RESEARCH REPORT
PORK QUALITY

Title: Evaluating rapid evaporative ionization mass spectrometry (REIMS) as a real-time method for classifying pork quality in bacon and fresh pork bellies – **NPB #19-180**

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Industry Summary:

Rapid evaporative ionization mass spectrometry (REIMS) is a novel, *in-situ* analysis technology that measures the metabolomic profile in real time. The objective of this study was to evaluate REIMS as a real-time predictor of pork belly quality and fat composition to subsequently send market signals and value hogs based on objective measurements and ultimately improve the composition and quality of pork products. REIMS proved to be an effective technology in segregating individual pork bellies based on bacon yields, cook loss, and fatty acid composition. The use of metabolomic profiles collected in real-time and *in-situ* using REIMS characterized differences in degree of saturation of fat, which in turn, translated to higher quality bellies with a greater proportion of premium #1 slices. More remarkably, using the crudest and most conservative model building approach, REIMS was able to classify a test set of

samples into the predetermined quality groups with a high degree of accuracy. This study showed the ability of REIMS to accurately predict pork belly quality and fat composition to subsequently send market signals and value hogs based on objective measurements to ultimately improve the composition and quality of pork products.

Key Findings:

1. REIMS data were used to categorize individual samples into "Excellent", "Great", "Good", and "Poor" quality groups.
2. The samples grouped by REIMS had meaningful differences in overall fat quality and bacon performance.
3. The use of metabolomic profiles collected in real-time and *in-situ* using REIMS characterized differences in degree of saturation of fat, which in turn, translated to higher quality bellies with a greater proportion of premium #1 slices.
4. Using the crudest and most conservative model building approach, REIMS was able to classify a test set of samples into the predetermined quality groups with a high degree of accuracy.

Keywords: pork, bacon, REIMS, yield, fatty acid

Scientific Abstract:

Pork belly firmness, slice yield, smoke yield, and bacon cooking performance are all major considerations for overall belly quality, and, to date, there is no technology that exists to objectively measure these things simultaneously. Lab-based tests used to measure some of these belly characteristics are laborious, destructive, and take a considerable amount of time to complete. Rapid evaporative ionization mass spectrometry (REIMS) is a novel, *in-situ* analysis

technology that measures the metabolomic profile in real time. The objective of this study was to evaluate REIMS as a real-time predictor of pork belly quality and fat composition to subsequently send market signals and value hogs based on objective measurements and ultimately improve the composition and quality of pork products. Pork bellies (N=391) from pigs fed 3 diets with varying levels of predicted iodine value (IVP) were analyzed for bacon yield, fat shattering, slice distortion, cook loss, fatty acid composition, iodine value, and REIMS. The 3 diets altered bacon yield, fatty acid composition and calculated IV of pork bellies, ultimately creating a diverse population for the evaluation of the metabolomic profile as acquired by REIMS. Canonical correlations of fatty acids and REIMS mass bins were used to create a model, and scores derived from REIMS mass bins were used to categorize individual samples into "Excellent", "Great", "Good", and "Poor" quality groups. Those groups exhibited differences in proportion of #1 slices and #2 slices ($P<0.01$), shatter score($P<0.01$), and degree of saturation as described by the proportion of saturated fatty acids (SFA; $P<0.01$), monounsaturated fatty acids (MUFA; $P<0.01$), and polyunsaturated fatty acids (PUFA; $P<0.01$) as well as calculated iodine value (IV; $P<0.01$). More notably, the model trained from the metabolomic profile was able to categorize a test set of data into the same quality groups with an overall accuracy of 70% and only 1 individual being misclassified by greater than 1 quality group difference (0.003%). These data suggest the ability of REIMS to improve the composition and quality of pork products by sending market signals based on objective measurements of product quality and composition resulting from feeding practices and genetics.

INTRODUCTION

Pork belly firmness, slice yield, smoke yield, and bacon cooking performance are all major considerations for overall belly quality and value, and, to date, there is no technology that exists to objectively measure them simultaneously and in real-time. Many rely on lab-based tests, such as iodine values or fatty acid profiles, to measure some of these belly characteristics, but these tests are laborious, destructive, and take a considerable amount of time to complete. An instrument that could assess each of these factors *in situ* in an online application, would allow for the industry to more appropriately assess the value of individual animals or lots of animals so that they could be valued and purchased on the basis of objective quality measurements. Additionally, products could be sorted into quality classifications prior to fabrication and processing procedures. For several decades, the United States pork industry has evaluated opportunities to increase production efficiency, consumer demand, product quality and consistency. Producers and packers have also pushed for new technology that could objectively assess pork quality factors. This information could result in new avenues to increase value in the pork industry, such as opportunities for producers to receive premiums in return for meeting certain quality standards, However, these industry-changing possibilities depend on the introduction of a technology that could provide instant, objective feedback. Rapid Evaporative Ionization Mass Spectrometry (REIMS) is a novel technology that is currently used in the human medicine and biological science fields and has immense potential to be implemented in the pork industry.

Using time-of-flight (TOF) mass spectrometry, REIMS profiling provides *in situ*, real-time molecularly resolved information by ionizing biological samples without any sample preparation. Sample acquisition generally takes only a few seconds and can provide histological

tissue identification with 90 to 98% correct classification performance (Balog et al., 2013). Recent studies in species authentication, eating quality prediction, and residue testing have shown that REIMS can provide promising results when analyzing meat products (Balog et al., 2013; Guitton et al., 2018; Verplanken et al., 2017). Verplanken et al. (2017) even successfully segregated pork carcasses with and without boar taint, further indicating potential of REIMS in meat eating quality prediction. Guitton et al. (2018) successfully identified several porcine muscles from animals fed ractopamine with accuracies greater than 95%. A hand-held iKnife sampling device, developed by Waters Corporation (Wilmslow, UK), allows for tremendous mobility in the sampling procedure. With respect to the pork industry, for the first time, this technology could allow for meat quality attributes such as functionality, composition, flavor, tenderness, and freshness to be measured and characterized in real time. Unlike other metabolomic approaches that require tedious sample preparation and analysis times, this technology could be further developed as an on-line system in the processing environment to enable meaningful sorting of pork products into categories reflecting tangible differences in processing and eating characteristics.

The pork industry has not yet found a real-time, objective measure to quantify pork quality that could provide meaningful industry feedback. Accordingly, the inability to measure variation in carcass fat composition and the functionality of pork products used for further processing, such as bellies, results in inconsistencies in product performance and quality. These quality concerns increase economic losses associated with fabrication difficulties, reduce product yields, and decrease consumer acceptability (Gatlin et al., 2002). Many quality concerns can be attributed to diet and overfeeding highly unsaturated feed ingredients, such as distillers dried grains (DDGS), which influences fatty acid composition and can result in soft bellies. At

processing, it is believed that softer bellies possess slicing integrity problems when commercial, high-speed slicers are used and the blade fails to make clean individual slices (McClelland et al., 2012). As bacon represents a significant portion of the processed meat products in the retail case and is prominently used in restaurants (Wright et al., 2005), research to assess bacon and fat quality has value. While laboratory derived measurements, such as iodine values (IV), are being used to assess the fatty acid composition and firmness of fat, they are not efficient enough to identify these traits at production speeds. Thus, the ability to further provide market signals via premiums or discounts for desired fat composition does not currently exist without extensive investment in time and resources. The objective of this study was to evaluate REIMS to be developed as a real-time predictor of pork belly quality and fat composition, and to provide the opportunity for the pork industry to send market signals and value hogs based on objective measurements, ultimately improving the composition and quality of pork products.

Objectives:

1. Explore the ability of REIMS as a real time predictor of pork belly quality and pork fat composition.
2. Develop REIMS as a technology to send market signals and value hogs based on objective measurements of product quality and composition resulting from feeding practices and genetics.
3. Evaluate the ability of REIMS to be developed as a tool to improve the composition and quality of pork products.

MATERIALS & METHODS

Institutional Animal Care and Use Committee approval was not required for this study as samples were obtained from federally inspected harvest facilities in which humane handling and harvest practices were implemented and monitored.

