ATR-FTIR EVALUATION OF IMPORTANT FATTY ACID PROFILE IN JAPANESE BLACK CATTLE BEEF

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Abstract – A rapid method for evaluating important fatty acid profile, especially mono-unsaturated fatty acid (MUFA), saturated fatty acid (SFA), omega-3 and omega-6 fatty acids in Japanese black cattle beef were developed. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was applied to subcutaneous, intermuscular and intra-muscular adipose tissues and lipids extracted from those tissues. Partial least-squares (PLS) regression models were developed from combined spectra regions of 3050-2800 and 1500-1000cm^{-1} with reference data of fatty acid compositions determined by gas chromatography (GC). The PLS models predicting the contents of MUFA, SFA, omega-3 and omega-6 fatty acids showed higher correlation with GC data for extracted lipid than that for adipose tissue. The coefficients of determination in cross-validation (R^2) ranged from 0.59- 0.94.

Key Words – MUFA, SFA, omega-3 and omega-6 fatty acids, PLS, wavenumber selection.

I. INTRODUCTION

Japanese black cattle beef has the characteristics of fatty, well-marbled texture and palatability. The beef quality such as marbling, tenderness and flavor has relationship with fatty acid composition. A higher content of MUFA in beef fat provides desirable flavor, meanwhile long-chain saturated fatty acids (SFA) and linoleic acid cause unpleasant waxy taste and flavor [1]. Omega-3 and omega-6 fatty acids are essential fatty acids and have health functions. A ratio of omega-6/omega-3 is important for homeostasis and normal development [2]. Therefore, the fatty acid profile of Japanese black cattle beef is of importance to beef production and distribution. GC analysis has been prevalently applied as a standard method for the determination of fatty acid composition of edible oils and fats. However, GC measurement is time-consuming and requires hazardous chemicals and trained users. Near infrared (NIR) and mid-infrared (MIR) spectroscopy has been proven to be an effective alternative to qualitative and quantitative measurement in food. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) was applied for determining the potential adulterant of olive oil [3], fatty acid composition of pork fat [4], relative amount of omega-3 and omega-6 polyunsaturated fatty acid species in vegetable oils and oil seeds [5]. The objectives of this study were to develop ATR-FTIR for determining fatty acid composition of adipose tissue in beef fat and extracted lipid.

II. MATERIALS AND METHODS

Meat samples from 38 Japanese black cattle up to 30 or 31 months of age were tested within 3 weeks after slaughter. Fat specimens (about 2.0g) were taken from subcutaneous, inter- and intra-muscular adipose tissues of the carcass cross-section between 6th-7th ribs respectively. The adipose tissue specimens were tested for ATR-FTIR measurement and subsequently preserved at -20℃ until the further analysis. The total lipid of each specimen was extracted by chloroform-methanol and washed by 0.88% (w/w) potassium chloride solution according to Folch method [6]. An aliquot of extracted lipid was used for measuring FTIR spectrum immediately and another aliquot was used for fatty acid analysis by GC. Triplicate spectra for each specimen were measured between 4000 and 600 cm^{-1} at a resolution of 2 cm^{-1} with 32 scans with Bomem MB 3000 (ABB Co. Ltd., Japan) spectrometer mounted with a MIRacle-single horizontal ATR accessory (ZnSe, PIKE Technologies Co. Ltd., WI, USA) and a high-pressure clamp. Specimens with two different preparation methods were applied to FTIR detections: direct measurement of non-processed

adipose tissue and solvent-extracted lipid. Fatty acid composition of the three kinds of tissue (subcutaneous, inter- and intra-muscular adipose tissues) was determined by GC analysis. The extracted lipid was esterified by modified BF_3-MeOH method (AOCS Method Ce 1b-89). A standard fatty acid methyl-esters mixture (Supelco FAME Mix, RM-6) and samples were then analyzed on a GC-2014 (Shimadzu) equipped with an OmegawaxTM 250 capillary column (30m*0.25mm*0.25μm film thickness) and a flame ionization detector (FID). The temperatures of column, injection and detector were 180℃, 260℃ and 250℃ respectively. A split ratio of 1:100 was used in the analysis.
Principal component analysis (PCA) and partial least-squares (PLS) regression were applied by PLS_Toolbox with the Matlab software. Criteria for evaluating validity of calibration and its leave-one-out cross-validation models were the RMSEC (V) (Root Mean Square Error of calibration/validation), the R^2 (coefficient of determination).

