Journal of Food Quality

From Farm to Fork: New Strategies for Quality Evaluation of Fresh Meat and Processed Meat Products

Lead Guest Editor: Gonzalo Delgado-Pando
Guest Editors: Carlos A. García and Lara M. Lobato

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Editorial

From Farm to Fork: New Strategies for Quality Evaluation of Fresh Meat and Processed Meat Products

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Meat production has increased globally over the past decades and is expected to keep growing. At the same time, consumers have become more demanding with respect to the quality of meat and meat products. Producing high quality meat consistently is a big challenge for meat producers, processors, and retailers due to the intrinsic variability of the raw material, but it also generates the necessity to develop, improve, and upgrade the current quality analyses by faster and more reliable ones. Precisely, as results of the recent technological and biotechnological advances, a plethora of new possibilities have been opened for the meat production and processing sectors, and a vast improvement of the quality assessment and assurance throughout the whole processing could now be a reality. This special issue aims to cover the recent advances on quality assurance and assessment of fresh meat and meat products.

Eight different articles were finally accepted for publishing, after the editorial and peer-reviewing process. The average number of authors for each paper is 5.6, and the authors' affiliated institutes are from a wide variety of countries, such as New Zealand, Germany, Vietnam, China, Poland, Spain, and Ireland. This special issue covers a variety of topics that can be associated in different groups: consumer acceptance studies, emerging analytical methods, or effect of processing conditions.

With regard to the animal production line, A. Albrecht et al. studied the influence of an alternative production system based on a slow-growing, corn-fed, and antibiotics-free chicken line compared with conventional poultry production, on the quality and shelf life of poultry meat. This paper clearly represents an opportunity towards a more sustainable poultry production due to an extensive husbandry system without antibiotics, a slower growth, and enhanced animal welfare advance in line with latest consumer demands.

Insights on the manufacturing and processing procedures to improve meat quality and safety have been given by other authors. Freezing is one of the most commonly used methods for preservation in the meat industry, and thus its effect on the quality of the final product is paramount. D. Li et al. investigated the effect of freezing conditions on mutton meat quality and structure. The importance of this study lies not only on the effect of freezing on meat microstructure but also on their innovative approach and the techniques used in the study. The paper by R. Zhang et al. aimed also to investigate the effect of freezing, but in this case on dry-aged beef meat. The use of freezing on this type of meat is not common, but it proved to be an interesting approach to extend the shelf life of the final products; however, the effect of freezing on quality parameters should be carefully assessed in this high-value product. A. Augustyńska-Prejsnar et al. completed this area with the study of the effect of different marinades on meat quality and microbiological safety of pheasant, relatively under the studied type of meat. In general, these studies highly contributed to ideas for innovation on the meat industry especially for that based on gourmet products such as pheasant or dry aged-beef.

M. M. Farouk et al. delved into the consumer preference field. They designed an acceptability survey of one of the most consumed meat products for Chinese, Korean, and

Japanese people: hot pot beef with several fat percentages. The study purposed by M. M. Farouk et al. goes beyond meat quality itself and brings knowledge of the effect of different productive, processing, and cooking methodologies on beef meat digestibility. These studies are not very common, and the information they provide is key from a nutritional and consumer marketing point of view.

The present special issue is finally completed with the in-depth review paper from this editorial group (C. Álvarez et al.), and the one presented by M. A. Cook and P. Duc Phuc. The former focuses on the measurements that are used or can be used during the rigor period in order to predict eating quality early. This comprehensive review is particularly interesting in the field since it deepens in the high number of unstudied measurements that can be easily applied during this stage and the lack of relationship of many of the used methods with final meat quality, highlighting the need of further studies. Finally, M. A. Cook and P. Duc Phuc reviewed the major pathogens and hazards identified in Southeast Asia related to the consumption of pork. This study focuses on food-borne disease impact and incidence due to pork consumption. The paper is especially interesting due to the necessity to address the high impact of food-borne disease on low- and middle-income countries.

In summary, we are pleased to introduce this special issue covering meat quality aspects from the animal production line to the consumer preference and the effect of the manufacturing processes. We sincerely hope that the readers will find this special issue interesting and informative.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

Acknowledgments

Finally, we would like to acknowledge the authors of the papers for their contributions as well as all the reviewers for their valuable participation in the evaluation process.

Gonzalo Delgado-Pando
Carlos Álvarez
Lara Morán

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Research Article

Visible Fat Content of Hotpot Beef Acceptability by New Zealand Chinese, Japanese, and Korean Consumers

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Guest Editor: Carlos Álvarez

Hotpot meat is a popular way of meat consumption in some Asian countries. This study was carried out to investigate the effect of visible fat content on consumer acceptability of hotpot meat. Hotpot beef with six visible fat levels (11%–35%) was produced and digitally photographed, and the images were ranked by panels of New Zealand Chinese (110), Japanese (145), and Korean (118) consumers. For all the nationalities, a preference for visible fat was influenced by both gender and age. The Chinese preferred the visibly fattiest hotpot beef, whereas the Koreans preferred the leanest with the Japanese preferring the two fat extremes equally. For individuals in the age range of 19–30 years, both Chinese males and females preferred the higher fat meat (35%), while their Japanese and Korean counterparts preferred the second visibly leanest (14%). For those over 50 years, Chinese females preferred the lower fat meat compared to their male counterparts, whereas there was no gender difference at this age for the Japanese and Koreans preference for the visibly lean hotpot beef. This study indicates that there are subtle differences between nationalities in terms of their preference for the fat content of hotpot beef that may have implications in meat merchandising, product development, and health policies.

1. Introduction

One of the popular forms for eating red meat is hotpot in China or its variants *shabu-shabu* and *jeongol* in Japan and Korea, respectively [1, 2]. Hotpot meat is a rolled, thinly sliced, whole-tissue, or restructured meat, often sold frozen in order to maintain the rolled appearance and form (Figure 1). The meat is usually cooked from frozen in a metal pot containing a simmering soup made of vegetables, spices, and seafood; the cooked pieces are then removed and eaten with a dipping sauce using chopsticks [1].

Any cut of meat can be used for making hotpot, although the cheaper, fattier, or well-marbled cuts are generally used. With an increasing awareness of health-related issues concerning fat consumption and the growing availability of cheaper lean bull/buffalo beef, there is the potential to replace the fattier cuts with lean meat. If this is the case, there is an opportunity for exporting countries to add value to lean meat by turning it into hotpot logs for the domestic Asian markets and for export to Asian countries [3, 4]. For this goal to be achieved, the first question that needs to be addressed is as follows: What is the consumers' preference for hotpot meat in terms of leanness in those countries? And as meat cut/product aesthetics and intrinsic qualities, such as fat content, colour, and appearance, are also important to consumers [1, 5], what would the impact of visible fat

Figure 1: Stages of hotpot meat production and consumption. (a) Restructured/formed hotpot beef log being sliced using a hotpot slicer; (b) rolled hotpot slices ready for cooking; (c) hotpot meat pieces being dipped in a simmering hotpot soup.

content be on the acceptability of hotpot meat by Asian consumers? Hence, the objective of this study was to determine the preference of New Zealand Chinese, Japanese, and Korean consumers in terms of the visible fat content of hotpot beef. The long-term goal is to provide insight regarding the preference for hotpot meat that exporting countries could use to add value to lean meat for export to China, Japan, Korea, and other Asian markets where hotpot and thinly sliced meat are acceptable formats for meats.

2. Materials and Methods

2.1. Sample Preparation and Imaging. Frozen boxed 65–95 CL (chemical leanness) manufacturing beef from bulls, cows, and steers of normal pH (5.6-5.8) were thawed overnight at 10°C, sorted, and combined to obtain a range of visible fat content. It was restructured into a log by orienting the muscle fibres and fat to provide a sliced hotpot beef appearance typical of that obtained in hotpot butcheries and grocery stores. The fat content of the logs was varied to produce sliced hotpot with a range of visible fat (Figure 1).

The logs were frozen to −18°C and held at that temperature until ready to be sliced. The logs were then tempered to −3 to −5°C and sliced (2-3 mm thick) using a hotpot slicer (Figure 1). The sliced hotpot was placed on white polystyrene trays, overwrapped with an oxygen-permeable film, and held at −18°C until used.

The packaged hotpot slices were photographed using a Canon EOS 550D digital camera. All the samples were photographed at the same distance, with the camera fixed perpendicularly to the surface of the packaged rolls. To ensure even lighting, the samples were placed under an opaque shell, illuminated by indirect light on the outside. The images were transferred from the camera and stored as JPEG files.

2.2. Survey Questionnaire. Survey questionnaires in English, which was then translated into Mandarin, Japanese, and Korean by native speakers (these translations were then checked by two other native speakers), were developed around the images of the packaged sliced servings (Figure 2). To create the questionnaires, the selected images were imported into Corel Photo-Paint X7 V17.1, cropped to a uniform size, and laid out with the respective language questionnaires for printing. The images were not retouched or separately manipulated prior to layout for printing.

The questionnaire included demographic questions about the gender and age, frequency of consumption of hotpot, and the type of meat preferred for hotpot. Consumers were also asked to rank the 6 hotpots photos (from A to F) by preference, from the most preferred to the least preferred.

2.3. Population Surveyed. Members of the Chinese, Japanese, and Korean communities from all walks of life were surveyed, mainly from three New Zealand cities. The survey was carried out face to face at various settings including churches, clubs, campuses, and fairs where participants were directly approached and asked whether they would like to complete the questionnaire. We ensured that participants understood the questions and explained that they must concentrate on only the visible fat content in the photographs and not take any cues about meat quality from other attributes, such as number of slices, roll shape, or colour. Participation was completely optional, and respondents were not pressured or rewarded to respond before or after the survey.

A total of 115, 145, and 121 Chinese, Japanese, and Korean consumers, respectively, completed the questionnaires. Five of the Chinese and three of the Korean responses were discounted because respondents failed to rank the samples properly.

