RESEARCH REPORT
PORK QUALITY

Title: Influence of iodine value and packaging type on shelf life of HRI packaged bacon slices – **NPB# 13-127**

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Industry Summary:

Over the last decade, a great deal of research has been done investigating how diet manipulation influences pork quality. Most studies, regardless of dietary treatments, show that increasing the mono and polyunsaturated fatty acid ratios in carcass fat will result in decreasing pork fat quality. In the pork industry, fat quality is generally expressed in terms of iodine value. While not all studies calculate an iodine value, it has been demonstrated it is possible to use a variety of feed sources and feeding methods to attain similar iodine values. Most commonly, the location of the feeding operation dictates the economics of what feed ingredients are included in the diet, meaning that iodine values will always be a source of variation. Commercial diet manipulations will always be at the mercy of economics and least cost formulation of swine diets. Diet manipulation/substitution has occurred off and on over the last decade because dried distillers grains with solubles have been used in diet formulations to reduce costs when they are cheaper than traditional feed stuffs. Thus, it is a reasonable expectation that variation in iodine value and pork quality will continue to occur in the future due to the changing economics of diet formulation.

Due to observed variation in carcass iodine values it would be beneficial to investigate ways to manage incoming fat quality. This is especially important for belly processing as bellies contain compositionally high amounts of fat that has been shown in literature to vary in fatty acid composition. A concept that is often forgotten in bacon quality literature is the fact that not all bacon is manufactured and stored under traditional retail vacuum storage conditions. Bacon is inherently prone to oxidation due to its high fat content, low nitrite content, and high salt content. These properties when combined with freezing and oxygen exposure, which are

known pro-oxidants, could potentially be a source of concern when bacon is packaged using an aerobic format such as bulk packaging schemes currently utilized for food service applications. Food service or Hotel, Restaurant, and Institutional (HRI) bacon is typically frozen and bulk packaged under atmospheric oxygen containing systems (non-vacuum). The management of lipid oxidation is undoubtedly much more challenging when utilizing a frozen, aerobic packaging environment compared to vacuum packaged retail bacon. As a result, no literature exists documenting how lipid oxidation promoters such as freezing and aerobic packaging impact the shelf life of products that have different iodine values. Therefore, the main objective of this study was to determine the shelf life characteristics of bacon slices from bellies with different iodine values that are aerobically and anaerobically packaged during extended frozen storage. A secondary objective was to evaluate how bacon morphology differs from bellies with different iodine values.

This study was conducted in a commercial swine harvest facility, where NitFom™ technology (Near-Infrared-Transmission spectroscopy) was used to identify the iodine value of pork carcasses for Low, Intermediate, and High iodine value categories. The iodine value sampling site was taken over the shoulder of the carcass and confirmed in plant using fat cores from the same location using a benchtop Near Infrared Reflectance (NIR) unit. Bellies were selected from 72 carcasses with 24 carcasses representing each of three iodine value categories representing a Low, Intermediate, and High iodine value. The Low iodine value population had an average NitFom™ iodine value of 64.9, Intermediate an average iodine value of 70.5, and High an average iodine value of 76.5. After chilling, bellies were removed from the carcass and shipped to a commercial plant to be processed into bacon. After slicing, bacon slices were packaged and shipped to the Kansas State University Meat Laboratory to be packaged in either vacuum or aerobic bulk food service packaging in a multiple sheet layout. The samples were then stored at 0 ^{o}F and evaluated at day 0, 28, 56, 70, 84, 98, 112, 126, 140, and 154 for color and lipid oxidation.

Belly measurements were recorded for thickness, length and width. Additionally, belly firmness was measured using the belly "bend" test. Bacon was analyzed for pH, instrumental fat color, proximate composition, fatty acid composition, oxidative rancidity, collagen content, fat cell size and fat cell number.

It was possible to sort carcasses into Low, Intermediate, and High iodine value categories utilizing NitFom™ technology. However, the resulting composite iodine values for bacon samples analyzed using gas chromatography showed that the High iodine value was the only iodine value group that was shown to be statistically different when compared to the Intermediate and Low iodine value groups. However, this did not prevent the detection of belly firmness differences between the categories. As expected, objective firmness measurements displayed a decrease in belly firmness as the iodine value increased. Belly dimensions (length, width, and thickness) did not differ between the iodine value categories.

Bacon from the High, Intermediate, and Low iodine value treatments did not statistically differ in proximate composition (fat, moisture, and protein) or pH. As expected, bacon from the High iodine value category had higher percentages of linoleic, linolenic, and total polyunsaturated fatty acids compared to the other two iodine value categories. Also, bacon from the Low iodine value category was higher in the percentages of myristic, palmitic, stearic, and total saturated fatty acids compared to the High iodine value treatment.

Iodine value had minimal impact on fat color or lipid oxidation in both the vacuum packaged samples and the aerobically bulk packaged samples. The one exception with regards to iodine value was that fat from the Low iodine value category was lighter in color than fat from the Intermediate and High iodine value categories regardless of storage length. This would indicate that as iodine values increase, bacon fat becomes darker in color. Color was most affected by packaging scheme, as aerobically bulk packaged HRI products had lighter and less red fat color.

Lipid oxidation was significantly higher in aerobically packaged samples indicating much higher concentrations of lipid degradation by-products compared to vacuum packaged samples. In fact, all iodine value levels packaged aerobically were much higher in lipid degradation products than vacuum packaged samples after just 28 days of frozen storage. Additionally, most of the oxidation that occurred over the 154 day shelf life occurred in the first 28 days of frozen storage for the aerobically packaged treatments. Bacon fat color also became more yellow with increased storage length for the aerobically packaged samples regardless of iodine value category. Surprisingly, there was no difference in oxidation between iodine value categories within each packaging treatment.

