Title: Determining Pork Fat Quality as Measured by Three Methods with an Industry Standard Marketing Plan for Pigs Fed 20% DDGS – **NPB #12-045**

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Industry Summary

Fat quality affects the entire pork chain as both fresh and further processed products are subject to fat oxidation, color change, and shortened shelf-life in light of poor fat quality. The literature indicates fat quality has been important for decades, but as economic indicators encourage pork producers to use non-traditional fat sources, such as dried distiller's grain with solubles (DDGS), in swine diets, the impact of fat quality has created challenges for end users of pork chain products. Export markets for fresh pork and domestic markets of valued added pork such as sausage and bacon are especially influenced by fat quality as these are opportunities for increased profit margin and require the delivery of high quality products to meet consumer expectations.

According to research within our lab and reports of others, the fat source in the diet is a significant driver of pork fat profile and ultimately fat quality in the final product. We expect the timing of marketing pigs to interact with dietary fat source and this relationship could alter feeding strategies of certain fat sources to optimize fat quality in pork. Therefore, in the following study, we sought to determine the fatty acid profile and iodine value of jowl and belly fat from hogs fed a diet including 20% DDGS and marketed in three cuts from a commercial facility. Iodine value was determined using three methods; chemical titrations, calculated from a fatty acid profile and using in-plant near infrared (NIR) spectroscopy. Specifically, we want to understand how

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traditional marketing cuts from finishing facilities affect early maturing pigs in relation to their fat profiles and how these pigs differ from their later marketed contemporaries.

Eight hundred and eighty crossbred growing finishing hogs (PIC genetics) were raised in a commercial facility with 22 pigs allotted to each pen. Twenty pens were fed a control corn and soy based diet and the remaining 20 pens were fed a diet containing 20% DDGS (Tables 1 and 2). Animal growth and performance was measured by pen body weight, average daily gain, average daily feed intake and gain to feed ratio. The heaviest hogs were marketed first removing 4, 8 and 12 head per pen in the first, second and third marketing cuts, respectively. Belly and jowl fat samples were collected 1 day postmortem from chilled carcasses in a commercial slaughter facility. Lab analysis was performed at the University of Missouri Meat Science Laboratory.

Growth performance was unaffected by the inclusion of DDGS in the diets of growing and finishing hogs. However, marketing cut changed growth parameters, specifically ADG. The hogs marketed in the second cut have a clear advantage over counterparts in the first and third cuts. By removing the fast growing, early maturing hogs in the first cut, feeder and floor space expanded, allowing the remaining hogs to more closely meet their genetic potential. The genetically superior animals grew faster and gained more thereby surpassing the slow growing, late maturing hogs left in the third cut.

Dietary fat is largely reflected in the fatty acid (FA) profiles and iodine value (IV). Fatty acid composition is more saturated in pigs fed the control corn diet, while pigs fed 20% DDGS, have a greater proportion of unsaturated fatty acids. Iodine values, regardless of determination method, mimic the fatty acid profiles. Pigs fed 20% DDGS had higher IV (greater degree of unsaturation) when compared to control counterparts and jowl fat had a higher IV then belly fat. These results suggest that feeding 20% DDGS increases IV in pork fat, but does not impede growth performance. As expected, timing of marketing will change the pen dynamics and feeding behavior to alter growth performance as well as fat tissue composition. Fat sampling depot differs for animals at varied stages of growth and should be considered when managing pork carcasses for fat quality.

III. Keywords: DDGS, fat quality, pork, iodine value, fatty acid profile

IV. Scientific Abstract

An experiment was performed to evaluate the effects of dried distiller's grain with solubles (DDGS) and marketing cuts in a commercial swine facility on growth performance, fat quality and the relationship between iodine values determined by three methods in two fat depots. Pen (n=40) was the experimental unit with 20 replications per treatment and 22 pigs per pen. Pigs were randomly allotted to 1 of 2 dietary treatments in a 2 x 3 factorial arrangement of treatments with 2 levels of DDGS (0 and 20%) and chosen for 1 of 3 marketing cuts. The first, second and third cuts removed 4, 8 and 12 head from each pen, respectively. Carcasses were sampled for fat tissue at the anterior tip of the jowl and posterior to the sternum on the belly edge 1 day postmortem. The inclusion of 20% DDGS in the diet did not affect growth performance. Marketing cut significantly ($P<0.0001$) affected final BW, ADG, ADGI and G:F. Total SFA ($P<0.0001$) and MUFA ($P<0.0001$) concentrations were lower in belly and jowl fat from control diet hogs. Total PUFA ($P<0.0001$) and PUFA:SFA ($P<0.0001$) increased with 20% DDGS in the diet in belly and jowl fat. Inclusion of DDGS significantly increased iodine value (IV) in belly and jowl fat regardless of method of determination. Belly fat had significantly ($P<0.0001$) lower IV compared to jowl fat for two methods (titration and GC) suggesting pigs have varied degrees of physiological maturity at specific fat depots during the finishing phase. These results suggest that feeding 20% DDGS increases IV, but does not slow growth performance and time of marketing impacts growth and IV of pork lipid tissue in the jowl and belly.

V. Introduction

Fat quality is important in meat products as it can influence further processing characteristics and pork export potential (Carr, 2005). The main components of fat quality include composition, titer (hardness), color, impurities, and stability (Azain, 2001). Recent industry trends have closely associated iodine value (IV) to the definition of fat quality as well. The composition of a sample refers to the percentage of each individual fatty acid and is measured through gas chromatography (Azain, 2001). The fatty acid composition of the sample can also impact other fat quality characteristics based on the quantity of saturated and unsaturated fatty acids present. Unsaturated fatty acids have much lower melting points than saturated fatty acids, with the *cis* double bond configuration having a decreased melting point when compared to the *trans* configuration. Additionally,

short chain fatty acids have lower melting points than longer chain fatty acids, because the association between fatty acid chains increases as chain length increases.

The firmness/hardness of fat is determined by the composition, as different fatty acids have different melting points (Wood et al., 2003; Wood et al., 2008). The chain lengths and degree of unsaturation determine both titer and iodine value (Azain, 2001). Although fat color has no association with nutritional quality, it may indicate both dietary and anatomical fat source and fatty acid composition (Azain, 2001). The stability of a sample refers to the sample's ability to resist breakdown in the presence of oxygen (Azain, 2001). Oxidative rancidity is an important factor of fat quality and must be monitored during processing and retail display as it can determine the shelf-life of meats.

Dietary fat influences the fat profile of pork. The inclusion of dried distiller's grains with soluble (DDGS) in swine diets has increased over the past 10 years, due to a rise in ethanol production and greater availability of byproducts for incorporation in livestock diets (Stein and Shurson, 2009). DDGS from modern ethanol plants contain are a highly concentrated source of protein and energy (in oil form) and contain high contents of digestible phosphorus, amino acids and energy (Shurson et al., 2003). Typically, DDGS contain 6-12% oil, made up of a high percentage of unsaturated fatty acids, (approximately 81% with 54% linoleic acid), and a low percentage (13%) of saturated fatty acids (Xu et al., 2010).