2.4. Image Analysis to Estimate Visible Fat Content. Evaluation of digital images of the hotpot slices was performed in Matlab 2014b (MathWorks) using the Image Processing Toolbox. Each image was initially cropped to reduce the viewing area of the images, and the meat was then manually delineated to create a mask (Figure 3(a)). This mask was multiplied with the cropped image to create a starting point for image processing and to reveal the total area of the meat in pixels. The colour image was converted to grayscale and then contrast adjusted to improve discrimination between fat and lean regions. An automatic threshold was then applied to convert the image to black and white, identifying fat as well as many small white regions that were not of interest. To remove this speckling effect, a filter was applied that morphologically opened (erosion followed by

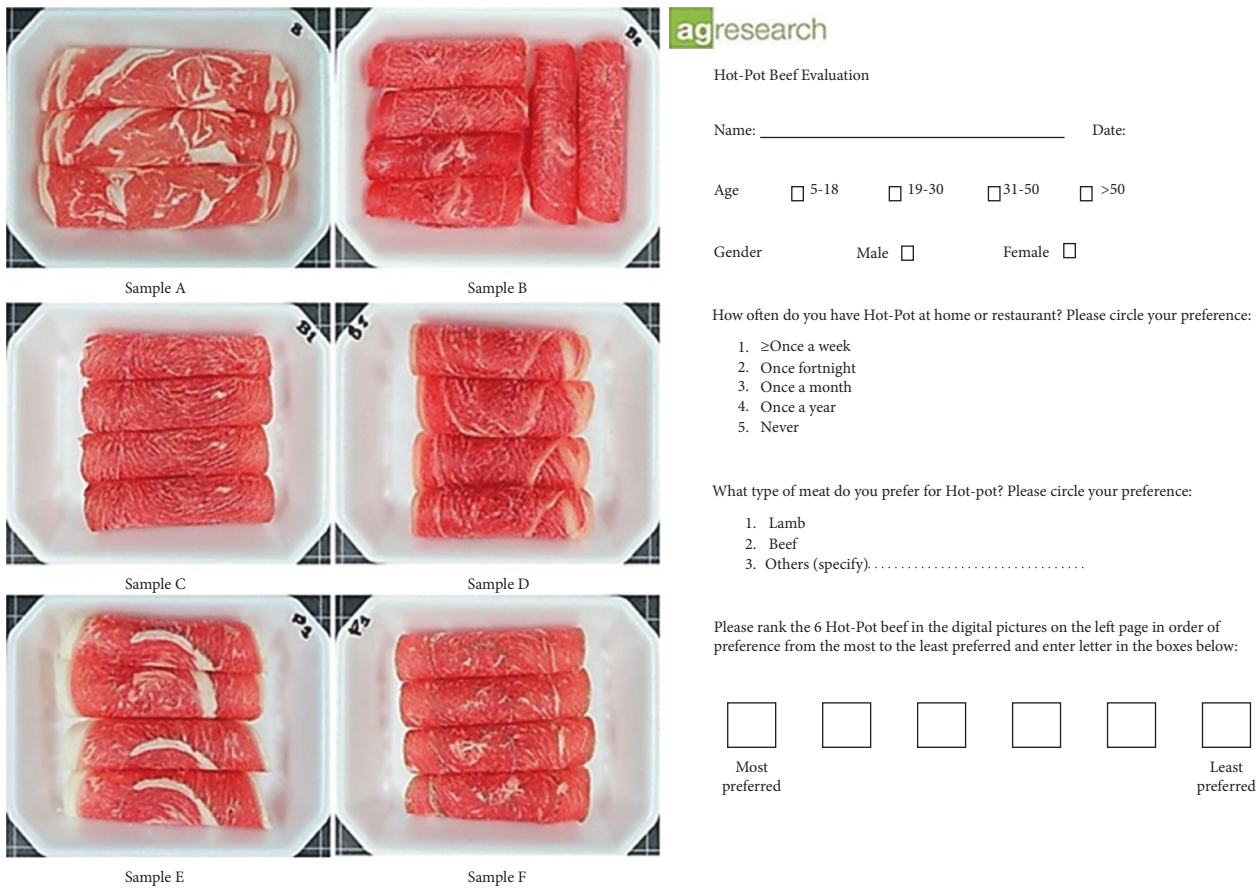


Figure 2: The English version of the questionnaire used in the survey of the three consumer groups.

dilation) the image using a disk-shaped structuring element with radius 4 pixels, followed by a close operation (dilation followed by erosion) with a larger disk-shaped structuring element having a radius of 6 pixels. The number of white pixels at this point represents the fat content in pixels and can be divided by the total pixel area found earlier to calculate the percentage of fat within the object. Following this, the perimeter of the white regions was identified and dilated to join up any gaps and overlaid onto the original cropped image to enable visual checking of the fat detection sensitivity (Figures 3(a) and 3(b)). Using this analysis (Figure 3(b)), the percent visible fat content of the hotpot samples was A (34.57), B (13.54), C (10.75), D (23.77), E (27.57), and F (14.81).

2.5. Statistical Analysis. The preference for the six hotpot beef samples for each person and ethnicity was given a ranked order, in which the most preferred sample was given a score of 6 and the least preferred was given a score of 1.A mixed-effects model was used to compare the responders' preferences. The full model included the fixed effects for ethnicity, age, gender, samples and their interactions. The random effect was the responder ID. There was a significant four-way interaction between sample, gender, age, and ethnicity ($P = 0.027$). Therefore, pairwise differences in the main effects within each ethnicity and two-way interactions using the standard error of differences from the full model were discussed. All analyses were performed in R [6] using "lmer" [7].

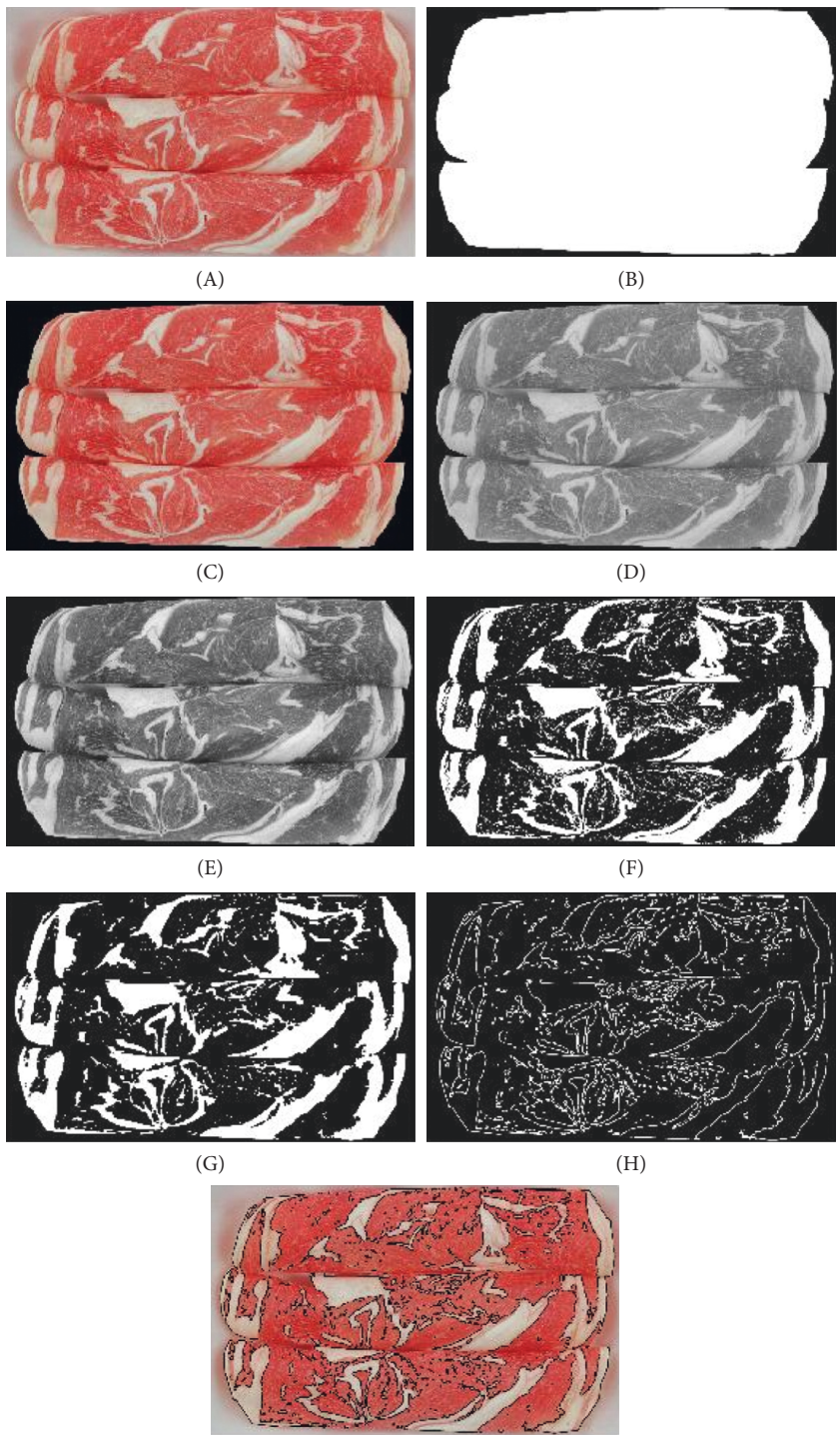
3. Results

3.1. Frequency of Hotpot Meat Consumption. Among the 110 Chinese consumers whose responses were analysed, none indicated that they consumed meat cooked in a hotpot every week, 6% reported that they consumed hotpot cooked meat once every fortnight, 30% once a month, 28% twice to 10 times a year, and 32% about once every year. 3% had never eaten hotpot cooked meat.

Of the Japanese consumers who responded to this question, no one consumed hotpot cooked meat every week, 2% had it fortnightly, 20% monthly, 46% about twice to 10 times a year, and 32% once a year.

None (0%) of the Korean consumers indicated they consumed meat cooked in hotpot every week (once a week), 20% of them consume hotpot cooked meat only once every fortnight, 48% once a month, and 31% about once every year.

