sorbent (ODS, octadecylsilica). Carbon dioxide (N48 grade, Alfax, Malmö, Sweden, or 4.8 grade, AGA gas AB, Sundbyberg, Sweden) was used as the extraction medium. Carbon dioxide (N40 grade, Alfax, or 4.0 grade, AGA) was the cryo gas, required for cooling different zones in the SFE apparatus. Hewlett-Packard standard 7 mL thimbles were used throughout this work. In most experiments a 10 min static extraction step was performed at 329 bar, with a chamber temperature of 60 °C (density, 0.85 g/mL), prior to a dynamic extraction. The dynamic extraction step was normally performed at 115 bar and 40 °C, corresponding to a density of 0.7 g/mL. The flow rate was set to 2.5 or 4.0 mL/min. Trap and nozzle temperatures were set at 40 and 45 °C, respectively. After complete extraction, the trap, containing the analyte, was rinsed with 1.5 mL of cyclohexane, pumped at 1.0 mL/min. During the rinse procedure the trap temperature was 40 °C. The extract was collected in standard vials, to which 1 μg of internal standard (5α-androstan-3-one) had been added.

Two different GC–MS systems were used. One equipment consisted of a GC 8000 MS TRIO 1000 and a A200S autosampler (Fisons Instruments, Manchester, U.K.). The other equipment consisted of a Hewlett-Packard 7673 GC/SFC autosampler/injector (Hewlett-Packard, Waldbronn, Germany) connected to a Hewlett-Packard 5890 Series II gas chromatograph interfaced to a Hewlett-Packard 5972 mass spectrometer. A 30 m × 0.32 mm column coated with 0.25 μm film of DB-1 (J&W Scientific, Folsom, CA) was used in the Fisons equipment. The column in the Hewlett-Packard system was a HP-5 MS (Hewlett-Packard), of the same dimensions and with the same film thickness as the DB-1. Helium was used as the carrier gas (N55 grade, Alfax, or 5.6 grade, AGA) in both systems. The flow rate was set to 2.0 mL/min in the Fisons Instruments GC and to 1.0 mL/min in the Hewlett-Packard GC. In both systems, the temperatures of the injector and the GC–MS interface were 270 and 250 °C, respectively. The split/splitless injectors on the two instruments were used in the splitless mode with the vent valve closed for 90 s after injection. The separation was carried out using the same temperature program in both systems. After an initial isothermal period of 1 min at 100 °C, the temperature was raised to 300 °C at a rate of 20 °C/min and kept there for 10 min. The mass spectrometers were used in the SIM (single ion monitoring) mode, recording the sum of the molecular ions of the target analyte and the internal standard, i.e., m/e = 272 and 274 for 5α-androst-16-en-3-one and 5α-androstan-3-one, respectively. For quantitative determinations, the integrated areas of these two peaks were compared. Mass spectra of the target analyte and the internal standard are shown in Figure 1.

Chemicals. Structures of the investigated compounds are shown in Figure 2. The target steroid 5α-androst-16-en-3-one [18339-16-7] (CAS Registry Numbers have been provided by the authors.) and the internal standard 5α-androstan-3-one [1224-95-9] were purchased from Sigma (St. Louis, MO). In the following the steroids will be referred to as androstenone and androstanone, respectively. The alcohols, 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol, were also delivered by Sigma. Cyclohexane (p.a.) was purchased from LabScan Ltd. (Dublin, Ireland).

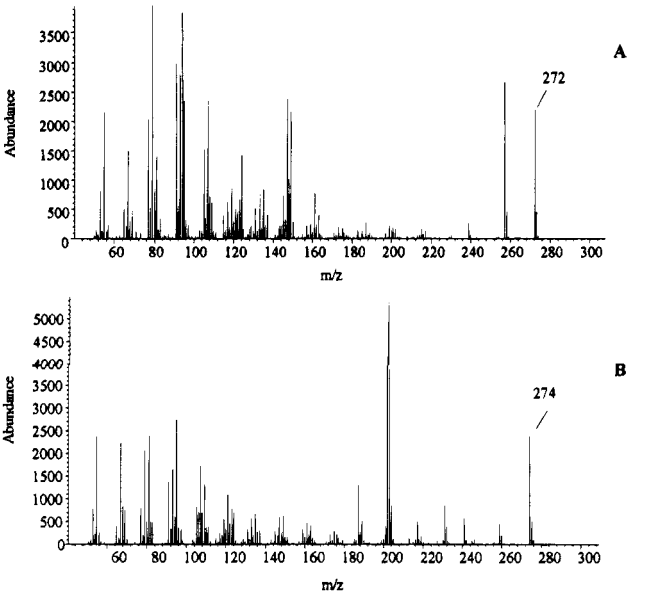

Figure 1. Mass spectra of (A) 5α-androst-16-en-3-one and (B) the internal standard 5α-androstan-3-one. Experimental parameters are described in the text.