In a study conducted by Benz et al. (2010), pigs were fed increasing levels of DDGS (0, 5, 10, 15, or 20%) for 57 or 78 days prior to slaughter. For pigs fed DDGS for 57 days, increasing levels of dietary DDGS led to a linear increase in C18:2n-6, C20:2, total PUFA, PUFA:SFA, and IV and a linear decrease in C16:0, C18:1n-7, and total MUFA for belly fat, back fat, and jowl fat samples. For belly fat and back fat samples from pigs fed for 57 days, increasing DDGS levels led to a linear decrease in C16:1 and C18:1n-9 cis and a linear increase in C18:3n-3. Total SFA was decreased in belly fat samples and C18:0 was decreased in jowl samples with increasing levels of dietary DDGS for 57 days. When the feeding duration was increased to 78 days, a linear decrease in C16:0, C18:1n-9 cis, and total MUFA was observed and a linear increase in C18:2n-6, total PUFA, PUFA: SFA, and IV was seen for belly fat, back fat, and jowl fat samples.

Iodine value (IV), which measures the degree of unsaturation via presence of double bonds, has been well documented to increase with increased levels of DDGS in the diet. Notably, IV can be measured from several locations or fat depots on a carcass including but not limited to the anterior tip of the jowl, the belly from a location on the midline posterior to the sternum and anterior to mammary tissue, subcutaneous back fat from the 10th rib or intermuscular fat or marbling. Some studies have focused on distinguishing the differences between fat depots on a carcass and even tried to find correlations between less valuable regions such as the jowl and regions that are preferred left intact such as the belly.

Fattening patterns of food animals appears to be from the distal ends and toward the visceral cavity. These patterns would indicate that finishing pigs would likely deposit fat earlier in the jowl and over the front shoulder prior to deposition of fat in the loin and belly region (Hammond, 1932). If pigs are harvested at similar market weights, but differing maturities, it seems logical that the total fat content and fatty acid profiles would differ by fat depot. Therefore, pigs that are closer to their physiological maturity at market weight could be expected to have fat profiles that are more similar across depots when compared to pigs that are still accumulating muscle at a rapid rate versus fat tissue accumulation (Wiegand et al., 2011).

VI. Objectives

1. Determine the fatty acid profile and iodine value of jowl and belly fat of pigs fed 20% DDGS and marketed in three cuts from commercial facilities.
2. Determine the relationship between three methods of determining iodine value in pork fat (In-plant NIR, gas chromatography, and iodine absorption by titration).

VII. Materials and Methods

A. Animals

The University of Missouri Animal Care and Use Committee approved animal care and experimental protocols prior to initiation of this experiment. A total number of 40 pens containing 22 crossbred hogs (initial body weight = 43.17 kg, PIC genetics) were housed in a typical commercial grow finish facility, with fully slatted concrete floors, stainless cup waters and a four hole stainless feeders with 50 in of linear feeder space. Water was available in each pen at all times. The pens were 8 feet wide and 19 feet long providing a total of 152 square feet of floor space (6.9 ft^2 initial floor space per pig before first marketing cut).

B. Diets

Twenty pens were randomly assigned to receive a control corn-soy diet (Table 1) and 20 pens were fed a diet containing 20% DDGS (Table 2). All phases of feeds were mixed at a central feed mill prior to delivery to the facility. Ractopamine was included during phase IV (220-market) of both control and DDGS diets. Feed was augured in from the main outside feed tanks (separate tanks were used for each treatment feed) into the equipment room, where it was pre-weighed in batch form via the Howema mixer prior to its distribution to the individual pen. Feed was issued three times per day and feed issue amount at each feeding was recorded via the computerized feed system. Feed was offered *ad libitum* throughout the study. Feed weigh backs were taken each time pigs were weighed in order to calculate interim performance.

C. Growth Performance

Pen body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F) were calculated on days 20, 38, 70, 89, 95, 109 and 116. Finished hogs were marketing in three cuts on days 95, 109 and 116. The heaviest hogs, with appropriate finish, were marketed first removing 4, 8 and 12 head per pen in the first, second and third marketing cuts, respectively. For block 1, pen density improved from 6.91 sq. ft. per pig to 8.44 sq. ft. after first cut and to10.86 sq. ft. after the second cut. For block 2, pen density improved from 6.91 sq. ft. per pig to 8.00 sq. ft. after first cut and to 10.13 sq. ft. after the second cut.

D. Sample Collection

At each marketing cut, hogs were transported to Farmland Foods, Inc. in Milan, Missouri where hogs were humanly slaughtered following standard U.S. pork industry practices and USDA/FSIS inspection criteria. Jowl and belly tissue samples were collected from chilled carcasses. Belly samples were taken from a region on the midline posterior to the sternum and anterior to any mammary tissue and jowl samples were taken from the anterior tip of the jowl at the site of the head removal. Samples were sealed in Ziplock® bags and transported to the University of Missouri Meat Science Laboratory where samples were labeled and frozen until sample analysis.

E. Sample analysis

i. Fatty Acid Analysis

All samples were separated from any muscle, skin and/or lymph gland tissue and ground prior to fatty acid analysis. Fatty acid profiles were determined according to modified methodologies by Folch *et al*. (1957) and Morrison and Smith (1964). Approximately 100 mg of adipose tissue was homogenized in chloroform:methanol ($CHCl_3$:CH_3OH, 2:1, v/v) in a glass tube to extract lipids. Dehydrated samples were filtered through a sintered glass funnel fitted with a Whatman 2.4 cm GF/C filter.

A volume of 8 ml of 0.74% KCl was added to each sample and after two hours, two distinct layers formed. The upper phase was removed and discarded while the lower phase was evaporated to dryness with nitrogen in a water bath. At the point of dryness, 1 ml of 0.5 N KOH was added to each tube and heated for 10 min. in a 70°C water bath. The addition of KOH initiates the saponification reaction, which hydrolyzes fatty acids from a triglyceride molecule. Following this, 1 ml of 14% BF_3 in MeOH was added, samples were flushed with nitrogen and heated in the water bath for 30 min. Boron trifluoride is highly volatile and acts as an acid catalyst in the transesterification reaction that methylates the acid group on free fatty acids removing the net negative charge. The remaining molecule is known as a fatty acid methyl ester (FAME).

FAMEs are liquefied by adding 2 ml of HPLC grade hexane and 2 ml of NaCl. Two distinct layers are formed; the upper layer is removed and added to ~800 mg of Na_2SO_4 to remove any moisture in the sample. At this point, 2 more ml of hexane was added to the tube containing NaCl and once more, the upper layer was removed and added to the tube containing Na_2SO_4. The hexane portion was removed from the salt and added to a labeled scintillation vial. The salt was rinsed once more with 1 ml of hexane and the liquid was added to the vial. Samples were evaporated to dryness in a water bath at 70°C under nitrogen flow. Lastly, samples were reconstituted with 1 ml HPLC grade hexane and transferred to gas chromatograph vials.

