

Air versus water chilling of chicken: a pilot study of quality, shelf-life, microbial ecology, and economics

Ariel D Belk

Colorado State University <https://orcid.org/0000-0001-9925-6986>

Toni Duarte

University of California Davis Department of Animal Sciences

Casey Quinn

Colorado State University Department of Mechanical Engineering

David A. Coil

University of California Davis Genome Center

Keith E. Belk

Colorado State University Department of Animal Sciences

Jonathan A. Eisen

University of California Davis Genome Center

Jason C. Quinn

Colorado State University Department of Mechanical Engineering

Jennifer N. Martin

Colorado State University Department of Animal Sciences

Xiang Yang

University of California Davis Department of Animal Science

Jessica L. Metcalf (✉ jessicametcalf@gmail.com)

Colorado State University <https://orcid.org/0000-0001-8374-8046>

Research

Keywords: chicken, meat, chilling methods, spoilage, shelf life, 16S rRNA gene, pseudomonas, technoeconomics, energy, microbiome

Posted Date: July 10th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-40342/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at mSystems on April 27th, 2021. See the published version at <https://doi.org/10.1128/mSystems.00912-20>.

Abstract

Background.

The United States' large-scale poultry meat industry is energy and water intensive, and opportunities may exist to improve sustainability during the broiler chilling process. After harvest, the internal temperature of the chicken is rapidly cooled to inhibit bacterial growth that would otherwise compromise the safety of the product. This step is accomplished most commonly by water immersion chilling in the United States, while air chilling methods dominate other global markets. A comprehensive understanding of the differences between these chilling methods is lacking. Therefore, we assessed the meat quality, shelf-life, microbial ecology, and technoeconomic impacts of chilling methods on chicken broilers in a university meat laboratory setting.

Results.

We discovered that air-chilling (AC) methods resulted in superior chicken odor and shelf-life, especially prior to 14 days of dark storage. Moreover, we demonstrated that AC resulted in a more diverse microbiome that we hypothesize may delay the dominance of the spoilage organism *Pseudomonas*. Finally, a technoeconomic analysis highlighted potential economic advantages to AC when compared to water-chilling (WC) in facility locations where water costs are a more significant factor than energy costs.

Conclusions.

In this pilot study, AC chilling methods resulted in a superior product compared to WC methods and may have economic advantages in regions of the U.S. where water is expensive. As a next step, a similar experiment should be done in an industrial setting to confirm these results generated in a small-scale university lab facility.

Background

Currently, the United States is the largest producer and second-largest exporter of poultry meat worldwide [1]. The poultry industry in the United States has seen a five-fold production increase in the last 40 years and currently produces more than 50 million pounds of live birds annually [2]. As a result of increased production, the poultry industry has also seen a tremendous rise in energy expenditures and water depletion [1]. As demands for poultry meat continue to rise [3], novel approaches for reducing the environmental impact of poultry production, while not sacrificing poultry safety or quality, need to be considered.

Temperature control during production and processing is a critical point in ensuring the safety and quality of poultry products. Thus, it is common for broiler production systems to reduce the internal temperature of chicken meat from 40 °C to 4 °C within one to two hours following harvest. This step, though critical to maintaining the safety of the product, is time-consuming and requires significant investments in energy and water, depending on the chilling method utilized [4]. Water immersion chilling (WC) and air chilling (AC) are the two most common chilling methods globally. Water immersion chilling is the most widely used chilling method in the United States, while AC is predominant in Europe, Brazil and Canada [5, 6]. During WC, eviscerated chicken carcasses are submerged in cold water that is often supplemented with antimicrobials intended to inhibit microbial growth. These organic acids, combined with a continuous clean water system, result in notable reductions to the total bacterial population. However, cross-contamination, retained water on the carcass, consumer perception, water consumption, and wastewater management issues are a few challenges associated with WC [7–9]. As mentioned, AC is widely utilized across Europe, Brazil, and Canada involves the chilling of poultry carcasses by forced air in a cold room. Some studies have shown enhanced microbial quality and less exudative packaging in AC broilers when compared to WC systems [7, 10]. However, these microbial investigations were limited to culture-dependent techniques that focused on just a few microbes and investigations with more robust sampling methods are warranted.

The rapid reduction in carcass temperature during poultry processing provides a unique thermodynamic challenge that requires significant energy inputs. Although AC results in a carcass with a slightly reduced yield (due to evaporative water loss), it has been estimated to require almost 50 times less gross energy than water chilling systems, when entire energy expenditures are considered [11, 12]. In that regard, although WC methods are the most commonly utilized in the U.S., the opportunity for significantly reducing water use and energy expenditures by converting to AC systems exists. However, before this transition can be made, it is imperative to assess how the conversion will affect the holistic quality of the final product in addition to the economic viability of the pathway.

To address meat quality, shelf-life, microbial, and techno-economic impacts of chilling methods, we conducted an experiment at the University of California, Davis (UC Davis) Meat Science Laboratory, in which chickens were either chilled via AC or WC. Novel to this experiment was the assessment of the impacts each chilling system had on the microbiome of poultry products, and how that may relate to the quality of products

from each system. This experiment yielded results that enhance the current knowledge regarding not only the quality of broiler meat produced using either chilling method, but also the important techno-economics, which may guide industry investment or utilization in either system.

Results

Experimental Results

On average, carcasses chilled using the WC system gained 5% of the pre-chilled weight, whereas carcasses chilled using the AC system lost 1.6% of the pre-chilled weight (**SI table 1**). This difference in weight change is similar to what has been reported in other studies [12]. There was no difference in pH between AC and WC chicken breasts ($P > 0.05$; data not presented).

Quality and shelf-life implications of chilling strategy

The WC chicken had fewer ($P < 0.05$) psychrotrophic bacteria, organisms capable of growing at low temperatures, (1.05 log Colony Forming Units/g; CFU/g) prior to fabrication than the AC chicken (2.12 log CFU/g). However, no difference in mesophilic bacteria, organisms that grow best at moderate temperatures, was observed between the two treatments for pre-fabrication samples (**SI table 2**). On day 7, an approximately 1-log difference in psychrotrophic bacteria was observed between AC (5.56 log CFU/g) and WC (6.59 log CFU/g) breasts, regardless of fabrication type (**Table 1**). WC and AC boneless breasts had lower total microbial counts throughout storage and display than the bone-in samples. Regardless, by day 14 of storage, chicken breasts from both chilling methods (WC and AC) and fabrication types (BI and BL) had aerobic bacteria populations greater than 7 log CFU/g, a threshold commonly associated with the end of shelf-life [13].

Instrument assessments of color demonstrated that the International Commission on Illumination (CIE) a^* and b^* values were greater ($P < 0.05$) for AC breast than WC breasts throughout the display period, indicative of more desirable red and yellow tones within the muscle of AC breasts (**Figure 1A**). Similarly, panelist evaluations indicated the boneless chicken breasts were more desirable than bone-in breasts during the 3-day display period following 7 days dark storage. Although there were notable differences in instrument color between chilling method, the difference was not observed in consumer preference. During the 3-day display following 14 days dark storage, panelists considered the color and odor of all samples unacceptable regardless of the chilling method or fabrication type. Chilling method and fabrication type did not have an impact on texture selection ($P > 0.05$). Additionally, trained sensory panelists detected no differences in flavor or texture attributes between chilling methods ($P > 0.05$) (**SI table 3**)

Time	Bone-In		Boneless	
	Air-Chilled	Water-Chilled	Air-Chilled	Water-Chilled
Pre-Fabrication (D00)	2.12 ^{by} (0.15)	1.05 ^{ax} (0.15)	2.12 ^{by} (0.16)	1.05 ^{ax} (0.16)
Post-Fabrication (D0)	1.90 ^{by} (0.16)	1.81 ^{by} (0.15)	0.61 ^{ax} (0.16)	0.68 ^{ax} (0.16)
7 day – storage (D7)	5.86 ^{cx} (0.15)	6.96 ^{dy} (0.16)	5.26 ^{cx} (0.16)	6.22 ^{dy} (0.16)
End of Display (D10)	7.0 ^{dy} (0.15)	6.92 ^{dy} (15)	6.44 ^{dy} (0.16)	6.42 ^{dy} (0.16)
14 day – storage (D14)	8.71 ^{ey} (0.15)	9.0 ^{ey} (0.15)	8.51 ^{ey} (0.16)	8.67 ^{ey} (0.16)
End of Display (D18)	9.11 ^{ey} (0.15)	9.41 ^{ey} (0.16)	8.63 ^{ey} (0.16)	9.24 ^{ey} (0.16)

Table 1. Least square means of psychrotrophic bacterial counts (log CFU/g) for bone-in and boneless chicken breast cooled by either air chilling or water chilling pre and post fabrication, pre and post chilling, during storage, during and after display.

^{a-f} Least square within a column with different superscripts differ ($P < 0.05$)

^{x-y} Least square means within a row with different superscripts differ ($P < 0.05$)

As expected, lipid oxidation increased as the time after chilling progressed (**Figure 1B**). Lipid oxidation levels, as indicated by measurement of thiobarbituric acid reactive substances, were similar across chilling method and fabrication type on the initial day of processing, 7d of dark storage, 14 days of dark storage, and 7 days of dark storage with 3 days of retail display ($P > 0.05$), though there were differences when comparing sampling groups across these days. The greatest differences between treatments were seen on the samples collected after 14 days of dark storage with a 3-day retail display. Among samples from this time point, the boneless WC breasts had a higher degree of lipid oxidation when compared with the boneless AC breasts ($P < 0.05$).

