

EVALUATING THE EFFECTS OF STORAGE TIME
ON GAS FORMATION FROM RETAIL GROUND
MEAT

By

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EVALUATING THE EFFECTS OF STORAGE TIME ON GAS FORMATION
FROM RETAIL GROUND MEAT

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Title of Study: EVALUATING THE EFFECTS OF STORAGE TIME ON GAS
FORMATION FROM RETAIL GROUND MEAT

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Abstract: The objective of this study was to evaluate greenhouse gas emissions (GHG), specifically carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) from raw and cooked ground beef. Shoulder clods were ground, formed into loaves, and displayed in a retail case. Following the retail display, the samples were collected for GHG analysis from raw and cooked samples (n = 4 replications). The samples were aged to either 7 or 14 d. Following aging, ground beef loaves were displayed under retail conditions for 3 days. Displayed samples were stored under dark at 4 °C (4, 8, and 11 days) to simulate meat storage conditions at home. Samples were cooked to 71.1 °C. Aerobic samples were sealed with atmospheric oxygen, and anaerobic samples were flushed with 100% nitrogen gas. During retail display, objective color measurements of *a** were recorded. Total plate count was conducted on days 4, 8, and 11. The aerobic condition had greater CO₂, CH₄, and N₂O formation compared with the anaerobic condition. Dark storage time had a significant effect on CO₂ formation, but not on CH₄ and N₂O. Aging time increased CO₂ and CH₄ formation (P < 0.05); however, the aging time had no effect on N₂O formation. Raw meat had greater greenhouse gas formation than cooked meat. Bacterial characterization identified *Carnobacterium divergens*, *Hafnia alvei*, *Lactobacillus sakei*, *Lactobacillus sakei*, and *Yersinia enterocolitica*. N₂O gas production was lesser from aged product, and cooked products had greater gas formation. The results suggest that incubation conditions, aging time, and storage time can impact GHG formation of ground beef products.

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CHAPTER I

INTRODUCTION

Enhancing the food system’s efficiencies is critical with the anticipated increase in global population combined with the need to source safe, healthy, and sustainable food. This tasks agriculturists and scientists to seek out new technologies and methods to prevent food waste. The demand for animal proteins will rise drastically with an expected 3.5% increase in global Gross Domestic Product (GDP) and combined with a 34% world population increase by 2030 (Fiala, 2008). Not only are developing countries now able to afford more meat, but their rates of consumption are also predicted to increase. The total global protein demand, accounting for a population of 7.3 billion, is 202 million tons (Henchion et al., 2017). However, with an increase in the production and consumption of animal proteins, the issue of increased food waste, particularly meat waste, possesses a potential negative economic and environmental impact.

It has been estimated in the United States, Canada, Australia, and New Zealand, that approximately 22% of total meat and poultry production is discarded annually (Gunders, 2012). Wastage of meat can result from a variety of factors, including losses during fabrication and processing, cooking or serving a larger portion than is consumed, expiration or over-purchase in the home or in food service, or from a lack of

22 marketability due to discoloration or inability to fulfill color expectations for consumers.
23 A recent report suggests that in 2020 the United States meat industry wasted 5.8 metric
24 tons of meat due to discoloration (Maia Research Analysis, 2020).

25 Meat discoloration can occur from various of factors such as higher retail display
26 case temperature, muscle-specific differences in stability, lipid oxidation, microbial
27 growth, and exposure to oxygen (Ramanathan et al., 2020; Mancini & Hunt, 2005). As a
28 result, discolored meat is typically marked down, reground, or thrown into a landfill. It
29 was reported in 2019 that meat waste due to discoloration resulted in a loss of \$3 billion
30 in the United States and \$14.2 billion in loss globally (Maia Research Analysis, 2020).

31 While there are substantial economic impacts of meat loss, there is also a potential
32 for irreversible environmental impacts. Various packaging types have made substantial
33 progress in extending meat color in a retail setting. Recently, companies have started
34 thinking about reducing the use of plastic in their packaging. For example, Perdue
35 Farms[®] has made a switch to water dissolving biodegradable foam tray, and several other
36 companies have replaced Styrofoam[™] trays with recycled cardboard (Kavilanz, 2020).
37 However, meat wastage can substantially contribute to greenhouse gas production in
38 landfills.

39 The Environmental Protection Agency reported that landfill gas, a natural
40 byproduct from the breakdown of organic matter, is composed of 50% methane and 50%
41 carbon dioxide and 15.1% of total human methane production comes from municipal
42 waste (United States Environmental Protection Agency, 2018). In comparison, various

43 studies have determined that pork production has generated 668 million tons of CO₂
44 equivalent, broiler chicken production accounts for 343 million tons of CO₂ equivalent,
45 and beef accounts for 2.9 gigatonnes of CO₂ equivalent from the production lifecycle
46 (MacLeod et al. 2013, Suszkiw, 2019). However, there is limited knowledge on the
47 impact of meat waste on greenhouse gas production.

48 The objectives of this study were to determine the combined effects of aging,
49 storage condition, and meat state (either raw or cooked) on greenhouse gas formation,
50 specifically carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) from ground
51 beef loaves. We anticipate the results will add missing details of greenhouse gas
52 formation from meat in the life cycle analysis and also could bring greater awareness to
53 consumers about meat waste.

54

CHAPTER II

55

56

57

REVIEW OF LITERATURE

58

59 **Food Waste and Environmental and Energy Impact**

60 Food waste is defined as food appropriated for human consumption that is
61 discarded. It can occur at the pre-harvest level in fruit and vegetables, post-harvest steps,
62 such as processing, transport, over-purchase, or at the consumer level, and it can post
63 detrimental environmental and energy impacts (Food and Agriculture Organization,
64 2013).

65 The United States Department of Agriculture Economic Research Service has
66 estimated that 133 billion pounds out of the 430 billion pounds of edible food produced
67 in the United States were not consumed in 2010. The retail and consumer losses were 43
68 and 90 billion pounds, respectively (Buzby et al., 2019). These losses are valued at over
69 \$161.6 billion USD, with the highest monetary losses resulting from meat, poultry, and
70 fish (Buzby et al., 2019). Consumers in the United States are losing approximately 1% of
71 their average disposable income, essentially losing 0.80 pounds of food per day,
72 equivalent to almost \$1 a day (Buzby et al., 2019). The Food and Agriculture
73 Organization of the United Nations has estimated one-third of global food production

74 deemed for human consumption is wasted every year (Food and Agriculture
75 Organization, 2013). With the pressing need to feed an expected 9 billion people by the
76 year 2050, the world cannot afford the massive amount of food waste, nor the
77 environmental and energy impacts it can bring.

78 Wastage varies across the world with differing climates, soil types, economies,
79 and food waste types. The overall quantity of wastage is not equally matched across the
80 world. Bluewater footprints and carbon footprints have to be considered with varying
81 threat levels globally. Bluewater footprints focus on the freshwater utilized to make a
82 product, industrial or agricultural, while the carbon footprints broadly reference carbon
83 production in greenhouse gases, whether they be from agricultural, industrial, or
84 residential production (Hoekstra et al., 2011, Environmental Protection Agency, 2020).