The stable FAMEs were loaded into a Varian 3,800 gas chromatographer (Varion, Pala Alto, CA) to determine fatty acid profiles. The column utilized was a fused silica capillary column (SPTM – 2,560; 100 m x 0.25 mm x 0.2 µm film thickness; Supelco, Bellefonte, PA). Temperature of the injector was held constant at 240°C and temperature of the flame-ionizer detector was held at 260°C. The oven operated at 140°C for 5 min (temperature programmed 2.5°C/min to 240°C and held for 16 min). Helium, the carrier gas, was maintained at

a constant pressure of 37 psi. Individual fatty acids were expressed as a percentage of the total area under the peaks.

Total saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acid (PUFA) contents were calculated according to the following equations: SFA = (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0); MUFA = (C14:1 + C15:1 + C16:1 + C17:1 + C18:1n9t + C18:1n9c + C18:1n7 + C20:1 + C22:1n9 + C24:1); PUFA = (C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C18:9c11t + C18:10t12c + C18:9c11c + C18:9t11t + C20:2 + C20:3n6 + C20:3n3 + C20:4n6 + C22:5n3 + C22:6n3). The ratio between PUFAs and SFAs was calculated using the equation: [(C18:2n6c) + (C18:3n3)]/[(C14:0 + C16:0 + C18:0)]. The following equations were used to calculated total omega 3 and omega 6 fatty acid content: total omega 3 = C18:3n3 + C20:3n3 + C22:5n3 + C22:6n3); total omega 6 = (C18:3n6 + C20:3n6 + C20:4n6). Finally, IV from fatty acid profiles were determined according to the equation described by AOCS (1998): IV = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1).

ii. Iodine value titrations

Iodine value titrations were performed according to a modified WIJS method (AOAC, 1984). Adipose tissue was melted and approximately 0.6-0.8 g were place in a 500 ml Erlenmeyer flask and dissolved in 15 ml of 1:1 cyclohexane:acetic acid. Samples were incubated for 30 minutes in 25 ml of WIJS (iodine) solution, which provided iodine molecules to bind double bonds of unsaturated fats. After the incubation period, the reaction was stopped with 150 ml of water and 15 ml of 15% potassium iodine was added to bind with any free iodine from the WIJS solution that did not bind double bonds in the adipose tissue. Samples were titrated with sodium thiosulfate and the volume needed to induce color changes was used to calculate iodine value.

iii. Iodine value by near infrared imaging

Rapid NIR determination of IV was performed using a Bruker® NIR system currently utilized by Farmland Foods, Inc. (Milan, MO) for in-plant measurement of the belly fat depot. The NIR equipment was used according to manufacturers recommended operating procedures for this application.

iv. Raman Spectral Analysis of Iodine Value

The experiment was conducted with fat samples obtained from 832 pigs. As to C-18 FA standards, stearic acid and linoleic acid were purchased from Sigma company (St. Louis, MI), while oleic acid and linolenic acids were purchased from Matreya company (Pleasant, PA).

Pork fat samples were melted at 50 ºC under control atmosphere (N_2). After filtering out the solids, the liquefied fat samples were stored at 4 ºC for 30 minutes for them to re-solidify. These fat samples were considered as homogeneous, and were subjected to subsequent analysis. Five small pieces of fat with 3 mm thickness were cut from each homogeneous fat sample and stored in -20ºC separately before measurement. Raman measurements were performed using a DXR Raman Microscope (14 mW, Thermo Electron, Madison, WI.) under 780nm diode excitation laser at ambient temperature (20-23ºC). Raman signals were collected with 10 s exposure time from 500 to 3100 cm-1 which contain the C-C, C=C, C-O, and C=O stretches and the C-H bends at a resolution of 1 cm-1. Five replicate spectra were obtained for every sample. The pork fat samples were placed directly at the focus of the laser beam without pretreatment. A total of 20,750 spectra were recorded.

The average spectrum of each sample was calculated from 5 replicated measurements of the five sub-samples cut at different spots of the same sample-layer. The data were analyzed using Omnic Software Suite (Thermo Electron, Madison, WI). All spectra were baseline corrected, smoothed, normalized and mean-centered, after conducting a fluorescence-removal procedure. LS-SVM modeling was performed using the LS-SVM ver 1.5 toolbox in Matlab (R2010a, The Mathworks Inc., Natick, MA, USA).

The LS-SVM is an optimized algorithm based on the standard SVM. The LS-SVM has the capability for both linear and nonlinear multivariate calibration, and it solves multivariate calibration problems in a relatively fast way. It has been widely used in fields of multivariate statistical analysis. To construct a predictive model for the determination of the iodine values and the fatty acids profiles, we first looked at the full spectra ranging from 500 cm^{-1} nm to 3100 cm^{-1}. The Raman intensity data at 13 peaks (Table 14) were applied as inputs to LS-SVM models to correlate to the iodine values and levels of three major fatty acids (C18:1, C18:2, C18:3). The prediction performance of the models was evaluated by correlation coefficient for calibration set (Rc) and correlation coefficient for validation set (Rp). The MSE (the average squared difference between the predicted

outputs and the targets) for calibration set and validation set were also determined to evaluate the performance of the predictive model.

F. Statistical Analysis

The experiment was defined as a 2 x 3 factorial arrangement. Growth performance data was analyzed using the PROC MIXED procedure of SAS (SAS Inst., Cary, NC) with pen serving as the experimental unit. Iodine values were analyzed using the PROC GLM procedure of SAS and correlations between iodine determination methods were calculated with PROC CORR procedure of SAS. The statistical model included the fixed effects of marketing cuts (95, 109 or 116 days on feed) and dietary treatment (control corn and soy diet or corn and soy with 20% DDGS). Fixed effects were arranged as factorials within a completely randomized design. Least squares means and standard errors were estimated. Level of significance was predetermined at $P < 0.05$.

VIII. Results

Growth Performance

Growth performance, shown in Table 3, was not significantly impacted by the inclusion of 20% DDGS in the diet. No difference was detected in final BW, ADG, ADFI or G:F between pigs fed control and diets fed 20% DDGS. However, final BW, ADG and ADFI were significantly impacted by marketing cut ($P < 0.0001$). The most notable difference, however, is the increase of ADG after the first marketing cut was removed from the barn. The pigs in the second marketing cut show a clear advantage in gain per day.

Fatty Acid Composition

The fatty acid composition of belly fat is presented in Table 4. Pigs fed the control diet had significantly higher levels of 16:0 (palmitic acid), 16:1 (palmitoleic acid), 18:0 (stearic acid) and 18:1 (oleic acid) in belly fat. The levels of 18:2n6c (linoleic acid), 18:3n3 (linolenic acid) and 20:4n6 (arachidonic acid) were significantly higher in the belly fat of pigs fed 20% DDGS. Overall, the belly fat of pigs fed the control diet had significantly higher levels of total SFA and MUFAs ($P < 0.0001$). Belly fat from pigs fed 20% DDGS had significantly higher levels of PUFAs ($P < 0.0001$), total n3 ($P = 0.02$) and total n6 ($P = 0.01$) as well as a higher PUFA:SFA ($P < 0.0001$).