Animals and Diets

Approximately 3,000 crossbred barrows and gilts (PIC Cambrough females × DNA 600 sire lines, Pig Improvement Company, Gowrie, IA) started 1 of 3 finishing diets at a mean initial body weight body weight of 63.5 kg. Each diet was comprised of 4 phases appropriate for meeting the nutritional requirements of the pigs for the stage of maturity within that feeding period (Reese et al., 2000). The duration that pigs were fed in phases 1, 2, and 3 was 16, 14, and 20 days, respectively. Phase 4 continued until the last day in the feeding period, lasting approximately 14 days, corresponding to three finishing sorts in which the first 1/3 of the pigs to reach approximate market weight were pulled to kill before those that reached approximate market weight later. Diets, formulated to a predicted iodine value (IVP), were used to produce a diverse sample population of differences in bacon yield, belly composition and metabolomic profile. Major components of the diet impacting IVP were relative proportions of corn, DDGS, soybean meal and added corn oil. Diet A was formulated to an average IVP of 68.6 intended to yield the most saturated or lowest IVP within the bellies. Diet B was formulated to an intended intermediate average IVP of 71.9. Diet C was formulated to an average IVP of 81.1 to yield the least saturated or highest IVP bellies. Representative feed samples from diet treatments were collected at the time of mixing and shipped to Texas Tech University Animal & Food Sciences (TTU AFS; Lubbock, TX) for proximate analysis.

Proximate Analysis of Feed Samples

Twelve diet samples (4 phases × 3 diets) were analyzed for dry matter (DM), ash, crude fat, starch, and gross energy (GE) at TTU AFS. Crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed at a commercial laboratory (ServiTech, Amarillo, TX). Diet subsamples were ground into a homogenous powder (Nutribullet Pro, Capital Brands, LLC, Los Angeles, CA), dried in a convection oven at 60°C until a constant weight was achieved, and stored in desiccators to maintain a constant DM. Diets were analyzed in duplicate for crude fat (method 2003.06 of AOAC, 2007), starch (Smock et al., 2021), and DM (method 930.15 of AOAC, 2007). Diets were analyzed in triplicate for NDF (Van Soest & J. B. Robertson, 1979) and ADF (Goering & Van Soest, 1907). The GE of diet samples was determined in duplicate using an automated isoperibolic bomb calorimeter (model 6400; Parr Instrument Co., Moline, IL) using Benzoic acid (6318 kcal GE/kg; Parr Instrument Co.) as the standard for calibration and was determined to contain 6316 ± 1.11 kcal GE/kg. Data summarizing the composition of diet treatments and proximate analysis of feed samples are summarized in Table 1.

Belly Identification and Bacon Collection

Bellies (N = 391; IMPS #408; NAMI, 2014) from hogs fed diet A, B, or C were collected at a large-scale, commercial pork processing facility located in the upper mid-west. Hogs were sorted into 3 separate finishing groups over a 3-week span to account for weight and condition of hogs at finishing, so that bellies from 50 hogs of each diet treatment were sampled during 3 different collection periods. After carcasses were split at harvest, an approximately 15 cm × 5 cm strip of the fresh belly was taken from the ventral midline just posterior to the location of the navel/sheath of the right side of each carcass. The samples were immediately frozen with dry ice and shipped frozen to the Gordon W. Davis Meat Laboratory (Lubbock, TX) for subsequent

REIMS, fatty acid composition, and iodine value analysis. Simultaneous to the collection of this fresh belly sample, the whole bellies from the left side of the carcass were labeled for collection during carcass fabrication post-chilling the following day. Bellies were collected at fabrication and shipped to a large-scale, commercial bacon-processing facility.

Bellies were processed using the standard procedures and using the proprietary brine formulations of the bacon processor, which represented a conventional bacon product. Bellies were weighed prior to brine injection to determine "green weight" and immediately following to obtain "pumped weight". After bellies were chilled (4h), smoked (5-6.5h), and frozen (20h), they were pressed and weighed to determine a "smoked belly weight" used to calculate bacon slice yield. Additional calculations used to evaluate belly performance are as follows: Percent Pump = ((pumped weight – green weight)/green weight) *100, Percent Smoked Weight Change = ((green belly weight – smoked belly weight)/green belly weight) *100.

Immediately after pressing, bellies were sliced on a CashinEDGE® slicer (Provisur Technologies, Chicago, IL) Institutional Bacon Slicing System where every belly was sliced to the same thickness (0.5588 mm). Sliced bellies were divided and characterized by plant graders as #1's (secondary lean, M. cutaneous trunci, was greater than 50% of the width of the slice and no measurement less than 1.9cm in profile thickness at any point), #2's (insufficient secondary lean characteristics- M. cutaneous trunci less than 50% of the width of the slice) and ends based on secondary lean characteristics and appropriate slice profile thickness. From each belly, ten #1 bacon slices and ten #2 bacon slices were then separately weighed, chilled to 4˚C and transported to Texas Tech University for subsequent analysis of fat shattering, slice distortion, and shrink.

Sample Designation

Upon arrival at TTU, fresh belly samples collected at harvest were individually thawed in ice water for approximately 1 min and divided in half so that one section would be allocated for subsequent REIMS analysis and the other section would be allocated to fatty acid analysis. Samples designated to fatty acid analysis were homogenized into a finely divided powder (Nutribullet Pro, Capital Brands, LLC, Los Angeles, CA), and both samples were stored at -80°C in individual bags until further analysis.

Bacon samples were repackaged at TTU, with each slice laid flat on parchment paper within the package to maintain slice integrity. Bacon samples were vacuum sealed, frozen and stored at -20˚C until being analyzed for fat shattering, slice distortion, and shrink analysis.

Rapid Evaporative Ionization Mass Spectrometry (REIMS)

Metabolomic profiling of fresh pork belly samples was performed using REIMS. Before analysis, REIMS samples were thawed at 0-4°C for 16-24 h. To ensure a consistent sample temperature at the time of analysis, vacuum-sealed samples were held in an ice-water bath for more than 10 min prior to being analyzed. Samples were analyzed using a Synapt G2 Si Q-ToF (TOF) running in negative sensitivity mode, fitted with a REIMS ionization source coupled with an iKnife sampling device (Waters Corporation, Milford, MA). Three 2 cm burns per sample were collected using the iKnife, with each burn lasting approximately 1 sec. The burns from each sample were collected across both fat and lean that was naturally present in each sample, simultaneously on a fresh cut surface representative of the ventral midline of the carcass and belly. Data were collected from the TOF in the mass range from 50-1,200 m/z. Data were preprocessed to include leucine enkephalin as lock mass correction and background subtraction. Leucine enkephalin has a molecular weight of 555.632 g/mol and its detection interfered with neighboring components; therefore, mass bin 554.25 was excluded from the data matrix.

Additionally, individual peaks were binned in intervals of 0.5 m/z resulting in a total of 2,300 variables. Data binning was used to account for minor errors during data collection.

Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) were prepared by a modified method described by O'Fallon et al. (2007). Approximately 1.0 g ± 0.02 g of raw, fresh belly homogenate was weighed into pre-washed, labeled and weighed glass Pyrex tubes. One mL of .5mg/mL of tridecanoic acid (C:13) standard and 0.7 mL of 10 N KOH were added to each glass tube, and samples were vortexed. After being vortexed, 5.3 mL of methanol was added, and tubes were placed in a water bath at 55°C for 1.5 h. The samples were shaken every 20 min. Tubes were then cooled to room temperature in a cold tap water bath, 0.58 mL of 24 N sulfuric acid was added and the samples were incubated in the water bath for another 1.5 h, shaken every 20 min. Once the tubes were cooled to room temperature, 6.0 mL of hexane was added, and samples were vortexed for 5 min before being placed in the centrifuge for 5 min at 3500 rpm. Samples were further diluted where 30 µL of the FAME hexane layer was diluted with 270 µL of hexane in an amber split insert gas chromatography vial which was sealed with a polypropylene lined cap (Fisherbrand; made in Mexico; 14-962-26G). Hexane quantity was increased in the extraction and dilution procedure from the method described by O'Fallon et al. (2007) to accommodate the higher fat content within samples and ensure full extraction of lipid. Separation of FAME was carried out by an Agilent 7890 GC instrument equipped with a flame ionization detector. Individual FAME were separated with a HP-88 capillary column (100m × 0.25 mm × 0.20 µm; Agilent Technologies, Palo Alto, CA). One microliter of sample was injected with a split ratio of 100:1, hexane: sample. The oven method was as follows: 35° C held for 2 min, increased to a temperature of 4 °C/min to 170 °C and held for 4 minutes. Then

increased 3.5 ˚C per minute to a final temperature of 240 ˚C for 2 minutes. The injector and FID were operated at 250 °C. Fatty acids were identified based on the similarity of retention times with GC reference standards (Nu-chek Prep, Inc., Elysian, MN). Fatty acid concentrations were calculated relative to initial wet sample weight (mg/g).