85 Global food waste ranks as the third-highest total carbon footprint in the world
86 and is only surpassed by the total carbon footprints of the United States and China (Food
87 and Agriculture Organization, 2015). The highest contributor to the Food Supply Chain-
88 Food Waste carbon footprint, specifically evaluating carbon footprint equivalents from
89 all stages of the supply chain such as production, processing, or retail, was found at the
90 consumer level. The average carbon footprint from food waste is about 500 kg CO₂ per
91 capita per year (Food and Agricultural Organization, 2013). Global water footprints in
92 2007, based on consumption and withdrawals, revealed the blue water footprint was
93 approximately 250 km³, and compared to all other countries sampled, the blue water
94 usage from food waste alone had the highest water footprint (Food and Agricultural

95 Organization, 2013). When focusing on arable land, land available for crops or suitable
96 for grazing, food wastage in 2007 was at almost 1.4 billion hectares, almost equivalent to
97 28% of total global agriculture land (Food and Agricultural Organization, 2013). With
98 such losses, the potential for irreversible global environmental impacts could be faced
99 (Food and Agriculture Organization, 2015).

100 **Meat Waste**

101 Meat is a nutrient-dense food. With an expected annual Gross Domestic Product
102 (GDP) increase of 3.5% and a 34% world population increase by the year 2030, it has
103 been predicted the demand for animal proteins will also drastically rise. For example,
104 beef is anticipated to have a 32% increase in demand, pork 73%, and chicken 110% by
105 2030 (Fiala, 2008). It has been estimated that in the United States, Canada, Australia, and
106 New Zealand, 22% of total meat production is wasted annually (Gunders, 2012). Wastage
107 of meat can result from a variety of factors, including cooking or serving a larger portion
108 than is consumed, expiration or over-purchase in the home or in food service, or from a
109 lack of marketability due to discoloration. Globally, total meat loss due to discoloration
110 or lacking color expectations for consumers for 2020 is anticipated to be over 5.8 metric
111 tons (Maia Research Analysis, 2020).

112 Meat discoloration can occur from a variety of factors such as higher retail
113 display case temperature, muscle-specific differences in stability, lipid oxidation,
114 microbial growth, and exposure to oxygen (Elroy et al., 2015; Mitacek et al., 2019;

115 Mancini & Hunt, 2005). It was reported in 2019 that meat waste due to discoloration
116 resulted in a loss of \$3 billion in the United States and \$14.2 billion in loss globally
117 (Maia Research Analysis, 2020).

118 Meat waste also poses a threat to the environment. Greenhouse gases are emitted
119 throughout animal life cycles during production and processing. Since meat is organic, its
120 wastage can result in greenhouse gases. While packaging studies and packaging types
121 have made substantial progress in extending meat color in a retail setting, meat and its
122 packaging are still piling up in landfills. However, Perdue Farms has made the switch to
123 water dissolving biodegradable foam tray, and several other companies have replaced
124 Styrofoam™ trays with recycled cardboard (Kavilanz, 2020). Despite these efforts, meat
125 wastage can substantially contribute to greenhouse gas production in landfills.

126 The estimated water footprint of meat was predicted to be almost one-third of the
127 total agricultural water footprint, taking into account the production of feed,
128 transportation, and yields of the animal (Gerbens-Leenes et al., 2013). Using beef as an
129 example, applying the average dressing percentages and carcass weights, the estimated
130 water usage in processing one beef carcass is 11 L per kilogram of boneless beef
131 (Legesse et al., 2018). If annual meat waste is applied at 22% combined with the
132 estimated demand for beef in metric tons by the year 2027, the world would lose not only
133 12,090 metric tons of beef but would also be wasting over 860 million liters of water just
134 in processing (Organization of Economic Co-operation Development and the Food and
135 Agricultural Organization, 2018, Fiala, 2008).

136 Recent research has utilized artificial intelligence and eye-tracking technology to
137 study consumer purchasing behavior to analyze time spent looking at the nutritional
138 information, labeling claims, or the product as a whole. Samant and Seo (2016) reported
139 participants with a high-level understanding of a meat product looked at sustainability
140 and processing claims for longer amounts of time compared to those without any prior
141 knowledge. Preferences and background knowledge can span gender, socioeconomic
142 factors, as well as region or country of purchase. For example, in a Portuguese
143 experiment, it was observed that females had a significantly higher attraction to beef
144 steaks with less external fat compared to males (Banovic et al., 2016), and further
145 research could prove significant to a greater understanding of global consumer habits.

146 **Greenhouse Gas Formation**

147 Greenhouse gases can be defined as gases that trap heat in the atmosphere, and
148 their typical composition consists of carbon dioxide (CO₂), methane (CH₄), nitrous oxide
149 (N₂O), and fluorinated gases such as hydrofluorocarbons, and each can pose different
150 environmental threats or have specific effects (Environmental Protection Agency, 2020).
151 The Environmental Protection Agency has reported that landfill gas, a natural byproduct
152 from the breakdown of organic matter, is composed of 50% methane and 50% carbon
153 dioxide, and 15.1% of total human methane production comes from municipal waste
154 (United States Environmental Protection Agency, 2018). Many food items will produce a
155 variety of greenhouse gases. The most commonly produced gases and those that are seen

156 in the greatest volumes are CO₂, CH₄, and N₂O. Considering a full life cycle analysis for
157 greenhouse gas formation, emissions from food wastage have been estimated to be
158 approximately 2.7 gigatons of CO₂ equivalent or Gt CO₂e (Food and Agriculture
159 Organization, 2014). Although the focus of previous research was on all food types,
160 limited knowledge is currently available on the impact of meat on greenhouse gas
161 formation.

162 CO₂ is already present in the atmosphere; however, with human-related
163 emissions, including the emissions from the breakdown of organic matter from food
164 waste, emissions have been on a steady incline, increasing 5.8% from 1990 to 2018
165 (Environmental Protection Agency, 2020). In 2018, CH₄ accounted for approximately
166 9.5% of all United States greenhouse gas emissions from human activities; however,
167 manure, livestock, wetlands, and wastewater are large contributors to total CH₄ emissions
168 (Environmental Protection Agency, 2020). Pertaining to wetlands, manure, or food waste,
169 bacteria breaking down the organic materials in the absence of oxygen will also produce
170 methane. N₂O accounts for approximately 6.5% of the United States greenhouse gas
171 emissions from human activities, and can result from various agricultural fertilizers,
172 manure management, or soil management, along with fuel combustion (Environmental
173 Protection Agency, 2020).

174 The Environmental Protection Agency has stated that food is the largest category
175 of waste in municipal landfills, where food waste emits a medley of greenhouse gases. In
176 2017 alone, only 6.3% of the 41 million tons of food wasted were used for composting

177 (Environmental Protection Agency, 2020). A visual of gas resulting from food waste in a
178 landfill would be similar to tying food in a plastic bag; the nutrients are never returned to
179 the soil, and the rotting food can produce CH₄ gas (Environmental Protection Agency,
180 2020).

