applied sciences

MDPI

Brief Report

Comparative Study of Fresh and Frozen Broiler Neck Skin Sampled for Process Hygiene Purposes

Nina Langkabel [1,*], Verena Oswaldi [1], Roswitha Merle [2], Janine Dzierzon [1] and Diana Meemken [1]

[1] Working Group Meat Hygiene, Institute of Food Safety and Food Hygiene, Department of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany; verena.oswaldi@fu-berlin.de (V.O.); janine.dzierzon@fu-berlin.de (J.D.); diana.meemken@fu-berlin.de (D.M.)

[2] Institute for Veterinary Epidemiology and Biostatistics, Department of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany; roswitha.merle@fu-berlin.de

* Correspondence: nina.langkabel@fu-berlin.de

Abstract: The objective of the study was to determine the effect of freezing broiler neck skin samples before their microbial analysis, compared to freshly examined samples regarding total viable count (TVC) and Enterobacteriaceae count (EC). For this, 300 neck skin samples were taken at a German commercial broiler abattoir and each neck skin sample was cut into two parts. One randomly selected part underwent microbial examination after storage at 4 °C overnight; the other part was frozen at −30 °C for eight weeks before analysis in the same laboratory. Log cfu/g values of TVC and EC were separately compared between the fresh and frozen neck skin samples. A difference up to 0.5 log values was set as acceptable, i.e., fresh and frozen samples with counts that differed by this amount were considered as not different. The differences between the grouped samples of fresh and frozen broiler neck skin regarding both TVC and EC levels were less than 0.5 log values. Thus, it can be assumed that broiler neck skin samples, both fresh and frozen for eight weeks, are suitable for microbiological examination, as the TVC and EC results showed equivalence. Therefore, freezing broiler neck skin samples can be an option to maintain viable bacteria levels in broiler neck skin samples taken for microbiological examination in process control, when freezing and later examination is necessary due to insufficient laboratory capacity for the examination of fresh neck skin samples.

Keywords: total viable count; Enterobacteriaceae count; equivalence test; microbial comparison

Citation: Langkabel, N.; Oswaldi, V.; Merle, R.; Dzierzon, J.; Meemken, D. Comparative Study of Fresh and Frozen Broiler Neck Skin Sampled for Process Hygiene Purposes. *Appl. Sci.* **2022**, *12*, 6701. https://doi.org/10.3390/app12136701

Academic Editors: Huhu Wang and Jun Qi

Received: 28 May 2022
Accepted: 28 June 2022
Published: 1 July 2022

1. Introduction

Broiler carcasses at poultry abattoirs and processing plants can be contaminated with different bacteria, e.g., Gram-negative Enterobacteriaceae like *Escherichia coli* and *Salmonella* spp. as well as other potential human pathogens such as *Campylobacter* spp. [1,2]. Other sources of bacterial contamination of broiler meat can be slaughter and processing plant equipment and environments as well as slaughter personnel, from which a severe risk of cross-contamination emanates [3–5]. To monitor the hygienic status of broiler meat, it is necessary to reliably evaluate the microbial status of broiler carcasses in a standardized way. Neck skin taken at slaughter can be seen as a pooled sample of the carcass' surface, as broilers are processed head down, with all fluids running over the neck skin [6]. Moreover, following the European Regulation (EC) No. 2073/2005 [7], the use of neck skin samples is required for process controls in broiler abattoirs. Neck skin tissue can be seen as the preferred sample type because neck skin can be taken easily without removing carcasses from the slaughter line [8]. Additionally, this carcass part has low nutritional and economic value, and taking this part of a broiler carcass as a sample for analysis is less destructive than other sample types/methods [9]. Furthermore, skin tissue removal

was previously shown to be the most precise and accurate sampling method representing the skin contamination of poultry carcasses [10].

Bacterial multiplication requires certain physical conditions depending on the species. One of these conditions is the right temperature. Most zoonotic pathogens are adapted to the human body temperature and multiply and grow within a temperature range of 20 to 40 °C [11]. Because low temperature measurably controls microbial activity, especially mitosis, freezing has become a very important procedure for food preservation [12,13]. The two conditions that do not allow microbial multiplication are appropriately low water activity (a_w) and low temperature [14]. In meat, microbial multiplication stops at temperatures lower than −10 to −12 °C [15–17], while the total inhibition of cellular metabolism in animal tissues occurs at −18 °C [16].