III. RESULTS AND DISCUSSION

GC Data Analysis

Measured fatty acid composition of subcutaneous, inter- and intra-muscular fats is presented in Table 1. The GC result of all sampled adipose tissue indicated that major fatty acid composing the fats were oleic acid (C18:1) and palmitic acid (C16:0). MUFA and SFA were 56.4% and 33.7% in subcutaneous fat, 54.4% and 38.1% in intermuscular fat, and 50.4% and 43.0% in intramuscular fat, respectively. Subcutaneous fat had the highest content of MUFA, followed by inter- and intra-muscular fats. For SFA, the content decreased in the same order. The ratio of MUFA and SFA also decreased in the same order.

Principal component analysis (PCA) was applied to the fatty acid compositions of the fats. In the score plot (Figure 1), the first principal component accounted for 70.66% of the variance in the data and the second component accounted for 19.52%. Discrimination among the fats might be difficult on the basis of principal components; however, subcutaneous fat showed lower values of first and second components than inter- and intra-muscular fats.

Table 1 Fatty acid composition of subcutaneous, inter-, intra-muscular fat

Fatty Acid	Intramuscular	Intermuscular	Subcutaneous
oleic acid(18:1)	46.6±3.8%	49.7±3.9%	49.6±3.8 %
palmitic acid(16:0)	28.3±3.1	24.7±3.9	24.7±3.4
stearic acid(18:0)	12.0±3.1	11.1±3.6	6.6±2.0
palmitoleic acid(16:1)	3.9±1.0	4.7±1.3	6.8±1.3
myristic acid(14:0)	2.7±0.5	2.3±0.5	2.5±0.6
linoleic acid(18:2)	2.2±0.5	2.3±0.5	2.1±0.4
MUFA	50.4±3.9	54.4±4.1	56.4±3.9
SFA	43.0±5.3	38.1±5.7	33.7±5.0
MUFA:SFA	1.17	1.43	1.67

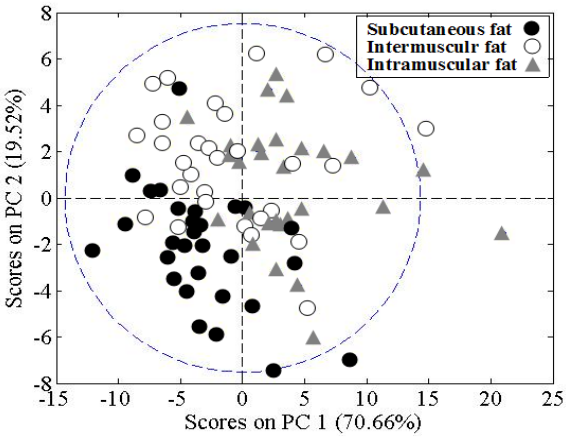


Figure 1. Score plot of PCA for FAC of the three extracted lipids by GC data.

ATR-FTIR spectra

Figure 2 shows FTIR spectra of extracted lipid (dotted line) and adipose tissue (solid line). The major peaks of extracted lipid's spectra were observed which comes from triglyceride functional groups around 2925cm^{-1} (C-H asymmetric stretching), 2856 cm^{-1} (C-H symmetric stretching), 1750 cm^{-1} (C=O stretching) as reported by Safar *et al*. [7]. The spectra also exhibited fat-related peaks at 1465 cm^{-1} (C-H scissoring bending), and 1163 cm^{-1} (C-O stretching and C-H bending) [8]. Besides, a small alkene peak was also observed at around 3000 cm^{-1} (Figure 2) and the peak position showed the shift from 3000 cm^{-1} depending on an amount and unsaturated degree of unsaturated

fatty acids [5]. In contrast, broad absorptions of water (around 3300 and 1650 cm^{-1}) and protein (around 1550 cm^{-1}) were observed in the spectra of adipose tissue [5, 9].