Nutritional content was very similar between chicken breasts regardless of chilling method (**SI table 4**). Dry matter measurements ranged from 26.7% to 28.3%, with an overall mean of 26.13%. Within this narrow range, the only difference between treatment groups was between bone-in and boneless breasts collected on the initial day of experimentation. Chilling method, dark storage, and retail display did not impact dry matter content of the chicken breasts ($P > 0.05$). The same pattern was detected in moisture, as moisture and dry matter are inversely related. Measurements of ash and crude protein revealed a few differences between treatment groups, but these did not reveal significant patterns (**SI table 4**). Crude fat values were, overall, low for all chicken products, ranging from 0.35% to 1.43% with a mean of 0.86%. Differences in these values were detected between fabrication types within a chilling method and sampling day ($P < 0.05$); carcass samples and bone-in breasts had higher crude fat content within almost all chilling methods and sampling days ($P < 0.05$; **SI table 4**). However, no differences were observed between WC and AC ($P > 0.05$). Following this trend, the relative abundance of fatty acids was not grossly different between chilling methods on any sampling day. However, among fatty acids with less than 10% relative abundance, linoleate methyl ester (C18 and C18:9c12c) were more abundant in WC than AC breasts after 7 days dark storage with 3 days retail display (**SI figure 1**).

Microbial ecology of chilled, fabricated, and packaged chicken

DNA sequencing resulted in a total of 3,837,564 demultiplexed reads. After denoising, quality filtering, read joining, and chimera removal via the DADA2 pipeline, **3,262,269 sequence reads** were assigned to 774 amplicon sequence variants (ASVs). Subsequently, we filtered out ASVs that were taxonomically identified as representing mitochondria and chloroplasts, resulting in 3,261,944 sequence reads and 752 ASVs. The commercial positive control sample (Zymo; Irvine, CA) resulted in the expected relative composition of microbes, indicating acceptable sequencing quality (**SI figure 2**). There were 24 negative/mock DNA extraction controls included in this sequencing run, which resulted in an average of 113 reads per negative control (range 1 - 1,086) compared to 15,195 sequences per sample for DNA recovered from chicken rinsate (range 3 - 51,660). This was considered an acceptable level for quality control. Additionally, samples were rarefied at 6,152 sequences for diversity analysis, which was above the highest negative control level. After rarefaction, all samples below this threshold were dropped from the diversity analysis, retaining 202 out of 259 rinsate samples. Before further analysis, negative and positive control samples were filtered out of the experimental dataset.

The alpha (within sample) bacterial diversity of chicken products was reduced during chilling and processing (**Figure 2A**). The hot carcass microbiome contained the highest mean alpha diversity (Shannon's = 2.29, Faith's = 9.96, observed ASVs = 50.6). During the chilling process, the mean Shannon's diversity and observed ASVs were reduced, significantly ($P < 0.05$) in AC. After fabrication, the alpha diversity increased, though not to the original levels. The high microbial diversity on chicken carcasses before product storage was associated with microbial communities dominated by organisms in the family *Enterobacteriaceae*. These communities also included bacteria at lower relative abundance from families *Clostridiaceae*, *Bacillaceae*, and *Pseudomonadaceae* (**Figure 2B**). There were no differences between products collected from products on the day of harvest based on the chilling method or fabrication method.

During storage and display, the alpha diversity of samples remained similar between treatment groups while the beta diversity showed clusters separated by chilling method. The greatest difference was seen between AC and WC samples collected after removal from dark storage on day 7. At this time point, the diversity of the WC samples was lower ($P < 0.05$) than the diversity of AC bone-in samples and AC boneless samples. After the 7-day time point, mean diversities were similar (Shannon's = 1.06 - 1.87, Faith's = 1.58 - 3.02, observed ASVs = 10.00 - 19.30). During these post-storage and post-display sampling points, *Pseudomonas* bacteria became the dominant group. For the chicken that was stored for 7 days, followed by 3 days of retail display, communities from WC chicken became dominated by *Pseudomonas* before AC chicken. When the beta diversity was calculated and visualized using Robust Aitchison Principal Components Analysis, samples separate initially by sampling day - all products that were stored, regardless of storage or display time, separated from samples that were not stored (**Figure 2C**). Then, within the stored product, the samples clustered by chilling method. When the ASV vectors that explain these separations are overlaid and evaluated, it is clear that the main ASVs that separate the stored microbiome are *Pseudomonas*-associated (**Figure 2C**). Moreover, the separation between the chilling methods was primarily associated with distinct *Pseudomonas* ASVs. The patterns of changes in the microbial communities were predictive of spoilage and quality outcomes (**SI Figure 3**). The microbiome could predict microbial spoilage, as defined by a psychrotrophic bacteria count of greater than 7 log CFU/mL, with an overall accuracy of 75%.

The WC-boneless samples were too low biomass, resulting in few DNA sequences, and therefore the samples were excluded after rarefying. B) Taxonomy of the bacterial microbiome of chicken products based on analysis with the SILVA database, segmented by sampling day and chilling method. Within a facet, samples are organized as carcass, bone-in and boneless. C) A biplot constructed using Robust Aitchison PCA

that demonstrates separation in beta diversity between samples. Points are colored by the day samples were collected, including samples collected before dark storage (not stored), after 7 and 14 days of dark storage, and after 3 additional d of retail display (10 day and 17 day). The shape represents the chilling method, including hot carcass (pre-chill), air chilled, and water chilled. The lines show ASVs that are important to the direction of the biplot and are colored by the taxon associated with the specific ASV.

Phylogeny, diversity, and spoilage potential of Pseudomonas

Pseudomonas ASVs (n = 33) come to dominate the microbial community during storage and display (**Figure 3**), and due to the importance of these ASVs we examined them in more detail. When placed into a phylogenetic tree containing 16S rRNA gene sequences from all *Pseudomonas* type strains, we reveal significant variation of branches associated with sequences from this genus. These ASVs are distributed throughout the phylogenetic tree and most likely represent multiple *Pseudomonas* species (**SI figure 4**). We also calculated the average percentage of reads in each sample that belonged to a *Pseudomonas* ASV (**SI table 5**).

In order to focus on particular ASVs, we performed an analysis of the composition of microbiomes (ANCOM) analysis at each sampling point (Days 0, 7, 14, 17) comparing the two chilling methods (pooling fabrication method). Prior to storage, none of the ASVs that were differentially abundant were ASVs assigned to *Pseudomonas*. At day 7, there was one *Pseudomonas* ASV which differed (ASV7: WC=.21%, AC=14.6%), At day 10 (7 days with 3-day retail display) there also one (ASV10: WC=16.45%, AC=1.8%), at Day 14 there were three (ASV7, ASV10, ASV20), and at day 17 (14 days with 3-day retail display) there were two (ASV7, ASV20).

Chilling system techno-economic analysis

Economic viability is a critical aspect of technology adoption. The levelized chilling cost (\$/tonne) for the baseline AC and WC models are shown in **Figure 4** with the total cost subdivided by capital cost, operational cost, and income tax. The costs for AC and WC are relatively similar, \$15.45 per tonne and \$14.15 per tonne, respectively. The operational costs dominate the total chilling cost for both the AC and WC systems. While the AC system has a higher capital cost than the WC system, the AC operational costs are less than the WC system. The primary difference between the two systems are the costs associated with electricity and water. The WC systems can use up to 10x more water than the AC system, but the electrical costs of the AC system are only 2x that of the WC system. Sensitivity analyses (**SI figure 5**) show there are scenarios where AC has the potential to be superior economically than WC, in particular for regions that might have lower electricity rates and high water purchase and treatment costs.

Discussion

Our study demonstrates that the method of chilling poultry carcasses not only influences the shelf-life and quality of chicken breasts, but also has important implications for energy and water usage. Overall, in this study, chicken breasts from carcasses chilled using air-chilling (AC) methods had superior odor and shelf-life (as assessed by psychrotrophic bacterial counts, consumer color and odor panels, and lipid oxidation patterns). Moreover, the microbial communities associated with AC products maintained diversity post-chilling, and therefore may be more favorable by slowing the growth of spoilage organisms such as *Pseudomonas*. Finally, our techno-economic analysis highlighted potential economic advantages to AC when compared to water-chilling (WC) with advantages in areas of limited water and low-cost electricity.

Throughout dark storage and retail display, quality and shelf-life attributes were impacted by the chilling method. While all product was spoiled after 14 days of dark storage, notable quality and physical differences were observed among chicken treated with different chilling methods after 7 days of dark storage and 3 days of retail display. In this time period (7 days of dark storage to retail display), chicken chilled using AC demonstrated more desirable quality attributes, including more yellow tones based and a lower abundance of spoilage-associated fatty acids including linoleate methyl esters. These fatty acids have been associated with odor, color, and shelf-life challenges in previous studies [14–16]. Differences in color and consumer appeal were similar to those reported in Jeong et al [17], who demonstrated that, while WC may reduce temperature more quickly, the use of AC resulted in superior color and juiciness. The color difference could be due to evaporative moisture loss drying the surface of the chicken, allowing for the breast muscle under the skin to become more visible [9]. However, they differ from other studies that showed no difference in color between AC and WC breasts [18, 19]. The spoilage patterns observed over time were similar to those previously reported by Katiyo et al [20]. *Taken together, the color and fatty acid differences suggest that WC breasts may reach a spoilage state more rapidly than AC breasts.*

We did not detect a significant impact on texture, flavor attributes or nutritive composition by chicken chilling method. Previous studies have shown mixed impacts of chilling method on flavor and sensory attributes. For example, Hale et al [21] suggested a flavor advantage in AC chicken compared to WC chicken, although their products were fried. Conversely, Ristic [22] suggested that WC can lead to flavor and texture

advantages. The lack of consistent impacts on sensory and nutritive variables, combined with the similarities observed in our study suggest that *consumer eating satisfaction and nutrient composition would not be influenced by chilling method selection*.