Following Rahman and Velez-Ruiz (2007) [12], up to 60% of a microbial population is killed during freezing, but the proportion of the remaining bacteria gradually increases during the freezing process [18]. A reduction of 60% of the aerobic mesophilic cell count occurred after long-term storage for one year at −30 °C [19]. The surviving microbial species found in thawed frozen meat were almost identical to those found before freezing, but they can occur in lower numbers. Moreover, after thawing, the surviving bacteria can multiply to such an extent that their count can reach the initial value [20]. Schmidt-Lorenz and Gutschmidt (1969) [19] found no influence on the quantitatively measured total viable count (TVC) after one year's storage at −30 °C, even though the proportions of Gram-positive and Gram-negative bacteria changed compared to their initial proportions. Speck and Ray (1977) [14] concluded that most bacteria are only damaged during frozen storage, but not killed. Influencing factors are the speed of freezing, the storage temperature, and duration and, in addition to the type of bacteria, the protective substances contained in the surrounding matrix [15,21].

If freezing occurs quickly, it is more likely that only small crystals form in the matrix surrounding the bacteria. When compared to large crystals, which occur during slow freezing, small crystals lead less frequently to the death of the bacteria present [14]. Higher temperatures, around −2 °C, were more likely to kill bacteria [12,22] during storage. In general, Gram-positive bacteria are more resistant to freezing death than Gram-negative bacteria, e.g., Enterobacteriaceae [12,15]. This was proven by several authors for different foods, such as milk, deep-frozen poultry meat, pork, rustic crossbred beef, frozen vegetables, and other food products [21,23–28]. However, after thawing, bacteria can multiply again [24], and even after storage at low temperatures around −80 °C, recovery rates of 98% were reported for cultures of Gram-negative and Gram-positive bacteria [29]. This phenomenon of growing after a freezing period is used for the storage of pure bacteria cultures in laboratories. For this purpose, storage at −80° C is usually used. The bacterial isolates are added to media that contain glycerol or other cryoprotective supplements such as dimethyl sulfoxide (DMSO). This procedure allows storage for up to ten years at −80 °C without relevant negative consequences [30,31].

Several authors investigated the effect of storing foods at different freezing temperatures on the bacteria present. They concluded that there was no change in the total bacterial counts in various foods, including vegetables, milk, raw sausages, or poultry, when foods were stored frozen for a few days or up to over twelve months at temperatures from −18 to −25 °C (mainly storage at −20 °C was examined) [24–26,32,33]. For high-fat foods stored at −20 °C for three days, Böhmler et al. (2008) [34] could not find significant changes in microbial counts. They concluded that this practice, as allowed in special cases in DIN EN ISO 7218:2014-02 (Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations) [35], can be used for the analysis of food samples.

Our study deals with the question of whether frozen then thawed samples of broiler neck skin are as suitable for hygienic assessment as fresh samples of broiler neck skin for process controls using TVC or Enterobacteriaceae counts. More precisely, is there a difference in the bacterial loads between fresh and frozen/thawed broiler neck skin samples, for

those bacteria that represent the microbial status of the samples? To answer this question, we compared the TVC and EC of broiler neck skin samples before and after a freezing period of eight weeks. Such information may be used in scientific works to evaluate the microbial status of skin samples of broiler carcasses.

2. Materials and Methods

2.1. Sampling

A total of 300 broiler neck skin samples were taken on six different days at a commercial broiler abattoir in eastern Germany. In this abattoir, broilers are stunned with carbon dioxide. For scalding, a counterflow scalding tank with pasteurization of the scalding water operating with water temperatures from 52 to 54 °C is in use.

Neck skin samples were taken with sterile forceps and single-use scalpels at the end of the slaughter line directly before the carcasses enter the chilling area. Each neck skin sample was split into two parts, and each part was placed in a separate sterile blender bag. Samples were chilled and transported to the laboratory directly after collecting. The weights of the 300 neck skin samples ranged from 3.14–27.82 g (mean weight per sample: 9.65 g). For the split parts (see immediately below), the weight ranges were: fresh samples 1.51−17.22 g; frozen samples 1.19–10.92 g

In the laboratory, one part of each neck skin sample was randomly chosen for freezing and was stored in a sterile blender bag (the type used for sampling) for eight weeks at −30 °C before analysis. The other part of each neck skin sample was stored in another sterile blender bag at 4 °C in a refrigerator until analysis started the following day.

2.2. Microbial Examination

Microbial analysis included the enumeration of the TVC by drop-plating according to the German standardized norm DIN 10161:2016-12 (Microbiological analysis of meat and meat products—Aerobic count at 30 °C—Drop-plating method) [36] and of the EC according to the German standardized norm DIN 10164-2:2019-06 (Microbiological examination of meat and meat products—Determination of Enterobacteriaceae—Part 2: Drop-plating method) [37].