The size of the fat cells and the total count of fat cells did not statistically differ between iodine value categories. However, the soluble, insoluble, and total collagen contents were higher in the High iodine value category than in the Intermediate or Low iodine value categories. The observed increase in collagen in the High iodine value group may give us insight into potential issues with bacon slice-ability as collagen is well known to be the most important component of meat tenderness.

In summary, the NitFom™ technology was able to sort belly quality via carcass iodine value. Iodine value did not dramatically affect quality parameters associated with vacuum packaged or aerobically, bulk packaged bacon. Lipid oxidation dramatically increased after only 28 days of frozen storage for the aerobically packaged HRI style bacon compared to vacuum packaged bacon. The fat histology was not significantly affected by belly iodine value. Finally, it appears that collagen content of bacon fat increases as the iodine value increases. This increase in collagen content may have an effect on bacon slicing yields.

This study is the first to demonstrate that there are serious quality considerations pertaining to lipid oxidation to be considered when bulk HRI packaging systems are to be used for extended frozen storage

periods. Lipid oxidation of fat results in increased off-flavors by bacon consumers. Therefore, it would be prudent to further study the effects of increased lipid oxidation on sensory traits of bacon packaged in aerobic packaging formats. It may also be fruitful to determine ways to increase bacon fat stability for food service applications by either adding additional antioxidants or possibly changing manufacturing/packaging methods. Finally, increases in collagen content due to elevated iodine values should be investigated due to their potential impact on bacon slicing yields.

For more information about the project please contact Terry A. Houser Ph.D., Associate Professor of Meat Science in the Department of Animal Sciences and Industry at Kansas State University.

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Scientific Abstract:

Pork carcasses were selected for iodine value (IV) using a NitFom™ sensor. Carcasses were sorted into three IV categories with the target IV range defined as 58-63 (Low), 68-73 (Intermediate), and 78-83 (High). Over the course of three sampling dates, 72 pork carcasses were identified. Bellies were removed from both the right and left sides of the carcass so that there was a total of 144 bellies in study with 48 bellies (24 carcasses) in each IV category. Bellies were processed via commercial methods into bacon. This experiment was organized into a generalized randomized complete block design with a split plot that had three IV treatments with an average carcass IV of 64.9 (Low), 70.5 (Intermediate), and 76.5 (High) and two packaging treatments (aerobic and anaerobic). Fresh bellies were analyzed for dimensional characteristics (weight, length, width, thickness) and belly firmness. Left and right bellies were randomly assigned to packaging treatment. Each individual sliced belly was divided into five zones and one slice taken from each zone was laid out on divider sheets to represent the whole belly. Ten sheets were laid out for each belly representing the ten pull dates (d 0, 28 56, 70, 84, 98, 112, 126, 140, and 154) for oxidation analysis. Slices were selected from zone two for histological and collagen analysis. Bacon slices were analyzed for oxidative rancidity and fat color (L*a*b*) for every shelf life pull date. After packaging, bacon slices were stored at -17.8 ºC for the remainder of the storage period. Day 0 bacon was analyzed for fatty acid composition, pH, and proximate composition. Bacon manufactured from the High IV category carcasses had a higher ($P < 0.05$) analyzed IV compared to the Intermediate or Low IV category with mean IV values of 76.9, 70.9, and 67.7 respectively. Belly weight, length, width, and thickness were not different ($P > 0.05$) between IV categories. Belly firmness significantly ($P < 0.05$) decreased as the IV category increased. Bacon slices were not different ($P > 0.05$) in proximate composition (fat, moisture, and protein) or pH. High IV bacon samples had greater ($P < 0.05$) percentages of linoleic acid, linolenic, and total polyunsaturated fatty acids and lower ($P < 0.05$) percentages of myristic, palmitic, stearic and total saturated fatty acids compared with the Low IV category. Aerobic and anaerobically packaged bacon from the High IV group had lower ($P<0.05$) L* compared with Low IV group. Aerobically packaged bacon had lower ($P<0.05$) a* values from day 0 to day 154. Anaerobically packaged bacon had higher ($P < 0.05$) a* values from day 0 to day 154. Increasing storage time from day 0 to day 154 increased ($P < 0.05$) b* values for both aerobic and anaerobic packaging treatments. Thiobarbituric acid reactive substances did not differ ($P > 0.05$) between IV categories. Aerobically packaged bacon had higher ($P < 0.05$) TBARS from day 0 compared to day 28. TBARS

values were also higher from day 28 to day 154 for aerobically packaged bacon. TBARS values for anaerobically packaged bacon did not increase (P>0.05) from day 0 to day 84. Soluble collagen, insoluble collagen, and total collagen were higher (P <0.05) in the High IV category than the Low IV category. No differences (P >0.05) were detected in fat cell size or the number of fat cells in bacon fat between IV categories. In conclusion, IV category had minimal impact on frozen bacon quality. However, frozen bacon stored in aerobic packaging resulted in rapid development of lipid oxidation and more pronounced changes in fat color compared with bacon stored in anaerobic packaging.

Introduction

From Shackelford et al. (1990) investigating bacon quality of finishing swine fed elevated monounsaturated fat to Apple et al. (2011) considering quality characteristics of bellies from pigs supplemented with 1-carnatine and three levels of corn oil, there has been a multitude of literature published discussing improving bacon quality through diet manipulation. In many of these studies, especially the ones concerning dried distillers grains with solubles (DDGS) inclusion, the impact of fatty acid composition on belly firmness and slice yields are a central topic of discussion. This has led researchers and industry to put an emphasis on expressing fat quality in terms of iodine value (IV). The impact of carcass fat IV has been researched in literature most commonly by feeding DDGS at various levels (0, 20, 30 and 45%) to achieve different IV (Goehring et al. 2010, Leick et al. 2010, Skaar et al. 2010, Whitney et al. 2006, Widmer et al. 2008, Xu et al. 2010). While these studies excellently detail the impacts on pork quality, there is variation in reported IV at certain levels of DDGS inclusion. Therefore, it would be more practical to make conclusions based off of specific IV as opposed to individual diets.