It appears that the physical properties of water versus air may result in distinct initial chicken carcass microbial ecologies. Immediately after chilling we found lower psychrotrophic plate counts in WC than AC carcasses, which suggests that WC is physically washing more cells from the carcass than AC. The difference in bacterial counts did not correspond to a difference in microbial diversity at this time point, similar to findings that demonstrated no difference in the presence of specific microorganisms between chilling methods [6, 23]. These initial post-chilling microbial communities were dominated by the families *Enterobacteriaceae*, *Clostridiaceae*, and *Bacillaceae*, in agreement with findings by Handley et al [24]. There were more obvious differences in the microbial assemblage patterns following 7 days of dark storage. At this point, there was no difference in psychrotrophic counts between AC and WC, but AC had a lower mesophilic count. These results are similar to those reported by Tuncer and Sireli [25], who concluded that the AC was superior to WC in terms of pathogen growth, though they did not specifically investigate the spoilage bacteria described in the current study. Additionally, after the 7-day dark storage period the AC microbial community was much more diverse than the WC community, which was dominated by *Pseudomonadaceae*. We hypothesize that by removing more bacterial cells during chilling, there is less microbial competition, which in turn allowed *Pseudomonadaceae* to grow more quickly in WC products. Competitive advantages associated with *Pseudomonas* were also found in Katiyo et al [20]. Furthermore, we observed more inter-sample diversity in the AC group than the WC. It is likely that, due to the nature of water chilling, there is more opportunity for the microbiomes to become homogeneous, while in air chilling the carcasses were kept separate and therefore microbes were not shared between carcasses. It has been well-documented that water chilling can provide an opportunity for cross-contamination of pathogens, and these results suggest that this trend holds for all bacteria [26–29]. However, there is evidence that this effect can be reduced or eliminated with the addition of antimicrobial compounds to the chill water, which was not done in this experiment [8]. *We hypothesize that the microbial ecology associated with AC delays the Pseudomonas bloom associated with spoilage and may extend the shelf life.*

The difference in microbial diversity of chicken breasts under different chilling methods was primarily due to different ASVs that assigned to *Pseudomonas*, further demonstrating that the population of *Pseudomonas* is likely a major driver of the microbial community structure and spoilage outcomes. Our phylogeny of *Pseudomonas* ASVs strongly suggests that the high number of *Pseudomonas* ASVs represents real biological variation.

While *Pseudomonas* species, in general, are thought to cause food spoilage [30], they are not often identified to the species level. *P. lundensis* and *P. fragi* are known to cause food spoilage in both dairy and meat [31–33] and were found in a clade with two of our ASVs (ASV24, ASV27), but these ASV were seen at similar abundance in both WC and AC samples (data not shown). Two other species of *Pseudomonas* known to be involved in spoilage, *P. fluorescens* and *P. putida* [31, 33], were not found in clades with any of the ASVs in this study. Furthermore, ASV7 (higher abundance in AC at d 7, 14, and 17), ASV21 (no difference), and ASV29 (no difference) were found in a clade with *P. argentinensis*, *P. straminea*, and *P. punonensis* which to our knowledge have not been previously shown to be involved in food spoilage. ASV10 (higher abundance in WC at d 10 and 14), ASV1 (no difference), and ASV11 (no difference) were found in a clade containing *P. lurida*, *P. poae*, *P. trivialis*, *P. palleronia*, *P. tolaasii*, *P. costantinii*, *P. extremorientalis*, and *P. simiae*. ASV20 (higher abundance in WC at d 14 and 17) and ASV14 (no difference) were found in a clade with *P. veronii*. *While none of these latter Pseudomonas species has been directly shown to be involved in food spoilage, we hypothesize based on these data that at least some of them likely play a role in chicken spoilage.*

Although our research suggests that AC may have shelf-life and quality advantages over WC, technoeconomics are more nuanced. The WC systems can use up to 10 times more water than the AC system, but the electrical costs of the AC system are two times that of the WC system. Our baseline models do not support the claims by two other studies that AC systems require almost 50 times less gross energy than WC systems, when entire energy expenditures are considered [11, 12]. Rather, our results agree with the conclusions of Northcutt et al., specifically, that WC and AC systems have similar total chilling costs [34]. However, Northcutt et al. also claimed that the large water requirements, which have been steadily increasing in the United States as a result of USDA regulations, can start to tip the balance for AC over WC systems especially if one considers the potential for changes in water purchase and treatment costs. *Therefore, AC may have an economic advantage over WC depending on the local price and availability of water resources.*

This experiment was designed to be a laboratory-based, pilot-sized representation of the larger process of chicken chilling, fabrication, storage, and retail display. We made efforts to represent industry conditions as accurately as possible in a small laboratory setting, however there were a few conditions we were unable to replicate. We were unable to reproduce an antimicrobial application, either in the WC chill water or sprayed on the AC carcasses, which could modify some of the microbiological results. Therefore, our experiment represents a worse-case scenario. Future experiments should confirm the current findings using real industry conditions.

Conclusion

The overarching goal of this study was to combine multi-disciplinary approaches to determine the impact of chilling method on the overall system efficiency and sustainability of chicken production. We were able to conclude that AC methods had an advantage in quality, spoilage,

and consumer appeal prior to 14 days of dark storage, that AC appeared to result in a more favorable, diverse microbial community, and that AC requires less gross energy and, depending on the local price of water, may be the more economically favorable system.

Materials And Methods

Experimental design

The experiment was conducted using a $2 \times 2 \times 2$ factorial design to evaluate three factors: chilling method (air chilling; AC vs. water immersion chilling; WC), fabrication method (bone-in vs boneless) and dark storage period (7 days vs. 14 days). Eviscerated, hot chicken carcasses ($n = 256$) were obtained from a commercial processing facility in California and transported to the USDA-inspected Meat Science Laboratory at the University of California, Davis (UCD; Davis, CA) within two hours of harvest. Carcasses were transported in sterile 150-quart coolers (MaxCold Cooler; Igloo Products Corp., Katy, Texas) at a mean temperature of 30.25°C . Upon arrival at UCD, carcasses were divided into sampling groups following the scheme in Fig. 1. Sixteen carcasses were identified for a taste panel and placed four each in the treatment groups that were placed under 7-day dark storage (AC-bone-in, AC-boneless, WC-bone-in, WC-boneless). Of the other carcasses, 20 were sampled immediately for hot carcass samples and the remainder were randomly and evenly assigned into either AC or WC ($n = 110$ birds/chilling method). Following chilling (described below), 10 carcasses from each AC and WC treatment group were sampled, and the remaining were evenly assigned to fabrication pathways ($n = 50$ birds/fabrication) yielding either bone-in or boneless chicken breasts ($n = 20$ breasts/fabrication pathway). Immediately following fabrication, 10 chicken breasts from each group were sampled, and the remaining breasts were placed on expanded polystyrene trays and overwrapped with polyvinyl chloride film (40-gauge, Berry AEP1504310). Overwrapped trays were placed in rigid cardboard boxes ($n = 8$ trays/box) and stored at 4°C ($3\text{--}6^\circ\text{C}$) for either 7 or 14 days. At each storage interval (7 or 14 days), individual packages of chicken breasts (bone-in and boneless) were removed from dark storage. Ten breasts from each group were sampled immediately after removal from storage, and the remaining packaged breasts were placed in a retail display case (Barker, Keosauqua, IA, average light intensity 1061 LUX) maintained at 4°C ($3\text{--}6^\circ\text{C}$) for 3 days.

Chicken processing

Procurement of Chicken Carcasses. A commercial chicken processing facility in California was utilized to procure hot chicken carcasses for this research. Live birds were subjected to standard poultry harvest protocols as implemented by the commercial processing facility. Carcasses used for this experiment were obtained following defeathering, evisceration, and application of an initial post-harvest antimicrobial carcass spray. Prior to chilling, the carcasses ($n = 256$) were removed from the processing line, placed in sterile plastic bags ($n = 30\text{--}32$ carcasses/bag), and bags placed in sealed sterile coolers for transportation to the Meat Science Laboratory at UC-Davis (Davis, CA). Additionally, temperature recorders (LogTag Tred30-16r; LogTag, Auckland, NZ) were placed in the coolers to monitor temperature during transportation.

Processing and Chilling of Chicken Carcasses. It should be noted that this process, while designed to mimic industrial systems, was performed on a much smaller scale. Upon arrival at the UC Davis Meat Science Laboratory, 20 chicken carcasses were randomly selected for initial evaluations (described below), while the remaining carcasses were randomly and evenly assigned to one of two chilling methods (AC or WC; Fig. 5A). Weights (g) of individual carcasses were obtained prior to chilling for comparison with weights obtained following chilling (described below). Sixteen carcasses were reserved for taste evaluation (described below) after seven days of storage and three days of retail display. This subset of carcasses was subjected to both chilling methods and fabrication methods (described below), leaving 240 carcasses for laboratory analyses. Carcasses designated for WC were submerged in one of two simulated water chill tanks (Fig. 5C). Simulated water chill tanks were constructed from commercial water tanks (Structural Form Stock Tanks, 150 gal, Rubbermaid), and a slurry of water and ice was formulated using potable water and commercial ice. Water temperature was monitored throughout chilling and birds were agitated while submerged using a paddle. Carcass temperature was monitored regularly using a thermometer (Multitrip Data Logger, Temprecord, New Zealand) probe inserted into the thickest portion of the chicken breast. When the average internal carcass temperature reached 4°C , the chicken carcasses were removed from the water chilling system and placed on sterile wire racks for 10 minutes. Post-chilling weight and temperature were obtained after the 10-minute holding period. Additionally, five carcasses were randomly selected for analyses (described below).