The day after sampling, the examination of the neck skin parts stored in the refrigerator was conducted. First, all samples were weighed and diluted 1:10 in Buffered Peptone Water (BPW, Merck KGgA, Darmstadt; article-ID: 1.07228.0500) and dilution series in sodium chloride peptone agar up to 10^{-6} for TVC and to 10^{-4} for EC were created. From each dilution, 0.05 mL was dropped on agar plates in duplicates. For TVC, Standard I agar (Standard I nutrient agar, Th. Geyer GmbH & Co. KG, Renningen, article-ID: 8152.0500) and for EC, VRBD agar (Violet Red Bile Dextrose agar) (Merck KGgA, Darmstadt, article-ID: 1.10275.0500) were used as culture media.

After an incubation period of 48 h at 30 °C, colony-forming units (cfu) were counted and transformed to logarithmic values to the power of 10 to achieve a normal distribution. Counts of 5 cfu and more were included in the results (according to DIN 10161:2016-12 and DIN 10164-2:2019-06 [36,37]). In cases of counts of 1–4 cfu, the value of log 3.0 cfu/g was used, and in cases of no cfu being visible in the lowest dilution, the log count was set to 2.3 log cfu/g according to the respective DIN [36,37]. These cases were highlighted in the data tables used for statistical analysis as "below minimum limit of detection".

After the freezing period of eight weeks, the frozen neck skin sample parts were thawed and afterwards examined in the same way as the fresh parts of the samples before. Thawing of the samples of frozen neck skin parts was achieved by completely defrosting them on a rack in a refrigerator at +4 °C for 30 min. Afterwards, a small batch of these samples was taken out of the refrigerator and kept at room temperature for weighing before diluting as described above. Then, TVC and EC were determined as described above for fresh samples following the appropriate DIN [36,37].

2.3. Statistical Analysis

Mean log levels for TVC and EC on fresh and frozen neck skin samples were tested for a normal distribution using descriptive statistics and visual inspection.

In a second step, results for TVC and EC in log cfu/g were tested for equality following Thrusfield (2008) [38]. For this, for each pair of TVC and EC from one sample, the difference was calculated. Then, the 99% confidence interval of the differences was assessed. Differences between the dependent samples including the 99% confidence interval were calculated. Following Hübner et al. [39] cited in [40,41], a difference of ±0.5 log cfu/g was set as the acceptable value of difference, i.e., if the difference between the fresh and frozen samples with regard to TVC or EC values was not larger then ±0.5 log cfu/g, it can be concluded that the bacteria level is equal on these two sample types. If the 99% confidence interval is completely within the range of ±0.5 log cfu/g, it can be assumed with 99% confidence that the two sample types have equal values, individually, of TVC and EC.

3. Results

Bacteria counts (log cfu/g) for all sample parts examined were normally distributed.

3.1. TVC and EC Levels

TVC levels on fresh neck skin ranged from 3.48 to 7.11 log cfu/g, with a mean level of 4.88 log cfu/g (SD = 0.484). On frozen-then-thawed neck skin samples (hereafter called frozen samples), TVC levels ranged from 3.08 to 6.49 log cfu/g with a mean level of 4.81 log cfu/g (SD = 0.470) (Figure 1).

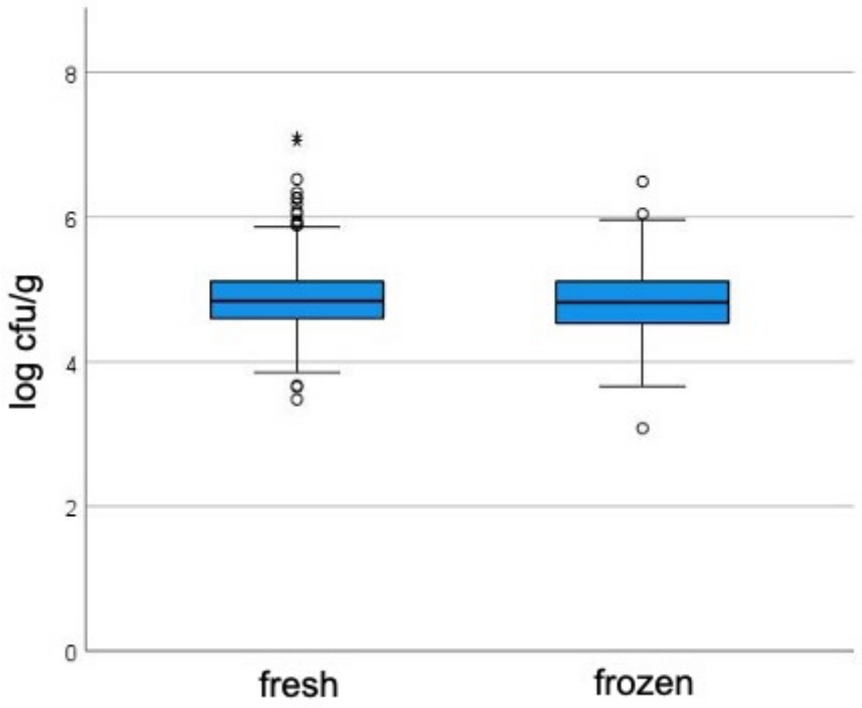

Figure 1. Boxplots of total viable counts for fresh and frozen broiler neck skin samples. (Asterisk and circles indicate outliers; asterisk: > or < 3 SD from mean, circle: > or < 1.5 SD from mean.)