When dealing with fat quality, most literature identifies lipid oxidation as a major concern when shifting porcine fat composition to higher concentrations of unsaturated fatty acids (Rickard et al. 2012). However, it is unclear how large the difference between IV levels have to be in order to notice a difference in fat quality and how severely that will impact lipid oxidation. Additionally, it is unclear the extent of the impact of IV on aerobically packaged bulk bacon used in food service. Muhlisin et al. (2011) investigated storage quality of bacon processed with organic acids (0.4%) and stored in modified atmosphere packaging (50% CO_2 MAP or 100% N_2 MAP). Muhlisin et al. (2011) reported no significant differences among treatments for lipid oxidation with TBARs values ranging from 0.12 to 0.27 over 14 day duration.

Another topic that has been ignored with these pork quality studies is the impact added ingredients have on raw pork materials that have different IV levels. This could pose a problem as a good proportion of pork produced is further processed in some way, with salt being commonly added. It is clear that shelf life studies of bacon have shown little difference, mainly due to storage under anaerobic conditions and low storage times. Therefore, it is essential to determine the implications of IV and how the magnitude between IV levels impact the shelf life of aerobic and anaerobically packaged bacon.

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Objectives

1. Determine the shelf life characteristics of frozen bacon slices from bellies with different iodine values that are aerobically and anaerobically packaged.

2. Evaluate how bacon morphology differs from bellies with different iodine values.

Materials and Methods

Belly Selection

In this study, fresh pork bellies were acquired from a commercial swine harvest facility (Farmland Foods, Milan, MO) over the course of three sampling dates. Target IV categories were defined before entering the plant at Low (58-63), Intermediate (68-73), and High (78-83). Using a NitFom™ (Near-Infrared-Transmission Spectroscopy) sensor unit (Carometec A/S, Herlev, Denmark), IV was measured on the left side of the pork carcasses above the scapula, and two inches from the mid line of the carcass as carcasses exited the kill floor going into the blast freezer. The IV of at least 200 carcasses were measured during each sampling date. NitFom IV measurements were validated by taking core samples from the same location on the carcass where the NitFom measurement was taken. Cores were taken using 2.5 mm core bit attached to a drill as carcasses exited the blast freezer. The core attachment was wide enough diameter to completely surround the sampling site of the NitFom. Core IV were measured in plant using a Bruker-NIR bench top device. Pork carcasses were stored overnight in carcass coolers which allowed researchers to identify the pork carcasses that best fit the three targeted IV category parameters. More pork carcasses were identified per IV category than was required to compensate for any bellies getting lost during the fabrication process or further processing into bacon. Table 1 describes the IV of bellies selected for each category across all three repetitions.

Fresh Belly Analysis

During pork carcass identification hot carcass weight, and carcass sex were recorded. After carcass fabrication, bellies from both the right and left side were collected. As pork carcasses exited the carcass cooler identification numbers were drawn on the skin surface of the belly with a wax crayon so that bellies could be

collected off the belly line. Belly length, width, and thickness were measured with a metal ruler. Belly length was measured by positioning the ruler down the middle of the belly starting from ham end. Three belly thickness measurements were taken on the dorsal edge throughout the length of the belly. Thickness measurements were taken 5 cm from the ham edge, 5 cm from the shoulder edge, and in the middle of the belly. Belly width was measured by placing the ruler 1/3rd the belly length from the ham edge and 1/3rd the belly length from the shoulder edge. Belly weight was measured using scales provided by the plant.

Objective belly firmness was measured by centering the belly perpendicular to the belly length over a round metal bar. Bellies were placed skin side down and measured on the dorsal edge. Firmness was quantified by measuring the distance between the bottom of the ham and shoulder edges. After fresh belly measurements were taken, the bellies were stacked in cardboard combos and sent for further processing at the Farmland plant in Denison IA.

Bacon Processing

Fresh bellies were commercially processed into bacon using Farmland's curing and smoking operating procedures. Before slicing the IV results from the Bruker-NIR were analyzed and bellies from carcasses that showed IV discrepancies between the NitFom and the Bruker-NIR were excluded from the study. At this point the 24 paired bellies (right and left sides) intended for this study were identified. A total of 48 bellies were acquired for each repetition totaling 144 bellies. Bellies were sliced using a high speed slicer set to a slice thickness of 4 mm. Line workers were instructed not to do any slice sorting so that all bacon slices were in continuous order from the shoulder end to the ham end. Bacon slices were bulk boxed so that there was only one belly per box and that the ends of the belly were clearly marked so that researchers could identify the anatomical origins of each slice in each belly. Bacon slices were transported to the Kansas State University Meat Laboratory (Manhattan, KS) to be assigned to packaging treatments.

Bacon Layout and Packaging

Upon arrival at the KSU Meat Laboratory, bacon from the paired bellies was randomly assigned to one of two packaging treatments. Packing treatments were aerobically packaged using either a poly-liner overwrap or an anaerobic 60.96 cm × 76.2 cm vacuum package pouche (3 mil standard barrier, Prime Source Vacuum

Pouches, Bunzl Processor Division, Koch Supplies, Kansas City, MO). For each packaging treatment, seven bacon slices were selected from each belly, laid out in a HRI single slice layout style on a paper divider sheet measuring 41.12 cm × 25.52 cm. The bacon slices were kept in chronological order from shoulder to ham end with the slices kept together so that the overall belly profile was retained. This allowed the sliced bacon slab to be divided lengthwise into five zones similar to Trussell et al. (2011). Each zone was 12 cm long with zone one starting near the shoulder end and zone five near the ham end of the belly.