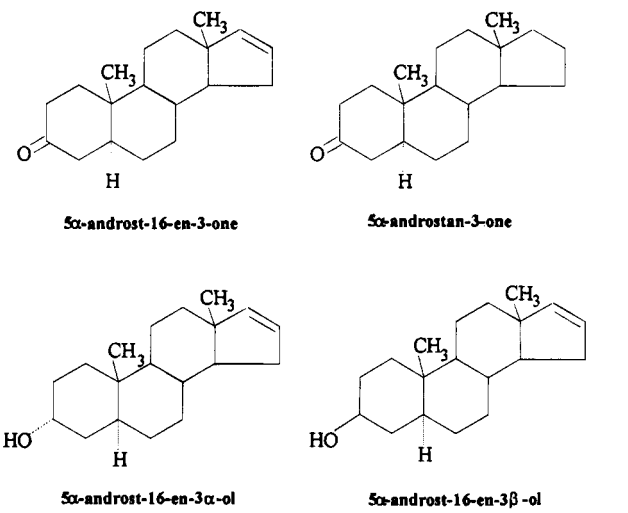

Figure 2. Structures of the investigated compounds.

Sample Preparation and Loading Procedure. Sow back fat, tested to contain negligible concentrations of androstenone and androstanone, was used as a matrix for the experiments with spiked samples. The sow fat was melted in a standard microwave oven and spiked with a cyclohexane solution of androstenone to a concentration of 1.0 μg/g.

In some introductory solubility experiments, the pure substances were extracted from filter paper (Munktells, diameter = 55 mm, Grycksbo AB, Stora Kopparberg, Sweden) placed in the extraction vessels. Fat samples were loaded on either dental tampons (No. 2, 50 × 10 mm, Sjukvårdsgrossisten Väst AB, Fritsla, Sweden) or aluminum oxide (Aluminiumoxid 90 aktiv, aktivitätsstufe I, basic, particle size 0.063–0.200 mm, Merck, Darmstadt, Germany). The fat samples were melted and pipetted onto the material in the extraction thimbles. The amount was determined by weighing the extraction thimble vessel before and after sample addition.

RESULTS

Steroid Recovery. To minimize contamination problems in the ion source of the mass spectrometer, the fat content of the injected samples should be kept as low as possible. The solubility of fat in supercritical carbon dioxide increases with density and temperature

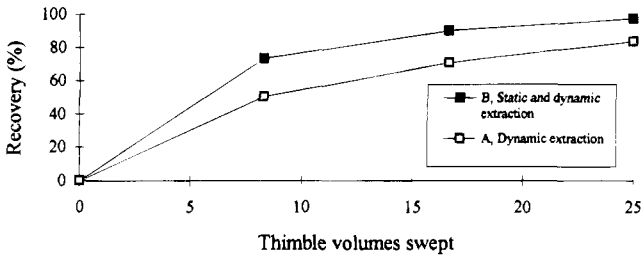

Figure 3. Extraction profile of androstenone using methods with and without a static extraction step. Conditions: sample, 0.3 g of sow back fat spiked with androstenone at 1 μg/g; extraction fluid, CO_2; trap, ODS; trap temperature, 40 °C; rinse solvent, cyclohexane; rinse solvent flow rate, 1.0 mL/min; vial volume, 1.5 mL.(A) Dynamic extraction with pure CO_2 (pressure, 115 bar; temperature, 40 °C; density, 0.70 g/mL; flow rate, 2.5 mL/min). (B) Static extraction with pure CO_2 (Pressure, 329 bar; temperature, 60 °C; density, 0.85 g/mL; time,10 min) followed by the same dynamic extraction step as in (A).