To simulate air chilling, an isolated cold room in the UC-Davis Meat Science Laboratory was outfitted with a high-velocity fan (Model # BF60BDORGPRO, Maxx Air; 60' fan with 19000 CFM, providing an airflow of 1.23 m/s .) as shown in Fig. 5C. Chicken carcasses were placed on sterile wire racks located approximately six m from the commercial fan. The wire racks were rotated throughout the chilling process to assure equitable exposure to the chilling conditions. Carcass temperature was monitored throughout by inserting a thermometer probe into the thickest portion of the breast, and once the average internal temperature reached 4°C , the carcasses were removed from the AC room. A post-chilling weight and internal temperature were obtained from individual carcasses. Additionally, 10 carcasses from each method were randomly selected for analyses (described below).

Fabrication of Chicken Carcasses, Packaging of Chicken Breasts, and Dark Storage.

Immediately following chilling, carcasses within each chilling method (AC and WC) were randomly and evenly assigned to one of two fabrication methods ($n = 50$ / fabrication method) for the generation of boneless (BL) and bone-in (BI) chicken breasts. Carcasses were fabricated by trained personnel in the UC Davis Meat Science Laboratory using sterile instruments and WC and AC carcasses were fabricated separately. Immediately following fabrication, 10 chicken breasts from each group were sampled, and the remaining breasts were placed on expanded polystyrene trays and overwrapped with polyvinyl chloride film (40-gauge, Berry AEP1504310). Overwrapped trays were placed in rigid cardboard boxes ($n = 4$ trays/box) and stored at 4 °C (3–6 °C) for either 7 or 14 days.

Retail display

At each storage interval (7 or 14 days), individual packages of chicken breasts (BI and BL) were removed from dark storage. Ten breasts from each group were sampled immediately after removal from storage, and the remaining packaged breasts were placed in a retail display case (Barker, Keosauqua, IA, average light intensity 1061 LUX) maintained at 4 °C (3 °C – 6 °C). Packages remained in the retail display case for three days. Instrumental meat color, measured via evaluating the lean color of the boneless samples and skin color of the bone-in samples, was taken using a portable spectrophotometer (MiniScan EZ; Hunter Association Laboratory Inc., Reston, VA) that was standardized before each use. A total of three readings of the International Commission on Illumination (CIE) L^* (lightness), a^* (redness) and b^* (yellowness) values were taken using an illuminant A/10° observer for each breast. Measurements were taken through the packaging material at three separate locations on the chicken breast and were averaged prior to analyses. Packages were rotated in the display case every 12 h to assure equitable temperature and light exposure.

Microbial sample collection/processing

At each sampling point, the microbial communities of the chicken products were collected using a rinsate method. At pre-fabrication timepoints, the entire chicken carcass was placed in a sterile collection bag (Whirl-Pak; Nasco, Fort Atkinson, WI) with 200 ml of phosphate-buffered saline (PBS; National Diagnostics, Atlanta, GA) and agitated for 60 seconds to dislodge surface bacteria. After this, the carcass was removed from the rinsate and saved for physicochemical analysis. At the post-fabrication timepoints, each chicken breast was divided in half. One half was placed in a sterile collection bag (Whirl-Pak; Nasco, Fort Atkinson, WI) with 50 mL of PBS and agitated for 60 seconds. The second half was reserved for physicochemical analysis. At all time points, the rinsate was collected from the sampling bag into 50 mL falcon tubes (Corning Science, Mexico) for analysis. A 10 mL aliquot of each sample was separated to be used for aerobic bacteria population analysis and the remainder was frozen to -80 °C and transported to Colorado State University (Fort Collins, CO) PI Metcalf Laboratory for microbial ecology analysis.

The rinsate sample collected for microbiome analysis was further divided into 30 mL aliquot before DNA extraction. Cells within the rinsate were concentrated into a pellet by centrifugation at 4,600 g for 15 minutes in a swing bucket rotor (Sorvall Legend X1R; Thermo Scientific, Waltham, MA). The supernatant was poured off and a portion of the pellet equivalent to approximately 600 μ L was used for analysis. DNA was extracted from the pellet following standard protocols utilizing the Qiagen PowerSoil DNA 96 well extraction kit (Qiagen, Hilden, Germany) following manufacturers protocol for low biomass samples, which included the additional step of allowing the EB solution to be heated to 65 °C before adding to the DNA plate wells for five minutes before eluting. DNA was eluted in two steps. Initially, 60 μ L was eluted and considered our more concentrated DNA extraction. Next, we eluted an additional 80 μ L of DNA. Each 96-well plate included 8 mock extractions (no sample added) and 1 positive control (ZymoBIOMICS D6300). The 16S rRNA gene (V4 region) was amplified using primers 515F and 806R universal primers with the forward primer barcoded to allow for multiplexing during sequencing, following the Earth Microbiome Project protocols (<http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/>). The forward primer 515F included the unique sample barcode following Parada et al. [35], and both primers included degeneracies as described in Parada et al. and Apprill et al. [35, 36]. Two PCR reactions using Invitrogen Platinum Hot Start PCR 2x Mastermix (Invitrogen, Carlsbad, CA) with 1 μ L of DNA and a final concentration of 0.2 μ M primer were run for each sample and combined to a total of 75 μ L. The PCR product was quantified and then pooled into a single pool in equimolar concentrations with the exception of samples that did not meet a minimum concentration, in which case 25 μ L were added (this allowed the inclusion of mock extraction controls in the sequencing run). The resulting pool was cleaned using a Minelute PCR purification kit (Qiagen) and sequenced with a Miseq reagent v2 500 cycle kit at the CSU Next Generation Sequencing Core on the Illumina Miseq platform.

After sequencing, microbiome data were analyzed using QIIME2 [37] and R software version 3.5.1. Sequences were demultiplexed and quality-filtered in QIIME2 using error-correcting Golay barcodes that prevent misassignment. Reads were trimmed to 250 bp, then amplicon sequence variants (ASVs) were inferred using DADA2 [38]. Taxonomy was then assigned using the QIIME2 feature-classifier plugin [39] against the SILVA-132 99% database [40]. Non-microbial sequences that assigned to mitochondria and chloroplasts were filtered from the dataset. Samples were rarefied to 6,152 sequences and diversity metrics were calculated using the QIIME2 core metrics pipeline. Statistical comparisons for alpha diversity were made using the Kruskal-Wallis test with an alpha of 0.05 and statistical comparisons for beta diversity were made using PERMANOVA with multiple testing correction and an alpha level of 0.05. The composition of the microbiomes was compared by testing the differential abundance of taxa using the ANCOM plugin in QIIME2 [41]. The ability of microbial communities to predict quality and spoilage outcomes was assessed using the QIIME2 sample-classifier classify-samples plugin [42, 43]. Models were trained and tested using k-fold cross-

validation and the Random Forest classifier with hyperparameter tuning. Visualizations were generated using QIIME2 and R software with ggplot2 [37, 44].

Phylogenetic trees

All *Pseudomonas* ASV sequences were extracted from the feature table by filtering based on assigned taxonomy. An alignment of all type strain 16S rRNA gene sequences for this genus was downloaded from the Ribosomal Database Project [45], along with an appropriate outgroup. A maximum likelihood backbone tree was generated using RAxML 8.2.12 [46] using the GTRGAMMA substitution matrix and 100 rapid bootstraps on the RDP alignment. An information file was then generated to be used in SATé-Enabled Phylogenetic Placement (SEPP) which was modified to fit the parsing parameters from pplacer v1.1.alpha13-0-g1ec7786 (removed 1 line according to documentation on the SEPP website <https://github.com/smirarab/sepp/issues/40>). SEPP 4.3.10 was then run with the following parameters (-P = 33 -A 10) to optimize the alignment breakdown using the ASVs file, the RAxML tree, the RAxML info file and the reference alignment as input.

Quality measurements

Aerobic Bacteria Populations. As described by Martin et al. (2013), aerobic bacterial populations are strong indicators of the end of shelf-life. Thus, quantifying the aerobic populations present—in addition to the characterization of the microbiome—will provide insight into the shelf-life impacts of the microbial population. At each sampling interval, the carcass of one sample from each chicken was rinsed using 200 ml PBS for the carcass and 50 ml for the breast. One milliliter of the rinsate was serially diluted in 0.1% buffered peptone water (BPW; Becton, Dickinson and Company, Sparks, MD) and plated in duplicate onto Petrifilm aerobic count plates (3M Microbiology, St. Paul, MN). Plates were then incubated at 7 °C for 10 days and 35 °C for 48 h.

Physicochemical Analysis. Numerous biochemical and physicochemical changes that affect shelf-life occur in post-harvest meat products [47]. Thus, an assessment of these changes during processing was conducted to obtain information regarding product quality. At each sampling point, after the rinsate was collected, the sample was fabricated to a boneless breast if it was not already, though the skin was left on for carcass and bone-in samples. Then, the breast was flash-frozen in liquid nitrogen and homogenized using a blender (Magic Bullet; Capital Brands, Los Angeles, CA). To evaluate the carcass samples and bone-in breasts, the breast meat was removed from the bone at the time of sampling. The frozen homogenate was then transported to the Colorado State University Center for Meat Safety and Quality (Fort Collins, CO) for physicochemical and compositional analyses.