In an effort to maintain continuity in slice sampling a standard was created measuring 60 cm in length with five 10 cm zones marked on it. This method was implemented to reduce sampling variation due to bellies being different lengths. An additional reason for selecting samples like this is the expectation that in a commercial HRI slicing set-up, the end pieces containing larger proportions of fat would be sorted from the majority of the HRI bacon. One slice was taken from each zone and two random slices were laid out on a divider sheet. The two random slices were laid out in order to allow easy stacking of the bacon sheets for storage. The divider sheet contained a label with the belly identification number and the pull date. The slices were laid down in chronological order with the bacon slice from zone one starting at the end of the sheet with the identification label and the two random slices at the end of the continuous string of bacon slices. The two random slices were included on the layout sheet as a precaution against bacon slices getting lost and to provide extra material for chemical analysis if needed. Each sheet of bacon originated from a single belly and ten different sheets were made per belly to correspond with ten different shelf life dates. All bacon sheet units that were assigned to day 0 were packaged in the same box and the same was done each of the other pull dates so that on each pull date, all belly samples were accounted for. Packaging treatments were stored in separate boxes at -17.8 ºC . Box dimensions were 44.6 cm × 28.4 cm × 11.3 cm (L×W×D).

Chemical Analysis

Bacon slices used for chemical analysis were taken from the day 0 pull date. For each belly, the five slices representing the five belly zones were cut into small pieces, and mixed into a composite sample, frozen in liquid nitrogen, and pulverized in a blender (Model 33B179, Waring Products, New Hartford, CT., U.S.A) and stored in whirlpak bags. Samples used for proximate, fatty acid, and pH analysis were removed from the

composite sample. Protein composition was analyzed using the AOAC 990.02 (1994) protocol, and moisture and fat with the AOAC PVM-1 (2003) protocol.

A pulverized fat sample was weighed into screw-cap tubes with Teflon-lined caps for fatty acid analysis. Fat samples were then mixed with 3 mL of methanolic-HCL and 2 mL of an internal standard consisting of 2 mg/mL of methyl tridecanoic acid in benzene. Samples were heated in a water bath for 120 min at 70°C for transmethylation. Tubes were vortexed at 45 and 90 min during the water bath period. After heating, 2 mL of benzene and 3 mL of K_2CO_3 were added to extract the methyl esters. Methylated fatty acids were analyzed by gas chromatography. Iodine values were calculated from the fatty acid composition using the AOCS (1998) protocol: (C16:1 × 0.95) + (C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785) + (C22:1 × 0.723). pH was evaluated by mixing 10 g of ground bacon in 90 mL of deionized water on a stir plat and measured with a S220 SevenCompact pH meter (Mettler Toledo; Switzerland) (Sebranek et al. 2001).

Lipid Oxidation

Thiobarbituric acid reactive substances (TBARS) were measured using the method described by Lowe et al. (2014). Five samples from each pull date were pulverized as described in the chemical analysis section. Five g of sample were mixed with deionized water (14 ml deionized water) and 1 mL of 0.5% Sulfanilamide in 20% HCL in a 50 ml centrifuge tube. The mixture was homogenized on ice for 15 seconds. From that slurry, 1 ml of homogenate was mixed with 50 µL of butylated hydroxytoluene (BHT 7.2%) and a 2 mL of a 0.02 M thiobarbituric acid/15% trichloroacetic acid mixture. Samples were then vortexed and incubated in 90°C water for 18 minutes to allow color development. After heating samples were cooled for 10 minutes in a cold water bath and centrifuged @ 3000 × g for 15 minutes at 21°C. One mL of the supernatant was transferred into a spectrophotometer cuvette and absorbance was measured on a spectrophotometer (EON265489, Biotek Instruments, Inc., Winooski, VT, U.S.A) at 531 nm against a blank cuvette containing 1 mL of deionized water, 50 µL of BHT, and 2 mL TBA/TCA solution. Thiobarbituric acid reactive substances values were expressed as milligrams of malondialdehyde per kg of wet tissue.

Fat Color Analysis

Instrumental color was measured using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10° standard observer; Hunter Associates Laboratory, Reston, VA). For every shelf life date (d 0, 28 56, 70, 84, 98, 112, 126, 140, and 154) fat color was measured on five bacon slices from every belly. The five bacon slices represent zones 1 – 5. Three samples were taken on every slice and L* (lightness), a* (redness), and b* (yellowness) were recorded.

Histochemistry

One 0.5 cm thick bacon slice was taken from the line between zone 2 and zone 3 of the sliced slab of bacon. This sample profile included an *Abdominus* muscle, a fat layer, the *Cutaneous Trunci*, and the bottom fat layer. The *Abdominus* muscle was included in the sample so that it was possible to identify the location (above or below the *Cutaneous Trunci*) of each picture taken under the microscope. Samples were preserved in 10% formaldehyde and sent to the Kansas State University Veterinary Histopathology Laboratory to be paraffin embedded. Two 10-µm thick sections were collected on positively charged slides (Fisher Scientific, Pittsburgh, PA) from each sample.

To remove the paraffin wax, slides were heated in an incubator at 55°C for 20 min and positioned to allow drainage of the melting paraffin. Slides were immersed in xylene twice for10 min and were then submerged in 100, 95, 70, 50, and 30% ethanol solutions for one minute each, with the exception of the 100% ethanol (2 min). To finish the deparaffinization process, slides were submerged in a 0.85% NaCl solution and 1× phosphate buffered saline (PBS) for 2 min each.

Samples were stained with Harris Hematoxylin and Eosin-Y (Fisher Scientific). Slides were submerged in Harris Hematoxylin for 1 min and then removed and rinsed with water until the runoff water was clear. Then slides were submerged in Eosin-Y for2 min and then rinsed as previously stated. The staining protocol was concluded by dehydrating the sections in ascending alcohol solutions (50, 70, 80, 95 and 100%) for 1 min each and then submerging the sections in Xylene for 2 min. After staining, samples on the slides were covered with 10 µL of 9:1 glycerol in PBS and were cover-slipped for imaging. Sections were imaged using a Nikon Eclipse TI-U inverted microscope equipped with a Nikon DS-QiMc digital camera at a 10× working distance

magnification (Nikon Insturments Inc., Melville NY). For each slide, four representative photomicrographs were taken in each fat layer of each section.