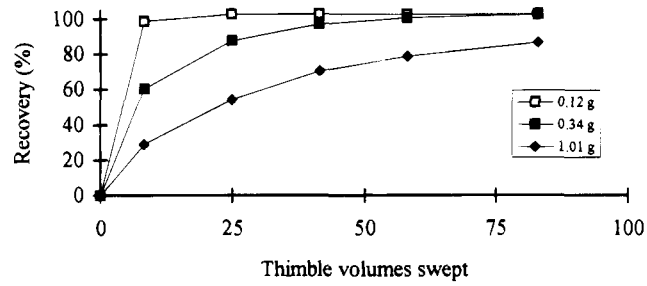

Figure 4. Extraction profiles of different amounts of fat samples spiked with 1 μg/g androstenone. Other conditions are as in Figure 3, plot B.

(Gere et al., 1993). In general, solute solubility is considerably increased above its melting point (King and France, 1992). Thus, the extraction of the target analyte in a fat matrix should preferably be performed at the lowest possible temperature and density. The melting point of the fat matrix investigated here is around 40 °C. Hence, to minimize bulk fat extraction, 40 °C was used as the chamber temperature in all experiments, except in the selectivity experiments with alumina as adsorbent, for which the temperature was varied.

To elucidate the solubility of the pure target analyte in supercritical carbon dioxide, androstenone was loaded on filter paper in the extraction thimble and extracted dynamically for 30 min at a flow rate of 2.5 mL/min. At 40 °C, a density of 0.7 g/mL (115 bar) was needed to achieve a full recovery of the pure substance. Thus, the spiked fat samples were extracted at a density of 0.7 g/mL. For these samples, the kinetics of the process was investigated by monitoring androstenone recovery using different extraction times. The results are shown in Figure 3, where target analyte recovery has been plotted versus the number of thimble volumes swept with carbon dioxide.

The extraction of androstenone is rather slow (Figure 3, plot A), and full recovery is not reached, even after 25 thimble volumes, which corresponds to approximately 50 min. This value has been obtained by recalculating the flow rate of 2.5 mL/min set at the pump, operating at a density of 0.92 g/mL, to the flow rate in the thimble, where the density was 0.70 g/mL. In this case the sample (0.3 g) was pipetted onto a dental tampon inserted in the extraction thimble. However, by using a 10 min static extraction step at 60 °C (at which the fat is melted) and 329 bar (density, 0.85 g/mL) prior to the dynamic extraction phase, the extraction was more efficient, giving full recovery of the target substance after 25 thimble volumes (Figure 3B). The conditions during the static step are such that the solubility of fat in the supercritical fluid is high, leading to a more uniform distribution of the sample in the thimble. Prior to the dynamic step the temperature is decreased and the fat is spread out onto the tampon. This increases the area of the sample, which promotes a fast mass transfer of the analyte, leading to the observed improvement in extraction time. This opinion is supported by the fact that a static extraction step performed at 40 °C and 211 bar (0.85 g/mL) prior to the dynamic step did not significantly improve extraction efficiency, compared to a dynamic extraction without a static extraction phase.

With 2.5 mL/min as the flow rate of the extraction fluid during the dynamic extraction phase, the total processing time (including the static extraction step) for one sample is about 1 h. Since it was desirable to decrease this time for the present application, the flow rate was raised to 4.0 mL/min in the dynamic extraction phase. The extraction volume profile (recovery versus thimble volumes swept) for 4.0 mL/min was shown to almost overlap the 2.5 mL/min plot in Figure 3, indicating that the kinetics of the extraction was sufficiently fast to allow similar recoveries with both flow rates.

The extraction volume profile is also affected by the amount of sample loaded in the extraction thimbles. In Figure 4, extraction profiles for three different amounts are presented. If the concentration of androstenone in the sample is sufficient, a sample weight of 0.12 g allows 100% recovery after approximately 8 thimble volumes. With a flow rate of 4.0 mL/min, this corresponds to a total extraction time (including the static extraction phase) of 20 min, which at present is the shortest time achievable. Static extraction times shorter than 10 min resulted in a less efficient extraction, requiring a longer dynamic extraction phase.