181 When analyzing gas formation from municipal waste, temperature can also play
182 an effect on the amounts of gas produced. A study observing the production of methane
183 and nitrous oxide from compost consisting of municipal food waste at set temperatures of
184 40, 55, and 67 °C found carbon dioxide equivalents from methane were higher than from
185 nitrous oxide except for the composts run at 67 °C (Ermolaev et al., 2015). In another
186 study, utilizing the EX-ACT, a model to account for multiple environmental practices,
187 greenhouse gases, and carbon pools, it was found in those developing countries that
188 processing, transport, and storage inefficiencies were responsible for the food waste. This
189 suggests that their supply chain was more responsible for gas contribution in municipal
190 waste, compared to more developed nations whose gases are a result of excess at the
191 consumer and retail level (Galford et al., 2020).

192 NASA's Global Climate Change has observed a simple molecule in the
193 atmosphere, the hydroxyl O.H. radical can act as a self-recycling detergent in the
194 atmosphere (Gray, 2018). CH₄'s current atmospheric lifecycle is estimated to be nine
195 years, but the lifecycle can be cut down and regulated by this detergent. Nitrogen oxides
196 aid the detergent in the self-recycling process, as the breakdown of methane products
197 react with the nitrogen oxides for the O.H. to be recycled back into the atmosphere (Gray,

198 2018). However, Global Warming Potentials (GWP) should be considered when
199 calculating the given effect of gases in the atmosphere as it allows the comparison of
200 different gases. A larger GWP is indicative that a given gas warms the Earth more
201 compared to CO₂, typically measured over a time span of 100 years (Environmental
202 Protection Agency, 2020). Since CO₂ is a reference gas, its GWP will remain at a
203 constant of 1, and its increase in concentration can last thousands of years. CH₄'s GWP is
204 28-36 over 100 years due to its ability to absorb energy greatly, and N₂O's GWP is 265-
205 298 times that of CO₂, remaining in the atmosphere for over 100 years (Environmental
206 Protection Agency, 2020).

207 The Environmental Protection Agency has reported greenhouse gases (GHG) trap
208 outgoing energy produced by the Earth and retain heat in the atmosphere which can
209 disrupt the radiative balance of the Earth (Environmental Protection Agency, 2020).
210 Greenhouse gases have the potential to alter climate and weather patterns, which is more
211 commonly referred to as global warming (Environmental Protection Agency, 2020). With
212 a minuscule change in overall global temperature, it has been predicted that sea levels
213 could rise, population displacement and a disruption of the food supply could occur, as
214 well as increased chances for flooding and infectious diseases (Feldscher, 2011).

215 **Bacteria in Meat**

216 Spoilage bacteria assist in the breakdown of organic matter and can produce
217 greenhouse gases. More specifically, carbon, nitrogen, and hydrogen atoms in organic

218 matter are utilized by bacteria in the decomposition process (Utah State University,
219 2020). During decomposition, energy can be released through heat from oxidation of
220 carbon. When materials are piled onto each other the temperature can range from 72.2-
221 77.7 °C. A previous study observing temperature and moisture variations effect on the lag
222 phase of the bacterial growth curve found higher temperatures, in this case, 30 °C,
223 produced shorter lag periods even though it was not ideal for growth (Nicola & Baath,
224 2019). However, if higher temperatures were investigated, there may have been a much
225 shorter lag phase, as the USDA's Food Safety and Inspection Service reports
226 temperatures above 60 °C will destroy bacteria (United States Department of Agriculture
227 Food Safety and Inspection Service, 2013). With temperatures exceeding 60 °C, it is
228 possible to destroy bacteria's growth that can assist in protein decomposition. However,
229 thermophilic bacteria, which can withstand temperatures of over 80 °C, will breakdown
230 proteins and can sustain growth in a landfill environment (Suzuki et al., 2006).

231 Animal-based proteins are much more difficult to degrade. Incineration has
232 previously been used to dispose meat products; however, some researchers have
233 suggested shifting focus to thermophilic bacteria to assist in meat breakdown. Prions,
234 extracellular matrix proteins, and keratins can be difficult to break down, and across
235 multiple industries, their rigid structures resist proteases, but thermophilic bacteria are
236 capable of breaking down their structural composition. With an elevated temperature
237 range in which thermophiles thrive, proteins can be weakened and are made more
238 susceptible to break down, and specific microbes with strong proteolytic activity spread

239 bacterial toxins over the proteins and can break through the extracellular matrix (Suzuki
240 et al., 2006). Potential use for thermophilic degradative enzymes could decompose other
241 pathways or mechanisms and could even be used in the treatment of neurological
242 degenerative diseases (Suzuki et al., 2006).

243 When landfills reach their capacity, they are typically capped with a layer of clay,
244 reducing the amount of water let in and oxygen exposure, creating an anaerobic
245 environment. Anaerobic conditions can greatly slow decomposition, and with the CH₄
246 gas produced being trapped by the clay barrier, it must be burned or released to avoid
247 hazards from its flammable and explosive properties (Utah State University, 2020). The
248 production of CH₄ and CO₂ can result from fermentative microbes, referred to as
249 acidogens, hydrogen producing acetogens, and methane producing methanogens. For
250 hydrolysis and acidogenesis, sugars, amino acids, and fatty acids are results from
251 microbial degradation of biopolymers that are metabolized by fermentation products and
252 other enzymes from microbial species and can be fermented to produce carbon dioxide
253 and hydrogen (Food and Agriculture Organization, 1997). Through anaerobic digestion,
254 methanogens can produce methane utilizing acetate or hydrogen or carbon dioxide, and if
255 they utilize hydrogen or carbon dioxide for their production, they can limit atmospheric
256 carbon dioxide production (Food and Agriculture Organization, 1997). Through secretion
257 of enzymes and hydrolyzing of polymeric materials, acetogenic bacteria will convert
258 volatile fatty acids to hydrogen, CO₂, or acetic acid, and methanogens will convert the
259 previous products to either CH₄ or CO₂ (Food and Agriculture Organization, 1997).

260 **Greenhouse Gas Quantification**

261 While various studies have determined GHG emissions throughout meat animal
262 and poultry production life cycles (MacLeod et al., 2013, Suszkiw 2019) there is limited
263 knowledge on the impact of meat waste alone on GHG production. The greenhouse gas
264 emission quantification differs across sectors, such as from industry or natural resources.
265 There are several approved methods to measure and analyze gas composition. Direct
266 emissions are defined as those of carbon dioxide from combustion fossil fuels as well as
267 those of non-combustion from process emissions (Environmental Protection Agency,
268 2008). Indirect emissions are measured as carbon dioxide emissions from the generation
269 of electricity by the specific sector (Environmental Protection Agency, 2008).

270 Out of all sectors measured for GHG emissions food and beverage ranked sixth,
271 with fossil fuel combustion and electricity posing the highest CO₂ emission, and non-
272 combustion for CH₄ emissions (Environmental Protection Agency, 2008). Utilizing fuel
273 consumption from estimated and purchased electricity for production combined with
274 emission factor data, the combustion, non-combustion, and purchased electricity gas
275 generation was calculated. The data revealed over 50 million metric tons of CO₂
276 equivalents from fossil fuel combustion and purchased electricity was barely under 50
277 million metric tons of CO₂ equivalent (MMTCO₂E) (Environmental Protection Agency,
278 2008).