On fresh neck skin, EC levels ranged from 2.30 to 6.32 log cfu/g, and the mean level was 3.25 log cfu/g (SD = 0.440). EC on frozen neck skin ranged from 2.30 to 5.28 log cfu/g, and the mean level was 3.10 log cfu/g (SD = 0.415) (Figure 2).

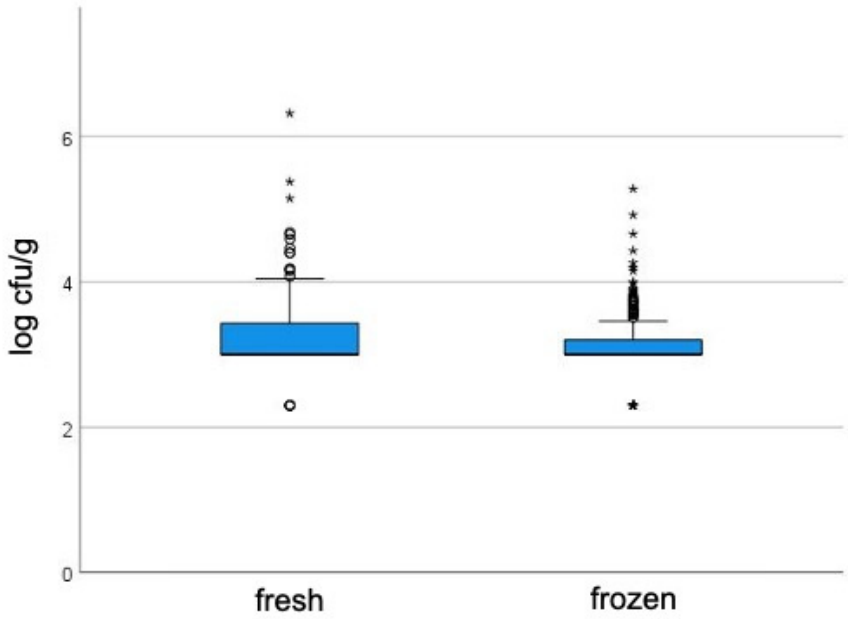

Figure 2. Boxplots of Enterobacteriaceae counts for fresh and frozen broiler neck skin samples. (Asterisk and circles indicate outliers; asterisk: > or < 3 SD from mean, circle: > or < 1.5 SD from mean.)

3.2. Tests for Equivalence

As expected, mean bacteria levels on frozen neck skin were lower than levels on fresh neck skin. Confidence intervals (99%) were −0.004 to 0.149 log cfu/g for TVC and 0.069 to 0.209 log cfu/g for EC. The mean pairwise differences in TVC (0.072 log cfu/g) and EC (0.150 log cfu/g) between the fresh and frozen neck skins and calculated confidence intervals are shown in Figure 3. Since this difference was within the range of ±0.5, equality between the pairs for TVC and EC (separately, and in log counts) can be assumed.

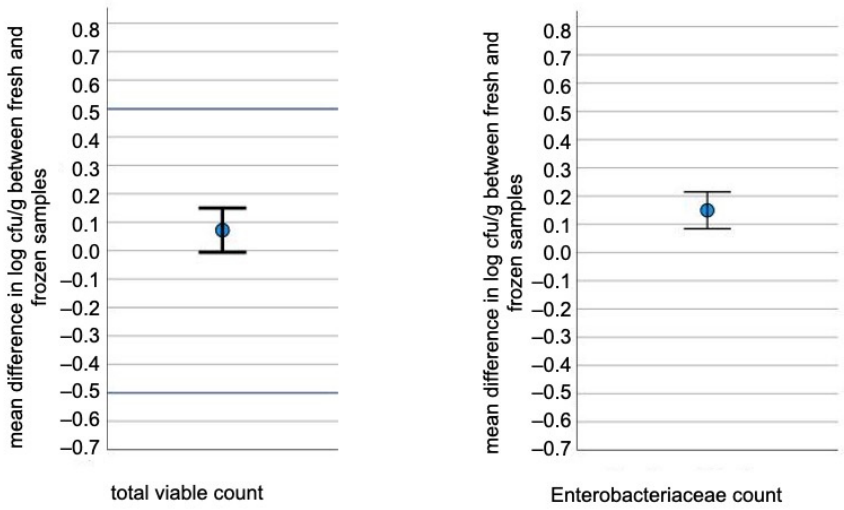

Figure 3. Pairwise differences and 99% confidence intervals for total viable count and Enterobacteriaceae count of fresh and frozen broiler neck skin.