The repeatability, with 0.3 g of spiked sow fat loaded in each thimble and at a flow rate of 2.5 mL/min, was 3.3% RSD ($n = 6$). This value was obtained using a 10 min static extraction step followed by a dynamic step with a total carbon dioxide volume corresponding to 25 thimble volumes, giving a recovery of 99%. The linearity with these parameters was checked by analyzing fat samples spiked at different concentrations in the range 0.1−5.0 μg/g. Within this range, a straight calibration plot was obtained (slope = 1.01 ± 0.11, intercept = −0.038 ± 0.29 at the 95% confidence level, $R = 0.9994$).

Selectivity. When the extracts are analyzed in full-scan mode, several matrix peaks are discovered. In Figure 5A a typical full-scan chromatogram is shown, with some matrix peaks identified. The analyte and internal standard peaks, both with retention times of approximately 11 min, are barely visible in the full-scan mode. However, using SIM mode, as in Figure 5B, the target analyte can easily be identified and quantified.

The excellent selectivity obtained in the SIM mode does not exclude the fact that it is desirable to suppress extraction of bulk fat components. It is, as mentioned above, important to avoid contamination of the ion source. The degree of coextraction of bulk fat components is dependent on the parameters used for the extraction. Alumina has been shown to retain lipids in environmental samples (Johansen et al., 1992; Gere et al., 1993), and it was tested whether it could serve the same purpose here.

Table 1. Influence of the Support Material on Selectivity at Different Extraction Conditions (Loaded Amount, 0.3 g; Thimble Volumes Swept, 8.3)

support	dynamic extraction parameters	androstenone recovery (%)	remaining fat in the thimble (%)
dental tampon	ϱ = 0.7 g/mL, 40 °C, 115 bar	77	89
alumina	ϱ = 0.7 g/mL, 40 °C, 115 bar	0	90
alumina	ϱ = 0.8 g/mL, 40 °C, 164 bar	18	84
alumina	ϱ = 0.9 g/mL, 40 °C, 281 bar	89	31
alumina	ϱ = 0.7 g/mL, 60 °C, 187 bar	7	78
alumina	ϱ = 0.7 g/mL, 80 °C, 260 bar	31	69
alumina	ϱ = 0.7 g/mL, 100 °C, 334 bar	47	37

The sample, 0.3 g of sow fat spiked (1 μg/g) with androstenone, was loaded on two different materials in the thimbles, dental tampons and alumina. As before, 1 μg of internal standard was added in each vial. Measurements were made after a carbon dioxide volume corresponding to 8.3 thimble volumes, giving 75% recovery according to Figure 3.

The recovery of androstenone is listed in Table 1 together with data showing the extraction selectivity toward fat using different extraction parameters and support material. When using alumina as the support material, an increase in density or temperature is required to reach the same target analyte recoveries as when dental tampons were used (Table 1). Unfortunately, with the type of alumina material investigated in this work, bulk fat extraction becomes significant at these conditions. On the other hand, the alumina matrix strongly retains components as oleic acid and cholesterol, giving cleaner extracts. The striking difference between the obtained TIC chromatogram is shown in Figure 6. Thus, if the amount of fat collected in the vial after the trapping step could be reduced by introducing a fractionating step, the addition of this type of alumina to the extractor could be very attractive. This feature is currently being investigated.

These results also show that with the strong retaining capability of alumina for polar compounds, a careful investigation for each new analyte is needed. In this respect dental tampons present a more neutral alternative, requiring less rigorous optimization.

Employing the parameters used in the validation of the androstenone method, as described below, it was possible to analyze also 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol. As shown in Figure 7, the alcohols are not individually separated with the current temperature programming. A slower rate was shown to resolve also the two alcohols.