279 To quantify soil GHG emissions, a study by the University of Vermont calculated
280 greenhouse gas emissions and tested the carbon storage capabilities of soil from a variety

281 of farms in Vermont. With manure present, N₂O and CO₂ emissions were increased with
282 little impact from tillage, and high impact from temperature and nitrate levels in the soil
283 (Goeschel, 2016). Farms selected varied in their soil management practices, including
284 aerated, non-aerated, to-till, conventional, strip, vertical, and conventional tillage
285 (Goeschel, 2016). This study utilized a 1412 infrared-photoacoustic-spectroscopy gas
286 analyzer, and it was found that manure injection increased N₂O fluxes and aeration
287 decreased them, and no-till decreased CO₂ the most (Goeschel, 2016).

288 Another method of GHG quantification revolves around metrics and calculations
289 of GWP. The CO₂ equivalent is also a metric used to compare gas emissions based on
290 their GWP and convert amounts of gases to MMTCO₂E. The GWP of CH₄ is 28-36 and
291 would indicate 1 million metric tons of CH₄ is equivalent to 25-36 metric tons of CO₂
292 (Eurostat, 2017). The GWP of N₂O is 265-298 times that of CO₂ and would be equivalent
293 to 265-298 metric tons of CO₂ (Eurostat, 2017).

294 The Varian gas chromatograph, a method for reading specific headspace
295 concentration of any sample, manufactured by Agilent Technologies, has two methods of
296 quantifying GHG emissions. The first method consists of single-channel that utilizes dual
297 detectors for analysis of CO₂, CH₄, N₂O and sulfur hexafluoride (SF₆) in samples (Wang,
298 2010). The second method uses two channels and three detectors for wide concentration
299 levels, allowing for lower levels of CO₂ to be converted to CH₄ and higher levels to
300 remain as CO₂ in the samples (Wang, 2010).

301 The objectives of this study were to determine the effects of aging, storage
302 condition, and meat state, either raw or cooked, on greenhouse gas formation, specifically
303 carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) from ground beef loaves.
304 More specifically, the objectives were:
305 (1) to determine the effects of aerobic and anaerobic conditions on greenhouse gas
306 formation from raw ground beef loaves
307 (2) to compare greenhouse gas formation from raw and cooked ground beef when
308 incubated at aerobic conditions.

309

310

CHAPTER III

311

312

313

MATERIALS AND METHODS

314

315 **Product Collection and Storage**

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A flow-chart showing sample allocation is included in Figure 1. Eight beef shoulder clods (clods are considered a large muscle system and include *infraspinatus*, *teres major*, and *triceps brachii*, IMPS 114, North American Meat Processors Association, 2002) were purchased from Creekstone Farms in Arkansas City, Kansas. Clods were transported on ice to the Food and Agricultural Products Center at Oklahoma State University. The samples were purchased within 3 d of harvest and remained in vacuum bags in dark storage at 4 °C until 7 d postmortem. Of the eight clods, four clods were randomly assigned to 7-d aging, and the remaining four were assigned to 14-d aging.

325

Grinding and Packaging

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After 7 or 14 d aging, four clods packaged were opened, cut into chunks, and coarsely ground with a ½-inch stainless steel grinder plate (BIRO Model meat grinder,

328 Biro Manufacturing Co., Marblehead, OH). Proximate analysis was performed with a
329 FOSS FoodScan™ (FOSS Analytics North America, in Eden Prairie, MN). The ground
330 samples were hand-mixed to ensure lean and fat particles did not congregate. After the
331 desired protein fat ratio average was met (85% lean), meat from each clod was finely
332 ground with a ³/₁₆-inch grinder plate.

333 Fine ground samples were hand-formed into eight loaves (approximate weight
334 was 454 g; Mettler-Toledo scale, Mettler-Toledo, Columbus, OH). Meatloaves were
335 placed into Styrofoam™ trays wrapped with a polyvinyl chloride (PVC) (oxygen-
336 permeable polyvinyl chloride fresh meat film; 15,500 to 16,275 cm³ O₂/ m²/24 h at
337 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies,
338 Kansas City, MO) and heat sealed (Intertek Heat Seal, model 600A, Intertek USA Inc.,
339 Houston, TX).

340 **pH**

341 The pH of the ground clods was measured on day 7 and 14 using a Hanna pH
342 meter (model HI 99163, Hanna Instruments Inc., Smithfield, RI) by inserting the pH
343 meter. The pH measurements were recorded in triplicates and averaged for statistical
344 analysis.

345 **Retail Display and Instrumental Color Analysis**

346 Packaged trays were placed in a coffin style retail case (Husmann IM1SL,
347 Bridgeton MO) set at 2.5°C (average temperature of 3.13°C; EL-USB-2-LCD

348 temperature data logger, LASCAR Electronics Erie, PA). The product remained in the
349 case for three days. The retail case was lit with Philips LED T8 Lamps (model number
350 9290011240B-453597, Niles, OH).

351 Instrumental color readings were recorded in three random locations on the
352 product's surface every 24 h of retail display (0, 1, 2, and 3 d) using a HunterLab
353 MiniScan spectrophotometer (HunterLab MiniScan®E.Z. spectrophotometer, model
354 4500L, Reston, VA). CIE L^* and a^* values were measured to represent lightness and
355 redness. A greater L^* value indicates a lighter product, and a greater a^* values indicate a
356 redder product. The instrument was standardized with white and black tiles before use.

357 **Sample Preparation for Greenhouse Gas Analysis**

358 After 7- or 14-day aging (Figure 1) and 3 days of retail display, each loaf was
359 divided into three sections and assigned to 4, 8, and 11 days for storage in Ziploc® bags
360 (to simulate storage of meat in the refrigerator at home). The days (4, 8, and 11) represent
361 from the initial fine grind. The samples assigned to 4, 8, and 11 days were utilized for
362 raw meat greenhouse gas analysis.

363 For cooking, approximately sixteen 100 g patties were hand-formed from the
364 eight loaves and cooked to an internal temperature of 71.1 °C using a George Foreman
365 Grill (Model GRP99 B, Beachwood, OH). The internal temperature was monitored using
366 a meat thermometer (Alpha Grillers, Instant Read Thermometer, Anchorage, AK). The
367 cooked patties were allowed to cool at 21.5 °C (room temperature) for 1 h. Five grams of

368 cooked patties that contain both interior and exterior meat were placed in 20 mL glass
369 vials headspace vials (Thermo Scientific™, Waltham, MA). Tubes were sealed with
370 atmospheric oxygen and were left at 21.5 °C to incubate for 24 h ± 0.50 h before analysis.

371 The raw product from the loaves after 4, 8, and 11 days of storage, also comprised
372 of a combination of interior and exterior meat, was weighed into either 5 g samples for
373 gas readings or 11 g samples for aerobic plate count analysis. The meat samples were
374 placed in vials and flushed with either nitrogen (to create anaerobic condition) or
375 atmospheric condition. Nitrogen vials were flushed with certified 100% nitrogen
376 (Stillwater gas, Stillwater, OK) gas for 30 s. Once gas tubes had been sealed and flushed,
377 they were placed in a Ziploc® baggie as designated and were left to incubate at 21.5 °C
378 for 24 h ± 0.50 h.