Fatty Acid Composition. Fatty acid composition was obtained using gas chromatography following methods described by Engle et al. and Kang and Wang [48, 49]. First, fat was extracted using the Folch method (Folch, 1957). One gram of the frozen homogenate was combined with 20 mL of 2:1 chloroform:methanol mixture and homogenized, then filtered using Whatman No. 1 filter paper (Fisher Scientific; Waltham, MA). Then, 4 mL of 0.9% NaCl solution was added per 20 mL chloroform:methanol and the solution was incubated at 4 °C overnight. During this time the solvent separated into two phases; the lower phase contained the lipid extract, which was separated and dried in a dry matter oven at 100 °C for 16 h. After this extraction, the lipid extract was methylated by adding 1 mL of 0.5 N KOH in MeOH and heated in a water bath. Samples were then prepared for gas chromatography by mixing with 2 mL HPLC-grade hexane and 2 mL saturated NaCl, which was then back-extracted and reconstituted to concentrate the fatty acids. The reconstituted lipid was measured by gas chromatography (Agilent 6890 plus; Agilent, Wilmington, DE) with standard fatty acid methyl ester mixtures and SUPELCO FAME standard (Millipore Sigma, Darmstadt, Germany) to calibrate. Fatty acids were identified by matching relative peak retention times to those of the standards, calculated as normalized area percentages of fatty acids.

Lipid Oxidation. Lipid oxidation was measured using the thiobarbituric acid assay (TBARs), as described in Yin et al. [50]. Briefly, 5 g of the frozen homogenate was mixed with trichloroacetic acid, homogenized using a standing homogenizer, and filtered using Whatman No.1 filter paper (Fisher Scientific; Waltham, MA). A 1 mL aliquot of the filtrate was mixed with 1 mL of 10 mM thiobarbituric acid and incubated at 25 °C for 20 h, after which the absorbance at 532 nm was measured using a spectrophotometer (UV-2401, Shimadzu Inc., Columbia, MD).

Proximate Analysis. Nutrient composition analysis (proximate analysis) was conducted to determine the dry matter, moisture, ash, crude fat, and crude protein composition within each sample. Dry matter and moisture were measured using the AOAC oven drying method, 950.46 and 934.01 [51]. Two grams of frozen homogenate were weighed and placed in a standard laboratory convection oven for 24 h at 60 °C, then re-weighed. Percent moisture was calculated using the formula: % moisture content = $[(\text{wet weight} - \text{dry weight}) / \text{wet weight}] * 100$. Percent dry matter was calculated as 100 - moisture content. Ash content was determined using the ash oven method as described in the AOAC 923.03 and 920.153 [52]. Approximately 1 g of the frozen homogenate was placed into a dry crucible, then inserted into a Thermolyne box furnace at 600 °C for 18 h. Percent ash was calculated using the formula: % ash = $(\text{ash weight} / \text{wet weight}) * 100$. Crude fat was measured using the Folch method, as described above. Finally, crude protein was measured following AOAC method 992.15 [53], which used a TrueSpec CN nitrogen determinator (LECO, St. Joseph, MI). Percent protein was calculated using the formula: % protein = total % N * 6.25. Results were represented on a dry matter basis. Statistical analyses on all physiochemical tests were conducted using ANOVA and the emmeans package [54] with a 2 × 2 × 2 factorial design with an alpha level was 0.05.

Sensory Analysis.

Eight untrained participants were asked to evaluate the acceptability of three sensory attributes (color, odor, texture) during retail display using a three-point sensory scale described by Lytou et al. [55]. In addition, subjective color (desirable, acceptable, unacceptable) and willingness to purchase (would purchase, would not purchase, would purchase at a discounted price) was evaluated by these untrained panelists every 12 h during retail display. At the end of each three-day retail display period (10 day and 17 day) the same participants were then asked to evaluate subjective odor, texture and purchase selection using the same approach. Evaluation scores were analyzed as continuous data using mixed procedures of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Participants were treated as random variables and the alpha level was defined as 0.05.

In addition to evaluation of chicken breast color, odor, and texture, trained taste panelists were asked to evaluate various palatability attributes (chicken flavor intensity, off-flavor intensity, springiness, cohesiveness of mass, and moistness) of bone-in and boneless chicken breasts representing both chilling methods following 7 days of dark storage. Panelists consisted of graduate students from the CSU Center for Meat Safety and Quality and were trained to recognize the aforementioned attributes using methods and references described by Solo [56]. Samples for evaluation were randomized and panels were conducted over two days to avoid sensory fatigue. Chicken breasts were cooked to an internal temperature of 76 °C and cut into 2.54 × 2.54-cm cubes before being served to panelists under red lights. Panelists then ranked each breast portion for each of the above attributes on a 100-point scale. Data were analyzed using an ANOVA and the emmeans package in R [54] with an alpha level of 0.05.

Chilling system techno-economic analysis

An economic evaluation of each chilling system, AC and WC, was also performed. The work included the development of baseline models of each system that allowed for a direct comparison on the metric of economics. The baseline models used the same system boundaries that limited this techno-economic assessment to the chilling process and used harmonized model inputs when possible for consistency. Some facilities include maturation as an extension of the chilling process; however, these baseline models don't include anything outside the chilling process. The models used standard n^{th} plant economic assumptions from literature and assume 10% internal rate of return (IRR), 20-year facility life, 8% loan interest rate on a 10-year loan with 40% equity, and the 2019 U.S. corporate tax rate of 21% [57–59]. The above economic assumptions were combined with capital costs, operational costs, linear depreciation, and poultry processing rate to perform a 20-year discounted cash flow rate of return (DCFROR) for each poultry chilling system. These models use the IRR as the discount rate to determine the minimum processing cost (\$/metric ton) associated with poultry chilling while providing a net present value (NPV) of zero. This minimum processing cost represents a levelized cost of chilling poultry carcasses that supports a 10% IRR over the 20-year life of the system.

All baseline values were taken from literature or acquired through communication with poultry chilling equipment manufacturers and poultry processing facilities. In particular, the system layout and energy consumptions reported in the literature were found to be antiquated and thus most of these data were acquired through communications with industry. The mutual baseline inputs were plant operation (250 d per year, 16 h per day), poultry processing rate (16.5 tonne/hour), water treatment (\$1.5/m³; [9, 34, 60, 61]) and electricity (\$0.10/kWh) prices, and fixed annual maintenance cost (5% of total capital cost). While AC and WC fixed and variable labor requirements might vary slightly, the labor requirements were assumed to be equivalent for the baseline cases for both systems. Based on input from a chilling equipment suppliers, the AC and WC models reflect the major differences between the two systems (WC, AC); floor space requirements (100 m², 500 m²), water use (3200 L/tonne; 300 L/tonne), and chilling energy costs (20.9 kWh/tonne, 31.4 kWh/tonne). Due to the complexity and high variability in WC and AC system designs and operational characteristics, sensitivity analyses were used to evaluate how system parameters can impact the levelized cost associated with chilling poultry carcasses. The sensitivity analysis varied the following model values by ± 50%: capital cost (\$/tonne), chilling floorspace (m²), processing rate (tonne/hour), chilling energy (kWh/tonne), worker capacity (tonne/hour), water use (L/tonne), water price plus water treatment cost (\$/L), and electricity cost (\$/kWh). The end result is a direct comparison of the two technologies in terms of costs with sensitivity used to highlight high impact variables.

Abbreviations

AC

Air Chilled

ASV

Amplicon Sequence Variant

BI

Bone-in

BL

Boneless

CIE

International Commission on Illumination

DCFROR

Discounted Cash Flow Rate of Return

IRR

Internal Rate of Return

PCR

Polymerase Chain Reaction

TBARS

Thiobarbituric Acid Assay

WC

Water Chilled

Declarations

Ethics approval and consent to participate

Human participation in taste and food quality evaluation was reviewed by the Institutional Review Board at Colorado State University with IRB ID 364-18H and declared to be exempt from the requirements of the human subject protections regulations.

Consent for publication

Not Applicable

Data Availability

16S rRNA gene sequencing data is available in QIITA study 12193. Additional data and analysis details can be accessed at <https://github.com/Metcalf-Lab/Air-versus-water-chilling-of-chicken>.

Competing interests

The authors declare that they have no competing interests

Funding

Funding for this work was provided by the Innovation Institute for Food and Health at UC Davis.

Authors contributions

AB and DC analyzed and interpreted the microbiome and phylogenetic data. AB and JNM analyzed and interpreted the physicochemical data including lipid oxidation, proximate analysis, fatty acid profile, and taste panel data. TD and XY analyzed and interpreted the microbiological data and color and odor panels. CQ and JQ performed the techno-economic analysis. AB and TD led manuscript preparation. JLM, JNM, DC, KB, and JE were responsible for securing funding and project resources. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank Caleb Sehnert and the staff of the UC Davis Meat Sciences Laboratory for use of their facilities and help with chicken processing. Additional thanks to Nathan Frazier, Jennifer Levey, Sam Ogden, Jaqueline Colin, Kaylah Marcello, Katherine Dahlhausen, Dennett Rodriguez and Karla Michelle Villatoro for help with chicken processing and sample collection. Thanks to the commercial donor of the chickens used in this study and their expertise in chicken processing. Thanks to Holly Archer and Andy Esterle for assistance with microbiome laboratory work.