A total of 128 boar samples (with androstenone

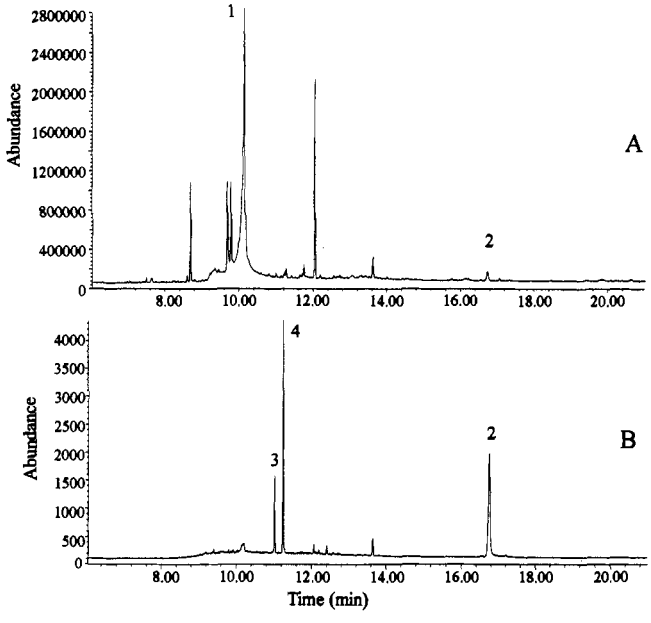

Figure 5. Chromatograms of an SFE extract of sow back fat recorded on a Hewlett-Packard 5972 MS. (A) Total ion current (TIC); (B) sum of m/e = 272 and m/e = 274. Other GC–MS parameters are described under Experimental Procedures. SFE conditions are as in Figure 3, plot B. Peak identification: 1, oleic acid; 2, cholesterol; 3, androstenone; 4, androstanone (internal standard). Analyte concentrations in injected solutions: androstenone, 0.51 μg/mL; androstanone, 0.67 μg/mL.

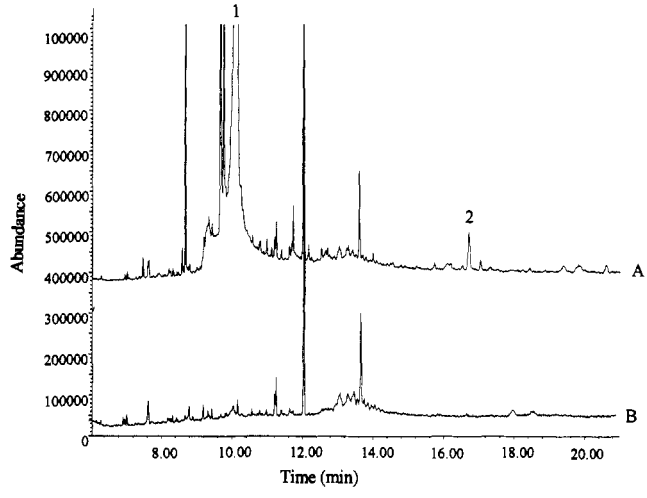

Figure 6. Chromatograms of an SFE extract of sow back fat in the total ion current (TIC) mode recorded on a Hewlett-Packard 5972 MS. GC–MS parameters are described under Experimental Procedures. Support material: (A) dental tampon; (B) alumina. SFE conditions: (A) extraction parameters as in Figure 3, plot B; (B) dynamic extraction density, 0.9 g/mL. Other parameters are as in Figure 3, plot B. Peak identification is as in Figure 3.

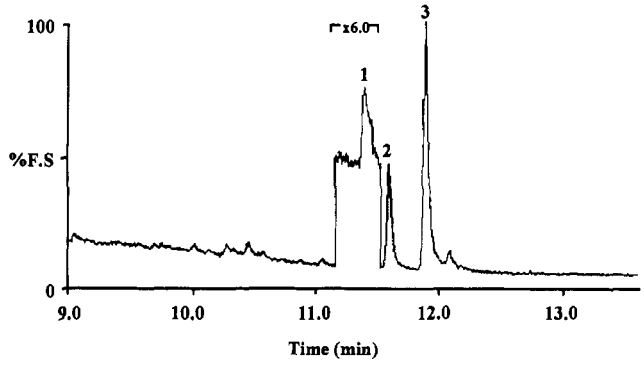

Figure 7. Chromatograms of an SFE extract of boar back fat recording the sum of m/e = 272 and m/e = 274 recorded on a GC 8000 MS TRIO 1000. GC–MS parameters are described under Experimental Procedures. SFE condition parameters are as in Figure 3, plot B. Peak identification: 1, 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol; 2, androstenone; 3, androstanone (internal standard). Analyte concentrations in injected solutions: 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol, 0.02 μg/mL; androstenone, 0.06 μg/mL; androstanone, 0.13 μg/mL.