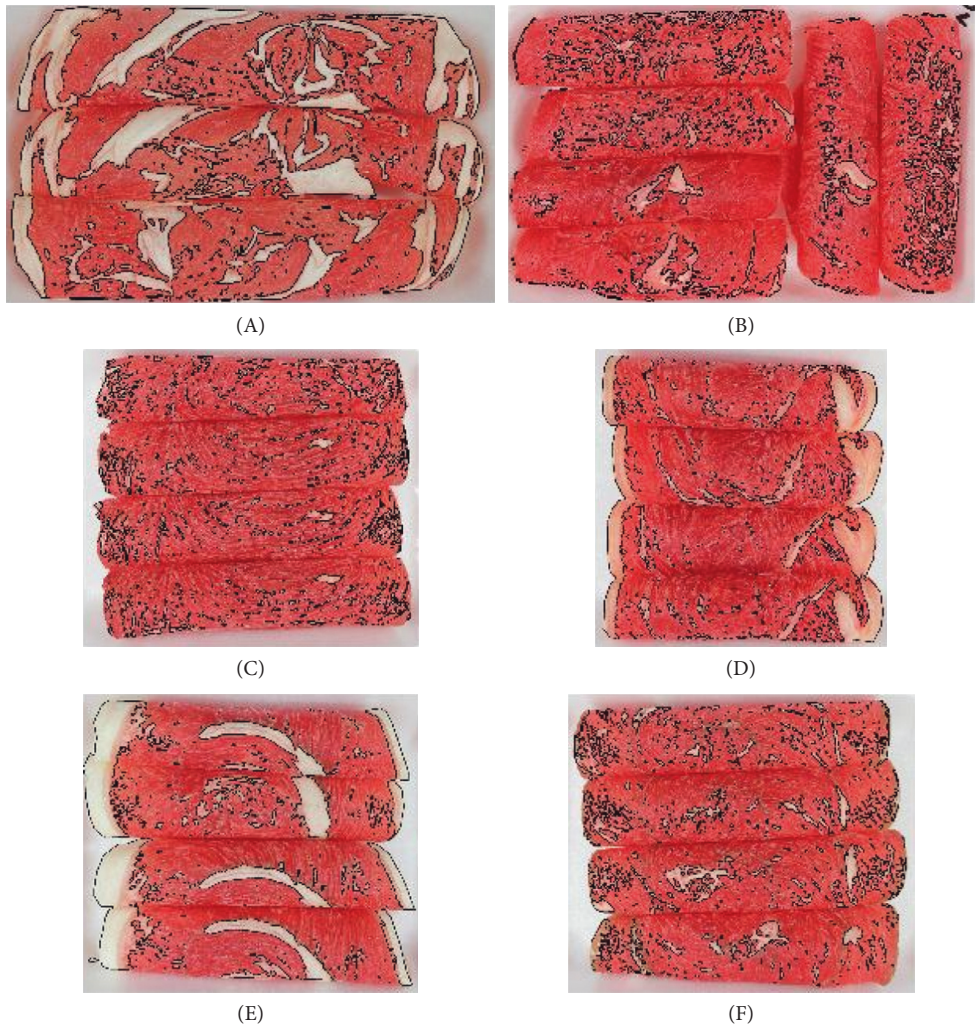
3.2. Hotpot Meat Type Preference. Among the NZ Chinese consumers surveyed, lamb/mutton was the most preferred meat for hotpot cooking (76%), followed by beef (14%) and



(A) (B) (C) (D) (E) (F) (G) (H) (I)

(a)

Figure 3: Continued.



(b)

Figure 3: (a) Images showing the processing steps for determining the percentage of visible fat within the samples: (A) cropped, (B) mask, (C) masked, (D) grayscale, (E) contrast adjusted, (F) B&W, (G) imopen and imclose, (H) identify perimeter, and (I) fat%: 34.57. (b) Images of the samples used in the survey and their percentages of visible fat: (A) 34.57, (B) 13.54, (C) 10.75, (D) 23.77, (E) 27.57, and (F) 14.81.

"others" (10%). Seafood, fish, vegetables, and tofu were included in the "others".

Fifty-five (55%) percent of the Japanese consumers preferred beef and 14% pork for hotpot. 10 and 9%, respectively, preferred a combination of beef and pork or beef and other meat. Only 2% preferred lamb and the remaining 10% preferred others.

Beef was the most preferred meat for hotpot cooking (86%) among the Korean consumers surveyed, followed by lamb/mutton (14%) and "others" included fish balls, pork, and chicken.

3.3. Visible Fat Content and Consumer Acceptability of Hotpot Meat. The representative images of the sliced hotpot meat for each of the six visible fat ranges used in the questionnaire are shown in Figure 3(b). Sample A had the highest percentage of visible fat at 35% and sample C had the lowest at 11%. There was a significant four-way interaction between samples, gender, age, and ethnicity for the preference of visible fat content of hotpot ($P = 0.027$). For this reason, the results for each ethnic group are discussed separately.

There were significant three-way interactions between samples, gender, and age ($P < 0.05$) and two-way interactions between samples and gender for the Chinese and Korean consumers, but not for the Japanese ($P > 0.05$). There were interactions between samples and age for all the three cultures ($P < 0.05$).

3.3.1. Chinese Consumers. Overall, NZ Chinese consumers preferred the sliced hotpot meat with the highest visible fat content while the rolls with the lowest visible fat content were the least favoured (Figure 4). The average rank given to sample A was 4.15, and this was significantly higher ($P < 0.05$) than the average ranks given to the other samples. When the individual preferences were separated into groups based on their choices of most preferred visible fat content,

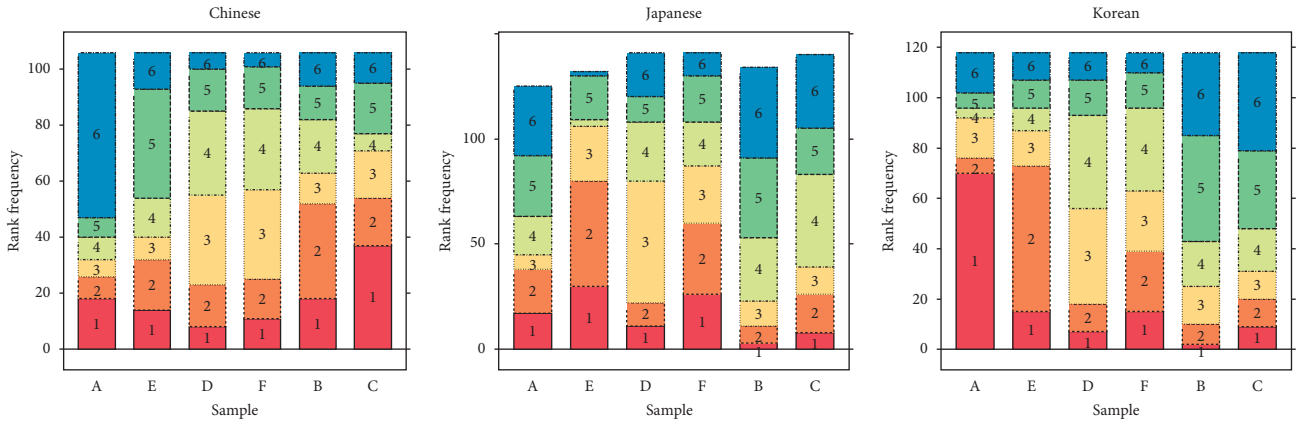


FIGURE 4: The frequencies of the rank for each sample, with the rank of 1 as the least preferred and 6 the most preferred. Samples are ordered from highest (A) to lowest (C) percentage of visible fat along the x-axis. A = 35%, E = 28%, D = 24%, F = 15%, B = 14%, and C = 11%.

the consumers that ranked sample A (35% visible fat content) as the most preferred also ranked sample C (11% visible fat content) as the least preferred and vice versa. This outcome strongly suggests that, overall, NZ Chinese consumers prefer fattier meats for their hotpot.

3.3.2. Japanese Consumers. Overall, regardless of gender or age, sample B (14% visible fat content) had the highest average rank of 4.2 and sample E (28% visible fat content) had the lowest of 2.33 (Figure 4), and this difference is significant ($P < 0.05$). Samples A and C also had high average ranks of 3.7 and 4.0, respectively, and there was no evidence that the average rank for sample B differs from that for samples A and C.

3.3.3. Korean Consumers. Korean consumers ranked sample A (35% fat) as the least preferred and sample C (11%) as most preferred overall (Figure 4), although similar number of people ranked the leaner looking samples B and C as the most preferred.

3.4. Influence of Consumer Gender on Hotpot Meat Visible Fat Acceptability

3.4.1. Chinese Consumers. Overall, the frequency of ranked samples was similar for both males and females (Figure 5). The difference in the average rank for the fattiest sample (A, 35% fat) did not significantly differ between males and females ($P > 0.05$) and the trend in ranking of the samples from least to most preferred was similar for both males and females. However, we noticed that more females than males indicated that the visibly fattiest sample (A) was their least preferred. Furthermore, more females than males indicated that the leanest sample (C, 11% fat) was their most preferred.

3.4.2. Japanese Consumers. The pattern in ranking appears similar for both males and females ($P > 0.05$). Slightly more females indicated sample A as their least preferred. Because there was no evidence of a statistical difference between males and females, these means are not discussed ($P > 0.05$).

3.4.3. Korean Consumers. The frequency of ranked samples was similar for both males and females (Figure 5). Slightly more females indicated sample A as their least preferred compared to males. Additionally, females indicated sample C was their most preferred compared to males who indicated sample B as their most preferred ($P < 0.05$). The trend in responders ranking of the samples from least preferred to most preferred was similar for both males and females.

3.5. Influence of Consumer Age on Hotpot Meat Visible Fat Acceptability

3.5.1. Different Ethnicities

(1) Chinese Consumers. The rank of the samples differed for older persons (50 years and older), the highest proportion of persons ranking sample A (35% fat) as the least preferred, with most in this age bracket ranking sample B (14% fat) as the most preferred. The average ranking for the fattiest sample (A, 35% fat) was 2.9 for older persons and this was significantly lower ($P < 0.05$) than the ranks of 4.8, 4.7, and 4.5 for the other age groups. Likewise, older persons gave the leanest sample (C, 11% fat) a higher average rank than the other age groups.

(2) Japanese Consumers. The number of persons ranking each of the samples was similar for the different age groups. For older persons (>50), there was a higher proportion ranking sample C (11% fat) as their most preferred.

(3) Korean Consumers. The 118 consumers surveyed were made up of 28% (19- to 30-year-olds), 30% (31- to 50-year-olds), and 42% (>50-year-olds). The average trend was similar for the different age categories (Tables 1–3). The rank

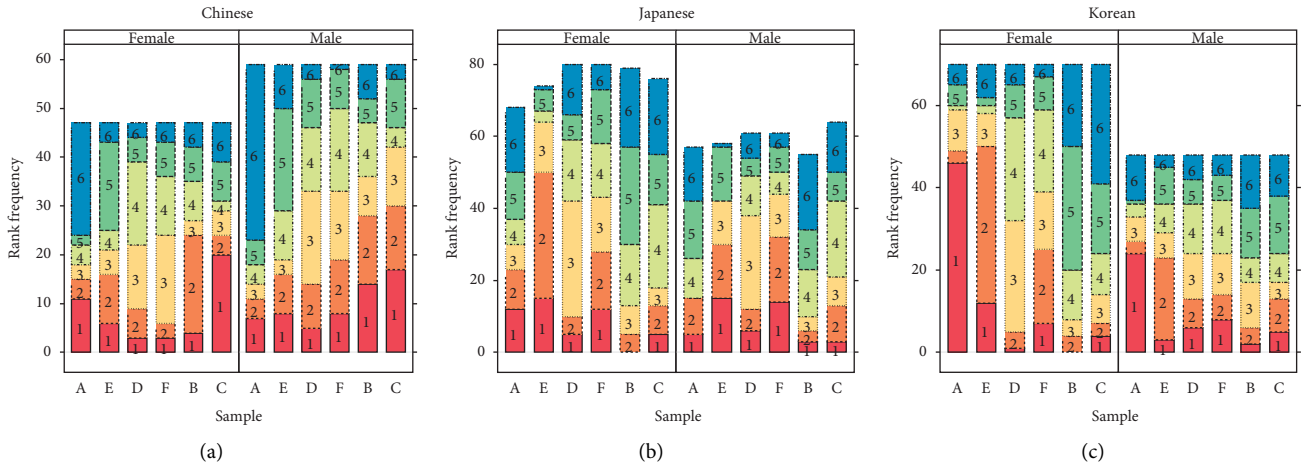


Figure 5: The frequency of ranking separated by gender, with the rank of 1 as the least preferred and 6 the most preferred. Samples are ordered from highest (A) to lowest (C) percentage of visible fat along the x-axis. A = 35%, E = 28%, D = 24%, F = 15%, B = 14%, and C = 11%.