3.3. Samples with Bacteria Counts for EC Below Minimum Limit of Detection

In total, 150 fresh neck skin samples and 196 frozen neck skin samples harbored bacteria levels below the minimum limit of detection for EC (1–4 cfu on the countable sectors or no growth (0 cfu)). Differences were seen between bacteria counts on fresh and frozen neck skin. No growth for EC was observed in four fresh neck skin samples and in 28 frozen samples. Comparing these four fresh neck skin samples without growth for EC with their respective fresh sample pairs, we observed growth in only two of these respective fresh samples.

From 146 fresh neck skin samples with 1–4 cfu in the lowest dilution (log 3.0), 119 of the paired frozen samples also had bacteria levels below the minimum limit of detection. In 20 samples thereof, no growth of bacteria could be seen at all. After freezing, EC numbers of another 77 neck skin samples were below the minimum limit of detection.

4. Discussion

Overall, our results showed that freezing and storing for eight weeks at −30 °C had no significant effect on the microbial load (TVC or EC) of broiler neck skin samples. The detected differences in bacteria levels on fresh and frozen neck skin samples were within the maximum accepted measurement of uncertainty in microbiology laboratory analyses. The observed differences can be considered to be methodologically and biologically irrelevant [39] cited in [40,41]. Similar results regarding the effect of freezing on TVC and EC were shown in other investigations on different food products, vegetables, milk, and meat products [21,32–34,42–44].

Freezing places bacteria in a dormant state, but is also a common method of storing bacterial cultures. Green et al. (2007) [29] showed recoveries of up to 98% for cultures of Gram-positive and Gram-negative bacteria after storage in −80 °C in glycerol for 564 days. Similar to glycerol or other media used for storage of highly concentrated bacterial suspensions [31], our results and those of other authors for frozen vegetables, meat products, and other food products showed that the food matrix seems to have a certain protective effect during freezing [21,33,44]. The bacterial load, TVC or EC, did not change when the foods were stored frozen. In our study, we suspect that the fat present in the neck skin had a protective effect, and, in addition, we observed that the samples were entirely frozen very quickly due to their low volume, so we presume that the bacteria present were not destroyed and could start proliferating again after thawing. This assumption is in concordance with Speck and Ray (1977) [14], who reported that rapid freezing resulted in small cellular ice crystals which did not damage the bacteria as much as slow freezing, which results in large ice crystals. Additionally, rapid thawing results in less lethal damage to bacteria [14]. These two factors can be assumed to be the case for the examined frozen neck skin samples in our study, as they had a low volume and mass and, thus, were frozen and thawed quickly.

Therefore, the temperature of −30 °C is suitable for freezing neck skin samples and for limited storage of such samples before further analysis, even if this temperature is not common in food storage or for laboratory purposes. However, freezing can result in the death of individual bacteria fractions while other fractions of bacteria can proliferate after thawing [19]. Consequently, the detectable numbers of cfu/g in quantitative TVC and EC studies can remain almost the same. Anyway, individual neck skin samples in our study with EC below the minimum limit of detection showed that Enterobacteriaceae (or Gram-negative bacteria in general) enumerated with the EC technique are more sensitive to freezing than Gram-positive bacteria. This is in concordance with other studies looking at the effects of freezing at temperatures around −20 to −25 °C on bacteria numbers in different kinds of foods [19,21,28,33,44]. Petzer et al. (2012) [45] showed that freezing at different temperatures for at least 16 weeks did not influence the survival of common mastitis pathogens in mastitis milk samples in South Africa. Lower detection rates for *E. coli* when compared to Gram-positive bacteria were observed, but the authors suggest that storing

milk samples at −20 or −196 °C can be used in South Africa for improving udder health management [45].

Altogether, we conclude that, in our study, the effect of freezing the broiler neck skin samples on the mean microbial loads was small, as the tests for equivalence, comparing mean loads on fresh and frozen samples, showed equality. Similar results were reported by Böhmler et al. (2008) [34] for the investigation of cream and bakery products with non-heated fillings. Thus, the deep freezing (at a minimum of −18 °C) of very-perishable samples (such as shellfish) or perishable samples (such as fish and raw milk) for transport, as allowed by DIN EN ISO 7218:2014-02 [35], can also be considered as an option for broiler neck skin samples when direct laboratory analysis is not possible. However, it should be noted that an influence, especially on Gram-negative bacteria, cannot be excluded.