In the present study, similar trends in the fatty acid composition are observed in jowl fat when compared to belly fat (Table 5). Jowl fat from pigs fed the control diet had significantly higher levels of 16:0, 16:1, 18:0 and 18:1. The levels of 18:2n6c, 18:3n3 and 20:4n6 were significantly higher in the jowl fat of pigs fed 20% DDGS. Feeding DDGS significantly increased PUFAs, total n3, total n6 and the PUFA:SFA of jowl fat while control diet samples had significantly higher SFAs and MUFAs.

Iodine Values

The iodine value of fat removed from the belly depot was determined via three methods and the values are presented in Table 6. Inclusion of 20% DDGS significantly increase iodine value of belly fat when obtained from titration ($P = 0.0004$), GC calculation ($P < 0.0001$) and NIR ($P < 0.0001$). Similar to the belly depot, the iodine value of jowl fat significantly increased with 20% DDGS in the diet when iodine value is found via titration ($P = 0.0004$) or GC calculation ($P < 0.0001$) (Table 7). When comparing belly to jowl fat, the iodine value of belly fat is significantly ($P < 0.0001$) lower regardless of what method is used to calculate iodine value (Table 8).

Methodology Correlation

Overall, moderate correlations exist between methods for determining iodine value (Table 9). Titration and NIR have the highest significant ($P < 0.001$) correlation of 0.68. However, when correlations are broken down by fat depot and diet, associations become less significant. In Table 10, the correlation of iodine values of belly fat from control diet pigs, the GC and NIR iodine values have the strongest correlation at 0.45, followed by titration and NIR at 0.44 and titration and GC at 0.19. Correlations of belly fat from pigs fed DDGS are the strongest between titration and NIR at 0.53 (Table 11). The weakest correlations between methods occur amongst jowl fat samples (Tables 12 and 13); in control diets, titration and CG IV have a 0.32 correlation, but in DDGS pigs, the correlation is 0.19.

The Raman Spectral Characteristics of Pork Fats

Raman spectra of typical pork fat samples are shown in Fig. 1, Raman bands observed in pork fat at 1750, 1660, 1441 and 1302cm-1 were assigned to the C=O stretching modes, C=C stretching modes, CH2 scissoring modes and CH2 twisting modes, respectively. The wavenumbers of the C=C stretching bands of

unsaturated fatty acids are very sensitive to the configuration around the C=C bond, trans and cis unsaturated fatty acids have the C=C stretching band in 1670–1680 cm-1 and 1650–1665 cm−1 regions respectively (Table 14).

In the oils and fats industry, classical methods based on wet chemistry or gas-chromatographic (GC) analysis are typically used to quantify cis and trans isomers and the total degree of unsaturation, whereas X-ray studies using single-crystal, powder diffraction and scattering, and differential scanning calorimetry have been used to provide detailed information on the polymorphism or arrangement of triacylglycerols and diacylglycerols. Raman spectroscopy now has the potential to replace or at least complement the classical, time-consuming methodologies, and thus could be used as rapid screening methods for quality control purposes and also for basic research on the factors affecting polymorphic transitions and stability.

Standard Calibration Curve to determine the number of carbon double bonds in a fat sample

C=C stretching (cis) mode is represented by a Raman band at ~1657 cm-1, the intensity of this band therefore is proportional to the number of (C=C)cis functional groups in a sample. A linear correlation was established between the intensity of the 1657 cm-1 band and the double bond counts. To generate a standard calibration curve, samples with different double bond counts were measured (C18 fatty acids, stearic acid, oleic acid, linoleic acid, and linolenic acid) and a linear correlation of peak intensity at 1657 cm-1 with Double Bond count is established, as illustrated in Fig. 2.

Prediction of Iodine values of pork fat samples using Raman Probe

Spectral data from 500 randomly selected samples were pooled as a training set to construct a LS-SMV model to correlate the 13 Raman peaks to the IV of the sample. The leave-one-out validation tests were conducted to evaluate the accuracy of the predictive model. The result was shown in fig.3. The model was very good, with a $R^2 = 0.9948$ between the predicted IV values, and GC-determined IV values.

The model was then used to calculate IV of 332 samples that were not included in the training set as an independent test. The results were shown in Fig.4. With a $R^2 = 0.9837$, a good linear correlation existed between the predicted IVs and the GC-determined IVs. The correlation line was overlapping almost perfectly with the P=E line, suggesting good agreement between predicted and measured IVs for the 332 samples.

Fig. 5 shows the error of prediction for the training set and the test set, respectively. The error of prediction is defined in equation 1.

$$\text{Error} = \frac{IV_{exp} - IV_{pred}}{IV_{exp}} \times 100\%$$

Where IV_{exp} is the experimental IV, IV_{pred} is the predicted IV.

For the training set, the maximum error is 1.53%, and the average error is 0.49%; for the test set, the maximum error is 2.15%, and the average error is 0.74%. As expected, the error of prediction is smaller for the training set. However, the small (<1%) error of prediction for the training set suggests that the predicted iodine values show no significant differences comparing to the measured Iodine values, and the Raman spectroscopic method is a good alternative to achieve rapid and replicable measurement of unsaturation levels in pork fat samples.

Differences between Iodine values of frozen and fresh samples

Experimentally, iodine values were determined for both fresh and frozen fat samples. Since the spectral acquisition was only conducted with frozen samples, it is of interest to see whether the spectral data can be used to also predict iodine values of fresh samples with reasonable accuracy. Fig. 6 shows the correlation between frozen and fresh sample IVs acquired by GC. It is obvious that the deviation in IVs between frozen and fresh samples is quite significant. Fig.7 shows the correlation between the predicted IVs (calculated using spectral data from frozen samples) and the measured IVs for fresh samples. The deviation was at the same level as that of the GC-measured values. Fig. 8 shows the error of prediction and difference between fresh/frozen GC IVs. Although the error of prediction is slightly larger for the spectral-based IVs, they are at the same level. It should be pointed out that the error of prediction is much larger for the fresh samples. The reason for the quite significant difference between IVs of frozen and fresh samples is not clear.

IX. Discussion

Growth performance was unaffected by the inclusion of DDGS in the diets of growing and finishing hogs. This finding agrees with studies by Dahlen *et al*. (2011), Widmar *et al*. (2008, 2007), Drescher *et al*. (2008) and Duttlinger *et al*. (2008) who reported inclusion of 20% DDGS in the diet did not negatively impact

initial or final BW, ADG, ADFI or gain efficiency. However, marketing time significantly influenced growth parameters, specifically ADG. The hogs marketed in the second cut have a clear advantage over counterparts in the first and third cuts. By removing the fast growing, early maturing hogs in the first cut, feeder and floor space expanded, allowing the remaining hogs to more closely meet their genetic potential. The genetically superior animals grew faster and gained more thereby surpassing the slow growing, late maturing hogs left in the third cut.