Table 1: The Chinese, Japanese, and Korean mean preference (1 = least preferred; 6 = most preferred) of the samples for the age range of 19–30 years separated into females (F) and males (M).

Sample	Visible fat (%)	Sex	Ethnicity/mean preference		
			Chinese	Japanese	Korean
A	34.6	F	4.6	3.2	2.7
E	27.6	F	3.9	1.9	3.2
D	23.8	F	3.7	3.9	3.5
F	14.8	F	3.7	2.9	3.3
B	13.5	F	2.8	4.4	4.5
C	10.8	F	2.3	4.0	3.8
A	34.6	M	4.9	3.8	2.9
E	27.6	M	4.1	2.5	3.3
D	23.8	M	3.2	3.3	3.4
F	14.8	M	3.0	2.8	3.3
B	13.5	M	2.9	4.7	4.4
C	10.8	M	2.9	3.7	3.7
		SED	0.7	0.6	0.7
		Females (n)	18	12	20
		Males (n)	23	22	15

SED = standard error of difference between means within each ethnic group.

Table 2: The Chinese, Japanese, and Korean mean preference (1 = least preferred; 6 = most preferred) of the samples for the age range of 31–50 years separated into females (F) and males (M).

Sample	Visible fat (%)	Sex	Ethnicity/mean preference		
			Chinese	Japanese	Korean
A	34.6	F	3.9	3.2	1.4
E	27.6	F	3.7	2.5	2.5
D	23.8	F	3.5	3.3	3.7
F	14.8	F	3.3	3.3	3.4
B	13.5	F	3.3	3.7	5.0
C	10.8	F	3.2	4.3	5.0
A	34.6	M	5.2	5.0	2.8
E	27.6	M	4.4	2.8	3.3
D	23.8	M	3.6	2.8	3.4
F	14.8	M	3.0	4.2	3.6
B	13.5	M	2.7	3.8	2.7
C	10.8	M	2.2	2.8	2.2
		SED	0.6	1.2	0.7
		Females (n)	22	13	17
		Males (n)	26	18	18

SED = standard error of difference between means within each ethnic group.

of the samples did differ slightly for the age groups. Notably, the older persons (50 years and older) had the highest proportion of persons ranking sample C (the visibly leanest sample, 11% fat) as the most preferred. Younger persons (19–30) had equal numbers ranking sample A (the visibly fattiest sample, 35% fat) as the most preferred compared to the leanest sample C (11%).

3.5.2. Different Age Groups

(1) Age ≤ 18. Few younger persons under the age of 18 completed the survey. Among the Chinese consumers, females gave sample A (35% fat) the highest ranking (5.50) and sample C (11% fat) the lowest (1.50). In contrast, the average rank of males for sample A was 4.00 and for sample C was 4.60. Only 4 females and 5 males answered the survey in this age range, leading to very wide confidence intervals, and we cannot conclude if this difference between males and females was significant. There was evidence, however, to suggest that the change in rank from sample A compared to sample C for the females within this age category was significant ($P < 0.05$).

For the Japanese consumers, sample B had the highest mean rank of 4.8 in this age category; this was significantly ($P < 0.05$) higher than samples E, D, F, and C.

None of the Korean consumers who completed the survey were 18 years or younger.

(2) Age 19–30. The average rankings for hotpot visible fat content for responders aged 19–30 years are shown in Table 1.

Table 3: The Chinese, Japanese, and Korean mean preference (1 = least preferred; 6 = most preferred) of the samples for the age range of 50+ years separated into females (F) and males (M).

Sample	Visible fat (%)	Sex	Ethnicity/mean preference		
			Chinese	Japanese	Korean
A	34.6	F	2.0	4.2	2.0
E	27.6	F	1.6	1.5	2.2
D	23.8	F	3.0	3.6	3.9
F	14.8	F	4.2	3.0	3.0
B	13.5	F	5.0	4.4	4.9
C	10.8	F	5.2	4.3	5.1
A	34.6	M	3.2	3.2	2.5
E	27.6	M	2.3	2.6	2.9
D	23.8	M	2.8	3.8	3.6
F	14.8	M	4.7	3.0	3.3
B	13.5	M	4.7	3.2	4.7
C	10.8	M	3.3	5.2	3.3
		SED	1.3	1.1	0.6
		Females (n)	5	7	34
		Males (n)	7	5	16

SED = standard error of difference between means within each ethnic group.

Both the male and the female Chinese consumers in this age range ranked sample A higher than sample C. The mean ranks for sample A were 4.6 and 4.9 and those for sample C were 2.3 and 2.9 for females and males, respectively. There was no significant difference between the preferences of males and females in this age category ($P > 0.05$).

The Japanese consumer in this age range preferred sample B (14% fat) which had the highest mean rank of 4.6, and this was significantly higher than samples E, D, F, and C.

For Korean consumers, there was evidence that for both the males and females in this age range, sample A had a lower rank than sample B. The mean rank for sample A was 2.7 and 2.9 for females and males, respectively, and the mean rank for sample B was 4.5 and 4.4 for females and males, respectively. There was no evidence of a difference between males and females' preferences.

(3) Age 31–50. The average ranks for responders aged 30–50 are shown in Table 2. For the Chinese females, the average rank for sample A was 3.9, which was lower ($P < 0.05$) than the average rank for males (5.2). The average rank for sample C was 3.3 and 2.2 for females and males, respectively, showing no difference between males and females in their preferences for the leanest sample C. The difference in the ranking between A and C for the females in this age category was not significant but was for the males ($P < 0.05$).

Sample C had the highest mean rank of 4.1 among the Japanese consumers, and this was significantly higher than sample E ($P < 0.05$). For the Korean females in this age category, the average rank for the fattiest sample A was 1.4, which was lower than the average rank for males of 2.8. There was evidence that this difference was significant ($P < 0.05$). The average rank for samples B and C was 5 for females which was higher than the average rank of 3.8 and 4.2 for males for samples B and C, respectively. There was evidence that these differences are significant ($P < 0.05$). There was evidence that for females, the increase in ranking from sample A to sample C was significant ($P < 0.05$).

(4) Age over 50. The average ranks for responders aged over 50 years are provided in Table 3. The average rank of sample A for Chinese females was 2.0, which increased significantly to 5.2 for sample C. There was no significant difference in average ranking between samples A and C for the males ($P > 0.05$). The older Japanese consumers ranked the leanest sample C the highest (mean rank of 4.6), and this was significantly higher than samples E and F ($P < 0.05$). Sample E (28%) had the lowest mean rank of 1.9, which was significantly lower ($P < 0.05$) than samples A, D, B, and C.

The average rank of sample A for Korean females in this age category was 2.0 and this increased to an average rank for sample C of 5.1. There was evidence that this increase was significant ($P < 0.05$). The average rank for sample A for males was 2.5 and this increased to 4.8 for sample B. There was evidence that this increase was also significant ($P < 0.05$). There was no evidence of a difference between males and females for each sample.

4. Discussion

The conversion of beef farms to dairying in some of the major meat exporting countries is producing an abundance of bulls. Bulls are fast growing, and by 18 months old, they are ready for slaughter [8]. The meat from bulls is inherently leaner than that of steers, heifers, or cull cows. For export-oriented countries, process for adding value to this product category for both local and export markets is a highly regarded proposition. In the past, the focus was on how to deliver products to suit Western consumers in Europe. However, the focus is now shifting to Asia, particularly China, Indonesia, Japan, Korea, Malaysia, Thailand, and Vietnam where the volume of trade in meat is increasing [9–12]. Countries in this region such as Japan and Korea have strong preference for well-marbled beef [13, 14], and eating thinly sliced meat cooked in hotpots is popular in the region particularly in China where the tradition originated. This is why meat prepared for this form of cooking was chosen for the present study, for use in determining the acceptability of lean beef for hotpot. The aim was to understand the preference of consumers for hotpot meat in China, Japan, and Korea in terms of visible fat content at the point of purchase, an exercise that is imperative if the goal of exporting the right lean bull beef cuts to these countries for use as a hotpot were to succeed [15].

One of the hurdles we faced was how to get enough local Chinese, Japanese, and Korean consumers to assess the hotpot beef in the frozen rolled form that the product is normally sold, without running the risk of thawing and collapse, thereby losing its form and familiar characteristics. To circumvent this problem, we prepared the products and captured their digital images. These images were used for the consumer evaluation. The use of digital photographs instead of direct product samples to evaluate the visual acceptability of meat and other products has recently been validated

[16–18]. Passetti et al. [18] compared the outcomes of consumer visual acceptability of bull beef steaks directly in trays with sequential and random digital photos of the same and found that digital images could be used to evaluate beef colour. Furthermore, this method of evaluation resulted in more consistent outcomes compared to visualizing the steaks directly in trays. Holman et al. [19] also used standardised photographs of beef in a web-based survey to successfully determine the consumer acceptability of beef colour. The importance of visual estimation of fat content on the choice of beef has also been underscored by a recent study [20].

The results of the present study indicated that meat is consumed in hotpot about only once a month by about 36, 22, and 68 percent, respectively, of the New Zealand Chinese, Japanese, and Korean residents surveyed and that the majority of Chinese surveyed preferred lamb for hotpot cooking over beef which was most preferred by the Japanese and Koreans.