To the best of our knowledge, this study is the first to compare both TVC and EC on paired fresh and thawed broiler neck skin samples from the same animals. To sum up, the analysis of fresh and frozen then thawed broiler neck skin samples showed they had comparable levels of both TVC and EC. Therefore, for microbial examination in the laboratory, we suggest that freezing broiler neck skin samples (taken for process hygiene purposes) at −30 °C is an option when laboratory analysis can only be performed after a delay. Nevertheless, this storage technique cannot be standard for all process hygiene analyses, as outcomes likely also depend on freezing conditions such as the temperature, time, and sample matrix.

Author Contributions: Conceptualization and methodology, N.L. and D.M.; N.L., D.M. and V.O.; formal analysis, R.M.; investigation, V.O. and J.D.; data curation: V.O.; writing—original draft preparation, V.O., N.L., R.M.; writing—review and editing, N.L., V.O., R.M., J.D. and D.M.; supervision, D.M. and N.L.; project administration, N.L.; funding acquisition, Reinhard Fries (see Acknowledgements). All authors have read and agreed to the published version of the manuscript.

Funding: The study was performed within the framework of the joint project "Integhof". This research was supported by funds from the German Government's Special Purpose Fund held at Landwirtschaftliche Rentenbank (www.rentenbank.de) (grand ID: 313-06.01-28-RZ-3-72.049).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Acknowledgments: The authors thank Reinhard Fries for the acquisition of the funding of the joint project "Integhof" before his retirement. The authors acknowledge the support and help of the broiler abattoir. We thank Thomas Alter (Freie Universität Berlin) for his help by conceptualization and identification of the methodology. Additionally, we thank him and Matthias Upmann (OWL University of Applied Sciences and Arts) for their help by discussing our results and interpretations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Musgrove, M.; Cason, J.; Fletcher, D.; Stern, N.; Cox, N.; Bailey, J. Effect of cloacal plugging on microbial recovery from partially processed broilers. *Poult. Sci.* **1997**, *76*, 530–533.
2. Kotula, K.L.; Pandya, Y. Bacterial contamination of broiler chickens before scalding. *J. Food Prot.* **1995**, *58*, 1326–1329.
3. Corry, J.E.; Allen, V.; Hudson, W.; Breslin, M.; Davies, R. Sources of Salmonella on broiler carcasses during transportation and processing: Modes of contamination and methods of control. *J. Appl. Microbiol.* **2002**, *92*, 424–432.
4. Ellerbroek, L.; Lienau, J.A.; Klein, G. *Campylobacter* spp. in broiler flocks at farm level and the potential for cross-contamination during slaughter. *Zoonoses Public Health* **2010**, *57*, e81–e88.
5. Izat, A.; Gardner, F.; Denton, J.; Golan, F. Incidence and level of *Campylobacter jejuni* in broiler processing. *Poult. Sci.* **1988**, *67*, 1568–1572.
6. Ellerbroek, L.; Lox, C. Untersuchungen zur Eignung der Halshaut für die mikrobiologische Prozesskontrolle von frischem Geflügelfleisch mit dem Biolumineszenzverfahren. *DTW. Dtsch. Tierärztliche Wochenschr.* **2004**, *111*, 181–184.