Iodine Value Analysis

Iodine value (IV) titrations were performed according to a modified Wijs method (AOAC, 1984). Approximately 0.70 g of fresh belly homogenate were placed in a 500-mL Pyrex™ Erlenmeyer flask (Corning™ Pyrex ™, Corning, NY) and dissolved in 15 mL of chloroform. Samples were incubated for 30 min in 25 mL of Wijs solution, and reaction was stopped by adding 20 mL 15% potassium iodine (KI) solution (15 mL KI dissolved in 85 mL D-H_2O) and 100 mL of D-H_2O. Within 30 min of stopping the reaction, samples were titrated with 0.1 N sodium thiosulfate (Na2S2O3). The volume needed to produce colorless ions was recorded and IV was calculated using the following equation: IV = [(titration volume of blank − titration volume of sample) × molarity of Na2S2O3 × 12.69]/sample weight. Accuracy of titrated IV was determined by running a Pearson Correlation of titrated IV by the IV calculated from the FA composition of the sample. The IV determined from the FA composition was calculated using the following equation: IV = [16:1 (0.95) + 18:1 (0.86) + 18:2 (1.732) + 18:3(2.616) + 20:1 (0.785) + 22:1 (0.723)] (AOCS, 1998). The results of the Pearson Correlation determined a 0.25 correlation, indicating inaccuracy in the titration method. Therefore, the results of the titrated samples were excluded.

Bacon Fat Shattering, Slice Distortion, and Shrink

Bacon samples designated for fat shattering, slice distortion, and shrink were thawed at 4˚C for 12 h. At 4 ˚C, 5 #1 slices were analyzed per belly for fat shattering by 2 trained panelists

using a modified version of the method described by Mandigo (2002). Panelists scored slices for fat shattering on a continuous 100mm line scale where 0 indicated no shatter marks occurring in the fat perpendicular to the length of the slice, and 100 indicated shatter marks occurring throughout the entire slice. Slices were rolled on a 15mL High-Clarity Polypropylene Conical Tube (Falcon®, Corning Science, Mexico) to expose shatter marks. Panelists were provided a calibration sample of #1 slices collected at the same time as experimental slices at the beginning of fat shattering analysis and after every 100 bellies. All data was recorded on an electronic ballot generated by an online survey software (Qualtrics, Provo, UT, USA). Ratings for fat shattering were averaged across panelists and slices for each belly sample. Before cooking, a total weight was recorded for the same 5 slices assessed for fat shattering, and each slice was measured for length using a standard ruler. Slice weights and lengths were averaged per belly. Bacon slices were then cooked at 177˚C on a flat grate (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) in a combi-oven (Model SCC WE 61 E; Rational, Landberg am Lech, Germany) with 0% humidity at low fan speed for 8 minutes. Immediately following removal from the oven, bacon slices were removed from the hot grate and blotted with paper towels. Subsequent total cooked weight and individual slice lengths were obtained to determine cook loss and slice distortion, respectively, per belly. Values for percent cook loss and percent slice distortion were determined using the following calculations: percent cook loss = [((raw slice weight – cooked sliced weight)/raw slice weight) x 100], percent slice distortion = [((raw slice length – cooked slice length)/raw slice length) x 100].

Statistical Methods

All data were analyzed using R statistical software, version 1.4.1106 (R. Core Team, 2021) and significance level was set at $\alpha=0.05$ for all analyses.

Estimated marginal means were computed for all response variables aside from REIMS mass bins by using the lm() function from base R to fit a linear model with diet and sex or REIMS group as main effects. An analysis of variance (ANOVA) was conducted using the anova() function from the lmerTest package (Kuznetsova, Brockhoff, and Christensen, 2017). Using the emmeans package, comparisons were tested for significance using Tukey adjusted pairwise comparisons and significance at $\alpha = 0.05$ (Russell et al., 2020).

Assessment of REIMS Data

Data from REIMS were pre-processed and cleaned before analysis. Using AMX software, intensities of spectra between 50.0 and 1200.0 m/z were extracted into bin intervals of 0.5 m/z for each of 2 burns of a sample, applying background subtraction. Further processing of data was completed in R statistical software. Intensities of each bin interval were averaged across burns within each sample, and resulting intensities were normalized to sample sum intensity by dividing the intensity of each bin interval by the sum intensity. Mean normalized intensity for each bin interval was calculated across all samples. Bin intervals were sorted in descending order by mean normalized intensity, and cumulative intensity was calculated by adding the intensity of each bin interval to the sum of intensity of bin intervals ordered before it. Only bin intervals – ordered by intensity – needed to reach a cumulative intensity of approximately 0.80 (80% of total intensity) were retained for further analysis. This procedure retained 328 variables (of 2300 total) with the greatest average intensity across samples, effectively removing variables with little to no magnitude of contribution to the metabolomic profile. Intensities for selected bin intervals were re-normalized to the new sum intensity for each sample. Through a series of data exploration techniques, it was determined that day and cleaning interval of sample acquisition affected metabolomic profiles. Therefore, data were

subset by a combination of their day and cleaning interval of sample acquisition, and bin intervals within each subset of data were mean centered to 0. After centering, data subsets were joined together for further analyses.

Factor analysis was conducted on cleaned REIMS data to identify constructs of shared variance among certain bin intervals. The psych package was used to conduct a 10-factor principal factor analysis with varimax rotation on all 328 REIMS bin intervals. Bin intervals with the top 10 strongest loading values (either positive or negative) on each factor were interpreted to identify shared variance on each factor. Scores on each of 10 factors were extracted for each sample.

Canonical correlations were conducted to measure relationships between REIMS factors (created from factor analysis of REIMS bin intervals) and fatty acid composition measured on GC-FID. The independent variable dataset (X) comprised of fatty acids with a mean percentage across all samples of greater than 1.0%: 18:1-n9, 16:0, 18:2-n6, 18:0, 18:1-n7, 16:1-n7, 14:0, and 18:1-trans. The dependent variable dataset (Y) comprised of 10 REIMS factors. Both datasets X and Y were passed to the cancor function of the candisc package to produce canonical correlations. Figure 1 demonstrates the division and distribution of individual samples into REIMS Groups based on their first canonical variate scores for 10 REIMS Factors. Scores for the first Y canonical variate (Y1; created from the 10 REIMS factors) were extracted and used to categorize samples into 4 groups: “Excellent” – Y1 score less than negative 1, “Great” – Y1 score greater than negative 1 but less 0, “Good” – Y1 score greater than 0 but less than 1, and “Poor” – Y1 score greater than 1.