379 After incubation, cooked and raw tubes were analyzed using a headspace analyzer
380 (Agilent Technologies Inc., Santa Clara, CA), to determine carbon dioxide (CO₂),
381 methane (CH₄), and nitrous oxide (N₂O). Standard tubes were filled with 10% and 4%
382 CO₂ gas combinations, and ambient air was also utilized for standardization against the
383 samples being read.

384 **Total Aerobic Plate Count**

385 The samples assigned to d 4, 8, and 11 were utilized for total aerobic plate count
386 (APC). The samples were taken from vials incubated at 21.5 °C for 24 h ± 0.50 h. After
387 open each vial, 10 g samples from each treatment were homogenized in 90 mL of sterile

388 0.1% peptone water in a sterile stomacher bag and paddled for 30 sec at 230 rpm utilizing
389 a Stomacher 400 Circulator (Seward Laboratory Systems Inc., in Bohemia, NY).
390 Microbial growth was determined by plating 1 mL of the sample homogenate (3M™
391 Petrifilm™ Aerobic Count Plate, St. Paul, MN, USA). The plates were incubated for 48 h
392 at 37 °C and then counted, reporting the colony-forming units (CFU) per cm². Plates
393 were counted in accordance with the 3M™ Petrifilm™ Aerobic Count Plate
394 Interpretation Guide.

395 **Statistical Analysis**

396 The data were analyzed based on the objectives. A split-split-plot design was
397 utilized to determine the effects of incubation conditions (aerobic vs. anaerobic) and
398 effects of raw and cooked ground beef on greenhouse gas formation.

399 Objective 1: The whole plot consists of eight shoulder clods randomly assigned to
400 either 7 or 14 aging periods (n = 4 at each aging period) and ground beef loaves were
401 repeatedly measured to determine color during retail display. Within the subplot, ground
402 beef loaves were assigned to raw and cooked patties. Within the sub-sub plot, raw and
403 cooked samples were assigned to 4, 8, and 11 days of dark storage at 4 C. During dark
404 storage, samples were collected at each dark storage time point for greenhouse gas
405 emission analysis. The fixed effects for the whole plot consist of aging period and the
406 random effect included error A (aging x unit). The fixed effects for the subplot was raw
407 or cooked and the random effect was error B (aging x state x unit). The fixed effects for

408 subplot include aging, state, dark storage, and their interactions. The unspecified
409 residual error was used for the subplot random effect.

410 Objective 2: The whole plot consists of eight should clod randomly assigned to
411 either 7 or 14 aging periods ($n = 4$ at each aging period) and ground beef loaves were
412 repeatedly measured to determine color during retail display. Within the subplot, raw
413 samples were assigned to 4, 8, and 11 days of dark storage at 4 °C. During dark storage,
414 samples were collected at each dark storage time point for greenhouse gas emission
415 analysis. Within the subplot, raw ground beef samples were incubated at either aerobic or
416 anaerobic conditions. The fixed effects for whole plot consist of aging period and random
417 effect included error A (aging x unit). The fixed effects for subplot was dark storage time
418 and the random effect was error B (aging x dark storage x unit). The fixed effects for
419 subplot include aging, incubation conditions, dark storage, and their interactions. The
420 unspecified residual error was used for the subplot random effect.

421 For both objectives, Type-3 tests were performed using the Mixed Procedure of SAS
422 (SAS 9.3; SAS Inst. Inc., Cary, NC). Least squares mean for the highest-order
423 interactions determined to be significant will be presented. Least squares means were
424 separated using the PDIFF option and were considered significant at $P < 0.05$.

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CHAPTER IV

430

431

432

RESULTS

433

434

435 **Proximate Analysis and pH**

436

437

438

There were no differences ($P > 0.05$) in the fat, protein, or moisture percentages between 7- and 14-days aged products (Table 1). The pH values on day 14 was greater ($P < 0.05$) than that of day 7 (Table 1).

439

Color Analysis

440

441

For 7 days aged, retail day 0 and 1 were significantly different ($P < 0.05$) from d 2 and 3 (Table 2).

442

Total Aerobic Plate Count and Microbial Classification

443

444

445

There were no differences in APC between 14 d aged patties that were stored for 4 d and the 14 d aged and stored for 8 d. However, samples stored for 11 d had greater ($P < 0.05$) APC than 4 and 8 d (Table 3).

446

447

Bacteria in ground beef samples were characterized using a proteomic based approach (MALDI-Biotyper). Following bacteria were characterized under the aerobic

448 condition: *Carnobacterium divergens* (very large amount), *Hafnia alvei* and
449 *Lactobacillus sakei* (small amounts), *Lactobacillus sakei* and *Yersinia enterocolitica*
450 (*trace amount*) (Table 4). However, no anaerobic bacterial growth was detected in the
451 culture.

452 **Effects of incubation condition (aerobic or anaerobic) on greenhouse formation**

453 Table 6 indicates a significant difference between dark storage d 4 and 8 for CO₂.
454 However, there was no difference in CO₂ formation between dark storage 8 and 11. There
455 were no differences observed for CH₄ and N₂O among dark storage time (Table 6).

456 Aging time had an effect on CO₂ and CH₄ formation ($P < 0.05$); however, the
457 aging time had no effect on N₂O formation (Table 7). The aerobic condition had greater
458 CO₂, CH₄, and N₂O formation compared with the anaerobic condition (Table 8). There
459 was a storage time and incubation time interaction for carbon dioxide (Table 9). Aerobic
460 condition on day 4 had greater CO₂ formation than an anaerobic condition on day 4. In
461 both aerobic and anaerobic conditions, dark storage time increased CO₂ formation.

462 There was aging time and dark storage time interaction for CO₂ formation (Table
463 10). Ground beef aged for 7 days and displayed for 4 d had greater ($P < 0.05$) CO₂
464 formation than aged 14 d and stored for 4 d. In both d 7 and 14 aging, dark storage time
465 increased CO₂ formation.

466 There was an aging time and condition of incubation interaction for CO₂, CH₄
467 formation (Table 10). Ground beef aged 14 days and under aerobic conditions had greater
468 ($P < 0.05$) CO₂ and CH₄ formation than aged 14 d and anaerobic condition. Aging time

469 did not increase CO₂ and CH₄ formation under anaerobic conditions but increased for the
470 aerobic condition.

471 There was a dark storage x aging x incubation condition interaction resulted for
472 nitrous oxide formation. Ground beef aged for 7 days, stored for 4 days and incubated at
473 aerobic condition had lower N₂O than ground beef aged for 14 days and stored for 4 days
474 under aerobic condition. Ground beef aged for 14 days, stored for 4 days and incubated at
475 aerobic condition had greater N₂O than ground beef aged for 14 days and stored for 11
476 days under aerobic condition.