7. Commission, E. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* **2005**, *50*, 1–26. Available online: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073 (accessed on 15 June 2022).
8. Hutchison, M.; Walters, L.; Mead, G.; Howell, M.; Allen, V. An assessment of sampling methods and microbiological hygiene indicators for process verification in poultry slaughterhouses. *J. Food Prot.* **2006**, *69*, 145–153.
9. Gill, C.; Badoni, M. Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. *Food Microbiol.* **2005**, *22*, 101–107.
10. Avens, J.; Miller, B. Quantifying bacteria on poultry carcass skin. *Poult. Sci.* **1970**, *49*, 1309–1315.
11. Barer, M.R. 4 Bacterial growth, physiology and death, In *Medical Microbiology. A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control*, 18th ed.; Greenwood, D., Barer, M., Slack, R., Irving, W., Eds.; Churchill Livingstone/Elsevier: Edinburgh, NY, USA, 2012; pp. 39–53.
12. Rahman, M.S.; Velez-Ruiz, J.F. Food preservation by freezing. In *Handbook of Food Preservation*; CRC Press: Boca Raton, FL, USA, 2007; pp. 636–665.
13. Zhou, G.; Xu, X.; Liu, Y. Preservation technologies for fresh meat—A review. *Meat Sci.* **2010**, *86*, 119–128.
14. Speck, M.; Ray, B. Effects of freezing and storage on microorganisms in frozen foods: A review. *J. Food Prot.* **1977**, *40*, 333–336.
15. ICMSF. Temperature. In *Microorganisms in Foods 3: Microbial Ecology of Foods*; Silliker, H.J., Elliott, R.P., Baird-Parker, A.C., Bryan, F.L., Christian, J.H.B., Clark, D.S., Olson, J.C., Roberts, T.A., Eds.; Academic Press Inc.: New York, NY, USA, 1980; pp. 1–37.
16. Perez-Chabela, M.; Mateo-Oyagüe, J. Frozen meat: Quality and shelf life. In *Handbook of Frozen Foods*; Hui, Y.H., Cornillon, P., Legaretta, I.G., Lim, M.H., Murrell, K.D., Nip, W.-K., Eds.; Marcel Dekker Inc: New York, NY, USA, 2004; pp. 201–214.
17. Rose, A. Physiology of Micro-organisms at Low Temperatures. *J. Appl. Bacteriol.* **1968**, *31*, 1–11.
18. Dave, D.; Ghaly, A.E. Meat spoilage mechanisms and preservation techniques: A critical review. *Am. J. Agric. Biol. Sci.* **2011**, *6*, 486–510.
19. Schmidt-Lorenz, W.; Gutschmidt, J. Mikrobielle und sensorische Veränderungen gefrorener Brathähnchen und Poularden bei Lagerung im Temperaturbereich von −2,5° bis −10° C. *Fleischwirtschaft* **1969**, *8*, 1033–1041.
20. Mossel, D.A.A. *Microbiology of Foods: The Ecological Essentials of Assurance and Assessment of Safety and Quality*, 3rd ed.; University of Utrecht, Faculty of Veterinary Medicine: Utrecht, The Netherlands, 1982; 188p.
21. Reinartz, M. Einfluss des Tiefgefrierens und der Tiefkühllagerung auf die Mikroflora von vier handelsüblichen Tiefkühlprodukten. Doctor of veterinary medicine, Freie Universität Berlin, Berlin, Germany, 9 November 2011. Available online: https://refubium.fu-berlin.de/handle/fub188/5243 or https://refubium.fu-berlin.de/bitstream/handle/fub188/5243/Reinartz_online.pdf?sequence=1&isAllowed=y (accessed on 30 June 2022).
22. Haines, R. The effect of freezing on bacteria. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **1938**, *124*, 451–463.
23. Fries, R.; Eggerding, B. Bacterial reduction in deep-frozen sterile poultry meat. *Arch. Lebensm.* **1997**, *48*, 121–144.
24. Vieira, C.; Diaz, M.; Martínez, B.; García-Cachán, M. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Sci.* **2009**, *83*, 398–404.
25. Alrabadi, N.I. The effect of freezing on different bacterial counts in raw milk. *Int. J. Biol.* **2015**, *7*, 9–12.
26. Hubáčková, M.; Ryšánek, D. Effects of freezing milk samples on the recovery of alimentary pathogens and indicator microorganisms. *Acta Vet. Brno* **2007**, *76*, 301–307.
27. Medić, H.; Kušec, I.D.; Pleadin, J.; Kozačinski, L.; Njari, B.; Hengl, B.; Kušec, G. The impact of frozen storage duration on physical, chemical and microbiological properties of pork. *Meat Sci.* **2018**, *140*, 119–127.
28. Schukken, Y.; Smit, J.; Grommers, F.; Vandegeer, D.; Brand, A. Effect of freezing on bacteriologic culturing of mastitis milk samples. *J. Dairy Sci.* **1989**, *72*, 1900–1906.
29. Green, H.P.; Johnson, J.A.; Furuno, J.P.; Strauss, S.M.; Perencevich, E.N.; Lautenbach, E.; Lee, D.; Harris, A.D. Impact of freezing on the future utility of archived surveillance culture specimens. *Infect. Control Hosp. Epidemiol.* **2007**, *28*, 886–888.
30. Regnath, T. Stammhaltung. In *Mikrobiologische Diagnostik: Bakteriologie-Mykologie-Virologie-Parasitologie, Teil II: Mikrobiologische Untersuchungsmethoden*; Neumeister, B., Geiss, H.K., Braun, R., Kimmig, P., Eds.; Georg Thieme Verlag: Stuttgart, Germany, 2009; pp. 171–173.
31. Schrödel, A. Wie werden Bakterien optimal gelagert? *Biol. Unserer Zeit* **2009**, *39*, 372–372.
32. Redmond, G.; Gormley, T.; Butler, F. The effect of short-and long-term freeze-chilling on the quality of mashed potato. *Innov. Food Sci. Emerg. Technol.* **2003**, *4*, 85–97.
33. Redmond, G.; Gormley, T.; Butler, F. The effect of short-and long-term freeze-chilling on the quality of cooked green beans and carrots. *Innov. Food Sci. Emerg. Technol.* **2004**, *5*, 65–72.
34. Böhmler, G.; Seide, K.; Thiem, I. Einfrieren von Lebensmittelproben—Ermittlung des Einflusses auf den Keimgehalt. In Proceedings of the 49. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene—Dreiländertagung, Garmisch-Partenkirchen, Germany, 29 September–2 October 2008; p. 135.
35. DIN EN ISO 7218:2014-02: Microbiology of Food and Animal Feeding Stuffs—General Requirements and Guidance for Microbiological Examinations (ISO 7218:2007 + Amd 1:2013); German Version EN ISO 7218:2007 + A1:2013 (Mikrobiologie von Lebensmitteln und Futtermitteln—Allgemeine Anforderungen und Leitlinien für Mikrobiologische Untersuchungen (ISO

