RAPID ANALYSIS OF FATTY ACID PROFILES IN PORK USING NIRS WITH A DIODE ARRAY DETECTOR

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Abstract—**A near infrared (NIR) spectrometer with a diode array detector was used for determination of fatty acids in backfat and in intramuscular fat (IMF) of the longissimus dorsi muscle. Samples (n=135) were obtained from different German crossbred as well as pure bred pigs. The measurements were made by direct application of the spectrometer onto the backfat or the loin without any treatment or manipulation of the sample. Statistical calculations were performed with partial least squares regression for complete spectra, or for spectral subsets.**

Fatty acid estimation with the applied fast NIR system produced promising results. For backfat, calibration had coefficients of determination of R^2=0.61–0.92, with standard errors of 0.09–1.12. For validation, coefficients of determination were R^2=0.56–0.89, with standard errors of 0.11–1.20. For IMF, estimation results showed lower coefficients of determination and higher standard errors, due to a small range of fat content.

The method is suited for online application and has high potential for an optimization of rapid fatty acid estimation.

Index Terms—**diode array, fatty acids, NIR spectroscopy, pork**

I. INTRODUCTION

The fat content of pig carcasses as well as the fatty acid composition of the adipose tissue are important quality factors, both for the processing industry and for the consumer. Pork fat is a major component for processed meat products. Its composition determines the quality of the final product. Meat processors demand firm pork fat because of its high oxidative stability. Consistency and oxidative stability of fat result from the relative composition of fatty acids. For example, a high proportion of saturated fatty acids (SFA) results in firm fat. In contrast, increased levels of monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) are susceptible to oxidation and rancidity, and the fat has a soft, greasy and oily texture (Wenk, Hauser, Vogg-Perret & Prabucki, 1990). On the other hand, MUFA and PUFA are connected with superior health properties (Hugo & Roodt, 2007). Thus, both meat producers and processing industry have to deal with these contrasting demands. In this respect, the composition of fatty acids in adipose tissue is of primary importance, both in backfat and in muscles.

The routine measurement of fatty acids is performed with laborious gas chromatography. But several studies showed that near infrared (NIR) technology is possibly suited as a rapid method to estimate the fatty acid profile in various tissues (Gonzalez-Martin, Gonzalez-Perez, Alvarez-Garcia & Gonzalez-Cabrera, 2005; Galian, Freudenreich & Fischer, 2005; Müller & Scheeder, 2008; Perez-Marin, Sanz, Guerrero-Ginel & Garrido-Varo, 2009). The aim of our study was to evaluate if fatty acids can be determined in backfat and muscles with an NIR system suited for rapid online application. The NIR system used has a photo diode array. The advantage of this detector is its very fast measurement within milliseconds, compared to scanning spectrometers with measurement times of several seconds or even minutes.

II. MATERIALS AND METHODS

Animals and sampling

Meat (M. longissimus dorsi, LD) and backfat samples were obtained from 61 male pigs and 74 female pigs of different crossbreeds as well as pure breeds. Animals were reared at the Training and Research Centre for Pig Production of the Bavarian State Research Centre for Agriculture (LVFZ Schwarzenau). Pigs were fed with a standardized diet of the LVFZ (13.64 MJ ME/kg) and slaughtered at an average age and weight of 170 d and

107 kg, respectively. LD and backfat samples were taken after overnight chilling and stored deep frozen at -21°C for reference analysis. Data are analysed as relative percentages of total fatty acid content.

Analytical methods and statistics

Near infrared (NIR) absorption spectra were recorded with an NIR system from NIR-Online GmbH (Walldorf/Baden, Germany) with a photo diode array with a silicium detector for the visible range (VIS) and an indium-gallium-arsenide detector for the NIR range. Average reflectance spectra were recorded from 400 nm to 1900 nm at 10-nm intervals. Measurements were performed 24 h after slaughter and took 5 seconds per sample. No homogenization or pre-treatment of the samples were required.