Data were split into training and testing sets to assess the accuracy of REIMS to classify samples into established quality groups. This method to determine predictive accuracy is the

crudest and most conservative approach available because prediction of quality group in the testing dataset is completely independent from its values for variables used to create the predictive model in the training dataset. Correspondingly, a predictive model developed for one dataset is tested for its accuracy on a separate dataset – and vice versa – with no guidance, unlike other modeling approaches such as cross-validation or leave-one-out. All data (N = 391) were randomly split into 2 datasets of approximately equal proportion (N_1 = 195; N_2 = 196). Each data subset was analyzed with canonical correlation between fatty acids and REIMS factors, and scores for the first Y canonical variate of REIMS factors were used to classify each data subset into 1 of 4 "actual" quality groups (same approach discussed in previous paragraph for all data combined). From each canonical correlation of both datasets, coefficients for each of 10 REIMS factors used to derive scores for the first canonical variate were extracted. Extracted coefficients from one dataset were used to calculate predicted scores from REIMS factors in the other dataset, and vice versa. This was completed by multiplying the coefficient for each REIMS factor by the value of each sample within that respective REIMS factor and summing resulting values for each sample to produce a predicted score. Predicted scores were used to classify samples into 1 of 4 "predicted" quality groups using the same criteria discussed previously. Thus, within each dataset, each sample was characterized by 2 different types of classes: 1) an "actual" class, based on values derived from the relationship between fatty acid composition and REIMS factors within that dataset; and 2) a "predicted" class, based on values derived from REIMS factors alone within that dataset and their coefficients derived from the other dataset. Thus, modeling of the training dataset is completely independent of prediction. For each dataset, a misclassification matrix was created to demonstrate classification of samples into their "actual"

and “predicted” classes. Accuracy was calculated as the proportion of correctly classified samples.

RESULTS & DISCUSSION

Bacon Yield and Belly Composition

Estimated marginal means of the concentrations of fatty acids quantified and the calculated IV of fresh belly samples from barrows and gilts fed three diet treatments are presented in Table 2. In this study, the experimental diets created significant differences ($P < 0.05$) in the fatty acid profile of the fresh bellies of market ready pigs. Experimental diets significantly affected ($P < 0.01$) the total proportion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). It has been well documented that the fatty acid composition of pork closely mimics the fatty acid composition of the diet (Benz et al., 2010; Browne et al., 2011; Ellis & McKeith, 1999; Gatlin et al., 2002; Larsen et al., 2009; Maw et al., 2003; Rentfrow et al., 2003; Whitney et al., 2006); therefore, it is not surprising that in this study, increasing DDGS and corn oil, more polyunsaturated fat sources, decreased total SFA ($P < 0.01$). Major compounds contributing to total SFA are 10:0 (capric acid), 12:0 (lauric acid), 14:0 (myristic acid), 16:0 (palmitic acid), and especially 18:0 (stearic acid; $P < 0.01$). Total MUFA were similar in diets A and B, but decreased in diet C ($P < 0.01$). Cromwell et al. (2011) presented similar findings in the analysis of pork backfat. Aside from 13:1 and 22:1 (erucic acid), all individual MUFA exhibited this relationship, where 18:1n9 (oleic acid) exhibited the greatest contribution to total change in MUFA composition ($P < 0.01$). The greatest change in fatty acid composition because of diet is observed in the total PUFA, which increased as predicted IV of the diet increased ($P < 0.01$). These findings are in support of many other published works relative to dietary impact on fatty acid composition, noting 18:3n6 (γ-Linolenic) and 18:3n3 (α-Linolenic) as prominent factors contributing to change in total PUFA (Benz et al., 2008; Larsen et al., 2009; Leick et al., 2010; Rentfrow et al., 2003; Shircliff

et al., 2015; Xu et al., 2010). No differences existed in fatty acid composition because of sex, however a trend was observed where gilts exhibited a higher percentage of 14:1n5 (myristoleic acid). Additionally, the interaction of diet and sex resulted in a trend in 18:0 (stearic acid) and 18:1n7 (*cis*-vaccenic acid). A difference also existed because of the interaction of diet and sex in 22:1 (erucic acid), where gilts exhibited a greater amount of 22:1 than barrows ($P < 0.01$).

The calculated IV observed in this study supports the changes in fatty acid composition observed this study. Diets A and B demonstrated lower IV than C, which generated a much higher IV ($P < 0.01$). This is sensible, as IV increases with level of unsaturation and as SFA decreases. This coincides with the differences observed in bacon slice ability in this study. Whitney et al. (2006) and Widmer et al. (2008) also observed higher IV with increasing inclusion of feeds high in unsaturated fatty acids, such as DDGS, which negatively impacts bacon slice ability (Benz et al., 2011; Ellis & McKeith, 1999; Seman et al., 2013).

The estimated marginal means of bacon yield of bellies derived from barrows and gilts fed 3 diets are presented in Table 3. Diet significantly affected bacon shatter scores, cook loss %, and the proportion of #1 and #2 bacon slices ($P < 0.01$). As the diet increased in level of unsaturation, as indicated by IV (Table 1), cook loss % decreased while the percentage of #1 slices decreased and #2 slices inversely increased. In this study, diets A and B did not differ ($P > 0.05$) from one another in the proportion of #1 (and #2) slices and cook loss %, but the diet with the highest IV, diet C, was the lowest ($P < 0.01$). These findings indicate that as the diet becomes more unsaturated, cook loss % declines in a favorable fashion, and pigs fed a more unsaturated diet produced softer fat with a lesser amount ($P < 0.01$) of fat shattering in bacon slices. Even though a decrease in cook loss % and decreased shatter scores seem to be more favorable for pigs fed a more unsaturated diet, the proportion of premium, #1 slices was the

lowest for the most unsaturated diet. In partial agreement with these findings, McClelland et al. (2012) reported that increased DDGS in the diet created softer, more malleable bellies which were more resistant to shatter. In addition, a trend ($P = 0.06$) in pump retention was observed where bellies from diet A exhibited the least pump retention, bellies from diet B exhibited intermediate pump retention, and diet C exhibited the greatest pump retention. No differences were observed in smoked weight loss because of diet ($P = 0.35$). While there was no difference in slice distortion because of diet ($P = 0.81$), a trend existed in slice distortion because of sex ($P = 0.06$). Cook loss was also affected by sex in which bellies derived from barrows exhibited greater cook loss than those from gilts ($P < 0.01$). In contrast to these findings, some studies have suggested that sex and dietary inclusion of DDGS created no difference in slice cook loss (Heymann et al., 1996; Kelley & Rentfrow, 2020; LaBerge et al., 2011; McClelland et al., 2012; Rentfrow et al., 2003; Widmer et al., 2008; Xu et al., 2010). However, Leick et al. (2010) reported increased cook loss as DDGS increased in the diet, and Little et al. (2014) suggested that bellies from barrows exhibited greater cook loss than gilts. This relationship can be further explained by results from Brewer et al. (1995), which discussed an increase in cook loss of sliced bacon due to an increase in belly thickness, corresponding to an increase in fat, which is the main component lost during cooking. Accordingly, Mann et al. (2002) reported that bacon from barrows was found to have a higher fat content than that from gilts.

These findings suggest that increasing the predicted IV of diets altered bacon yield, fatty acid composition and calculated IV of pork bellies, ultimately creating a diverse population for the evaluation of the metabolomic profile as acquired by REIMS.

Rapid Evaporative Ionization Mass Spectrometry (REIMS) Model Building and Groups

The 10 mass bins with the greatest absolute loading value onto each factor are presented in Table 4. Interestingly, similar ranges of mass bins loaded onto the same factor, indicating that a narrow range of masses within the spectra was responsible for precise variation within the data set. This step was necessary to understand which compounds covary together which suggested composition differences in samples. For instance, the mass bins loading most strongly on Factor 1 were compounds with a smaller m/z and a range of 100 m/z (112.25-212.25 m/z). In other Factors (such as Factors 4-7), several of the top 10 bins were compounds with nearly equal m/z. Overall, this provides an indication of which mass range of the REIMS spectra were responsible for variation in each dimension of the data. Additionally, Factors are presented in the order of variance explained, where Factor 1 explained the greatest amount of variance (28%), and Factor 10 explained the least amount of variance (3%). However, cumulatively, all factors explained 99% of the variation in the data set.