Collagen Content

Collagen content was analyzed by using a methodology modified from Hill (1966). The amount of hydroxyproline was quantified in order to estimate total collagen content of fat in bacon slices. Three bacon slices were taken from the line between zone 2 and zone 3 of the sliced bacon slab. The fat was carefully separated from the lean of the bacon, and frozen in liquid nitrogen, and pulverized in a blender (Model 33Bl79, Waring Products, New Hartford, CT., U.S.A). Samples were then lyophilized and weighed out into a 50 mL (29 mm ×122 mm) Pyrex round bottom centrifuge tube (Sigma-Aldrich, St. Louis, MO). Twelve milliliters of 1/4 strength Ringer's Solution was added to samples and were incubated at 77°C for 80 min with gentle shaking every 10 min. Upon removal from the hot water bath, test tubes were centrifuged at 3000 rpm for 12 min at 20°C. The supernatant was siphoned off with serological pipettes and transferred to 25 mm ×150 mm glass test tubes for soluble collagen analysis. Three milliliters of Ringer's Solution was added to the test tubes containing the insoluble pellet and the tubes were centrifuged as described previously. After centrifugation the 3 mL of Ringer's solution was transferred to the soluble fraction test tube. Three milliliters of concentrated H_2SO_4 were added to the soluble fraction test tubes, and 30 mL of 3.5 molar H_2SO_4 were added to the insoluble fraction. All test tubes were incubated at 105°C for 16 h.

After hydrolysis, samples were removed from the incubator and allowed to cool for 30 min. The insoluble hydrolysate was transferred to a 500 mL volumetric flask and brought to volume with 1MΩ H_2O. The soluble hydrolysate was transferred to a 250 mL volumetric flask and brought to volume with 1MΩ H_2O. The volumetric flasks were poured into 600 mL beakers and mixed with a magnetic stir bar on a stir plate. After mixing, samples were gravity filtered through Whatman 541 filter paper (125 mm diameter) into 16 mm × 25 mm disposable glass culture tubes. Ten milliliters of the sample was transferred to 15 mL glass containers and immediately analyzed using a hydroxyproline assay.

Hydroxyproline determination was carried out following the procedures outlined by Bergman and Loxley (1963) using a BioTek Eon spectrophotometer (Winooski, VT) to read absorbance at 558 nm. The

spectrophotometer was calibrated using a distilled water blank sample, and readings were quantified by standard curves prepared for each day of analysis. Total and fractional collagen content was determined by multiplying the hydroxyproline content of the soluble fraction by 7.25 and the insoluble fraction by 7.52 (Cross et al., 1973).

Statistical Analysis

The data were analyzed as a randomized complete block design with a split plot using the MIXED Procedure of SAS (SAS Institute, Inc., Cary, NC) with each belly serving as the experimental unit. Fixed effects were the packaging treatments (aerobic and anaerobic) and IV categories (Low, Intermediate, and High). An α-level of 0.05 was used to assess significance among means.

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Results

Objective 1. Determine the shelf life characteristics of bacon slices from bellies with different iodine values that are aerobically and anaerobically packaged.

Carcass and Belly Characteristics

No significant differences ($P > 0.589$) were found between the hot carcass weights of pork carcasses assigned to the three IV categories (Table 1). There was a statistically detectable difference ($P<0.05$) between the iodine values of the pork carcasses between each IV category.

There were no statistical differences ($P < 0.05$) between belly weight, length, width or thickness between IV categories (Table 2). Bellies were firmer ($P < 0.05$) for the Low IV category compared with both the Intermediate and High IV categories. The High IV category had the softest bellies of all IV categories.

Belly IV in the High IV category was significantly higher ($P < 0.05$) than the Intermediate and Low IV categories (Table 3). Proximate composition (moisture, fat, and protein) and pH were not different ($P >0.05$) between IV categories.

The percentage of saturated fatty acids contained in the bacon slices including myristic, palmitic, stearic, and total saturated fatty acids were higher ($P <0.05$) for the Low IV category compared to the High IV category (Table 4). The percentage of unsaturated fatty acids in bacon slices including linoleic, linolenic, eicosadienoic, and total polyunsaturated fatty acids were higher ($P <0.05$) for the High IV category compared to the Low IV category.

Frozen Bacon Fat Color

No significant ($P >0.05$) three way interactions between belly IV category, packaging type, and storage duration were found in this study for color characteristics of bacon slices. However, an interaction between packaging type by IV category was detected ($P <0.05$) for L*, a*, and b* values. L* values were higher ($P <0.05$) for the Low IV category compared with the Intermediate and High IV categories for the aerobically packaged samples and the anaerobically packaged samples. However, the incremental increase in lightness was much more pronounced in the aerobically packaged samples compared with the anaerobically packaged

samples. Frankly, while significant differences were found in anaerobically packaged samples, the L* value differences between treatments is not of practical significance.

Redness values (a*) were significantly ($P < 0.05$) higher in aerobically packaged bacon from the High IV category compared with the Intermediate and Low IV categories (Table 6). Contrastingly, the Low and Intermediate IV categories had significantly higher a* values compared with the High IV category. It should be noted that none of the aforementioned changes in a* values have practical implications.

Much like a* values, yellowness values (b*) were significantly ($P < 0.05$) higher in aerobically packaged bacon from the High IV category compared with the Intermediate and Low IV categories (Table 7). However, the Low and Intermediate IV categories had significantly higher b* values compared with the High IV category. It should be noted that none of the aforementioned changes in b* values have practical significance due to the very small changes in values.