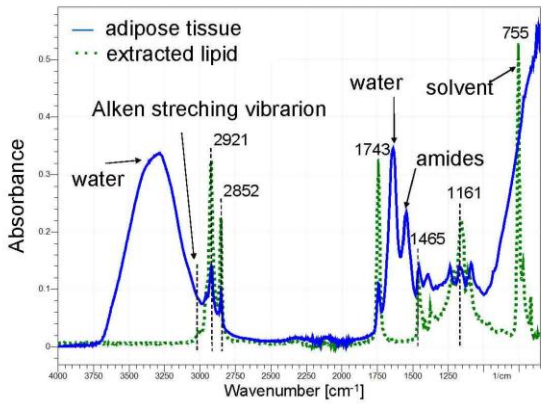
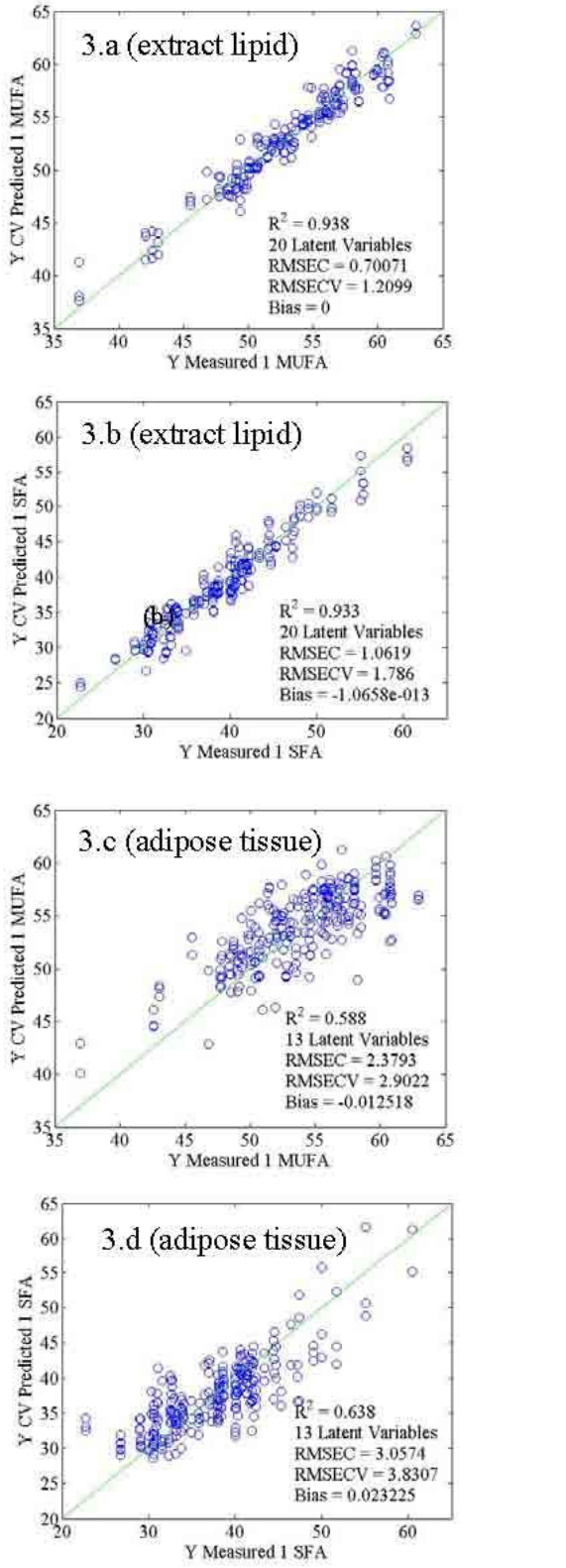


Figure 2. FTIR spectra of extracted fat and fat depot in the full region (4000-600 cm^{-1}).

PLS regression analysis

Wavenumber regions at 3050-2820 and 1500-1000 cm^{-1} were selected to develop a PLS regression model. Baseline correction, mean-centered normalization, and a second-order derivative with a 9-points Savizky–Golay derivative function were applied. Figures 3a-d show the prediction performances of MUFA and SFA by the PLS models developed from spectra of extracted lipid and adipose tissue. The models derived from extracted lipid were able to predict the actual contents of MUFA and SFA with higher determinant coefficients (>0.93); however, the predicted values from adipose tissues shows lower determinant coefficient (<0.64) and with larger RMSEC and RMSEV. FTIR spectra of extracted lipids have distinctive peaks originating from functional groups of lipid molecules, whereas adipose tissue containing water, protein and other components shows more complex spectra than those coexisting substances. Therefore, selection of the wavenumber region should be conducted more precisely. From GC analysis, alpha-linolenic acid (ALA) was found to be a major component of omega-3 fatty acids and gamma-linolenic acid (GLA, omega-6) was below detectable limits in tested fats. Thus, linoleic acid was regarded as a major omega-6 fatty acid.

Figure 3. PLS calibration models for predicting the contents of MUFA and SFA for extracted lipid and adipose tissue.



Therefore, specified wavenumber region for predicting ALA and linolenic acid were adopted. As a result, the determinant coefficients of the PLS models predicting ALA and linolenic acid were obtained around 0.823 and 0.91 even from the FTIR spectra of adipose tissues.

IV. CONCLUSION

ATR-FTIR spectroscopy was applied to evaluate a fatty acid profile of Japanese black cattle beef having high marbling. PLS regression model could predict the contents of MUFA, SFA, omega-3 and omega-6 with determinant coefficients of 0.94, 0.93, 0.83 and 0.94 for extracted lipid and 0.59, 0.64, 0.82 and 0.91 for adipose tissue. With no sample pretreatment, coexisting substances such as water, protein and others, except fat, in adipose tissue would reduce the accuracy of PLS model. Therefore, the selection of wavenumber region in the model development should be conducted precisely.

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