7218:2007 + Amd 1:2013, Korrigierte Fassung 2014-04-15); Deutsche Fassung EN ISO 7218:2007 + A1:2013), 2014. Available online: https://www.beuth.de/de/norm/din-en-iso-7218/216839549 (accessed on 15 June 2022).

36. DIN 10161:2016-12: Microbiological Analysis of Meat and Meat Products—Aerobic Counts at 30 °C—Drop Plating Method (German Norm, Original Title: DIN 10161:2016-12 Mikrobiologische Untersuchung von Fleisch und Fleischprodukten—Bestimmung der Aeroben Keimzahl bei 30 °C—Tropfplattenverfahren). 2016. Available online: https://www.beuth.de/de/norm/din-10161/263799680 (accessed on 15 June 2022).
37. DIN 10164-2:2019-06: Microbiological Examination of Meat and Meat Products—Determination of Enterobacteriaceae—Part 2: Drop Plating Method (German Norm: Original Title: Mikrobiologische Untersuchung von Fleisch und Fleischprodukten—Bestimmung von Enterobacteriaceae—Teil 2: Tropfplatten-Verfahren), 2019. Available online: https://www.beuth.de/de/norm/din-10164-2/302005342 (accessed on 15 June 2022).
38. Thrusfield, M. 17 Clinical trials. In *Veterinary Epidemiology*, 4th ed.; Thrusfield, M., Christley, R., Eds.; John Wiley & Sons Ltd: West Sussex, UK, 2018; pp 361–382.
39. Hübner, P.; Gautsch, S.; Jemmi, T. In house validation (single laboratory validation) of microbiological methods. *Mittelungen Aus Lebensm. Und Hyg.* **2002**, *93*, 118–139.
40. Anonymous. PLQML-5010-09 Entscheidungsregeln nach DIN EN ISO-IEC 17025-2018. Allgemeines Vorgehen, 2020. Available online: https://www.oslab.de/fileadmin/content/pdf/diverse_Scheine/PLQML-5010-09_Entscheidungsregeln_nach_DIN_EN_ISO-IEC_17025-2018._Allgemeines_Vorgehen.pdf (accessed on 9 May 2022).
41. SAS. Leitfaden zur Validierung Mikrobiologischer Prüfverfahren und zur Abschätzung der Messunsicherheit im Bereich Lebensmittel-und Umweltmikrobiologie, Dokument 328dw. Available online: https://www.sas.admin.ch/dam/sas/de/dokumente/Wie%20wird%20meine%20Stelle%20akkreditiert/Pr%C3%BCflaboratorien%20STS/328.pdf.download.pdf/328d.pdf (accessed on 9 May 2022).
42. Kluth, I.-K.; Teuteberg, V.; Ploetz, M.; Krischek, C. Effects of freezing temperatures and storage times on the quality and safety of raw turkey meat and sausage products. *Poult. Sci.* **2021**, *100*, 101305.
43. Krischek, C.; Teuteberg, V.; Kluth, I.-K.; Plötz, M. Gefrieren von Fleisch. *Fleischwirtschaft* **2021**, 11, 89–94.
44. Georgsson, F.; Þorkelsson, Á.E.; Geirsdóttir, M.; Reiersen, J.; Stern, N.J. The influence of freezing and duration of storage on Campylobacter and indicator bacteria in broiler carcasses. *Food Microbiol.* **2006**, *23*, 677–683.
45. Petzer, I.-M.; Karzis, J.; Van der Schans, T.J.; Watermeyer, J.C.; Eloff, S.; Fosgate, G.T. Comparing effects of freezing at −196 °C and −20 °C on the viability of mastitis pathogens. *Onderstepoort J. Vet. Res.* **2012**, *79*, 1–6.