The preference of sheep meat for hotpot cooking over beef by the Chinese is most likely due to the long tradition in China of mutton hotpot consumption compared to beef. Wu et al. [1] reported that the consumption of mutton hotpot and instant-boiled mutton was influenced by the Chinese royal custom since the Qing Dynasty (1644–1912AD) and by the nomadic culture of the Mongols. Mao et al. [2] reported that hotpot is one of the top preferred cooking methods for sheep meat for both at home and away-from-home consumption in China. The Japan Livestock Products Export Promotion Council in its Wagyu promotional material traced the origin of *shabu-shabu*—a Japanese variant of hotpot—to the Chinese hotpot using mutton. The preference of mutton over beef for hotpot by the NZ Chinese is not a direct reflection of the level of consumption of the two meats in China. However, China's net import of mutton in 2012 was 119 thousand tons compared to 49 thousand tons of beef [10]; the per capita consumption of beef at home and away from home was higher than that of lamb/mutton inside China [21, 22]. It is probable that this reflects the overwhelming preference of lamb/mutton for hotpot cooking and is not indicative of overall meat type preference. This could be seen in the fact that pork—highly consumed in China—was hardly mentioned by the Chinese consumers surveyed in the list of the "others" meats for hotpot, since pork does not appear to be a traditional hotpot meat. Comparatively, the Japanese and Koreans mentioned pork among the meats preferred for hotpot, although their main preference for that type of cooking is beef. The higher preference of beef over sheep meat for hotpot cooking by the Japanese and Koreans could be due to their stronger culture of beef consumption compared to their Chinese counterparts [23].

Our results showed that, overall, the NZ Chinese surveyed preferred hotpot beef with the highest fat content and Koreans with the least and the Japanese in between (Figure 4). Those among the Chinese who preferred the highest visible fat content in hotpot also preferred the hotpot beef with the lowest fat content the least. The preference for high visible fat content could be due to the fact that across cultures, fat is often associated with good eating quality such as higher tenderness, juiciness, flavour, and satiety [13, 24–26]. It could also reflect the traditional use of mutton—a fattier meat—in hotpot by the Chinese compared to beef by Japanese and Koreans, leading to a higher fat content being specifically associated with positive hotpot eating quality. The specific association of fatness with hotpot may be true in the light of the fact that a recent study [5] found that fat is a dominant intrinsic meat quality cue for the Chinese consumer (explaining 78% of choice variance) and that consumers in China prefer leaner pork ribs compared to fattier ones, indicating that the preference for fattier meats could be specific to product types. Results of this study (Figure 5) indicate that although fattier hotpot meat was preferred overall by the Chinese consumers surveyed, more females than males ranked the fattiest hotpot beef sample (A) as their least preferred and the visibly leanest sample (C) as their most preferred, suggesting a gender difference in the preference for visible fat content of hotpot meat, with females preferring lesser fat in hotpot beef than their male counterparts. Previous studies have shown that consumer preference and perception of fat are affected by the gender of the consumer with females preferring less fatty foods than males [27, 28]. In an eye-tracking study comparing male and female consumers in Portugal, females were found to pay more attention to red meat and required less time to choose products with less fat content than men [20]. Wang et al. [29] analysed data of fatty and lean red meat consumption of 16,822 Chinese adults aged 18–75 years and found that males consumed more fat than women.

The average trend for the ranking of hotpot meat visual fat acceptance was generally similar for the age groups of the Chinese, Japanese, and Korean consumers surveyed (Tables 1–3). However, among older consumers (≥50 years), a higher proportion ranked sample A, with the highest visible fat content, as the least preferred. The older consumers in this study may have been more aware of and concerned about the health issues related to fat consumption than the younger ones [30]. Olsen [31] found health concerns related to seafood consumption among Norwegians to be higher in older persons compared to younger ones, whereas Russel and Cox [32] found that meat products were perceived in similar ways by the young, middle-aged, and older Australian consumers with only subtle differences between the age groups.

The findings of this study have some limitations and are preliminary. First, the consumer survey was carried out in New Zealand, although the main aim of the study was to determine consumers' acceptance of the visual fat content of hotpot beef, so that value can be added to lean bull beef through manipulation of the fat content of beef for export to Asian countries mainly China, Japan, and Korea. Thus, the results may not truly reflect the situation in those countries, and hence, the study would need to be repeated there. Secondly, the number of consumers from the three ethnicities surveyed was only 382, some of whom may be long-term residents of New Zealand, which could affect the information on their attitude and perception compared to the consumers' resident in their home countries. Furthermore,

the current study did not collect comprehensive demographic information on those surveyed in order to indicate how representative of their populations those surveyed were in terms of income, education, and frequency of meat consumption. However, this study has a number of strengths including (1) the novelty of the study, given we are not aware of any previous study of its kind in the published literature; (2) the outcomes provide a good understanding of the diaspora Chinese, Japanese, and Korean consumer attitude towards the visible fat content of beef which could be used by the meat industry in adding value to meat for consumers from these cultures residing in some of the red meat exporting countries; (3) the study also validated the use of digital images for consumer surveys and could specifically be adopted for use where meat products are difficult to evaluate due to attribute changes; (4) the role of females in food purchasing and preparation among the cultures and the increasing proportion of elderly in the population [33] should be considered when developing products for these countries in the light of the outcomes of the present study regarding the attitudes of these two demographic groups towards the visible fat content of meat; and (5) some of the interactions between age and gender found in the present study could be useful for designing dietary fat-related health policies and marketing strategies in countries with a sizeable population of Chinese, Japanese, and Koreans.

5. Conclusions

Hotpot is a widely acceptable format for eating meat among the Chinese, Japanese, and Koreans. The Chinese surveyed overall prefer fattier sliced hotpot beef compared to Koreans who preferred the leaner equivalents with the Japanese falling in between, with their males and the younger among them preferring more fat than the females and the elderly over 50 years old. The outcomes of this study have many implications for value adding to meat for the New Zealand Chinese, Japanese, and Korean consumers. It is also novel in the sense that it is the first of its kind to determine the effect of one of the most important consumer meat quality point of purchase cues—visible fat content—using the hotpot format of meat preparation and merchandising and using digital images rather than tangible products.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Factors Affecting the Digestibility of Beef and Consequences for Designing Meat-Centric Meals

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The impact of the following on beef digestibility was determined by static *in vitro* methods: (1) age of cattle; (2) muscle rigor state, ultimate pH, and mincing/particle size; (3) muscle/meat cut; (4) organ meats; and (5) meat accompaniments. Results indicate that beef is more digestible from older compared to younger cattle; prerigor compared to postrigor meat; higher compared to lower ultimate pH meat; cuts with lower compared to higher collagen contents; finely compared to coarsely minced/ground meat; and organ (liver and kidney) compared to muscle meat. Beef digestibility is enhanced when cooked with mushroom and pumpkin and reduced with starchy foods such as rice and potatoes. The outcomes of this study provide a base for the scientific design of meals with beef as a central ingredient and digestibility as the main functionality of interest.

1. Introduction

For years, the field of meat science has prioritised research on meat as a standalone entity, with the aim of describing its table and manufacturing qualities, composition and nutrient density. Much less is known about its attributes in the context of whole meals where meat is just one of the several ingredients or in meat-rich foods that are designed with specific functionality or consumers in mind. Incentives for understanding how meat performs in complex matrices are increasing due to recent growth of the "meal kit" industry and demand for all in one meal solutions at retail outlets. Information about this central ingredient would assist in the design of meals to optimise processing and packaging requirements and to deliver better eating experiences and nutrition.

A key functionality of meat, whether eaten alone or in food combinations, is its digestibility. This has relevance to consumers across a wide range of demographics, physiologies, and lifestyles. Indeed, all desirable nutritional benefits of meat hinge first on adequate digestion. This is not a fixed attribute, as it is influenced by intrinsic and extrinsic factors such as animal rearing method [1], cooking methods and temperatures [2–5], oxidation [3], as well as ageing and pH [6]. Our current work extends research on the digestibility of muscle and organ meats by considering the inherent characteristics of those tissues and cuts, and the effects of accompaniments that are most commonly served in meat-centric meals. Outcomes could be used by meat producers, butchers, and chefs to tailor the merchandising of meat dishes around digestibility and not solely on aesthetic-gustatory considerations.

In this paper, we describe a linked series of experiments on intrinsic and extrinsic factors that can affect the *in vitro* digestibility of cooked beef: (1) age of cattle; (2) muscle rigor state, ultimate pH, and mincing/particle size; (3) muscle/meat cut; (4) organ meats; and (5) meat accompaniments. Our goal is to provide data to support the rational design of meals suited to groups of consumers who might benefit from different levels of digestibility.

2. Materials and Methods

2.1. Main Characteristics of Animal Samples. All animals were sourced from commercial or research farms in New

Zealand, where feeding systems are typically free-range grazing on ryegrass/clover pastures with occasional supplements of conserved forages. The genetic background of the animals was primarily Friesian for the dairy-based livestock classes and Angus × Hereford for prime beef.

For these experiments, cows were end-of-service cull dairy cattle aged 6 years. Bulls were noncastrated male dairy cattle aged 18–24 months. Calves were unweaned male dairy cattle aged 4–14 days, which is considered "veal" in some markets. Prime beef steers and heifers were castrated male and unmated female beef cattle aged 24–30 months. All animals were slaughtered and processed at licensed commercial abattoirs.

2.2. Trial Details

2.2.1. Experiment 1. To investigate the effect of age of cattle, legs from seven cows, seven bulls, and seven calves were collected. The legs were held for 24 h at 8°C–10°C to achieve rigor and then kept in a chiller (−1.5°C) over the weekend. For each leg, the semimembranosus (SM) muscle was dissected and the postrigor pH measured. A 100 g sample was minced using a food processor, and the remaining muscle was frozen at −30°C. Pooled samples of cow, bull, and calf were prepared by mixing 10 g of the mince from each of the seven animals. Subsamples of the 21 individual muscles and the three pooled samples were set aside for protein determination, and the remainders were frozen at −30°C. The pooled and individual samples ($n = 24$) were used for *in vitro* digestibility measurements (gastric and intestinal phases) as described in Section 2.5.

2.2.2. Experiment 2. To investigate the effect of rigor state, *M. longissimus dorsi* (loin, LD) from 48 bulls was collected at approximately 50 min postmortem. For each LD, a slice was taken, chopped into 1 cm^3 pieces, placed on a plastic film, and then snap-frozen in liquid nitrogen. This material was transferred to plastic containers and kept under liquid nitrogen until transferred to a freezer (−30°C). The balance of each LD was held at approximately 7°C and sampled again at 80, 110, 140, 170, and 200 min by the above protocol. The remnant of each LD was transferred to a chiller (−1.5°C), and a final postrigor sample was taken at 48 h postmortem, at which time, ultimate pH was also measured.

To investigate the effect of ultimate pH, seven of the LD were selected based on their ultimate pH to provide a pH range of 5.6 to 6.9. Two were low pH (i.e. ≤5.8), two were intermediate pH (pH 5.81–6.2), and three were high pH (>6.2). For each of these, all seven timepoints (six prerigor and one postrigor) were used ($n = 49$). Samples were ground to a powder under liquid nitrogen using a SPEX Freezer/Mill® cryogenic grinder just prior to gastric phase digestibility measurements.