References

1. Putman B, Thoma G, Burek J, Matlock M. A retrospective analysis of the United States poultry industry: 1965 compared with 2010. *Agric Syst.* 2017;157 C:107–17. <https://ideas.repec.org/a/eee/agisys/v157y2017icp107-117.html>. Accessed 23 Apr 2020.
2. USDA - National Agricultural Statistics Service. - Charts and Maps - Poultry. https://www.nass.usda.gov/Charts_and_Maps/Poultry/index.php. Accessed 23 Apr 2020.
3. Food and Agriculture Organization of the United Nations. Meat Market Review: Overview of global meat market developments in 2018. FAO; 2019.
4. Zhuang H, Bowker BC, Berrang ME, Meinersmann RJ, Buhr RJ. Impact of eliminating the carcass chilling step in the production of pre-cooked chicken breast meat. *J Appl Poult Res.* 2017;26:431–6. <https://academic.oup.com/japr/article-abstract/26/3/431/3778202>.
5. Sams AR, Alvarado C, Owens CM. Poultry meat processing. CRC Press Boca Raton, FL; 2001. http://www.academia.edu/download/5696249/poultry_meat_processing_poultryvet.blogspot.com_.pdf.
6. Zhang L, Jeong JY, Janardhanan KK, Ryser ET, Kang I. Microbiological quality of water immersion–chilled and air-chilled broilers. *J Food Prot.* 2011;74:1531–5. <http://jfoodprotection.org/doi/abs/10.4315/0362-028X.JFP-11-032>.
7. Sanchez MX, Fluckey WM, Brashears MM, McKEE SR. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. *J Food Prot.* 2002;65:948–56. <http://jfoodprotection.org/doi/abs/10.4315/0362-028X-65.6.948>.
8. Smith DP, Cason JA, Berrang ME. Effect of fecal contamination and cross-contamination on numbers of coliform, *Escherichia coli*, *Campylobacter*, and *Salmonella* on immersion-chilled broiler carcasses. *J Food Prot.* 2005;68:1340–5. doi:10.4315/0362-028x-68.7.1340.
9. Huez R, Smith DP, Northcutt JK, Fletcher DL. Effect of Immersion or Dry Air Chilling on Broiler Carcass Moisture Retention and Breast Fillet Functionality. *J Appl Poult Res.* 2007;16:438–47. doi:10.1093/japr/16.3.438.
10. Barbut S. *The Science of Poultry and Meat Processing*. University of Guelph; 2015.
11. Pederson R. Advantages and disadvantages of various methods for the chilling of poultry. In: Rep. No. 189. Landbrugsministeriets Slagteri- og Konserverlaboratorium Copenhagen, Denmark; 1979.
12. James C, Vincent C, de Andrade Lima TI, James SJ. The primary chilling of poultry carcasses—a review. *Int J Refrig.* 2006;29:847–62. doi:10.1016/j.ijrefrig.2005.08.003.
13. Dainty RH, Mackey BM. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J Appl Bacteriol.* 1992;73:103 s – 114 s. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2672.1992.tb03630.x?casa_token=mjIPCjUFzIQAAAAA:AzqqBhDQWWho_7EJomgrpqqZw8oHH9WSY8WK8llCPYFWaRxPjw_RXQjtn0Yj2LXDxovCAc6Q18DHGZux
14. Lorenzo JM, Bedia M, Bañón S. Relationship between flavour deterioration and the volatile compound profile of semi-ripened sausage. *Meat Sci.* 2013;93:614–20. doi:10.1016/j.meatsci.2012.11.006.
15. Rivas-Cañedo A, Juez-Ojeda C, Nuñez M, Fernández-García E. Volatile compounds in ground beef subjected to high pressure processing: A comparison of dynamic headspace and solid-phase microextraction. *Food Chem.* 2011;124:1201–7. doi:10.1016/j.foodchem.2010.07.045.
16. St. Angelo AJ, Vercellotti J, Jacks T, Legendre M. Lipid oxidation in foods. *Crit Rev Food Sci Nutr.* 1996;36:175–224. doi:10.1080/10408399609527723.
17. Jeong JY, Janardhanan KK, Booren AM, Harte JB, Kang I. Breast meat quality and consumer sensory properties of broiler carcasses chilled by water, air, or evaporative air. *Poult Sci.* 2011;90:694,700.
18. Fleming BK, Froning GW, Yang TS. Heme pigment levels in chicken broilers chilled in ice slush and air. *Poult Sci.* 1991;70:2197–200. <https://academic.oup.com/ps/article-abstract/70/10/2197/1546826>.
19. Zhuang H, Savage EM, Smith DP, Berrang ME. Effect of dry-air chilling on sensory descriptive profiles of cooked broiler breast meat deboned four hours after the initiation of chilling. *Poult Sci.* 2009;88:1282–91. doi:10.3382/ps.2008-00325.
20. Katiyo W, de Kock HL, Coorey R, Buys EM. Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage. *LWT.* 2020;128:109468. doi:10.1016/j.lwt.2020.109468.
21. Hale KK, Stadelman WJ, Bramblett VD. Effect of Dry-Chilling on the Flavor of Fried Chicken1. *Poult Sci.* 1973;52:253–62. doi:10.3382/ps.0520253.
22. Ristic M. Influence of the water cooling of fresh broilers on the shelf life of poultry parts at -15 degrees C and – 21 degrees C. *Lebensmittel - Wissenschaft + Technologie = Food science + technology.* 1982;15. <http://agris.fao.org/agris-search/search.do?recordID=US201301995208>.
23. Berrang ME, Meinersmann RJ, Smith DP, Zhuang H. The effect of chilling in cold air or ice water on the microbiological quality of broiler carcasses and the population of *Campylobacter*. *Poult Sci.* 2008;87:992–8. doi:10.3382/ps.2007-00406.

24. Handley JA, Park SH, Kim SA, Ricke SC. Microbiome Profiles of Commercial Broilers Through Evisceration and Immersion Chilling During Poultry Slaughter and the Identification of Potential Indicator Microorganisms. *Front Microbiol.* 2018;9:345. doi:10.3389/fmicb.2018.00345.
25. Tuncer B, Sireli UT. Microbial growth on broiler carcasses stored at different temperatures after air- or water-chilling. *Poult Sci.* 2008;87:793–9. doi:10.3382/ps.2007-00057.
26. Mead GC, Hudson WR, Hinton MH. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible control measures. *Br Poult Sci.* 1994;35:345–54. doi:10.1080/00071669408417699.
27. Lillard HS. The Impact of Commercial Processing Procedures on the Bacterial Contamination and Cross-Contamination of Broiler Carcasses. *J Food Prot.* 1990;53:202–4. doi:10.4315/0362-028X-53.3.202.
28. Gumhalter Karolyi L, Medić H, Vidaček S, Petrak T, Botka – Petrak K. Bacterial population in counter flow and parallel flow water chilling of poultry meat. *Eur Food Res Technol.* 2003;217:412–5. doi:10.1007/s00217-003-0772-6.
29. Munther D, Sun X, Xiao Y, Tang S, Shimozako H, Wu J, et al. Modeling cross-contamination during poultry processing: Dynamics in the chiller tank. *Food Control.* 2016;59:271–81. doi:10.1016/j.foodcont.2015.05.007.
30. Raposo A, Pérez E, de Faria CT, Ferrús MA, Carrascosa C. Food Spoilage by *Pseudomonas* spp.-An Overview. In: Singh OV, editor. *Foodborne Pathogens and Antibiotic Resistance.* Hoboken: John Wiley & Sons, Inc.; 2016. pp. 41–71. doi:10.1002/9781119139188.ch3.
31. Tryfinopoulou P, Tsakalidou E, Nychas G-JE. Characterization of *Pseudomonas* spp. associated with spoilage of gilt-head sea bream stored under various conditions. *Appl Environ Microbiol.* 2002;68:65–72. doi:10.1128/aem.68.1.65-72.2002.
32. Morales PA, Aguirre JS, Troncoso MR, Figueroa GO. Phenotypic and genotypic characterization of *Pseudomonas* spp. present in spoiled poultry fillets sold in retail settings. *LWT.* 2016;73:609–14. doi:10.1016/j.lwt.2016.06.064.
33. Wang G-Y, Wang H-H, Han Y-W, Xing T, Ye K-P, Xu X-L, et al. Evaluation of the spoilage potential of bacteria isolated from chilled chicken in vitro and in situ. *Food Microbiol.* 2017;63:139–46. doi:10.1016/j.fm.2016.11.015.
34. USDA ARS Online Magazine. Vol. 56, No. 4. <https://agresearchmag.ars.usda.gov/2008/apr/chicken>. Accessed 16 Mar 2020.
35. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol.* 2016;18:1403–14. doi:10.1111/1462-2920.13023.
36. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol.* 2015;75:129–37. doi:10.3354/ame01753.
37. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019;37:852–7. doi:10.1038/s41587-019-0209-9.
38. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016;13:581. doi:10.1038/nmeth.3869 <https://www.nature.com/articles/nmeth.3869#supplementary-information>.
39. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome.* 2018;6:90. doi:10.1186/s40168-018-0470-z.
40. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2012;41:D590–6. doi:10.1093/nar/gks1219.
41. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis.* 2015;26:27663. doi:10.3402/mehd.v26.27663.
42. Bokulich NA, Dillon MR, Bolyen E, Kaehler BD, Huttley GA, Caporaso JG. q2-sample-classifier: machine-learning tools for microbiome classification and regression. *J Open Res Softw.* 2018;3. doi:10.21105/joss.00934.
43. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine Learning in Python. *J Mach Learn Res.* 2011;12 Oct:2825–30. <http://www.jmlr.org/papers/v12/pedregosa11a.html>. Accessed 30 Nov 2019.
44. Wickham H. *ggplot2: Elegant Graphics for Data Analysis.* New York: Springer-Verlag; 2009. <http://ggplot2.org>.
45. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* 2014;42(Database issue):D633–42. doi:10.1093/nar/gkt1244.
46. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics.* 2006;22:2688–90. doi:10.1093/bioinformatics/btl446.
47. Giménez A, Ares F, Ares G. Sensory shelf-life estimation: A review of current methodological approaches. *Food Res Int.* 2012;49:311–25. doi:10.1016/j.foodres.2012.07.008.
48. Engle TE, Spears JW, Armstrong TA, Wright CL, Odle J. Effects of dietary copper source and concentration on carcass characteristics and lipid and cholesterol metabolism in growing and finishing steers. *J Anim Sci.* 2000;78:1053–9. doi:10.2527/2000.7841053x.
49. Kang JX, Wang J. A simplified method for analysis of polyunsaturated fatty acids. *BMC Biochem.* 2005;6:5. doi:10.1186/1471-2091-6-5.