477 **Greenhouse gas formation from raw and cooked ground beef**

478 The raw ground beef had lower CH₄ than cooked when aged for 7 d. However,
479 raw ground beef when aged for 14 d had greater CH₄ formation than 7 d aged. There was
480 a storage time x aging interaction resulted for N₂O. When aged 14 d, there was no effect
481 on storage time. However, the dark storage of 11 d had greater N₂O compared with dark
482 storage of 4 d for 7 d aged samples. Interestingly, cooked ground beef stored for 8 or 11 d
483 had greater than 4 d stored samples.

CHAPTER V

DISCUSSION

Effects of incubation conditions on greenhouse gas emissions from raw ground beef

With the effects of aging, storage day, and anaerobic and aerobic conditions, various results were seen in levels of gas production of CO₂, CH₄, and N₂O. It was hypothesized that anaerobic conditions would produce greater gas levels as the Environmental Protection Agency reported in anaerobic bioreactor landfills with moisture in the waste, as with the moisture of raw meat samples, biodegradation would occur anaerobically and produce greenhouse gases (Environmental Protection Agency, 2019). However, for CO₂, CH₄, and N₂O, aerobically conditioned samples produced greater levels of gas.

Anaerobic samples were flushed with 100% nitrogen gas. Pure nitrogen has been found to be bactericidal with *Pseudomonas* and *Bacillus*, common bacteria found in meat, and with the death of these bacteria, degradation and gas production during decomposition could have been reduced (Munsch-Alatossava, P., & Alatossava, T., 2014). With the bactericidal capabilities of the nitrogen gas, the chances for growth and multiplication of these bacteria were very low. APC of this study revealed no significant differences ($P > 0.05$) in the aerobic or anaerobic conditions between the conditions of

aging period 7 and period 14, but the production between anaerobic 7 and 14 as well as aerobic 7 and 14 was statistically different ($P < 0.05$). Samples were anaerobically analyzed by the Oklahoma Animal Disease Diagnostic Laboratory and had no growth detected, while aerobic samples produced bacteria from a range of trace to very large (Table 4). Bacterial decomposition and no growth detected in anaerobic samples imply that the samples' state and bacterial community can play a large part in greenhouse gas production and is an indicator as to why the aerobic samples actually produced significantly higher levels of gas.

It was also observed the shortest aging time had greater gas production compared to the second and longer aging treatment in the production of CO₂. Although the mechanistic basis for lower gas production with aging time is not clear, it has well documented that an increased aging period will change metabolites (Mitacek et al., 2019) and increase proteolysis (Nair et al., 2018). Therefore, differences in the metabolite profile have favored less for gas production.

Greenhouse gas formation from raw and cooked ground beef

In the current research, cooked meat had lower greenhouse gas formation. The USDA recommended cooking temperature to destroy bacteria present on the meat product could significantly affect total gas production and gas formulation (Wagner Jr., 2008). The raw state was significantly higher in all gases except for CH₄. The current

research suggests that raw meat waste can contribute more to greenhouse formation than cooked meat.

Addressing food safety, the USDA designates the “danger zone” of meat to be between a temperature of 4.44 ° and 60 °C, and the bacteria found on the meat products at these temperatures can double in amount every 20 minutes with nutrients permitting (United States Department of Agriculture Food Safety and Inspection Service, 2011). When vials were incubated at room temperature, the meat both cooked and raw did have the potential for this great level of bacterial growth, however, with bacteria being destroyed by the cooking process, it is evident that raw product would have a greater amount of GHG formation resulting from high levels of bacteria.

For storage of leftover raw and cooked products, the meat in this study was identically stored in a walk-in cooler at 4 °C until it was taken out for its next pull day. The USDA instructs post-cooking, meat should be cooled again and refrigerated within 2 hours, which was performed in this study (United States Department of Agriculture Food Safety and Inspection Service, 2011). However, in future studies, cooked meat could be left out of the refrigerator to reintroduce bacteria to the product and imitate garbage conditions in order to see if this could affect gas production and have significant effects on all greenhouse gases that were analyzed in this study.

CHAPTER VI

CONCLUSION

With the need to feed the growing population with healthy and high-quality meat products, meat waste, and energy expenditures in its creation have to be reduced. The results of this study indicated that raw product in aerobic conditions produced higher levels of greenhouse gases (GHG) compared to anaerobic conditions and that raw product had greater gas formation compared to cooked products. Characterizing the factors influence greenhouse gas formation may help to minimize the impact of greenhouse gases on environment.