DDGS significantly increased the levels of PUFA and PUFA:SFA in belly and jowl fat. A cooperative study reported PUFA levels increased while SAT and MUFA decreased in subcutaneous fat with increasing (15%, 30% and 45%) levels DDGS in the diet (Cromwell *et al*., 2011). Additionally, Xu *et al*. (2010, 2008a) demonstrated PUFAs increased and SAT decreased in belly fat with increased levels of DDGS.

Iodine values, which measure the degree of unsaturation, directly reflect the fatty acid levels from fatty acid analysis. Pigs fed 20% DDGS had significantly higher (more unsaturated) IV when compared to control diet pigs. Whitney *et al*. (2006) and Benz *et al*. (2008) also reported GC calculated iodine value of belly fat increased with increasing levels of DDGS in the diet. In our study, all three IV determination methods revealed increased IV by the inclusion of DDGS in the diet. DDGS is a very highly concentrated source of protein and energy in the form of oil (unsaturated fat). The dietary fat is reflected in the fatty acid profiles as well as IV especially when pigs shift away from de novo fat synthesis and preferentially incorporate dietary fat.

Jowl fat consistently shows a higher iodine value, indicating jowl fat is physiologically more mature. As animals mature, they accumulate fat in areas such as the jowl and shoulder prior to areas such as the belly. Protein turnover slows and less de novo fatty acid synthesis occurs allowing for more dietary fat to accumulate in fat cells. Since de novo synthesis results in increased saturated fats, the slowing of this synthesis results in carcass fat that is closer to the fatty profile of the dietary constituents. In this case, the corn oil from DDGS is a likely contributor to the increased IV in fat depots displaying greater physiological maturity.

The iodine value of belly fat is not highly correlated between analysis methods therefore, drawing confident conclusions from the methods as a whole is difficult. The use of multiple determination methods may be applicable in the industry provided the methods are either correlated or rank samples the same. Yet, from the

current study we see methods are not highly correlated, nor do methods rank samples the same based on IV. These inconsistencies create issues when deciding which method to recommend for industry wide use. The basic scientist can use gas chromatography (GC) or iodine titration to obtain an IV. However, these methods are expensive, slow, and require specialized equipment that is not practical for application to pork processing facilities. Between the current "gold standards" of determining IV, the titration method is by far, the most time consuming and expensive method. Additionally, it was demonstrated that rapid, quantitative determination of unsaturation level in pork fat samples could be achieved using a Raman spectroscopic method, and good correlation between the Raman results and conventional iodine values was established.

From an industry perspective, control bellies have the most acceptable IV regardless of method of determination, the inclusion of DDGS increase IV regardless of depot. There is a low correlation between belly and jowl IV and using jowls would incorrectly sort carcasses into categories based iodine value. Finally, the Raman method implemented with a portable spectrometer could be incorporated into meat processing line for real time, onsite monitoring.

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Table 1. Composition of the grower-finisher control diets

	Phase I (70-120 lbs)	Phase II (120-170 lbs)	Phase III (170-220 lbs)	Phase IV (220-market)
SID Lysine, %	0.93	0.81	0.72	0.95
Corn	1503.97	1596.57	1660.47	1477.32
SBM 48	425.00	335.00	275.00	460.00
DDGS	0.00	0.00	0.00	0.00
CWG	20.00	20.00	20.00	20.00
Monocal	6.50	5.00	4.00	2.50
Limestone	24.00	23.75	22.00	21.00
Salt	10.00	10.00	10.00	10.00
L-Lysine	5.10	4.90	4.40	4.40
Alimet	0.85	0.20	0.00	0.65
L-Threonine	1.40	1.40	0.95	2.03
Vitamin Premix + Phytase	1.00	1.00	1.00	0.60
Trace Mineral Premix	2.00	2.00	2.00	0.80
Optiphos	0.18	0.18	0.18	0.20
Paylean	0.00	0.00	0.00	0.50
	2000.00	2000.00	2000.00	2000.00
NRC ME (Mcal/lb)	1.53	1.54	1.54	1.54
SID, Lysine %	0.93	0.81	0.72	0.95
Available P, %	0.28	0.26	0.25	0.20
Ca, %	0.58	0.55	0.50	0.50
SID M + C:Lys	58.25	58.24	60.88	58.13
SID Thr:Lys	17.42	65.21	65.07	68.10
SID Ile:Lys	63.55	63.74	65.21	65.60
SID Val:Lys	73.00	74.73	77.76	74.88

Table 2. Composition of the grower-finisher diets containing 20% DDGS

	Phase I (70-120 lbs)	Phase II (120-170 lbs)	Phase III (170-220 lbs)	Phase IV (220-market)
SID Lysine, %	0.92	0.80	0.71	0.95
Corn	1187.07	1275.47	1337.02	1162.42
SBM 48	350.00	258.00	200.00	375.00
DDGS	400.00	400.00	400.00	400.00
CWG	20.00	20.00	20.00	20.00
Monocal	0.00	0.00	0.00	0.00
Limestone	22.50	26.50	24.00	22.50
Salt	10.00	10.00	10.00	10.00
L-Lysine	6.10	6.00	5.40	6.00
Alimet	0.35	0.00	0.00	0.43
L-Threonine	0.80	0.85	0.40	1.75
Vitamin Premix + Phytase	1.00	1.00	1.00	0.60
Trace Mineral Premix	2.00	2.00	2.00	0.80
Optiphos	0.18	0.18	0.18	0.00
Paylean	0.00	0.00	0.00	0.50
	2000.00	2000.00	2000.00	2000.00
NRC ME (Mcal/lb)	1.52	1.52	1.52	1.52
SID, Lysine %	0.92	0.80	0.71	0.95
Available P, %	0.34	0.34	0.33	0.27
Ca, %	0.49	0.55	0.50	0.50
SID M + C:Lys	58.18	59.51	63.85	58.10
SID Thr:Lys	17.51	65.06	65.22	68.12
SID Ile:Lys	66.56	66.83	69.11	66.84
SID Val:Lys	78.52	80.68	84.95	78.43

Table 3. Growth performance least squares means of hogs fed 20% DDGS and marketed in three cuts

	Control			20% DDGS				P-Value		
Item	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut	SEM	Diet	Cut	D x C[1]
Final BW, kg	115.03	128.94	131.11	114.56	128.94	131.40	0.89	0.87	<0.0001	0.93
ADG, kg	0.79	1.11	0.88	0.78	1.16	0.87	0.03	0.83	<0.0001	0.73
ADFI, kg	2.01	2.80	3.03	2.02	2.97	3.12	0.05	0.08	<0.0001	0.22
G:F	0.39	0.39	0.29	0.39	0.39	0.28	0.12	0.12	<0.0001	0.99