50. Yin MC, Faustman C, Riesen JW, Williams SN. α -Tocopherol and Ascorbate Delay Oxymyoglobin and Phospholipid Oxidation In Vitro. *J Food Sci.* 1993;58:1273–6. doi:10.1111/j.1365-2621.1993.tb06164.x.
51. AOAC Authors. Reference data: Method 934.01; WATER. In: Official methods of analysis Proximate Analysis and Calculations Moisture (M) - item 105. Gaithersburg: Association of Analytical Communities; 2006.
52. AOAC Authors. Official methods of analysis Proximate Analysis and Calculations Ash Determination (Ash) Flour - item 49. Gaithersburg: Association of Analytical Communities; 2006.
53. AOAC Authors. Official methods of analysis Proximate Analysis and Calculations Crude Protein Meat and Meat Products Including Pet Foods - item 80. Gaithersburg: Association of Analytical Communities; 2006.
54. Russell L. Emmeans: estimated marginal means, aka least-squares means. R package version. 2018;1.
55. Lytou A, Panagou EZ, Nychas G-JE. Development of a predictive model for the growth kinetics of aerobic microbial population on pomegranate marinated chicken breast fillets under isothermal and dynamic temperature conditions. *Food Microbiol.* 2016;55:25–31. doi:10.1016/j.fm.2015.11.009.
56. Solo J. Meat Quality and Sensory Analysis of Broiler Breast Fillets with Woody Breast Muscle Myopathy. University of Arkansas, Fayetteville; 2016. <https://scholarworks.uark.edu/etd/1603/>. Accessed 14 Nov 2019.
57. Davis R, Markham J, Kinchin C, Grundl N, Tan ECD, Humbird D. Process design and economics for the production of algal biomass: algal biomass production in open pond systems and processing through dewatering for downstream conversion. National Renewable Energy Lab.(NREL), Golden, CO (United States); 2016. <https://www.osti.gov/biblio/1239893>.
58. Summers HM, Ledbetter RN, McCurdy AT, Morgan MR, Seefeldt LC, Jena U, et al. Techno-economic feasibility and life cycle assessment of dairy effluent to renewable diesel via hydrothermal liquefaction. *Bioresour Technol.* 2015;196:431–40. doi:10.1016/j.biortech.2015.07.077.
59. Cruce JR, Quinn JC. Economic viability of multiple algal biorefining pathways and the impact of public policies. *Appl Energy.* 2019;233–234:735–46. doi:10.1016/j.apenergy.2018.10.046.
60. Ricke SC. Achieving sustainable production of poultry meat Volume 1: Safety, quality and sustainability. Burleigh Dodds Science Publishing. 2017. doi:10.19103/AS.2016.0010.22.
61. Northcutt JK, Jones DR. A Survey of Water Use and Common Industry Practices in Commercial Broiler Processing Facilities. *J Appl Poult Res.* 2004;13:48–54. doi:10.1093/japr/13.1.48.

Figures

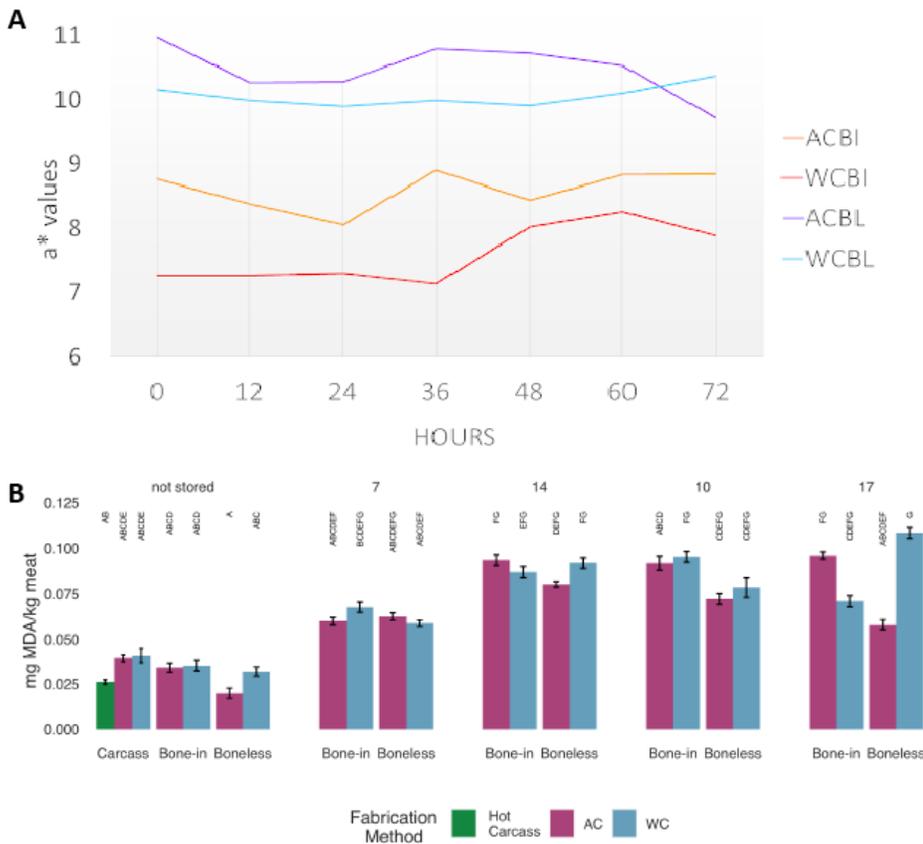


Figure 1

Figure represents changes in chicken quality over time. A) Least square means of a^* values for bone-in and boneless chicken breast chilled by either AC or WC following 7 days dark storage, during 3d retail display. CIE a^* represents the favorable redness of breasts. Chilling methods are represented by AC; air chilling and WC; water-chilling. Fabrication methods are denoted as BI; bone-in and BL; boneless. B) The average lipid oxidation levels within a treatment group as measured by thiobarbituric acid reactive substances (TBARS) assay. Bars along the x-axis refer to the chilling method and different colors represent the fabrication methods. As only chicken breasts were placed under dark storage, there were only carcass samples on the initial day of sample collection (not stored). Bars with the same letter were not statistically different ($P > 0.05$)

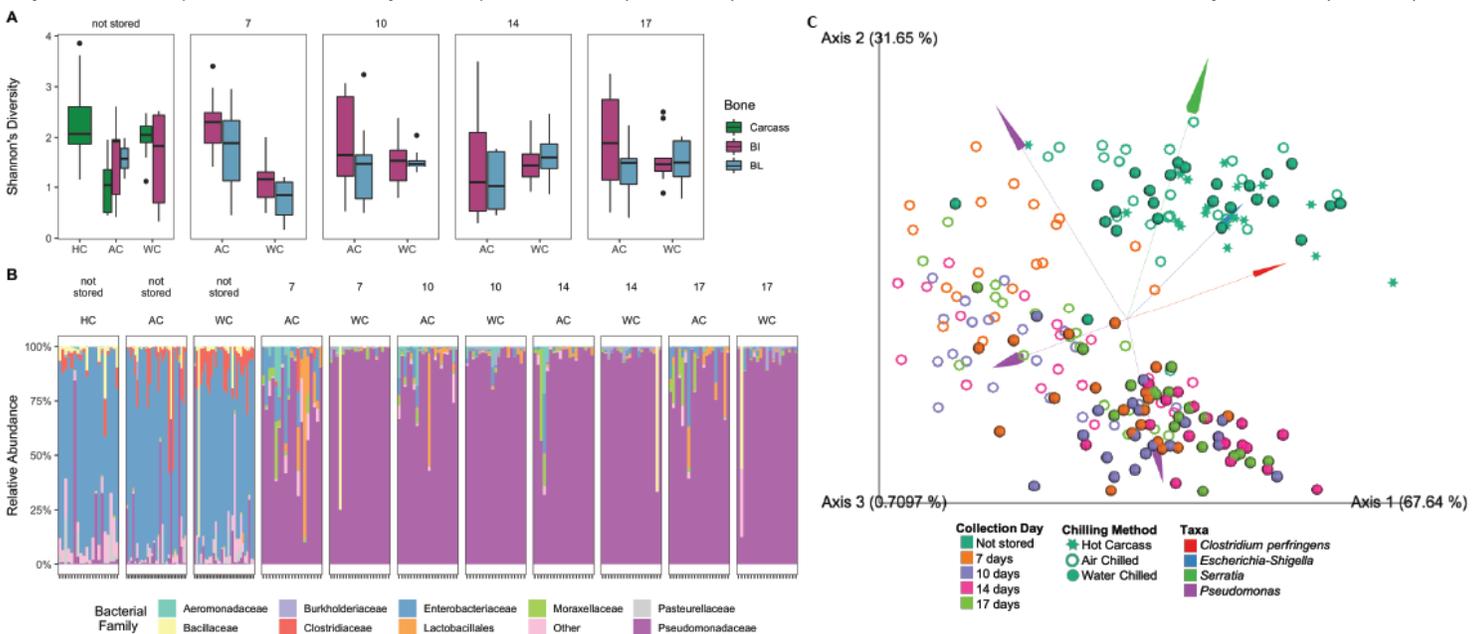


Figure 2

A) Shannon's diversity of the bacterial microbiome of chicken product samples, arranged by sampling day (7 and 14 days of storage plus 3 days retail display) and chilling method (Hot carcass, AC, WC) and colored by fabrication method (carcass, bone-in, boneless).