Canonical correlations of 8 fatty acids (14:0, 16:0, 16:1n7, 18:0, 18:1trans, 18:1n9, 18:1n7) combined to create the X matrix correlated with Y matrix composed of 10 Factors derived from REIMS bins are presented in Table 5. The relationship between REIMS factors and fatty acids was significant (canonical correlation not equal to zero) for both canonical correlation sets 1 and 2 ($P < 0.01$ and $P = 0.01$). The first correlation set, which compared the first linear combination of fatty acids to the first linear combination of REIMS factors had a correlation value of .68 and explained 77% of the variation in the data. Only canonical correlation set 1 was interpreted because it was the most highly significant and explained substantially more variation than any other canonical correlations set, regardless of significance. Correspondingly, only the scores for the REIMS factors were extracted. Subsequent correlation sets 3 through 8 were not significant ($P > 0.05$).

Figure 2 illustrates the loading values on each of the variates used in the canonical correlation responsible for segregation of bellies. The loadings from each factor on Y1 (REIMS Factors) were used to obtain scores for individual samples.

REIMS Groups exhibited differences in bacon processing and cooking yields as shown by the estimated marginal means presented in Table 7. REIMS groups were arbitrarily labeled as Excellent, Great, Good, and Poor for the purpose of clarity and ease of discussion. Green belly weights were different ($P < 0.001$) while Excellent and Great bellies were heavier than Good and Poor bellies. However, no processing differences were found among groups as pump retention and smoked weight loss were not different ($P \geq 0.33$). Percent of #1 slices was different ($P < 0.01$) among groups where numerically, Great bellies had the greatest percentage of #1 slices, followed by Excellent, Good, and Poor bellies. However, only Poor bellies had an increased percentage of #2 slices ($P<0.01$) among the other groups and the groups were not different in percentage of end slices ($P=0.16$). While slice distortion did not differ among groups ($P = 0.48$), Excellent and Great bellies had a greater shatter score ($P < 0.01$) and trended toward ($P = 0.08$) a greater cook loss than Good and Poor bellies.

Estimated marginal means of percent fatty acid composition and calculated IV are presented in Table 7. REIMS effectively sorted bellies by categorizing bellies into groups based on degree of saturation and calculated iodine values ($P < 0.01$). Bellies categorized as Excellent and Great were not different from each other in percent SFA but had a greater percent ($P < 0.01$) SFA (were less unsaturated) than Good and Poor bellies. Similarly, Excellent and Great bellies had greater percentages of MUFA than Good bellies which had a greater percentage MUFA than Poor bellies ($P < 0.01$). Conversely, Excellent and Great bellies had lesser percentage PUFA than Good, or Poor bellies, respectively ($P<0.01$). Finally, calculated IV agreed with the

percentage differences of classes of fatty acids where bellies were different among groups ($P < 0.01$) and, numerically, Excellent and Great bellies had the lowest IV, Good, and Poor bellies had the greatest numerical IV.

Table 6 summarizes the demographics of each REIMS group. Barrows made up 54% of the study and gilts comprised 46%. This was also true within each group- Excellent was comprised of 46% barrows, Great- 52%, Good-57%, and Poor- 63%. Sample collection days were also comparably represented across groups. However, diets were skewed in their representation in groups where Excellent and Great bellies had the highest proportion of diet A (57 and 51%, respectively). Additionally, 78% of diet B samples were contained in Great and Good bellies. Finally, 86% of diet C samples were categorized as Good or Poor bellies, and interestingly, 91% of Poor bellies were from diet C. This distribution of diet was sensible as it agreed with group differences caused by diet (Tables 2 and 3). REIMS group demographics, along with differences in bacon yield and belly composition (Tables 6 and 7), provide evidence of the ability of REIMS to categorize differences in belly quality regardless of diet or sex.

Validating the Ability of Rapid Evaporative Ionization Mass Spectrometry to Segregate Pork Belly Quality

After data were split into a training and testing dataset ($N_1 = 195$; $N_2 = 196$), a model was constructed as described above with the same 10 REIMS factors and 8 fatty acids, using only the data form the training dataset (Table 9). Then the testing dataset was used to create actual classes of data set 1 and predict data set 2, and vice versa. Overall accuracy was calculated by dividing total of correctly categorized samples by total sample size, which was 70% in both models, this similarity of the accuracy alludes to repeatability of the technique. Moreover, only 1 sample of all 391 samples was misclassified by greater than 1 class difference (1 Poor belly was classified

as Great; 0.003%). Interestingly, the greatest misclassification (lowest accuracy) occurred between Great and Good classification, meaning the model was more adept at separating belly classes shown to have greater differences in other variables indicative of quality (Tables 6 and 7). These results should be cautioned, as fresh belly samples were analyzed with REIMS in a chilled state, whereas samples analyzed in a practical setting would be hot. Furthermore, no study has ever used a train-test data set and segregated meat samples using the metabolomic profile as collected by REIMS to assess meat quality and achieved and accuracy of 70%. Therefore, this study displays the promise to develop REIMS as a tool to improve the composition and quality of pork products**.**

CONCLUSION

REIMS proved to be an effective technology in segregating individual pork bellies based on bacon yields, cook loss, and fatty acid composition. The use of metabolomic profiles collected in real-time and *in-situ* using REIMS characterized differences in degree of saturation of fat, which in turn, translated to higher quality bellies with a greater proportion of premium #1 slices. More remarkably, using the crudest and most conservative model building approach, REIMS was able to classify a test set of samples into the predetermined quality groups with a high degree of accuracy. This study showed the ability of REIMS to accurately predict pork belly quality and fat composition to subsequently send market signals and value hogs based on objective measurements to ultimately improve the composition and quality of pork products.

TABLES & FIGURES

Table 1. Composition of diets fed throughout growing phases.

	Phase 1[1]			*Phase 2*[1]			*Phase 3*[1]			*Phase 4*[1]		
	A[2]	B[2]	C[2]	A[2]	B[2]	C[2]	A[2]	B[2]	C[2]	A[2]	B[2]	C[2]
Ingredient composition, %												
Corn	65.88	56.53	53.27	66.24	59.33	56.56	70.95	64.91	54.74	75.32	61.22	58.34
DDGS	15.00	29.51	33.51	15.00	31.36	35.30	15.00	20.00	32.37	15.00	33.43	34.19
Soybean Meal	16.00	10.50	9.00	15.50	5.50	4.00	11.50	11.00	6.50	7.00	1.00	1.00
Animal Vegetable Blend	1.00	1.25	-	1.50	1.50	-	0.50	2.00	-	0.75	2.25	-
Limestone	0.96	1.01	1.02	0.94	1.02	1.03	0.92	0.93	0.96	0.91	0.96	1.08
Lysine HCl	0.42	0.50	0.51	0.27	0.46	0.48	0.40	0.43	0.49	0.37	0.47	0.47
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Threonine	0.12	0.12	0.12	0.03	0.09	0.08	0.15	0.15	0.15	0.12	0.12	0.12
VTM Premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
88% DL Methionine[3]	0.08	0.05	0.03	-	-	-	0.50	0.50	-	-	-	-
Enzyme Blend	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Tryptophan	0.04	0.05	0.05	0.01	0.04	0.05	0.03	0.04	0.05	0.03	0.05	0.05
Corn Oil	-	-	2.00	-	-	2.00	-	-	4.25	-	-	4.25
Calculated nutrients												
IV, predicted[4]	68.36	70.61	76.33	69.48	71.77	76.98	67.80	71.30	85.24	68.77	73.91	85.92
Analyzed Nutrients[7]												
GE, Mcal/kg	4.52	4.64	4.73	4.61	4.70	4.77	4.51	4.65	4.79	4.46	4.69	4.76
Starch, %	54.18	47.51	44.61	55.10	52.00	48.06	57.91	52.10	46.09	60.32	52.63	46.76
Crude Protein, %	19.22	20.10	20.87	18.33	19.13	18.52	17.28	17.88	18.78	14.51	17.01	17.99
NDF, %[5]	17.71	18.86	20.91	14.89	17.99	18.84	15.40	18.52	19.73	14.93	19.91	20.04
ADF, %[8]	5.56	6.97	6.89	4.79	5.77	6.96	5.15	5.79	7.47	4.60	6.54	6.87
Ash, %	4.07	4.01	4.29	3.89	4.10	4.10	3.77	3.70	4.07	3.21	3.67	3.69
EE, %[9]	2.21	4.22	5.57	3.92	5.13	5.34	2.38	4.25	8.15	2.94	6.32	5.01
ME, Mcal/kg[10]	3.65	3.69	3.66	3.84	3.77	3.78	3.75	3.73	3.76	3.75	3.70	3.75
NE, Mcal/kg[6]	2.63	2.64	2.62	2.81	2.74	2.74	2.74	2.72	2.75	2.80	2.73	2.71

[1]Four phases within each diet, formulated to meet nutrient energy requirements at respective stage of maturity.
[1]Diet: A: low iodine value (68.6), B: intermediate iodine value (71.9) and C: high iodine value (81.1)
[3]Alimet®, Novus International, Saint Charles, MO
[4]Predicted iodine value
[5]Percent neutral detergent fiber
[6]Net energy, Mcal/kg
[7]Calculations based on NRC 2012
[8]Percent acid detergent fiber
[9]Percent ether extract
[10]Metabolizable Energy, Mcal/kg

Table 2. Estimated marginal means[1] of concentrations of fatty acids quantified as a percent and calculated iodine value in fresh pork belly samples, representing three diets.