Figure 1: Summary of various treatment allocations

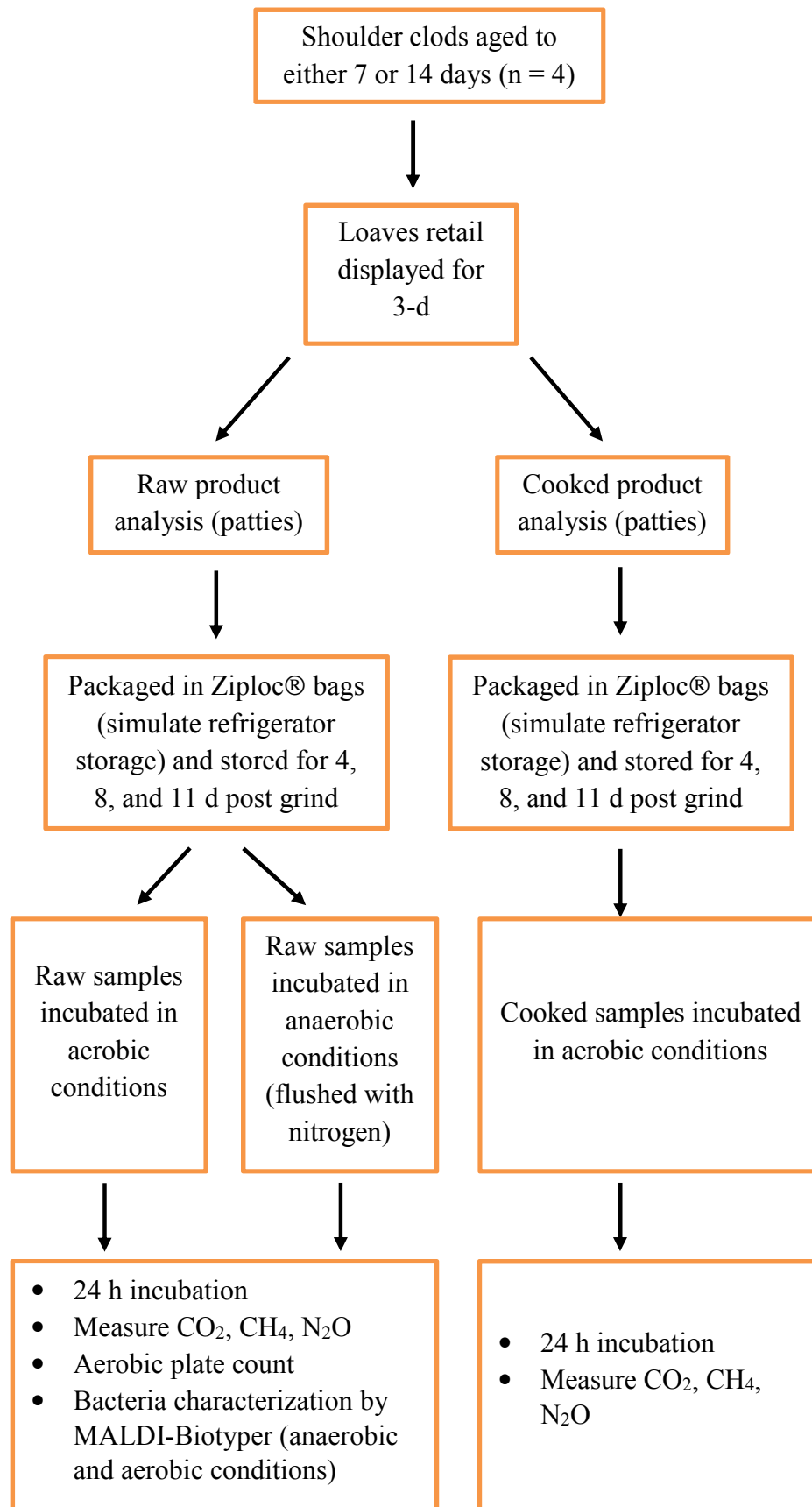


Figure 2: Pictorial representation of days allocation

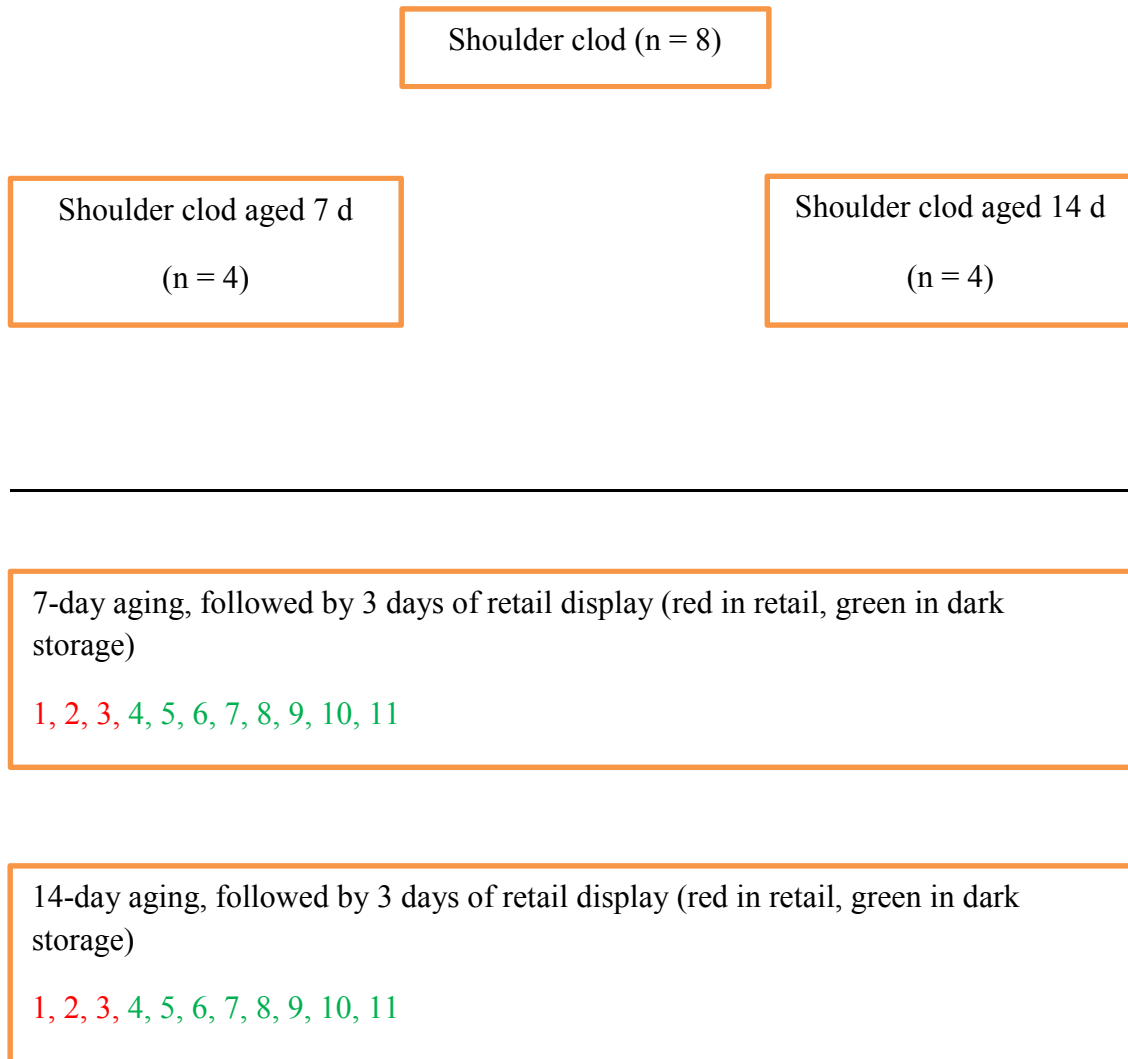


Table 1. Effects of aging on fat, protein, moisture, and pH from ground beef loaves.

Aging	Fat (%)	Protein (%)	Moisture (%)	pH
7	17.12	18.20	64.42	5.55 ^a
14	15.70	18.35	62.21	5.76 ^b

¹Aging: 7- and 14-d postmortem aged shoulder clods

Standard error: fat - 1.01, protein - 0.28, moisture - 0.71, pH - 0.03

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 2. Effects of retail display time and aging on a^* values from ground beef loaves.

Retail Display ¹	Aging ²	
	7	14
0	33.79 ^d	33.09 ^d
1	24.46 ^c	33.09 ^d
2	20.96 ^{ab}	21.66 ^b
3	17.65	18.51 ^b

¹Display: represents the displays of ground beef loaves in the retail display case

²Postmortem aging time

A greater a^* value indicates more red color

n = 4 shoulder clods with 2 loaves per clod

Standard error of retail display × aging: 1.23

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 3. Effects of storage, aging, and incubation conditions on total aerobic plate count formation from ground beef loaves.

Aging²	Condition³	Storage¹		
		4	8	11
7	Anaerobic	7.22	7.48	7.25
7	Aerobic	7.23	7.46	7.27
14	Anaerobic	4.98 ^a	5.08 ^{ab}	6.78 ^{bc}
14	Aerobic	4.92 ^a	5.07 ^{ab}	6.02 ^{abc}

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days' post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

³Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Unit: colony-forming units (CFU)

n= 4 shoulder clods with 2 loaves per clod

Standard error of storage × aging × condition: 0.73

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 4. Anaerobic and aerobic bacterial quantification and identification.