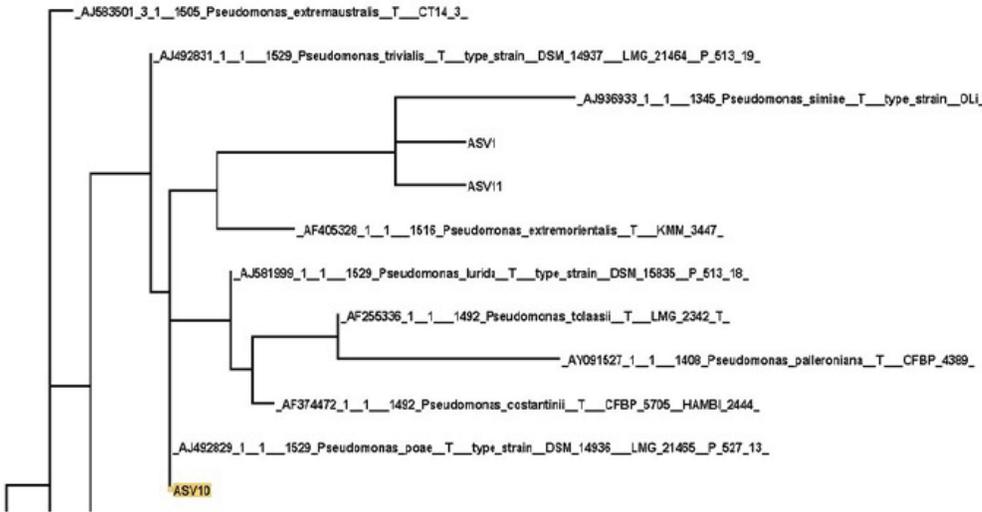


Figure 3

A portion of the detailed phylogenetic tree constructed from ASVs that assigned to Pseudomonas. The larger tree is included in the supplementary materials as Supplementary Figure 2.

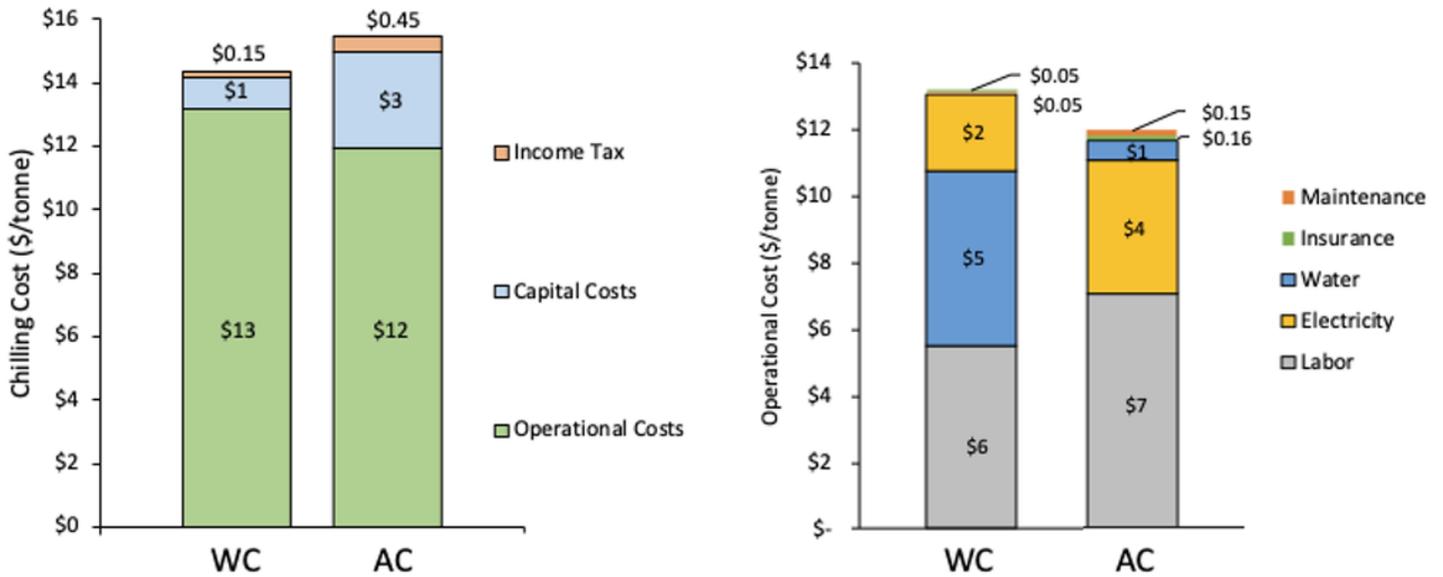


Figure 4

The levelized chilling cost (\$/tonne) for the baseline AC and WC models is shown in the left panel. The contributions of the capital costs, operational costs, and income tax to the total levelized chilling cost are displayed and demonstrate how the operational costs dominate the total chilling cost in both systems. The operational costs include the labor, electrical, water, maintenance, and insurance costs and are shown in the right panel for both the AC and WC systems.

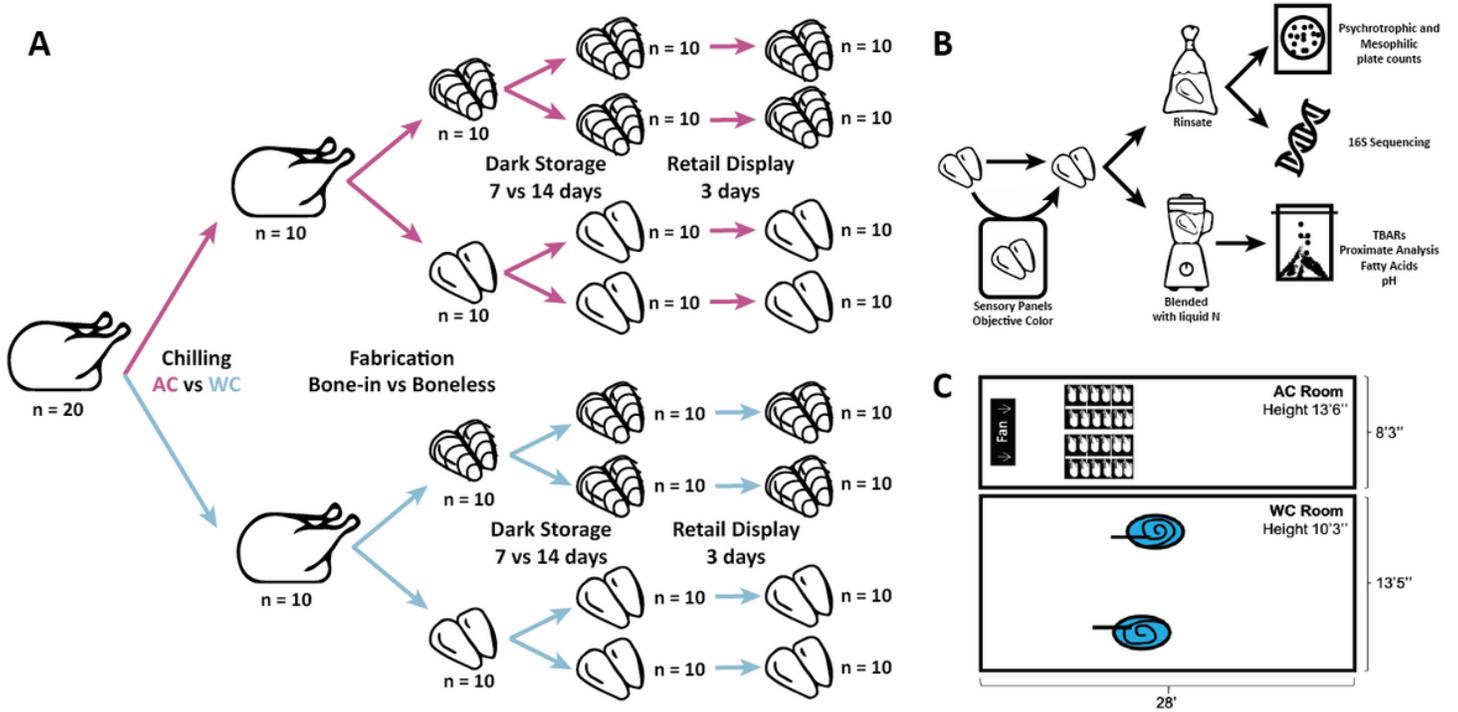


Figure 5

Representation of the experimental design. A) The processing scheme with time points for sampling during the experiment. B) The sampling process after the chicken product had been collected. The product was either sampled immediately or used for sensory panels. Microbial samples were taken via rinsate, which was then used for microbiological and microbiome analysis. The product was then flash-frozen in liquid nitrogen and powdered, then used for physicochemical analyses including pH, thiobarbituric acid assay (TBARs), proximate analysis, and fatty acid profiling. C) Representation of the two rooms used for carcass chilling.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)