	Diet[1]					Sex			
	A	B	C	SEM[2]	P-Value[3]	Barrow	Gilt	SEM[2]	*P*-Value[3]
SFA[4]	42.52[a]	41.10[b]	38.91[c]	0.36	<0.01	40.62	41.12	0.32	0.26
10:0	0.12[a]	0.12[a]	0.10[b]	<0.01	<0.01	0.11	0.11	<0.01	0.62
12:0	0.11[a]	0.10[a]	0.09[b]	<0.01	<0.01	0.1	0.1	<0.01	0.97
14:0	1.73[a]	1.71[a]	1.46[b]	0.03	<0.01	1.64	1.63	0.02	0.94
15:0	0.06[b]	0.07[a]	0.06[b]	<0.01	<0.01	0.06	0.06	<0.01	0.14
16:0	27.42[a]	26.49[a]	24.24[b]	0.29	<0.01	26.06	26.05	0.27	0.97
17:0	0.31[b]	0.36[a]	0.30[b]	0.01	<0.01	0.32	0.33	0.01	0.19
18:0	12.18[a]	11.66[b]	10.49[c]	0.15	<0.01	11.46	11.43	0.14	0.91
19:0	0.24[b]	0.21[b]	1.69[a]	0.30	<0.01	0.48	0.99	0.26	0.15
20:0	0.36[c]	0.38[b]	0.46[a]	<0.01	<0.01	0.4	0.4	0.01	0.72
MUFA[5]	42.27[a]	41.51[a]	36.41[b]	0.31	<0.01	40.29	39.81	0.33	0.28
13:1	0.0	0.0	0.0	<0.01	0.69	0.0	0.0	<0.01	0.18
14:1n5	0.03[a]	0.03[a]	0.02[b]	<0.01	<0.01	0.02[b]	0.03[a]	<0.01	0.04
16:1n7	2.53[a]	2.39[a]	1.74[b]	0.04	<0.01	2.22	2.22	0.04	0.99
18:1trans	1.66[a]	1.57[a]	1.31[b]	0.04	<0.01	1.56	1.46	0.04	0.06
18:1n9	34.77[a]	34.30[a]	30.76[b]	0.23	<0.01	33.46	33.07	0.24	0.23
18:1n7	2.59[a]	2.54[a]	1.92[b]	0.03	<0.01	2.36	2.34	0.04	0.69
19:1	0.05[c]	0.05[b]	0.06[a]	<0.01	<0.01	0.05	0.06	<0.01	0.13
22:1	0.64	0.62	0.60	0.04	0.75	0.61	0.64	0.04	0.61
PUFA[6]	15.21[c]	17.38[b]	24.68[a]	0.24	<0.01	19.08	19.07	0.37	0.97
18:2trans	0.03[a]	0.03[a]	0.03[b]	<0.01	<0.01	0.03	0.03	<0.01	0.16
18:3n6	0.04[c]	0.04[b]	0.05[a]	<0.01	<0.01	0.04	0.04	<0.01	0.80
18:2n6	14.96[c]	17.13[b]	24.44[a]	0.24	<0.01	18.84	18.82	0.37	0.97
18:3n3	0.18	0.17	0.17	<0.01	0.09	0.17	0.17	<0.01	0.57
IV[7]	63.3[b]	64.7[b]	72.9[a]	0.47	<0.01	67.3	67.3	0.39	0.94

[a-c] Estimated marginal means in the same row without a common superscript differ ($P < 0.05$) because of diet or sex.

[1]Diet: A: low iodine value (68.6), B: intermediate iodine value (71.9) and C: high iodine value(81.1).

[2]Standard error (largest) of the estimated marginal means.

[3]Observed significance levels for main effect of diet.

[4]SFA: Sum of total saturated fatty acids.

[5]MUFA: Sum of total monounsaturated fatty acids.

[6]PUFA: Sum of total polyunsaturated fatty acids.

[7]Calculated Iodine Value: IV = (0.95×[16:1])+(0.86×[18:1])+(1.732×[18:2])+(2.616×[18:3]), where [] indicate the concentration percentage.

Table 3. Bacon processing and cooking yields from barrows and gilts fed three diets.

	Diet[1]					Sex			
	A	B	C	SEM[2]	*P*-Value[3]	Barrow	Gilt	SEM[2]	*P*-Value[3]
Pump Retention, %	10.3	11.0	11.7	0.42	0.06	10.9	11.1	0.34	0.72
Smoked Weight Loss, %	0.6	1.4	0.6	0.67	0.65	1.1	0.7	0.57	0.65
#1 Slices[4], %	78.4[a]	77.8[a]	74.5[b]	0.59	< 0.01	76.8	76.9	0.48	0.86
#2 Slices[5], %	13.3[b]	14.2[b]	16.6[a]	0.63	< 0.01	14.9	14.5	0.51	0.58
End Slices[6], %	8.8	8.0	9.3	0.42	0.08	8.5	8.8	0.34	0.53
Shatter Score[7]	35.6[a]	31.4[b]	26.0[c]	0.74	< 0.01	31.6	30.4	0.61	0.14
Slice Distortion[8], %	23.6	23.6	23.9	0.47	0.81	24.2	23.2	0.38	0.06
Cook Loss, %	56.2[a]	56.0[a]	54.3[b]	0.42	< 0.01	56.4[x]	54.7[y]	0.35	< 0.01

[a-c] Estimated marginal means in the same row without a common superscript differ ($P < 0.05$) because of diet.
[x,y] Estimated marginal means in the same row without a common superscript differ ($P < 0.05$) because of sex.
[1]Diet: A: low iodine value (68.6), B: intermediate iodine value (71.9) and C: high iodine value (81.1).
[2]Standard error (largest) of the estimated marginal means.
[3]Observed significance levels for main effect of diet or sex.
[4]#1 Slices: slices that met requirements for secondary lean characteristics (M. cutaneous trunci greater than 50% of the width of the slice) and appropriate slice profile thickness (no measurement less than 1.9 cm in profile thickness at any point).
[5]#2 Slices: those that had insufficient secondary lean characteristics (M. cutaneous trunci less than 50% of the width of the slice).
[6]End Slices: Those slices not meeting ‘‘#1 slice’’ or ‘‘#2 slice’’ characteristics and slices from the cranial or caudal ends of bellies.
[7]Shatter Score: Trained panelists scored slices based on a continuous 100mm line scale where 0 indicated no shatter marks occurring in the fat perpendicular to the length of the slice, and 100 indicated shatter marks occurring throughout the entire slice.
[8]Slice Distortion, % = [((raw slice length – cooked slice length)/raw slice length) x 100].