[1] D x C = Diet by marketing cut interaction

Table 4. Interactive effects of 20% DDGS in the diet and marketing time on fatty acid (FA) composition of belly fat

	Control			20% DDGS				P-Value		
Item	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut	SEM	Diet	Cut	D x C[1]
Palmitic Acid (16:0)	23.53	24.17	23.52	22.60	23.30	22.50	0.28	<.0001	0.18	0.76
Palmitoleic Acid (16:1)	2.25	2.34	2.67	2.05	2.07	1.98	0.06	<.0001	0.66	0.62
Stearic Acid (18:0)	11.84	12.00	11.86	10.74	11.05	10.83	0.23	<.0001	0.14	0.98
Oleic Acid (18:1n9t)	0.12	0.20	0.39	0.11	0.13	0.09	0.09	0.02	0.15	0.04
Oleic Acid (18:1n9c)	40.14	41.61	40.56	38.22	40.7	38.03	0.40	<.0001	<.0001	0.06
Linoleic Acid (18:2n6t)	0.10	0.08	0.08	0.08	0.08	0.07	0.01	0.003	0.007	0.40
Linoleic Acid (18:2n6c)	14.06	13.78	14.09	18.41	18.58	19.35	0.52	<.0001	0.78	0.75
Linolenic Acid (18:3n3)	0.57	0.60	0.71	0.63	0.60	0.67	0.04	0.05	<.0001	0.30
Arachidonic Acid (20:4n6)	0.07	0.23	0.24	0.07	0.25	0.28	0.01	<.0001	<.0001	0.13
Total SFA	37.81	38.25	37.43	35.73	36.57	35.35	0.48	<.0001	0.62	0.89
Total MUFA	46.10	46.75	45.90	43.67	44.52	42.73	0.46	<.0001	0.44	0.56
Total PUFA	15.48	14.23	15.98	20.00	17.72	21.32	0.61	<.0001	0.23	0.76
PUFA:SFA	0.40	0.37	0.40	0.55	0.50	0.59	0.02	<.0001	0.40	0.86
Total n-3 FA	0.59	0.65	0.75	0.65	0.68	0.72	0.02	0.02	<.0001	0.20
Total n-6 FA	0.08	0.54	0.61	0.08	0.83	0.72	0.07	0.01	<.0001	0.09

[1] D x C = Diet by marketing cut interaction

Table 5. Interactive effects of 20% DDGS in the diet and marketing time on fatty acid (FA) composition of jowl fat

Item	Control			20% DDGS			SEM	P-Value		
	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut		Diet	Cut	D x C[1]
Palmitic Acid (16:0)	21.91	21.95	22.30	20.89	20.72	21.64	0.28	<.0001	0.18	0.76
Palmitoleic Acid (16:1)	2.25	2.21	2.19	2.02	1.94	2.03	0.06	<.0001	0.66	0.62
Stearic Acid (18:0)	10.38	10.41	10.84	9.62	9.48	10.09	0.23	<.0001	0.14	0.98
Oleic Acid (18:1n9t)	0.13	0.13	ND[2]	0.16	0.12	ND[2]	0.09	0.02	0.15	0.04
Oleic Acid (18:1n9c)	41.20	41.44	42.20	39.24	39.58	40.01	0.40	<.0001	<.0001	0.06
Linoleic Acid (18:2n6t)	0.06	0.06	0.08	0.07	0.05	0.07	0.01	0.003	0.007	0.40
Linoleic Acid (18:2n6c)	15.90	15.67	15.97	20.02	20.26	19.60	0.52	<.0001	0.78	0.75
Linolenic Acid (18:3n3)	0.65	0.96	0.71	0.71	1.04	0.74	0.04	0.05	<.0001	0.30
Arachidonic Acid (20:4n6)	0.10	0.26	0.23	0.11	0.28	0.25	0.01	<.0001	<.0001	0.13
Total SFA	34.61	34.41	35.34	32.76	32.11	33.87	0.48	<.0001	0.62	0.89
Total MUFA	47.02	46.75	47.53	44.56	44.25	45.01	0.46	<.0001	0.44	0.56
Total PUFA	17.63	17.97	16.48	21.99	22.86	20.44	0.61	<.0001	0.23	0.76
PUFA:SFA	0.50	0.50	0.45	0.66	0.68	0.60	0.02	<.0001	0.40	0.86
Total n-3 FA	0.67	1.08	0.84	0.74	1.20	0.87	0.02	0.02	<.0001	0.20
Total n-6 FA	0.16	0.45	0.41	0.17	0.50	0.44	0.07	0.01	<.0001	0.09

[1] D x C = Diet by marketing cut interaction

[2] Limits undetectable

Table 6. Least squares means for iodine values of belly fat determined by three different methods of analysis.

	Control			20% DDGS				P-Value		
Item[a]	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut	SEM	Diet	Cut	D x C[4]
Titration[1]	66.96[d]	66.91[d]	67.05[d]	73.47[b]	70.62[b]	68.93[c]	1.55	0.0004	0.11	0.50
GC[2]	63.01[d]	60.82[d]	66.33[b]	68.85[b]	61.33[c]	67.72[b]	1.52	<0.0001	0.14	0.13
NIR[3]	65.64[c]	67.89[e]	67.50[e]	71.10[c]	70.91[c]	73.16[b]	0.67	<0.0001	0.01	0.08

[a] LS means with in a row with similar superscripts do not differ at P<0.05.

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

[3] Iodine value calculated from NIR spectroscopy

[4] D x C = Diet by cut interaction

Table 7. Least squares means for iodine values of jowl fat determined by two different methods of analysis.

	Control			20% DDGS				P-Value		
Item[a]	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut	SEM	Diet	Cut	D x C[4]
Titration[1]	74.04[b]	70.47[c]	71.96[b,c]	74.49[b]	75.14[b]	73.44[b,c]	1.55	0.0004	0.11	0.50
GC[2]	67.29[d]	69.21[d]	69.59[d]	72.54[b]	75.31[b]	71.50[c]	1.52	<0.0001	0.14	0.13

[a] LS means with in a row with similar superscripts do not differ at P<0.05.

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

[4] D x C = Diet by marketing cut interaction

Table 8. Least squares means for iodine values of belly and jowl fat independent of diet

Item	Belly	Jowl	SEM	P-Value
Titration[1]	68.99	73.26	0.55	<0.0001
GC[2]	64.67	70.91	0.54	<0.0001

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

Table 9. Pearson Correlation Coefficients and P-values for three measures of iodine value

	Titration[1]	**GC[2]**	**NIR[3]**
Titration[1]	1.00	0.462	0.681
	-	<.0001	<.0001
GC[2]	-	1.00	0.431
	-	-	<.0001
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

[3] Iodine value calculated from NIR spectroscopy

Table 10. Pearson Correlation Coefficients and P-values for three measures of iodine value in belly fat of pigs fed a control diet

	Titration[1]	**GC[2]**	**NIR[3]**
Titration[1]	1.00	0.193	0.448
	-	0.065	<.0001
GC[2]	-	1.00	0.457
	-	-	<.0001
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

[3] Iodine value calculated from NIR spectroscopy

Table 11. Pearson Correlation Coefficients and P-values for three measures of iodine value of belly fat fed 20% DDGS

	Titration[1]	**GC[2]**	**NIR[3]**
Titration[1]	1.00	0.355	0.533
	-	0.0005	<.0001
GC[2]	-	1.00	0.321
	-	-	0.0027
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine values calculated from AOAC (1984) accepted titration method

[2] Iodine values calculated from fatty acid profile generated by GC

[3] Iodine value calculated from NIR spectroscopy

Table 12. Pearson Correlation Coefficients and P-values for two measures of iodine value in jowl fat of pigs fed a control diet.