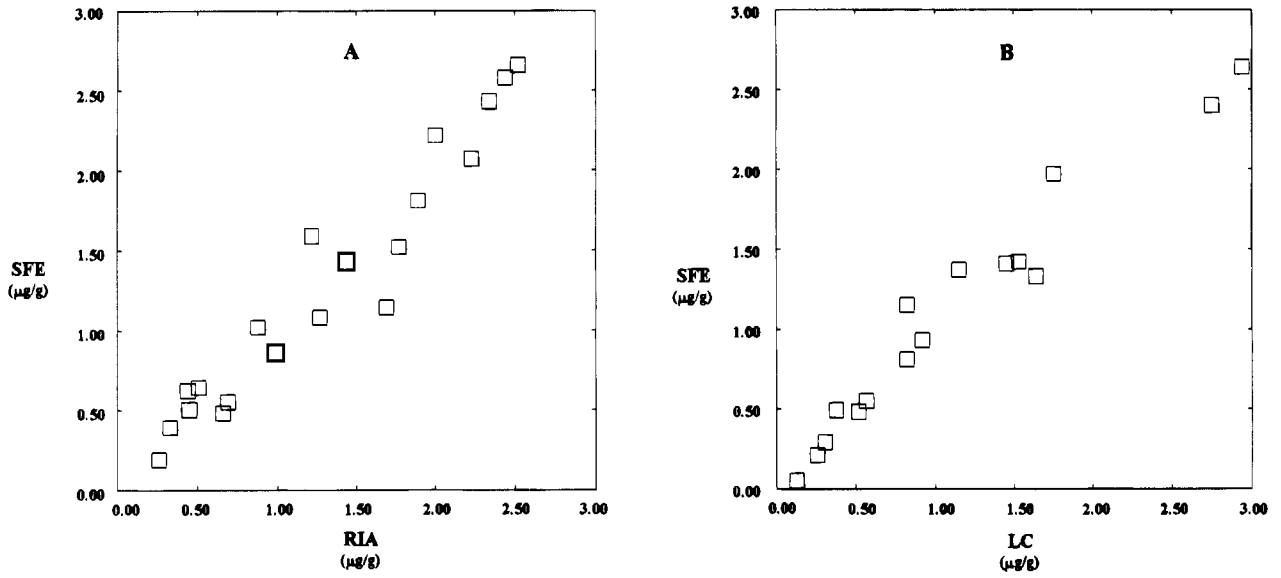

Figure 8. Comparison of the SFE/GC–MS method with other methods for the determination of androstenone in boar fat. Conditions: loaded amount: 0.5 g, 25 thimble volumes swept during the dynamic extraction step. Other SFE parameters are as in Figure 3, plot B. GC–MS parameters are described in the text. RIA and LC parameters are according to the literature (Claus, 1974; Hansen-Møller, 1994).

concentrations in the range 0.05–6.5 μg/g) were scanned for the presence of the alcohols. 5α-Androst-16-en-3α-ol and 5α-androst-16-en-3β-ol could be detected in 38 of the samples with total concentrations in the range 0.05–0.81 μg/g. The concentrations were calculated according to an expected recovery of 81%, determined by experiments with samples spiked with 1 μg/g 5α-androst-16-en-3α-ol. When the alcohol concentration (using the 38 samples in which the total concentration of 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol exceeded 0.05 μg/g) was plotted versus the androstenone concentration, a correlation coefficient of 0.6551 was found. Hence, no linear correlation was found between the concentration of the two alcohols and the androstenone concentration.

Method Validation. The SFE/GC–MS developed for the spiked samples was also validated on real (boar fat) samples by comparison with two other methods, one a radioimmunoassay (RIA) method performed as described in the literature (Claus, 1974) and one a recently developed LC method (Hansen-Møller, 1994) at two other laboratories. The RIA method was used at INRA, Station de Recherches Porcines, Saint-Gilles, France, and the LC method at the Danish Meat Research Institute, Roskilde, Denmark. In this case 0.5 g samples were used to ascertain that the amount of androstenone could be easily detected. To reduce the extraction time, 25 thimble volumes were used corresponding to an expected recovery of 80%, obtained from previous experiments on spiked sow fat. The results are shown in Figure 8, where values obtained using SFE are multiplied by 1.25 to correct for the less than 100% recovery. The values from the SFE/GC–MS method have been plotted versus the results obtained by RIA in Figure 8A and in Figure 8B versus results obtained with LC. The repeatability in SFE/GC–MS performed at the conditions given above was 6.6% RSD (n = 16). This is in agreement with data presented earlier (Claus et al., 1988), where an ELISA method was used to determine androstenone in the same concentration range and from the same matrix as are considered in this paper.