An interaction between packaging type by storage length was detected ($P < 0.05$) for L*, a*, and b* values. Lightness (L*) values were numerically higher for aerobically packaged bacon compared to anaerobically packaged bacon (Table 8). Additionally, L* values increased ($P<0.05$) from day 0 to day 154 for aerobically packaged bacon. Bacon packaged in anaerobic packaging had minimal changes over the 154 day storage period and were more stable compared to the aerobically packaged samples.

Redness (a*) values significantly decreased ($P < 0.05$) from day 0 to day 154 for the aerobically packaged bacon samples indicating that samples became less red as storage length increased (Table 9). Anaerobically packaged samples increased ($P < 0.05$) in redness from day 0 to day 28 but only changed slightly for the remainder of the storage period. Anaerobically packaged samples were much more stable in redness than aerobically packaged samples.

Both aerobically packaged bacon and anaerobically packaged bacon samples became more ($P<0.05$) yellow (b*) in color due to increased storage duration (Table 10). The anaerobically packaged treatments had a greater amount of numerical change in b* values due to storage length compared with the aerobically packaged bacon slices.

Lipid Oxidation

No significant (P >0.05) three way interactions between belly IV category, packaging type, and storage duration were found in this study for lipid stability of bacon slices. An interaction between packaging type and storage length was detected (P<0.05) and presented in Table 11. TBARS values increased rapidly during storage for the aerobically packaged bacon treatments. TBARS values increased from 0.42 mg malonaldehyde/kg of sample on day 0 to 1.07 mg malonaldehyde/kg sample on day 28 for the aerobically packaged bacon samples. TBARS values then increased at a more gradual pace from 1.07 mg malonaldehyde/kg of sample on day 28 to 1.66 mg malonaldehyde/kg of sample on day 126 for aerobically packaged bacon. The anaerobically packaged bacon also had increased (P <0.05) TBARS from day 0 to day 154 but were more gradual and were not over 1.0 mg malonaldehyde/kg of sample until day 126. In fact, no statistical change in TBARS values occurred in the anaerobically packaged samples from day 0 to day 70.

No significant interaction (P>0.05) was detected in TBARS values for IV and packaging type (Table 12). Additionally, no statistical differences (P >0.05) were detected for TBARS values due to IV category (Table 13).

Objective 2. Evaluate how bacon morphology differs from bellies with different iodine values.

No significant differences were observed in the size of the fat cells (P>0.05) or the number of fat cells (P>0.05) between the three IV categories (Table 14). However, the soluble collagen content in the High IV category was greater (P <0.05) than the collagen content in the Low IV category. There was a trend for the High IV category to have more soluble collagen than the Intermediate IV category (P = 0.096). The insoluble collagen content in the High IV category was higher (P <0.05) than the Low IV category and there was a statistical trend (P = 0.097) for the High IV category to have a higher insoluble collagen content than the Intermediate IV category. Following the pattern of the soluble and insoluble collagen content, the High IV category showed greater amounts of total collagen (P = 0.008) than the Low IV, and the High IV bellies tended to have more total collagen (P = 0.090) than the Intermediate category. There was no change in the percent soluble collagen between the IV categories (P >0.05).

Discussion

Results of this study show that there is considerable variation in carcass iodine values on any given day of in a commercial processing plant. It was possible to select a population of pork carcasses using NitFom technology and sort them into categories. However, this does not mean the IV of the bellies will fit perfectly in the carcass IV categories. Despite there being a smaller gap between the belly iodine values of the Intermediate and Low IV categories compared to the High and Intermediate categories, it was still possible to observe a difference in firmness between all three categories. In fact there was an equivalent decrease in firmness between the Low and Intermediate (31%) and the Intermediate and High (30%) IV categories. This would suggest one of two things: That an average difference of 3 IV units is enough to detect differences in belly firmness, or that the IV gradient within the belly is extreme enough to significantly influence fatty acid results if a composite sample is made from multiple sampling locations across the belly. By selecting average carcass weights in the range of 97.89 (SD of 5.0), it is possible to limit variation in belly dimensions (length, width, thickness, and weight).

Since calculated IV is dependent on changes in specific fatty acids it is no surprise that bacon from the High IV treatment had a higher percentage of unsaturated fatty acids and a lower percentage of saturated fatty acids compared to the Low IV treatment. As a result, we expected the changes that were reported in this experiment.

IV did not have a practical impact on color characteristics of frozen bacon fat samples. Most of the color changes reported were minor and less than 1.5 units of the respective color scale being used. Lightness values would be the one exception with High IV categories being darker than their Low IV counterparts. This may be due to increased collagen content found in the High IV treatments making the fat appear darker in color. If bacon with extremely high IV values would produce bacon with darker fat color, those raw bellies may be better suited for pre-cooked bacon so that consumers don't see the darker colored fat in the raw state.

The findings of this study indicate that the development of rapid oxidation of fat in sliced bacon is of major concern when using an aerobic packaging environment when frozen temperatures are held at -17.8 ºC for an extended period of time. It has been concluded by many researchers that lipid oxidation would be a major

problem with High IV bellies, however that was not the case in this study. These findings indicate that packaging environment has a much greater impact on fat stability than IV. It would be prudent for more bacon research to be conducted to try and stabilize bacon fat stored in aerobic packaging due to its popularity in food service applications. Very simply, few researchers have looked at aerobic packaging systems as it pertains to bacon fat quality.

As there were no differences in the size of fat cells or the number of cells in bacon fat, it is does not seem that this is a factor that might influence belly firmness. The animals selected in this study did come from various backgrounds and it is probable that there were different feeding regimens. Thus, it is possible that the question of fat morphology influencing belly firmness should be answered in a controlled diet manipulation study. Results from this study show that bellies with higher IV values have greater amounts of collagen in the fat depots. This could be due to the analysis method (Hill Method) which is known to produce variation in its process. A point to consider here is if the factors that control the IV gradient in the belly also cause a gradient in collagen content within the belly. It has been proven in studies investigating inter-muscular collagen turnover that control of muscle growth via animal nutrition and oxidative stress can influence collagen content. Again the question of collagen content in pork fat would be better answered in a controlled diet manipulation study. As the collagen content is different between High and Low IV categories it is possible that the collagen content plays a role in influencing belly firmness and may impact belly color and possibly slicing yields.