	Titration[1]	**GC[2]**
Titration[1]	1.00	0.320
	-	0.002
GC[2]	-	1.00
	-	-

[1] Iodine values calculated from AOAC (1984) accepted titration method
[2] Iodine values calculated from fatty acid profile generated by GC

Table 13. Pearson Correlation Coefficients and P-values for two measures of measures iodine value of jowl fat from pigs fed 20% DDGS.

	Titration[1]	**GC[2]**
Titration[1]	1.00	0.195
	-	0.062
GC[2]	-	1.00
	-	-

[1] Iodine values calculated from AOAC (1984) accepted titration method
[2] Iodine values calculated from fatty acid profile generated by GC

Table 14. Band assignment in the Raman spectrum of pork fat		
Peak #	**Raman Shift (cm^{-1})**	**Vibrational mode**
1	3000-3016	Asymmetric =C–H stretch
2	2925-2940	Asymmetric CH_2 stretch
3	2900-2920	Symmetric CH_3 stretch
4	2840-2860	Symmetric CH2 stretch
5	1730-1750	C=O stretch
6	1650-1665	*cis* C=C stretch
	1670-1680	*trans* C=C stretch
7	1430-1460	CH_2 scissoring
8	1295-1305	CH_2 twist
9	1250-1280	Symmetric =C–H *cis* in-plane hydrogen bend
10	1100-1135	$(C\text{-}C)_{ip}$ in phase aliphatic C-C stretch all *trans*
11	1080-1090	$(C\text{-}C)_{g}$ liquid, aliphatic C-C stretch in *gauche*
12	1060-1065	$(C\text{-}C)_{op}$ out of phase aliphatic C-C stretch all *trans*
13	800-920	(C-C), (C-O) stretch

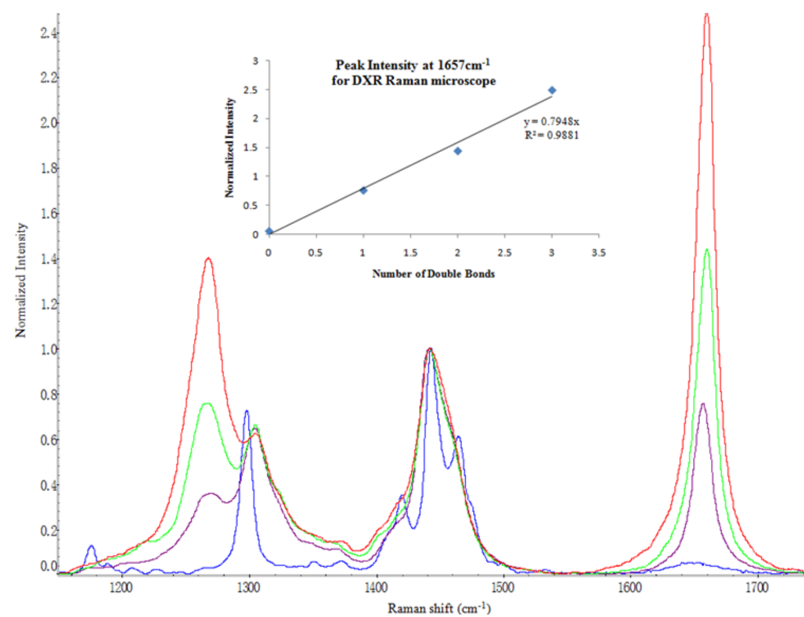

Fig. 1. Raman Spectra of stearic acid(blue), oleic acid(purple), linoleic acid(green), and linolenic acid(red) Inset: Calibration curve of –C=C- count at 1657 cm-1.

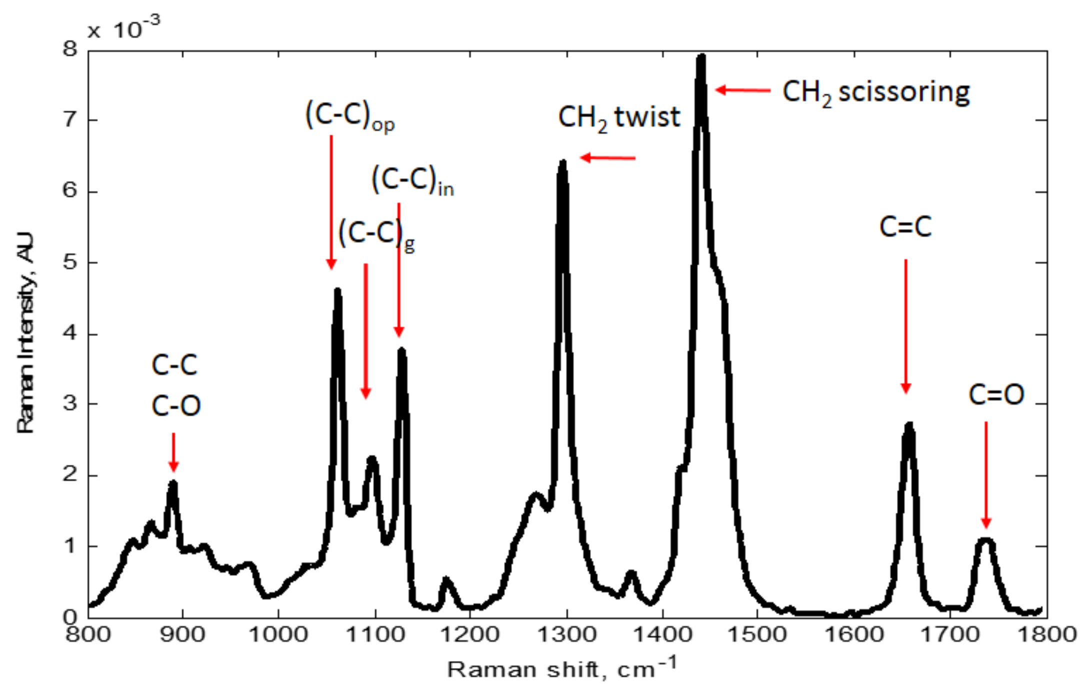

Fig. 2 Typical Raman spectrum of pork fat (IV = 57.34)