For reference, fatty acids were analysed by gas chromatography (GC). We used a Hewlett Packard 6890 series system with a J&W Scientific DB-23 capillary column (60 m x 0.25 mm, i.d. 0.25 µm; Agilent Technologies, Inc., US). Sample preparation was performed as described by Schulte & Weber (1989). In brief, backfat samples were homogenized and melted with butylated hydroxytoluol. For transesterification from fatty acids to methyl esters, an aliquot of the liquid fat was mixed with toluol and trimethylsulfonium hydroxide (TMSH). Fat content of muscle tissue was extracted with a mixture of methanol and dichlormethane and then transesterified with TMSH. Then, the sample was injected into the GC system.

Total LD intramuscular fat was determined by a modified method of §64 in the German code of law for food and animal feed (LFGB). Fat was extracted with petroleum benzene in the Soxhlet-system 810 of BÜCHI Labortechnik GmbH (Essen, Germany) without prior HCl-digestion.

Data were analysed with The Unscrambler 9.8 (CAMO Software AS, Olso, Norway). For calibration, we used partial least squares regression (PLS) with a full cross validation. Coefficients of determination (R^2), standard errors for calibration (SEC) and cross validation (SECV) are given. For optimum calibration and validation results, the spectral data were subsetted according to different selection criteria. On the one hand, the complete VIS/NIR spectrum was compared to the NIR interval only. On the other hand, either full spectra were used, or wavelengths were selected according to greatest influence, i.e. according to high regression coefficients.

III. RESULTS AND DISCUSSION

Differences in fatty acid composition between backfat and IMF of LD were small but significant for most fatty acids (Table 1). In both tissues, oleic acid had the highest percentage (37% and 35% for backfat and IMF, respectively), followed by palmitic acid with 23% and linoleic acid with 16% for backfat and 13% for IMF. Linolenic acid had mean values of only 1.6% for backfat and 0.7% for IMF. For some fatty acids, the range of percentages differed between the two tissues (Table 2). The difference was greatest for oleic acid. In backfat, the range was 10 %-points (31–41%) compared to 20 %-points in IMF (22%–42%). For groups of fatty acids – i.e. SFA, MUFA and PUFA – PUFA had the widest range with 16 %-points for backfat (13%–29%) and 25 %-points for IMF (10%–35%). This variability of ranges bears upon the estimability of fatty acid content because, in general, prediction results can become more accurate with a larger variation of reference values.

Table 1 Relative content (%) of selected individual and grouped overall fatty acids in backfat and intramuscular fat of LD

		backfat		IMF		P*
		Mean	SEM	Mean	SEM	
palmitic acid	C 16:0	22.8	2.0	22.7	2.0	0.66
stearic acid	C 18:0	12.5	1.1	11.8	1.0	<0.0001
cis9-oleic acid	C 18:1 n-9	37.3	3.2	34.7	3.0	<0.0001
cis9-linoleic acid	C 18:2 n-6	15.8	1.4	13.4	1.2	<0.0001
cis9-linolenic acid	C 18:3 n-3	1.6	0.1	0.7	0.1	<0.0001
SFA		37.3	3.2	36.4	3.1	<0.0001
MUFA		43.5	3.7	44.7	3.8	<0.0001
PUFA		19.1	1.6	18.9	1.6	0.15

*Wilcoxon signed rank test

Table 2 Range of relative content (%) of selected individual and grouped overall fatty acids in backfat and intramuscular fat of LD

		backfat		IMF	
		Min	Max	Min	Max
palmitic acid	C 16:0	19.9	25.9	20.3	25.3
stearic acid	C 18:0	9.4	15.4	9.5	14.5
cis9-oleic acid	C 18:1 n-9	31.4	41.2	22.4	41.6
cis9-linoleic acid	C 18:2 n-6	10.7	24.6	6.8	25.1
cis9-linolenic acid	C 18:3 n-3	1.1	2.1	0.4	1.0
SFA		32.1	42.4	32.3	41.1
MUFA		37.0	47.0	31.8	50.8
PUFA		13.3	28.7	9.8	35.3

Statistical parameters of prediction models for individual fatty acids as well as for SFA, MUFA and PUFA are summarized in Table 3 for calibration and for cross validation. For each model, the best prediction is given, based on spectral selection between the whole VIS/NIR spectrum (400–1900 nm) or the NIR spectrum only (700–1900 nm). Another possible selection was the limitation of wavelengths to those with high regression coefficients, i.e. to the range of wavelengths of greatest influence on the calibration. The number of factors for each calibration model was chosen according to the minimum root mean square error for validation based on full cross validation. Most estimations were calculated with the NIR spectrum only, but statistics for the unsaturated fatty acids achieved better results when both the VIS and the NIR spectra were included (expect for stearic acid C 18:0 in backfat).