Table 1. Mean hot carcass weights and iodine values from carcasses in the sample population[1]

	Iodine Value Category			
	High	Intermediate	Low	SEM[2]
Hot carcass weight, kg	98.1	96.6	98.2	2.58
Iodine value[3]	77.9[a]	72.6[b]	66.5[c]	1.89

[1]Treatment means with different superscripts are different (P < 0.05).
[2]Standard error of the mean
[3]Calculated using NitFom™ (Near-Infrared-Transmission Spectroscopy)

Table 2. Mean dimensional characteristics of bellies from the sample population[1]

	Iodine Value Category			
Characteristic	High	Intermediate	Low	SEM[2]
Belly weight, kg	7.8	7.8	8.4	0.57
Belly length, cm	74.8	75.2	75.5	1.21
Belly width, cm	32.6	32.5	32.1	0.68
Belly thickness, cm	3.45	3.58	3.67	0.12
Belly firmness, cm	5.55[a]	7.95[b]	11.5[c]	0.42

[1]Treatment means within a row with different superscripts are different (P < 0.05).
[2]Standard error of the mean

Table 3. Mean chemical characteristics of bacon samples from high, intermediate, and low iodine value categories.[1]

	Iodine Value Category			
	High	Intermediate	Low	SEM[2]
Belly/bacon iodine value[3]	76.9[a]	70.9[b]	67.7[b]	1.54
Moisture, %	45.0	44.0	41.5	1.81
Fat, %	36.4	38.3	41.8	2.38
Protein, %	13.6	13.0	11.8	0.58
pH	6.34	6.38	6.38	0.03

[1]Treatment means within a row with different superscripts are different (P < 0.05).
[2]Standard error of the mean
[3]Calculated from fatty acid analysis using AOCS (1998) protocol: (C16:1 × 0.95) + (C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785) + (C22:1 × 0.723)

Table 4. Mean fatty acid percentage for bacon samples from pigs of high, intermediate, and low iodine value categories.[1]

	Iodine value category			
	High[2]	Intermediate[3]	Low[4]	SEM[5]
Myristic acid (C14:0), %	1.31[b]	1.39[ab]	1.45[a]	0.03
Palmitic acid (C16:0), %	21.5[b]	23.2[a]	24.3[a]	0.45
Palmitoleic acid (C16:1), %	2.58	2.72	2.75	0.14
Margaric acid (C17:0), %	0.38	0.40	0.36	0.02
Stearic acid (C18:0), %	10.0[b]	11.4[a]	12.1[a]	0.36
Oleic acid (C18:1n9c), %	36.3	36.8	37.3	1.15
Vaccenic acid (C18:1n7), %	3.49	3.57	3.50	0.22
Linoleic acid (C18:2n6t), %	20.1[a]	16.5[b]	14.4[b]	1.20
α-Linolenic acid (C18:3n3), %	0.69[a]	0.55[b]	0.47[b]	0.07
Gondoic acid (C20:1),%	0.72	0.84	0.79	0.04
Eicosadienoic acid (C20:2), %	0.82[a]	0.74[b]	0.68[c]	0.02
Total SFA, %[5]	33.78[b]	36.82[a]	38.82[a]	0.69
Total MUFA, %[6]	43.50	44.25	44.72	1.08
Total PUFA, %[7]	22.72[a]	18.78[b]	16.46[b]	1.36

[1] Treatment means with different superscripts in a row are different ($P < 0.05$).
[2] High iodine value average 76.9.
[3] Intermediate iodine value average 70.9.
[4] Low iodine value average 67.7.
[5] Total saturated fatty acids, expressed as percentage of total fatty acids present.
[6] Total monounsaturated fatty acids, expressed as a percentage of total fatty acids present.
[7] Total polyunsaturated fatty acids, expressed as a percentage of total fatty acids present.

Table 5. Mean lightness (L*)[1] values of frozen bacon fat samples with different iodine value categories and packaging treatments[2].

	Iodine Value Category			
	High[3]	Intermediate[4]	Low[5]	SEM[6]
Packaging type				
Aerobic	81.4[c]	83.2[b]	84.2[a]	0.134
Anaerobic	80.5[c]	80.9[b]	81.4[a]	0.134

[1]L*, lightness, 0 = black, 100 = white
[2]Treatment means with different superscripts in a row are different ($P < 0.05$).
[3]High iodine value average 76.9.
[4] Intermediate iodine value average 70.9.
[5] Low iodine value average 67.7.
[6]Standard error of the mean

Table 6. Mean redness (a*)[1] values of frozen bacon fat samples with different iodine value categories and packaging treatments[2].

	Iodine Value Category			
	High[3]	Intermediate[4]	Low[5]	SEM[6]
Packaging type				
Aerobic	3.68[a]	3.27[b]	3.26[b]	0.061
Anaerobic	4.92[b]	5.10[a]	5.19[a]	0.061

[1]a*, redness, positive values = red, negative values = green
[2]Treatment means with different superscripts in a row are different (P < 0.05).
[3]High iodine value average 76.9.
[4] Intermediate iodine value average 70.9.
[5] Low iodine value average 67.7.
[6]Standard error of the mean

Table 7. Mean yellowness (b*)[1] values of frozen bacon fat samples with different iodine value categories and packaging treatments[2].