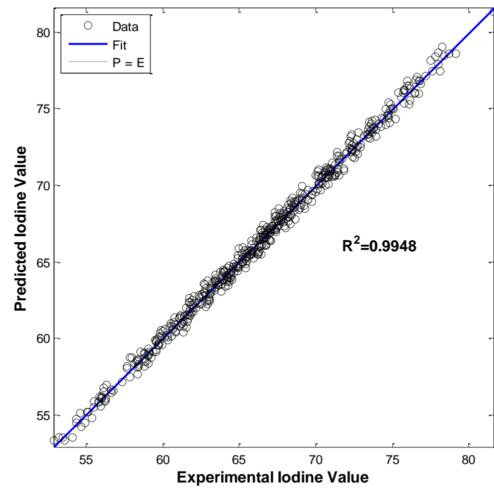

Fig. 3 Predicted Iodine value vs. experimental iodine value for the test set (500 samples)

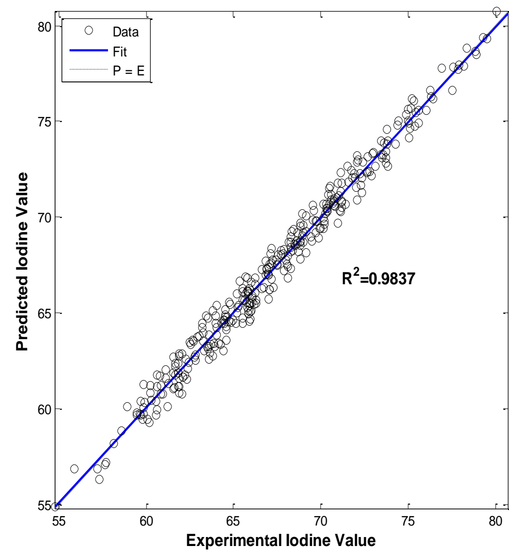

Fig. 4 Predicted Iodine value vs. experimental iodine value for the test set (332 samples)

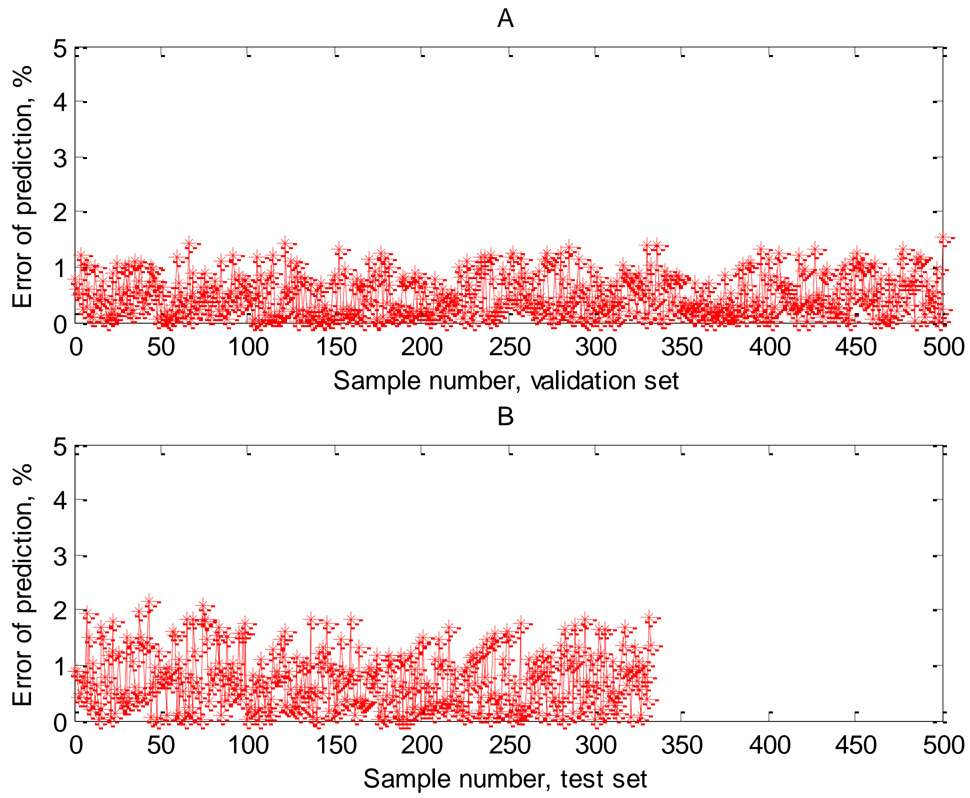

Fig. 5 Error of prediction. A. training set, 500 frozen samples; B. Test set, 332 frozen samples.

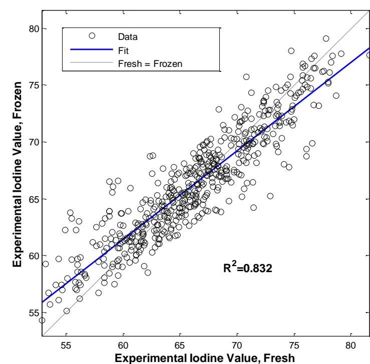

Fig. 6 Fresh vs. Frozen Iodine value, determined by GC, validation set

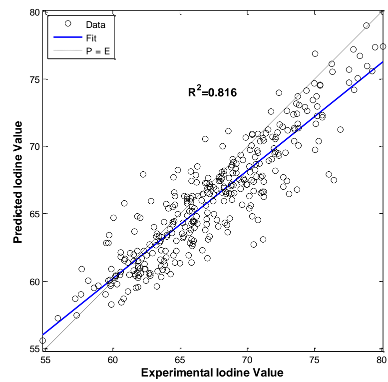

Fig. 7 Predicting Iodine value of fresh fat from predictive model built with Raman spectral data acquired from frozen fat samples.

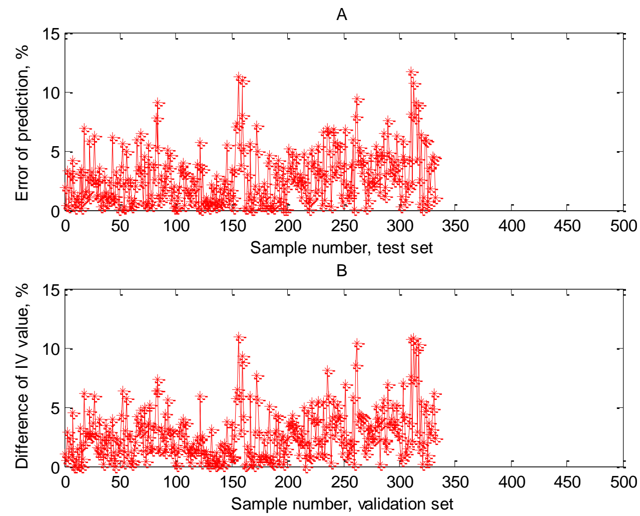

Fig. 8 Error of prediction. A= Raman IVs vs. GC IVs of fresh sample; Maximum = 11.66%, Mean = 2.79%; B=GC IVs of frozen sample vs. GC IVs of fresh sample, Maximum = 10.95%, Mean = 2.73%.