For backfat, the estimation of both individual and grouped fatty acids had medium to high coefficients of determination with R^2=0.61–0.92 for the calibration model (Table 3). As to be expected, validation coefficients of determination were slightly lower (R^2=0.56–0.89). Linoleic and linolenic acids had the highest coefficients of determination with calibration or validation R^2 between 0.81 and 0.92. This was reflected by the grouped PUFA with R^2=0.89–0.92. These good estimation results for PUFA probably result from the wide range of sample values. Table 3 also gives absolute estimation errors. Relative estimation errors (SECV, Table 3, in percent of mean, Table 1) were 3.0–6.9% for individual fatty acids and 2.0–5.8% for grouped fatty acids.

Table 3 Estimation statistics for selected individual and grouped overall fatty acids in backfat and in intramuscular fat of LD (standard errors of calibration, SEC, and of cross validation, SECV; coefficient of determination, R^2)

	spectral selection	calibration		validation	
		SEC	R^2	SECV	R^2
backfat					
C 16:0	VIS/NIR*	0.73	0.68	0.79	0.63
C 18:0	NIR*	0.74	0.70	0.81	0.65
C 18:1 n-9	NIR*	1.12	0.61	1.20	0.56
C 18:2 n-6	NIR	0.80	0.92	0.98	0.88
C 18:3 n-3	NIR	0.09	0.87	0.11	0.81
SFA	VIS/NIR*	0.85	0.87	0.96	0.83
MUFA	NIR*	0.86	0.74	1.06	0.62
PUFA	NIR	0.91	0.92	1.11	0.89
IMF					
SFA	VIS/NIR*	1.27	0.66	1.43	0.57
MUFA	NIR*	2.10	0.65	2.43	0.54
PUFA	NIR*	2.38	0.79	3.00	0.67
IMF	VIS/NIR*	0.23	0.77	0.26	0.69

* spectral selection for wavelengths with high influence (see Materials and Methods)

For IMF of LD, calibration models for individual fatty acids had rather poor predictive abilities. Therefore, only results for grouped fatty acids are given (Table 3). Their coefficients of determination ranged between 0.54 and 0.79. Also, standard errors for both calibration and validation were higher compared to the results for backfat. The poorer estimations of fatty acids in IMF may be explained by the low absolute contents of intramuscular fat ranging between 0.4% and 2.8%. This low content also explains that IMF can be estimated with medium precision only (R^2=0.69–0.77, Table 3). Hence, we propose to study also other muscles with a higher IMF for better prediction of fatty acids in IMF.

Already now, the calibration appears to be precise enough to allow the detection of small differences in fatty acid composition as revealed in this study. Backfat and LD differ in oleic, linoleic and linolenic acid by 2.6, 2.3 and 0.9 %-points, respectively (cf. Table 1), as determined by reference analysis. With prediction errors of 1.2, 1.0 and 0.1 %-points, respectively (Table 2), the NIR measurements can reveal these small differences between backfat and LD.

Some studies attained larger R^2 values than the results presented (Gonzalez-Martin, Gonzalez-Perez, Alvarez-Garcia & Gonzalez-Cabrera, 2005; Müller & Scheeder, 2008; Perez-Marin, Sanz, Guerrero-Ginel & Garrido-Varo, 2009). It is difficult to compare these studies with our approach directly. Differences in overall fat content affect the estimation results, e.g. as a consequence of pig breeds studied. Also, spectrometer technology differs. Furthermore, sample preparation has an important effect, in particular because of pre-treatments like homogenization. The advantage of the NIR-online system we used in our study is twofold. First, measurements can be taken directly on the carcass without prior treatment or tissue destruction. Second, the fast measuring time makes the system applicable under online conditions.

IV. CONCLUSION

The estimation of fatty acids with the NIR system evaluated is a promising alternative to conventional GC methods. It is a fast method without destroying the sample tissue and suited for online application. But further studies are necessary to optimize prediction results and to develop robust estimation models for practical use.

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