	Iodine Value Category			
	High[3]	Intermediate[4]	Low[5]	SEM[6]
Aerobic	8.87[a]	8.49[b]	8.46[b]	0.057
Anaerobic	8.35[b]	8.66[a]	8.61[a]	0.057

[1]b*, yellowness, positive values = yellow, negative values = blue
[2]Treatment means with different superscripts in a row are different (P < 0.05)
[3]High iodine value average 76.9.
[4]Intermediate iodine value average 70.9.
[5]Low iodine value average 67.7.

[6]Standard error of the mean

Table 8. Mean lightness (L*)[1] values of frozen bacon fat samples with different storage lengths and packaging treatments[2].

	Storage Date										
	0	28	56	70	84	98	112	126	140	154	SEM[3]
Packaging type											
Aerobic	82.3[de]	81.5[f]	82.5[cde]	82.9[c]	83.0[c]	83.0[c]	82.8[cd]	82.6[cde]	84.9[a]	83.9[b]	0.246
Anaerobic	81.9[a]	79.5[g]	80.2[f]	80.9[cde]	80.9[def]	81.5[bcd]	81.1[cde]	80.7[ef]	81.4[bcde]	81.1[cde]	0.244

[1]L*, lightness, 0 = black, 100 = white
[2]Treatment means with different superscripts in a row are different (P < 0.05).
[3]Standard error of the mean

Table 9. Mean redness (a*)[1] values of frozen bacon fat samples with different storage lengths and packaging treatments[2].

	Storage Date										
Packaging type	0	28	56	70	84	98	112	126	140	154	SEM[3]
Aerobic	4.41^{a}	4.01^{b}	2.98^{g}	3.47^{de}	3.29^{ef}	3.00^{fg}	3.67^{cd}	3.64^{cd}	2.81^{g}	2.78^{g}	0.112
Anaerobic	3.86^{g}	4.99^{def}	5.19^{cde}	5.15^{cde}	5.34^{abc}	4.95^{ef}	4.79^{f}	5.61^{a}	5.27^{bcd}	5.53^{ab}	0.111

[1]a*, redness, positive values = red, negative values = green
[2]Treatment means with different superscripts in a row are different (P < 0.05).
[3]Standard error of the mean

Table 10. Mean yellowness (b*)[1] values of frozen bacon fat samples with different storage lengths and packaging treatments[2].

	Storage Date										
Packaging type	0	28	56	70	84	98	112	126	140	154	SEM[3]
Aerobic	7.68^{e}	8.03^{d}	8.01^{d}	8.71^{bc}	8.04^{d}	8.54^{c}	9.73^{a}	9.65^{a}	8.83^{b}	8.86^{b}	0.106
Anaerobic	6.85^{i}	7.95^{h}	8.13^{gh}	8.33^{fg}	8.61^{def}	8.75^{cde}	9.06^{b}	8.92^{bc}	9.44^{a}	9.38^{a}	0.105

[1]b*, yellowness, positive values = yellow, negative values = blue
[2]Treatment means with different superscripts in a row are different (P < 0.05)
[3]Standard error of the mean

Table 11. Mean thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde[1]/kg sample) of frozen bacon samples with different storage lengths and packaging treatments[2].

	Storage Date										
Packaging type	0	28	56	70	84	98	112	126	140	154	SEM[3]
Aerobic	0.42^{f}	1.07^{de}	1.16^{cd}	1.19^{cd}	1.13^{cd}	1.45^{b}	1.25^{c}	1.66^{a}	1.66^{a}	1.61^{ab}	0.07
Anaerobic	0.41^{d}	0.41^{d}	0.46^{d}	0.45^{d}	0.68^{c}	0.63^{c}	0.92^{b}	1.45^{a}	0.69^{c}	1.05^{b}	0.07

[1]Product of lipid oxidation
[2]Treatment means with different superscripts in a row are different (P < 0.05).
[3]Standard error of the mean

Table 12. Mean thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde[1]/kg sample) for frozen bacon samples with different iodine value categories and packaging treatments[2].

	Iodine Value Category			
Packaging type	High[3]	Intermediate[4]	Low[5]	SEM[6]
Aerobic	1.3	1.2	1.3	0.033
Anaerobic	0.74	0.74	0.67	0.034

[1]Product of lipid oxidation
[2]Treatment means with different superscripts in a row are different (P < 0.05).
[3]High iodine value average 76.9.
[4]Intermediate iodine value average 70.9.
[5]Low iodine value average 67.7.
[6]Standard error of the mean

Table 13. Mean thiobarbituric acid reactive substances (TBARS) values for frozen bacon samples stored up to 154 days in anaerobic and aerobic packaging[1].

	Iodine Value Category			
	High[2]	Intermediate[3]	Low[4]	SEM[5]
Malonaldehyde[6] mg/sample kg	1.0	0.99	0.97	0.023

[1]Treatment means with different superscripts in a row are different ($P < 0.05$)
[2] High iodine value average 76.9.
[3] Intermediate iodine value average 70.9.
[4] Low iodine value average 67.7.
[5]Standard error of the mean
[6]Product of lipid oxidation

Table 14. Mean histology characteristics and mean collagen content of bacon fat from high, intermediate, and low iodine value categories.[1]

	Iodine Value Category			
Characteristic	High[2]	Intermediate[3]	Low[4]	SEM[5]
Fat cell size, μm	4016.2	4222.5	4369.0	175.43
Fat cell count, mm^2	246.0	233.0	223.3	14.99
Soluble collagen, mg/g	13.4[a]	12.2[ab]	11.2[b]	0.54
Insoluble collagen, mg/g	35.5[a]	30.2[ab]	24.9[b]	1.92
Total collagen, mg/g	49.0[a]	42.4[ab]	36.2[b]	2.31
% Soluble collagen	29.7	29.7	33.1	1.46

[1]Treatment means with different superscripts are different ($P < 0.05$).
[2] High iodine value average 76.9.
[3] Intermediate iodine value average 70.9.
[4] Low iodine value average 67.7.
[5]Standard error of the mean