To investigate the effect of particle size, the two LD with low ultimate pH (i.e. ≤5.8) were selected and their 50 min prerigor and 48 h postrigor timepoints were used. These were either ground to powder using the SPEX Freezer/Mill® or smashed coarsely with mortar and pestle just prior to digestibility measurements.

2.2.3. Experiment 3. To investigate the effect of muscle type or meat cut, the rhomboideus, infraspinatus, supraspinatus, and extensors/flexors from five prime heifers were collected. Each muscle/cut from each animal was separately minced through a 3 mm plate and thoroughly homogenised, then subsampled, and stored at −30°C until digestibility measurements.

2.2.4. Experiment 4. To compare organ versus muscle meats, heart, kidney, spleen, and liver meat and muscle meat (M. semitendinosus, eye of the round) from five prime steers were collected, and the surface fat was removed. The tissues were minced through a 3-mm plate and stored at −30°C until digestibility measurements.

2.2.5. Experiment 5. To understand digestibility of meat in the context of a meal, we first investigated which nonmeat (cereal or vegetable) accompaniments are most commonly served with red meat meals. An internet search of restaurants was carried out. A total of 101 and 120 restaurants in New Zealand and Australia, respectively, were selected that had a web presence, published their menus online, and served at least one dish in which accompaniments were cooked with a red meat or served alongside. Eligible meats were beef, lamb, calf veal, goat, venison, kangaroo, wallaby, and rabbit. The restaurants were distributed across the countries. In Australia, for instance, menus were studied from twenty restaurants in each of six cities: Brisbane (Queensland), Adelaide (South Australia), Melbourne (Victoria), Perth (Western Australia), Sydney (New South Wales), and Hobart (Tasmania). From the results of the survey, the most common accompaniments were tallied from the frequencies of their appearance in the menus (Figure 1). The top five accompaniments in the survey plus one additional vegetable of interest (pumpkin) were selected for consideration of their effects on the gastric and intestinal phases of beef digestibility.

Mushrooms (button, *Agaricus bisporus*), onions, potatoes (Nadine), rice (Calrose and SunRice Australian medium grain), tomatoes, and pumpkin (buttercup, *Curcubita maxima* kabocha) were purchased from a local supermarket. The raw foods were prepared as follows:

(i) Mushrooms: peeled, stalks trimmed, sliced, and blended in a food processor

(ii) Onions: peeled, chopped, and blended in a food processor

(iii) Potatoes: peeled, chopped, and blended in a food processor

(iv) Rice: ground to powder in a Waring blender

(v) Tomatoes: chopped and blended in a food processor (skin on)

(vi) Pumpkin: removed skin, deseeded, and blended in a food processor

The pooled samples from Experiment 1 of minced SM from either cow, bull, or calf were each combined with each of the accompaniments ($3 \times 6 = 18$ treatment combinations)

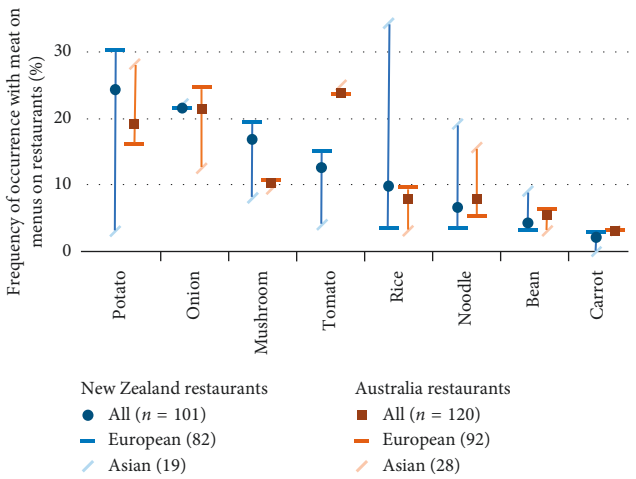

FIGURE 1: Experiment 5: summary of results from the online survey of accompaniments to meat, as described on menus in New Zealand and Australia restaurants.

in a 1 : 2 w/w ratio of meat and accompaniment. Unaccompanied meats served as controls. The various meats were weighed into 100 ml Schott bottles to give the equivalent of 875 mg protein (3.7 to 4.5 g of raw meat) and cereal/vegetable added at 2 × weight of meat. Total volume (including the volume of water contributed by the vegetables) was adjusted to 25 ml with deionised water. The mixture was homogenised using an IKA Ultra-Turrax® (13,500 rpm for 30 sec) and the shaft rinsed back into the Schott bottle with 5 ml of deionised water. The resulting slurry was placed on a laboratory shaker in the refrigerator overnight (4°C).

The following day, the slurries were cooked in a waterbath (100°C) for 30 min and then homogenised using the Ultra-Turrax to break up the lumps formed during cooking. Concentrated HCl was added to bring the acid content to 0.1 M. The slurries were diluted with 0.1 M HCL to a volume slightly less than that required to achieve a protein concentration of 23 mg/ml, then the pH was adjusted with 6 N NaOH to pH 1.9, and the total volume was topped up to reach 23 mg/ml.

2.3. pH Measurements. Ultimate pH of the meats used in Experiments 1 and 2 was measured using a Hanna pH meter (#HI99163) as previously described [6]. The pH of the meats used in the other experiments and for the slurries in Experiment 5 was measured using a Mettler Toledo MP 220 meter equipped with a Mettler Toledo InLab pH probe.

2.4. Protein Content of Meat. The protein content of muscles and organs in Experiments 1 and 4 was determined from total N using AOAC methods by a commercial analytical service (Eurofins Ltd, Hamilton, NZ).

2.5. Zymography Analysis of Protease Activity in Accompaniments. Proteolytic enzymes in the accompaniment foods of Experiment 5 may contribute to the efficiency of *in vitro* digestion of meat. The raw foods were prepared as above and enzymes extracted as described by Pirovani et al. [7]. In brief, 100 g of each raw material was suspended in 100 ml of prechilled extraction buffer (Tris-HCl 10 mM pH 7.5, Triton X-100 1%, and EDTA 5 mM) except for rice (200 ml buffer) and tomato (50 ml buffer). These samples were homogenised using the Ultra-Turrax at 19,000 rpm for 2 min in an ice bath. Thereafter, the samples were sonicated for 10 min, vortexed, and centrifuged at 10,000 × g for 10 min at 4°C. The supernatants were saved. All procedures were carried out in a refrigerated room (2°C–4°C).

Casein zymography was carried out on the extracts according to manufacturer's instruction. Four volumes of each extract were mixed with 1 volume of 5X sample loading buffer (50% glycerol, 10% SDS, 0.1% bromophenol blue and 150 mM Tris-HCl; pH 6.8). Gels were 4–16% Novex Zymogram Blue Casein prestained gels (Thermo Fisher Scientific). The extracts (30 μl each) and standard enzyme controls of approximately 0.1 μg each (protease, Sigma P-5255; protease calcium-activated, Sigma P-4523; papain, Sigma, P-3375) were loaded onto lanes and run at 125 V constant voltage with running buffer (192 mM glycine, 0.1% SDS, and 25 mM Tris) at 4°C. After 120 min, the gel was removed and incubated in zymogram renaturing buffer (2.5% Triton X-100) with slow shaking for 30 min. The renaturing buffer was discarded, and the gel was incubated in developing buffer 200 ml (50 mM Tris-base, 200 mM NaCl, 5 mM CaCl2, and 0.02% Brij-35, pH 7.5 adjusted with HCl) for 30 min with slow shaking. The buffer was then discarded, and the gel was incubated further in 200 ml of the developing buffer overnight with gentle shaking at ambient temperature. Finally, the gel was washed with deionised water. Areas of protease activity were identified by cleared zones in the prestained gels.

2.6. In Vitro Digestion. The digestibility of meat proteins was determined as previously described by Farouk et al. [6] using a static *in vitro* method that simulated the gastric and intestinal phases of digestion. This approach is widely employed, and the results obtained are indicative of outcomes of digestion *in vivo* [8, 9]. Gastric digestion (pepsin-based, representing the stomach) was applied to Experiment 2, while gastric and intestinal digestion (pancreatin-based, representing the upper gastrointestinal tract (GIT)) was applied to Experiments 1, 3, 4, and 5.

2.6.1. Gastric Digestion. For most muscle and organ meats, approximately 4.5 g of a sample was weighed into a 100 ml glass Schott bottle, sealed, and placed in a boiling waterbath for 15 min with intermittent mixing by swirling. The cooked sample was allowed to cool, covered with 34 ml of 0.1 M HCl, and then homogenised using the Ultra-Turrax for 1 min. The pH was adjusted to 1.9 with 6 N NaOH and made up to 36 ml with deionised water. Note that special attention was paid to the prerigor meat samples from Experiment 2 because these were susceptible to spontaneous glycolysis unless kept frozen until the moment of cooking. For these, approximately 4.5 g of a sample was weighed into a cold Schott bottle and immediately doused with 34 ml of boiling water and then placed in the boiling waterbath for 120 sec. The cooked

sample was allowed to cool and its concentration brought to 0.1 M with concentrated HCl and then homogenised using the Ultra-Turrax. The pH was adjusted to 1.9 with 6 N NaOH and made up to 36 ml with deionised water. For the meats combined with accompaniments from Experiment 5, the prepared slurries were already cooked, so these were used as is in their Schott bottles.

Samples were incubated in a shaking waterbath at $37 \pm 0.2^{\circ}$C for 15 min. Two ml of pepsin solution (Sigma P6887, 1.575 mg/ml; enzyme:substrate ratio 1 : 280 in 0.1 M HCL, equivalent to 12.5 U/mg protein) was added to start the proteolysis. Controls were prepared using cooked meat and 0.1 M HCL without pepsin. An aliquot of 500 μl was withdrawn immediately (T0) and again at planned timepoints. Enzyme activity in each aliquot was quenched by raising the pH to 8 by the addition of 1 M NaOH (37–39 μl). An equal volume of 2X Laemmli loading buffer containing 5% mercaptoethanol was added. The mixture was immediately vortexed, heated for 5 min in a waterbath at 95°C, and then stored at −20°C. After 120 min (T120), the remaining "digesta" in the Schott bottle was adjusted to pH 8 using 6 M NaOH to inactivate the pepsin and prepare the sample for intestinal phase digestion.

2.6.2. Intestinal Digestion. Pancreatin (Sigma P8096, 2.2 ml of 4 mg/ml, enzyme : substrate ratio 1 : 100 w/w in 0.1 M phosphate buffer, pH 8) was added to the Schott bottle and digestion allowed to proceed for further 2 h, with aliquots withdrawn at intervals. Enzyme activity in each aliquot was quenched by lowering the pH to 2 by the addition of 6 M HCl. Laemmli loading buffer protocol was then followed as above.