Condition	Organism ID
Aerobic	<i>Carnobacterium divergens</i> /+5
Aerobic	<i>Hafnia alvei</i> , <i>Lactobacillus sakei</i> /+2
Aerobic	<i>Carnobacterium divergens</i> , <i>Lactobacillus sakei</i> /+2
Aerobic	<i>Yersinia enterocolitica</i> /+1
Anaerobic	N/A
Anaerobic	N/A

Muscle sample: 4 samples, 2 analyzed as anaerobic, 2 as aerobic

Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Amount*: 0= no growth detected, +1= trace, +2= small, +3= medium, +4= large, +5= very large

Unit: colony forming units (CFU)

n= 4 shoulder clods

Table 5. Effects of storage time on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

Storage	Carbon Dioxide	Methane	Nitrous Oxide
4	92,591 ^a	3.18	0.52
8	117,329 ^b	2.65 ^a	0.54 ^a
11	124,660 ^b	2.66 ^a	0.33 ^a

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days' post grind

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 4,555.33, methane- 0.33, nitrous oxide- 0.15

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 6. Effects of aging time on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

Age	Carbon Dioxide	Methane	Nitrous Oxide
7	139,207 ^a	1.77 ^a	0.15
14	83,847 ^b	3.89 ^b	0.15

¹Aging: 7- and 14-d postmortem aged shoulder clods

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 4,729.13, methane- 0.37, nitrous oxide- 0.15

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 7. Effects of incubation conditions on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

Condition	Carbon Dioxide	Methane	Nitrous Oxide
Anaerobic	50,480 ^a	0.80 ^a	0.30 ^a
Aerobic	172,574 ^b	4.86 ^b	0.63 ^b

¹Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 3995.60, methane- 0.33, nitrous oxide- 0.14

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 8. Effects of storage, aging, and incubation condition on carbon dioxide gas formation from ground beef loaves.

Aging²	Condition³	Storage¹		
		4	8	11
7	Anaerobic	65,340 ^b	81,264 ^{bc}	81,059 ^{bc}
7	Aerobic	178,695 ^e	221,996 ^f	206,884 ^f
14	Anaerobic	22,253 ^a	18,725 ^a	34,237 ^a
14	Aerobic	104,078 ^c	147,329 ^d	176,460 ^e

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

³Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of storage × aging × condition: 16,369.99

Least squares mean with different letters are significantly different ($P < 0.05$)

Table 9. Effects of aging and incubation condition on methane gas formation from ground beef loaves.

Aging ¹	Condition ²	
	Anaerobic	Aerobic
7	0.48 ^a	3.07 ^b
14	1.13 ^a	6.64 ^c

¹Aging: 7- and 14-d postmortem aged shoulder clods

²Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error aging × condition: 0.43

Least squares mean with different letters are significantly different ($P < 0.05$)

Table 10. Effects of storage, aging, and incubation condition on nitrous oxide gas formation from ground beef loaves.

Aging²	Condition³	Storage¹		
		4	8	11
7	Anaerobic	0.11 ^a	0.71 ^{bc}	0.18 ^a
7	Aerobic	0.47 ^{abc}	0.82 ^{cd}	0.50 ^{abc}
14	Anaerobic	0.21 ^{abc}	0.51 ^{abc}	0.09 ^a
14	Aerobic	1.31 ^d	0.01 ^a	0.55 ^{abc}

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

³Condition: anaerobic- flushed with 100% nitrogen gas, aerobic- sealed with atmospheric oxygen

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × aging × condition: 0.22

Least squares means with different letters are significantly different ($P < 0.05$)

Table 11. Effects of storage time on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

Storage	Carbon Dioxide	Methane	Nitrous Oxide
4	73,822 ^a	4.96 ^a	0.68 ^a
8	139,071 ^b	4.43 ^b	1.44 ^b
11	152,485 ^c	4.29 ^b	0.98 ^a

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 5,814.97, methane- 0.32, nitrous oxide- 0.28

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 12. Effects of aging time on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

Age	Carbon Dioxide	Methane	Nitrous Oxide
7	94,916 ^a	3.69 ^a	1.45 ^b
14	148,670 ^b	5.43 ^b	0.62 ^a

¹Aging: 7- and 14-d postmortem aged shoulder clods

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 6,506.75, methane- 0.37, nitrous oxide- 0.31

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 13. Effects of incubation conditions on carbon dioxide, methane, and nitrous oxide gas formation from ground beef loaves.

State	Carbon Dioxide	Methane	Nitrous Oxide
Cooked	71,025 ^a	4.26 ^a	0.63 ^a
Raw	172,560 ^b	4.86 ^a	1.44 ^b

¹State: cooked- 71.1°C, raw- raw ground product

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error: carbon dioxide- 6,506.74, methane- 0.31, nitrous oxide- 0.32

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 14. Effects of storage and aging on carbon dioxide gas formation from ground beef loaves.

Storage ¹	Aging ²	
	7	14
4	91,871 ^b	55,772 ^a
8	182,238 ^d	95,904 ^b
11	171,899 ^d	133,072 ^c

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × aging: 8,223.24

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 15. Effects of display and incubation conditions on carbon dioxide gas formation from ground beef loaves.

Storage ¹	State ²	
	Cooked	Raw
4	6,271 ^a	141,372 ^d
8	94,012 ^b	184,129 ^e
11	112,792 ^c	192,179 ^e

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²State: cooked- 71.1°C, raw- raw ground product

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × condition: 8,223.45

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 16. Effects of storage, aging, and incubation states on carbon dioxide gas formation from ground beef loaves.

Aging ²	State ³	Storage ¹		
		4	8	11
7	Cooked	5,048 ^a	143,155 ^e	136,631 ^{de}
7	Raw	17,895 ^{fg}	221,321 ^h	207,437 ^{gh}
14	Cooked	7,494 ^a	44,870 ^b	89,224 ^c
14	Raw	104,050 ^{cd}	146,938 ^{ef}	176,920 ^{fg}

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

³State: cooked- 71.1°C, raw- raw ground product

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × aging × condition: 11,628.41

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 17. Effects of aging and incubation state on methane gas formation from ground beef loaves.

Aging ¹	State ²	
	Cooked	Raw
7	4.32 ^b	3.07 ^a
14	4.21 ^b	6.64 ^c

¹Aging: 7- and 14-d postmortem aged shoulder clods

²State: cooked- 71.1°C, raw- raw ground product

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of aging × condition: 0.42

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 18. Effects of storage and aging on nitrous oxide gas formation from ground beef loaves.

Storage ¹	Aging ²	
	7	14
4	0.46 ^a	0.90 ^a
8	2.46 ^c	0.41 ^a
11	1.43 ^b	0.54 ^a

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²Aging: 7 and 14 d postmortem aged shoulder clods

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × aging: 0.36

Least square means within a column with different letters are significantly different ($P < 0.05$)

Table 19. Effects of storage and incubation state on nitrous oxide gas formation from ground beef loaves.

Storage ¹	State ²	
	Cooked	Raw
4	0.46 ^a	0.89 ^{ab}
8	2.41 ^c	0.46 ^a
11	1.45 ^b	0.52 ^{ab}

¹Storage: samples of beef contained in airtight Ziploc® baggies 4-, 8-, and 11-days post grind

²State: cooked- 71.1°C, raw- raw ground product

Unit: parts per million (ppm)

n= 4 shoulder clods with 2 loaves per clod

Standard error of display × condition: 0.36

Least square means within a column with different letters are significantly different ($P < 0.05$)

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