Table 4. Top 10 bin intervals (and their minimum absolute loading value) onto 10 factors derived from factor analysis of rapid evaporative ionization mass spectrometry (REIMS) of fresh bellies.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10
REIMS bins (m/z) in descending order	112.25	353.25	311.25	642.25	327.25	377.25	293.25	253.25	670.75	151.25
	124.25	354.25	313.25	643.25	439.25	657.25	554.75	281.25	671.25	165.25
	138.25	382.25	325.25	687.75	715.25	683.25	555.25	282.25	672.25	177.25
	168.25	387.25	326.25	688.75	715.75	683.75	555.75	415.25	721.25	191.25
	169.25	394.25	339.25	771.75	716.25	684.25	556.25	751.25	723.25	193.25
	182.25	410.25	340.25	772.75	716.75	684.75	557.25	766.75	723.75	195.25
	184.25	438.25	341.25	773.75	717.25	685.75	666.25	767.75	776.75	205.25
	196.25	448.25	421.25	794.75	717.75	686.75	668.25	769.75	790.75	207.25
	198.25	467.25	422.25	795.75	744.75	729.75	1110.75	861.75	885.75	239.25
	212.25	481.25	653.25	797.75	745.75	730.75	1109.75	862.75	886.75	241.25
Minimum absolute value of loadings	0.95	0.80	0.77	0.69	0.42	0.67	0.80	0.59	0.50	0.39
Variance Explained	0.28	0.17	0.11	0.10	0.09	0.08	0.05	0.04	0.04	0.03

Table 5. Canonical correlation of 8 Fatty Acids variables (C18:1n9, C16:0, C18:2n6, C18:0, C18:1n7, C16:1n7, C14:0, C18:1trans) with composite Factors 1-10 comprised of rapid evaporative ionization mass spectrometry (REIMS) mass bins.

Correlation Set[1]	Canonical Correlation (r)[2]	Eigenvalue[3]	% Variance[4]	*P*-Value[5]
1	0.68	0.86	77.53	<0.01
2	0.30	0.10	8.90	0.01
3	0.26	0.07	6.61	0.20
4	0.18	0.03	3.01	0.75
5	0.14	0.02	1.67	0.87
6	0.13	0.02	1.65	0.84
7	0.08	0.01	0.61	0.95
8	0.02	0.00	0.03	0.99

[1]Correlation between first composite X variable composed of fatty acid variables and first composite Y variable composed of REIMS factors, then between second composite X and second composite Y, and so on.

[2] Value of the correlation between the composite fatty acid variables and REIMS factors

[3] Eigenvalue denotes the amount of variance in each direction of the data, where a larger number indicates greater variance

[4] Percent of total variance in data set explained by the canonical correlation and resulting eigenvalue in the same row

[5] H_0 : The canonical correlations and all that follow are zero, $\alpha = 0.05$

Table 6. Demographics of four belly quality groups separated by the metabolomic profile as collected by rapid evaporative ionization mass spectrometry (REIMS) on fresh bellies (N=391).

		REIMS Group[1]			
	Total	Excellent	Great	Good	Poor
N	391	56	150	123	62
Gilts, n	177	30	72	52	23
Barrows, n	214	26	78	71	39
Collection 1, n	146	22	57	47	20
Collection 2, n	102	14	41	36	11
Collection 3, n	143	20	52	40	31
Diet A, n	132	32	76	24	0
Diet B, n	129	23	58	43	5
Diet C, n	130	1	16	56	57

[1]Determined from canonical correlation of fatty acid profile and REIMS factors

Table 7. Bacon processing and cooking yields of four groups separated by the metabolomic profile as collected by rapid evaporative ionization mass spectrometry (REIMS) on fresh bellies (N=391).

	REIMS Group[1]					
	Excellent	Great	Good	Poor	SEM	*P*-Value
Green Belly Weight, kg	7.12[a]	9.90[a]	6.31[b]	5.99[b]	0.321	<0.001
Pump Retention, %	10.42	10.86	11.16	11.72	0.83	0.69
Smoked Weight Loss, %	1.1	0.9	1.6	0.6	1.0	0.33
#1 Slices, %[2]	78.00[ab]	78.27[a]	75.87[bc]	73.64[c]	1.01	<0.01
#2 Slices, %[3]	13.92[b]	13.70[b]	15.26[b]	18.96[a]	1.13	<0.01
End Slices, %[4]	8.02	8.03	9.50	8.64	0.76	0.16
Shatter Score[5]	36.05[a]	33.73[a]	28.07[b]	26.81[b]	1.30	<0.01
Slice Distortion, %[6]	22.73	23.30	24.01	24.13	0.82	0.48
Cook Loss, %	55.43	56.05	54.93	53.98	0.73	0.08

[a-c] Estimated marginal means in the same row without a common superscript differ (P <0.05) because of REIMS Group

[1]Determined from canonical correlation of fatty acid profile and REIMS factors

[2]#1 Slices: slices that met requirements for secondary lean characteristics (M. cutaneous trunci greater than 50% of the width of the slice) and appropriate slice profile thickness (no measurement less than 1.9 cm in profile thickness at any point).

[3]#2 Slices: those that had insufficient secondary lean characteristics (M. cutaneous trunci less than 50% of the width of the slice).

[4] End Slices: Those slices not meeting ‘‘#1 slice’’ or ‘‘#2 slice’’ characteristics and slices from the cranial or caudal ends of bellies.

[5] Shatter Score: Trained panelists scored slices based on a continuous 100mm line scale where 0 indicated no shatter marks occurring in the fat perpendicular to the length of the slice, and 100 indicated shatter marks occurring throughout the entire slice.

[6] Slice Distortion, % = [((raw slice length – cooked slice length)/raw slice length) x 100].

Table 8. Fatty acid composition (%) and calculated iodine value of four groups separated by the metabolomic profile as collected by rapid evaporative ionization mass spectrometry (REIMS) on fresh bellies (N=391).

	REIMS Group[1]					
	Excellent	Great	Good	Poor	SEM	*P*-Value
SFA[1]	42.59[a]	41.77[a]	39.85[b]	39.03[b]	0.55	<0.01
10:0	0.12[a]	0.12[a]	0.11[a]	0.10[b]	<0.01	<0.01
12:0	0.11[a]	0.10[a]	0.10[ab]	0.09[b]	<0.01	<0.01
14:0	1.68[ab]	1.72[a]	1.61[b]	1.42[c]	0.04	<0.01
15:0	0.06[b]	0.06[ab]	0.07[a]	0.06[b]	<0.01	<0.01
16:0	27.58[a]	26.85[a]	25.56[b]	23.74[c]	0.45	<0.01
17:0	0.33	0.32	0.33	0.33	0.01	0.94
18:0	12.24[a]	11.85[a]	11.15[b]	10.33[c]	0.23	<0.01
19:0	0.10[b]	0.37[b]	0.51[b]	2.50[a]	0.46	<0.01
20:0	0.37[c]	0.37[c]	0.42[b]	0.46[a]	0.01	<0.01
MUFA[2]	41.43[a]	41.75[a]	39.25[b]	36.43[c]	0.53	<0.01
13:1	0.00	0.00	0.00	0.00	<0.01	0.67
14:1n5	0.03[ab]	0.03[a]	0.02[b]	0.02[c]	<0.01	<0.01
16:1n7	2.44[a]	2.44[a]	2.09[b]	1.74[c]	0.07	<0.01
18:1trans	1.63[a]	1.57[ab]	1.47[ab]	1.38[b]	0.07	0.02
18:1n9	34.19[a]	34.44[a]	32.73[b]	30.77[c]	0.39	<0.01
18:1n7	2.49[a]	2.55[a]	2.26[b]	1.95[c]	0.06	<0.01
19:1	0.05[b]	0.05[b]	0.06[a]	0.06[a]	<0.01	<0.01
22:1	0.60	0.68	0.62	0.51	0.06	0.13
PUFA[3]	15.99[c]	16.48[c]	20.90[b]	24.54[a]	0.51	<0.01
18:2trans	0.03[a]	0.03[a]	0.03[ab]	0.03[b]	<0.01	<0.01
18:3n6	0.04[b]	0.04[b]	0.05[a]	0.05[a]	<0.01	<0.01
18:2n6	15.74[c]	16.23[c]	20.65[b]	24.30[a]	0.51	<0.01
18:3n3	0.17	0.17	0.17	0.16	<0.01	0.1
IV[4]	63.3[c]	64.2[c]	69.7[b]	73.5[a]	0.80	<0.01

[a-c] Estimated marginal means in the same row without a common superscript differ ($P < 0.05$) because of REIMS group

[1]Determined from canonical correlation of fatty acid profile and REIMS factors

[2]SFA: Sum of total saturated fatty acids.