2.7. Gel Electrophoresis. Proteins and peptides in the digesta collected during *in vitro* digestion were separated by 1D SDS PAGE as described by Farouk et al. [6]. For most experiments, the Laemmli-prepared aliquots were thawed and then centrifuged for 5 min at 9,300 × g at room temperature. The supernatants were loaded at 40 μg protein per lane onto Criterion TGX 10–20% gels (Bio-Rad #567-1114) running in Tris-glycine buffer at 120 V constant voltage. Gels were stained using Coomassie Blue R-250 followed by destaining with 30% methanol and 10% acetic acid in deionised water.

For Experiment 5 which combined beef with nonmeat accompaniments, 20 μg protein per lane was loaded onto Novex NuPAGE 10% Bis-Tris midi gels (#WG1202) and NuPAGE MES SDS running buffer (#B0002) with NuPAGE Antioxidant (#NP0005) added to the upper chamber. Gels were run at 200 V for 35–40 min and then stained using Coomassie Blue G-250 or SimplyBlue™ SafeStain (Invitrogen #LC6060) as per the manufacturer's protocol.

2.8. Gel Quantification and Statistical Analysis. To quantify the overall efficiency of *in vitro* digestion as well as compare and rank treatments, the SDS PAGE gels were scanned with a CS-900 densitometer and analysed by Image Lab software (Bio-Rad).

For experiments 1 and 4, we calculated the relative digestibility. This was calculated by summing the density of all bands in a gel lane >10 kDa and then normalising to (i.e., dividing by) the summed density of bands in the respective T0 lane (timepoint 0, prior to digestion activation). Analysis of variance (ANOVA) was performed on this point estimate using Genstat software (17th Edition, VSN International, 2014). Pairwise t-test comparison of the means was obtained using the standard error of a difference from ANOVA. For Experiments 2 and 5, we extracted the gel densities and created plots of the spectral signals of these to allow visual comparisons.

3. Results

The effects of intrinsic and extrinsic factors on the *in vitro* digestibility of proteins and peptides in beef muscle and organ meats were evaluated.

3.1. Animal Age. Figure 2 shows that beef protein was highly digestible regardless of the age of animal from which the meat was collected (4-day-old calf, 18- to 24-month-old bull, or 6-year-old cow). There were some differences in size and quantity of proteins that were extracted from the cooked meat during sample preparation. For instance, the lanes at T0 (prior to digestion) show that extractability of connective tissue proteins (collagen-related proteins) with molecular weight >260 kDa tended to decrease with age (calf > bull > cow), suggesting greater solubility of the connective tissue proteins in younger animals. There was a strong band of protein at 17 kDa that was present for bull and cow but absent for calf, suggesting underdeveloped myoglobin. At the end of the 120-minute gastric phase, overall digestibility was in the order calf < bull < cow (Figures 2 and 3).

3.2. Rigor State. The time of sampling of LD muscle (prerigor, from 50 min through 200 min postmortem) had little influence on the digestibility of beef proteins, and this was not markedly affected by rigor at 48 h. Typical results for T60 of the gastric phase are shown in Figure 4, in this case for two bulls that had low ultimate pH values of 5.63 and 5.71. Major muscle proteins were all well digested by 60 min, including myosin heavy chain, α-actinin, and actin (approximately 250, 100, and 42 kDa, respectively). Likewise, small proteins and peptides of 30 to 10 kDa were uniformly well digested. One minor difference was observed between the prerigor sampling times and the postrigor sample; a band near 40 kDa seemed less digested (darker) postrigor.

3.3. Ultimate pH. The proteins of high ultimate pH meat digested faster than their low ultimate pH equivalent. Typical results for T0 through T60 of the gastric phase are shown in Figure 5. Densitometry measurements of each gel lane were used to calculate digestion efficiencies and rates for each of the treatment combinations (n = 49, data not shown). Physiological and biochemical mechanisms

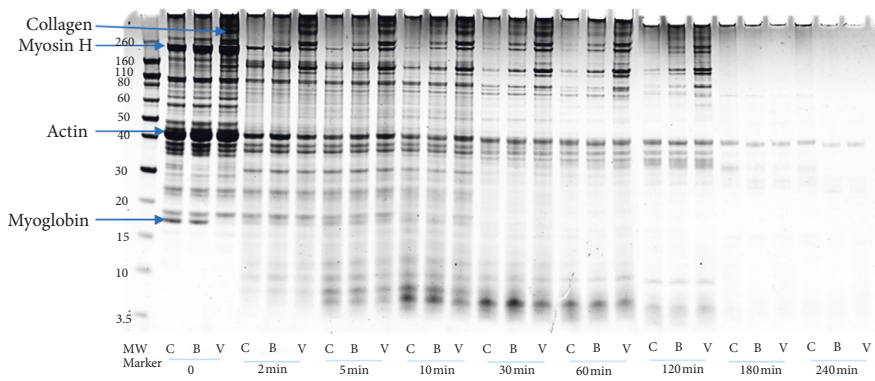

Figure 2: Experiment 1: SDS PAGE showing the effect of age of cattle on the digestibility of protein in cooked semimembranosus from cow (C), bull (B), and calf (veal, V). Results of gastric and intestinal phases for the pooled samples from 7 animals per age are presented as examples.

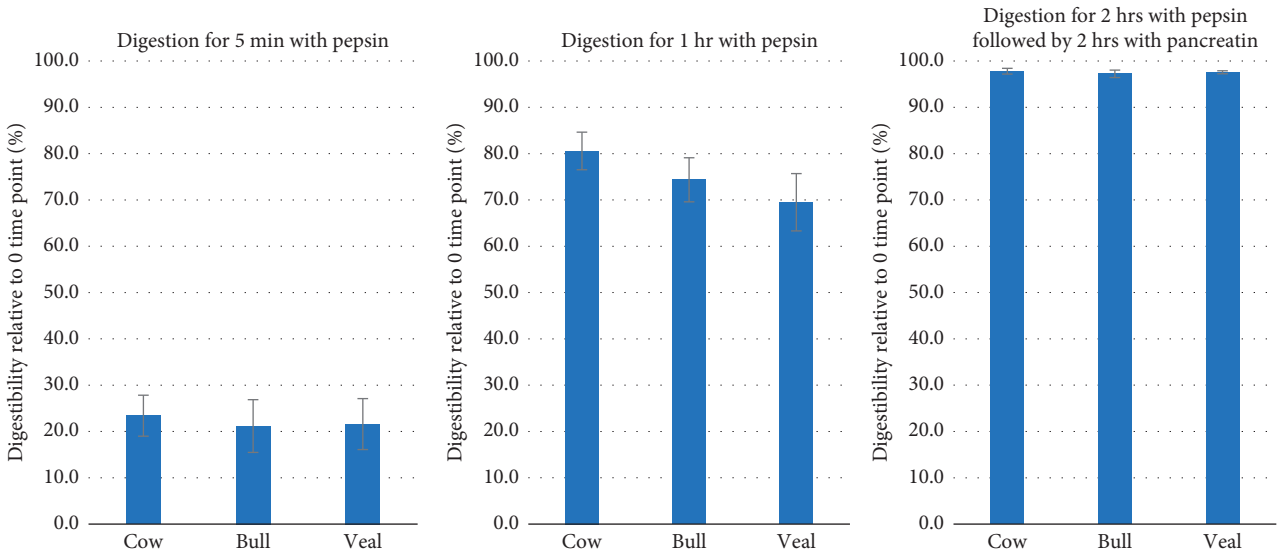

Figure 3: Experiment 1: the relative digestibility (see methods) of cooked semimembranosus from cow (C), bull (B), and calf (veal, V) at 5 min and one hour of gastric phase and two hours of intestinal phase. Results for the pooled samples from 7 animals per age are presented as examples.

underpinning the greater digestibility of high ultimate pH beef have been discussed by Farouk et al. [6].

3.4. Muscle/Meat Cut. The digestibility of proteins from different muscles and meat cuts from prime beef heifers was compared. Typical results for T0, T5, and T60 of the gastric phase and T240 of the intestinal phase are shown in Figure 6, in this case for four groups of muscle tissue. While there were few differences up to 5 min, by 60 min supraspinatus appeared more digested (fewer and fainter protein bands), suggesting that this cut might be faster and more thoroughly digested.

3.5. Mincing/Particle Size. There was little effect of particle size on the digestibility of cooked proteins in meat. Figure 7 shows the response of bull beef for subsamples of LD taken at 50 min prerigor and 48 h postrigor timepoints and either finely ground or coarsely smashed. Exposure of meat proteins to pepsin activity *in vitro* should have been much greater for the finely milled substrate, yet this did not markedly influence the rate or extent of proteolysis. Some protein bands, for instance, near 100 kDa and 40 kDa, did appear to digest more in the milled samples from T30 to T60; however, this was not consistent between animals.

3.6. Organ Meats. The structure and composition of organ meats is substantially different from muscle meat, and this has consequences for digestion. For instance, the protein content of the heart, kidney and spleen from prime steers was 10–27% less on a fresh-weigh basis (Table 1). Digestion commenced at a significantly faster pace for the kidney and liver compared to muscle, when evaluated as the relative digestibility of T5. However, by T60 and T240, there was no significant difference in digestibility between any of the tissues. The lower molecular weight and globular nature of the kidney and liver proteins likely contribute to their faster *in vitro* digestibility [10].

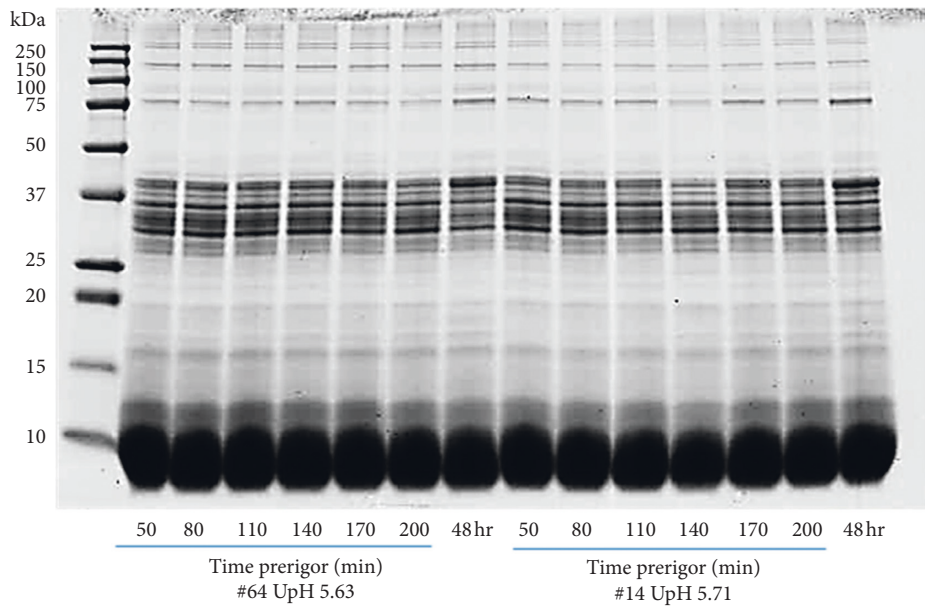

Figure 4: Experiment 2: SDS PAGE showing the effect of pre- and postrigor sampling time on the digestibility of protein in cooked M. longissimus dorsi from bull beef. Results at T60 of gastric phase for two of the 48 animals in this experiment are presented as examples.