The correlation coefficients were 0.965 and 0.977 for the RIA and LC plots, respectively. Statistical evaluation (Miller and Miller, 1984) showed that the slopes did not deviate significantly from 1 (0.99 ± 0.13 and 0.90 ± 0.12 for the RIA and LC slopes, respectively, 95% confidence level). Also, the intercepts did not deviate from zero (0.0028 ± 0.20 and 0.07 ± 0.17 for the intercepts in the RIA and LC plots, respectively). Hence, there are no significant differences between the SFE/GC–MS method developed here and the other two methods. However, there is a tendency for the LC method to give higher values than the SFE method. This maybr because the alcohols, which may be present, are not separated from androstenone in the LC method, leading to an overestimation.

The three methods are equal with respect to accuracy. Thus, other factors such as selectivity, use of organic solvents, sample throughput, the time needed for manual operation, and operator skill should be considered in the choice of analysis method.

DISCUSSION

In the experiments performed here it seems that the transport of analyte from the pores of the matrix into the major stream of supercritical fluid is the rate-determining step if the amount of sample is low enough so that the surface/volume ratio of the fat distributed onto the tampon or alumina matrix is sufficiently high. One problem here is that high surface area materials, which would be desirable to use, normally are porous and, since the pores in many cases are relatively small, a relatively large fraction of the analytes has to be transported by diffusion from a stagnant supercritical fluid. This situation differs from the one faced when sediment samples are extracted, where the mass transfer of the analyte often seems to a larger extent to depend on a slow mass transfer in the interior of the matrix (Bartle et al., 1993). One reason that fat samples behave in this manner may depend on the sample preparation step, after which the analytes probably are homogeneously distributed in the entire fat sample as a result of the melting procedure.

The low correlation found in this paper between androstenone concentration and the total concentration

of 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol indicates that variations in the concentration of the two alcohols will, if specificity is low, introduce unpredictable errors in androstenone determinations. A method comparison among the three methods used in the validation (SFE/GC–MS, RIA, and LC), and including also the enzyme immunoassay method proposed by Claus et al. (1988), reveals that the selectivity in the SFE/GC–MS method is high. The alcohols, which may interfere in the other three methods, are well separated from androstrenone and can, if needed, be separately determined.

The detection limit for the SFE method (0.05 μg/g fat using optimized conditions for the final analysis step with GC–MS) is comparable to that of the LC method (0.02 μg/g) but somewhat lower than that for RIA (0.15 μg/g) and for enzyme immunoassay (0.1–0.2 μg/g). However, all methods have sufficiently low values to permit meat quality control with respect to androstenone. Also, the precision in the different methods is sufficient in this respect, although the precision in the two methods based on chromatography (6.6% in the SFE/GC–MS and 4.5% in the LC method) is higher than when using the bioasssays.

All four methods discussed above follow the general trend of reducing the consumption of organic solvents. With the SFE method less than 2 mL is used per sample, the radio immunoassay uses 10 mL, and the other two methods use 3 mL per sample.

The sample throughput is highest in the enzyme immunoassay method: 64 samples per day (8 h workday) if the preparation of the plates is not included. The SFE method gives a throughput of 32 samples per day, running the extracts overnight in the GC–MS equipment. Corresponding figures are 16 for the RIA method and 40 for the LC method (prepared in 8 h and analyzed during 20 h).

The sample handling time is shortest for the semiautomated SFE/GC–MS method with fewer manual steps involved. The most time-demanding step, i.e. homogenization and weighing of the samples, takes less than 15 min for eight samples, which then are run in sequence for 20 min per sample. This means that a maximum of 15% of the working time is needed for sample handling. This can be compared with the enzyme immunoasssay method, for which the handling time is over 40% of the total analysis time. With automation follows another advantage: the skill of the operator does not need to be especially high, once the experimental parameters have been carefully optimized. In this respect the SFE method differs favorably from, for instance, the enzyme assay method, for which experience is needed to obtain reliable results (Claus et al., 1988).

CONCLUSIONS

In summary, with the described SFE/GC–MS method it is possible to selectively extract and determine androstenone in pig fat. The sample workup step is almost fully automated, and since the cycle time for each sample can be kept low, a high sample throughput is reached.

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