[3]MUFA: Sum of total monounsaturated fatty acids.

[4]PUFA: Sum of total polyunsaturated fatty acids.

[5]Calculated Iodine Value: IV = (0.95×[16:1])+(0.86×[18:1])+(1.732×[18:2])+(2.616×[18:3]), where [] indicate the concentration percentage.

Table 9. Misclassification matrix of metabolomic fingerprints acquired by rapid evaporative ionization mass spectrometry (REIMS) and fatty acid composition using canonical correlation on fresh bellies.

			Predicted					
			Excellent	Great	Good	Poor	Total	
Set 1	Actual	Excellent	19(73%)	7	0	0	26	
		Great	9	54(70%)	13	1	77	
		Good	0	13	41(68%)	6	60	
		Poor	0	0	9	23(72%)	32	Overall Accuracy
		Total	28	74	63	30	195	70%
			Predicted					
			Excellent	Great	Good	Poor	Total	
Set 2	Actual	Excellent	23(74%)	8	0	0	31	
		Great	7	51(70%)	15	0	73	
		Good	0	15	38(66%)	5	58	
		Poor	0	0	8	26(76%)	34	Overall Accuracy
		Total	30	74	61	31	196	70%

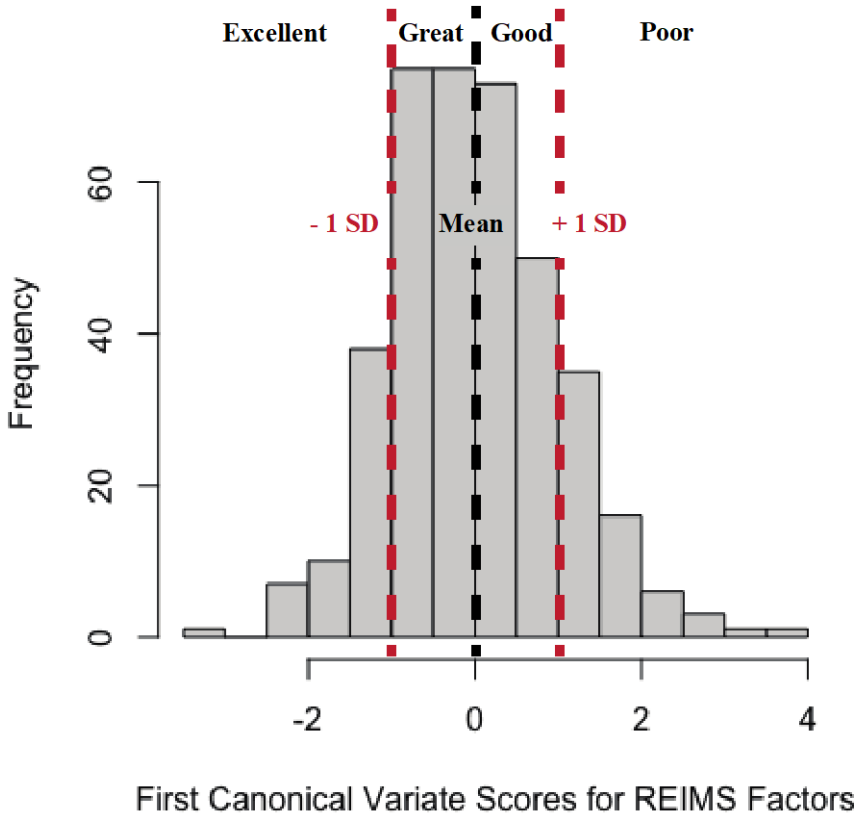

Figure 1. Categorization of fresh belly samples into quality groups by scores on first canonical variate of rapid evaporative ionization mass spectrometry (REIMS) factors.

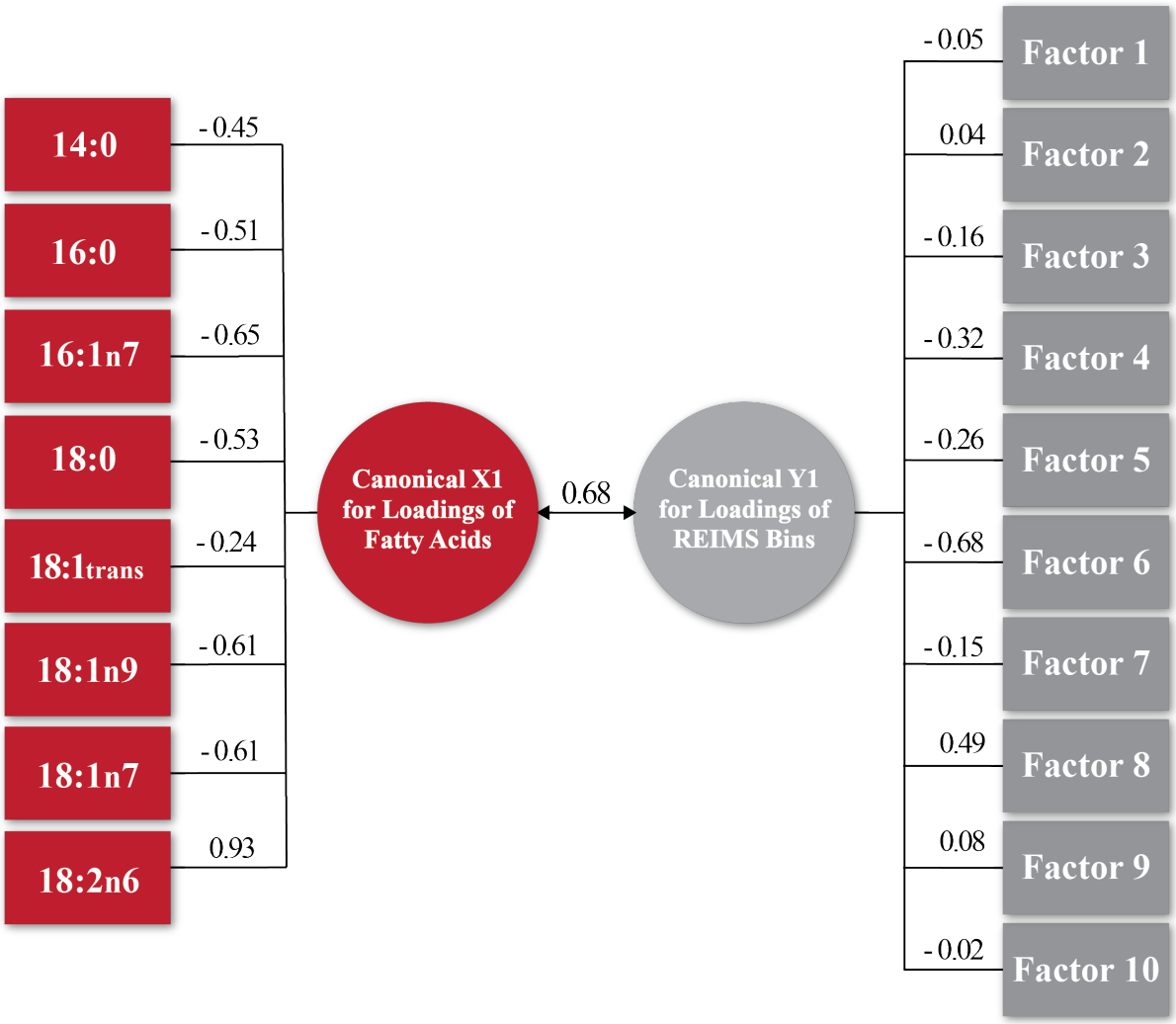

Figure 2. Relationship of fatty acids and rapid evaporative ionization mass spectrometry (REIMS) factors on the first canonical correlation variate.

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