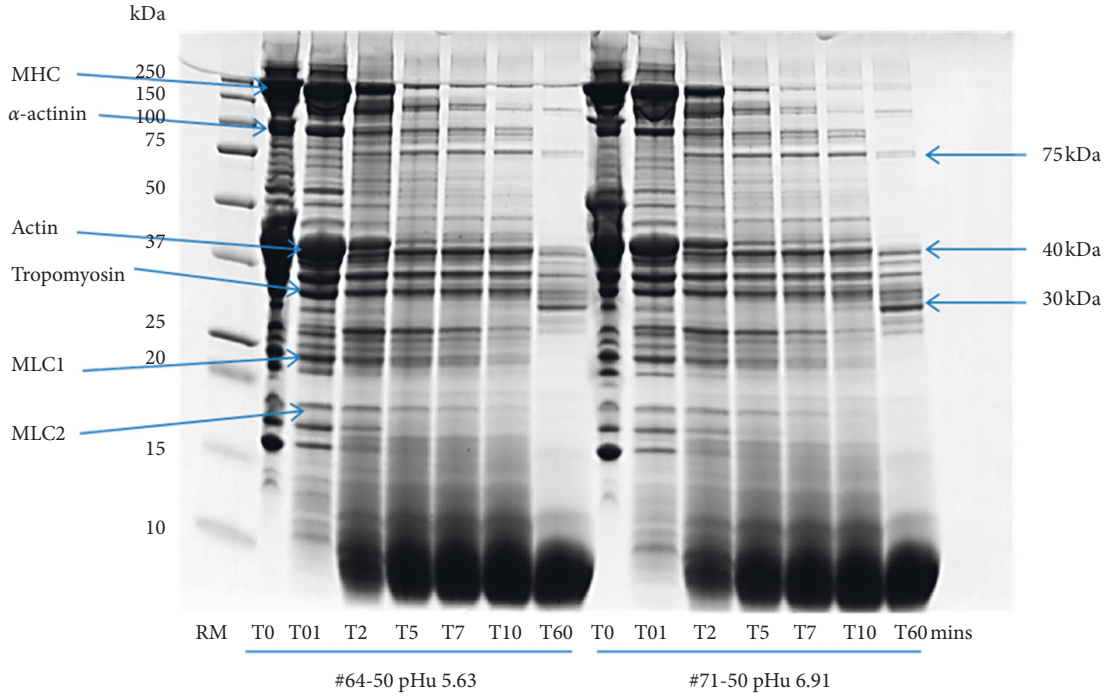

Figure 5: Experiment 2: SDS PAGE showing the effect of ultimate pH (pHu) on the digestibility of protein in cooked M. longissimus dorsi from bull beef. Results during the gastric phase for the sampling timepoint from two of the seven animals in this experiment are presented as examples.

3.7. Meat Accompaniments. The online survey of menus from New Zealand and Australia restaurants revealed that the most common accompaniments served with red meat were potato, onion, mushroom, tomato, rice, noodle, bean, and carrot (Figure 1). This varied slightly by country and markedly by cuisine. For instance, in New Zealand, noodle, rice, and bean were more popular at Asian restaurants, while potato, mushroom, and tomato were more popular with European cuisine.

SDS PAGE separation of proteins and peptides in the digesta of beef cooked with the top five accompaniments plus pumpkin showed that meats from all three age categories of animals (4-day-old calf, 18- to 24-month-old bull, or 6-year-old cow) were most digestible when cooked

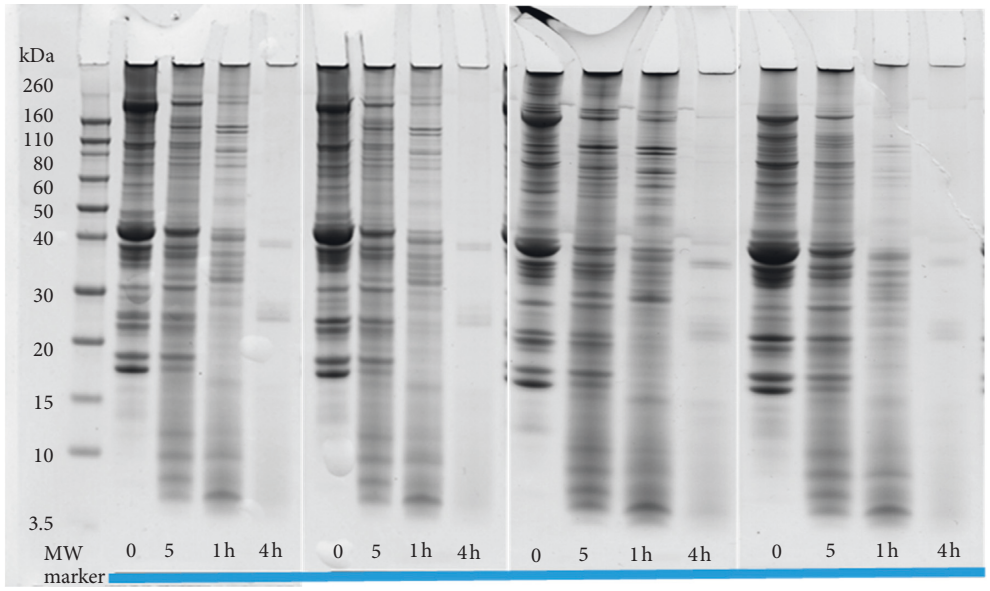

Figure 6: Experiment 3: SDS PAGE showing the effect of muscle type/cut on the digestibility of protein in cooked meat from prime heifers. Result at T0, T5, and T60 of gastric phase and T240 of intestinal phase for one of the five animals in this experiment are presented as examples. L to R extensors and flexors, rhomboideus, infraspinatus, and supraspinatus.

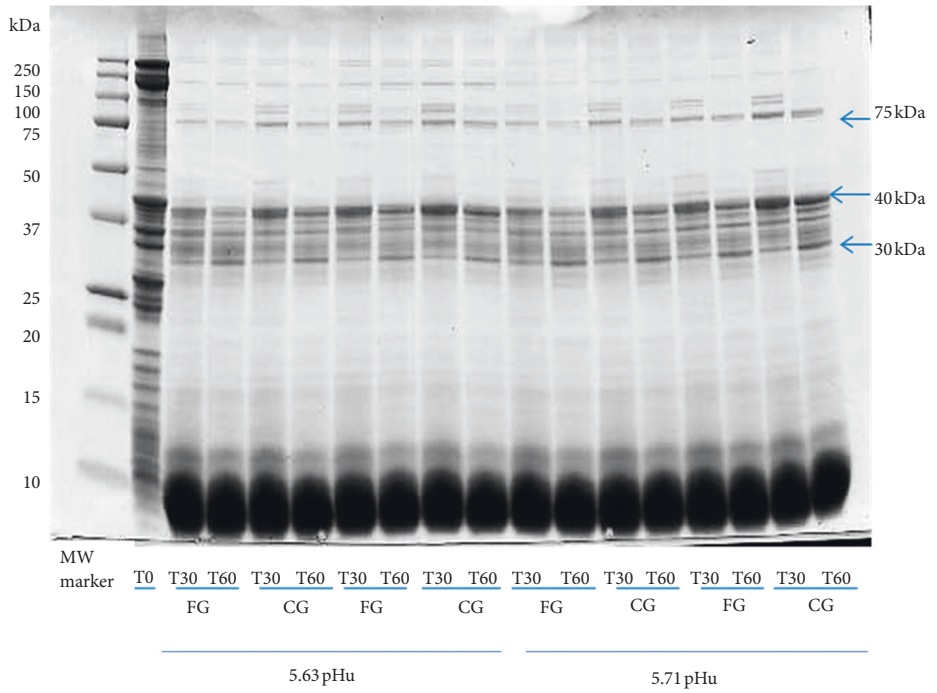

Figure 7: Experiment 2: SDS PAGE showing the effect of mincing/particle size on the digestibility of protein in cooked M. longissimus dorsi from bull beef. Results at T0, T30, and T60 of gastric phase for the two low pHu animals in this experiment are presented as examples. FG = finely ground; CG = coarsely ground.

with mushroom, whereas digestion was least efficient when the meats were cooked with rice and potatoes. Based on relative digestibility calculation and averaging over all animal ages, the rank order of protein digestibility was found to be mushroom > pumpkin > onion = tomato > rice > potato. Figure 8 presents an example with bull meat and shows that cooking meat with mushrooms was very effective in promoting digestion through the gastric and intestinal phases. In contrast, meat cooked by itself did not digest completely even after 240 min. Figure 9 shows the trace densities of beef cooked with the six accompaniments at T0 and T240 highlighting the extensive breakdown of the lower molecular weight proteins with mushroom and pumpkin compared to the other accompaniments.

Enhanced digestion from cooking with mushroom (and pumpkin) could be due to the presence of endogenous

Table 1: Experiment 4: protein content and digestibility of bovine organ meat and muscle meat.

Attributes	Organ meats				Beef	SED	P value
	Heart	Kidney	Spleen	Liver			
Protein (%)	18.43^{ab}	16.47^{a}	17.33^{a}	20.27^{bc}	22.73^{c}	1.21	0.001
RD @ 5 min	54.68^{a}	73.90^{b}	45.01^{a}	84.06^{b}	56.34^{a}	6.11	0.001
RD @ 1 h	79.86^{ab}	82.53^{ab}	75.55^{a}	86.43^{b}	75.84^{ab}	5.30	0.05
RD @ 4 h	95.33^{ab}	91.98^{a}	94.50^{ab}	96.32^{b}	95.09^{ab}	3.10	0.006

RD = relative digestibility; SED = standard error of difference; means in the same row bearing the same superscripts are not different ($P > 0.05$).

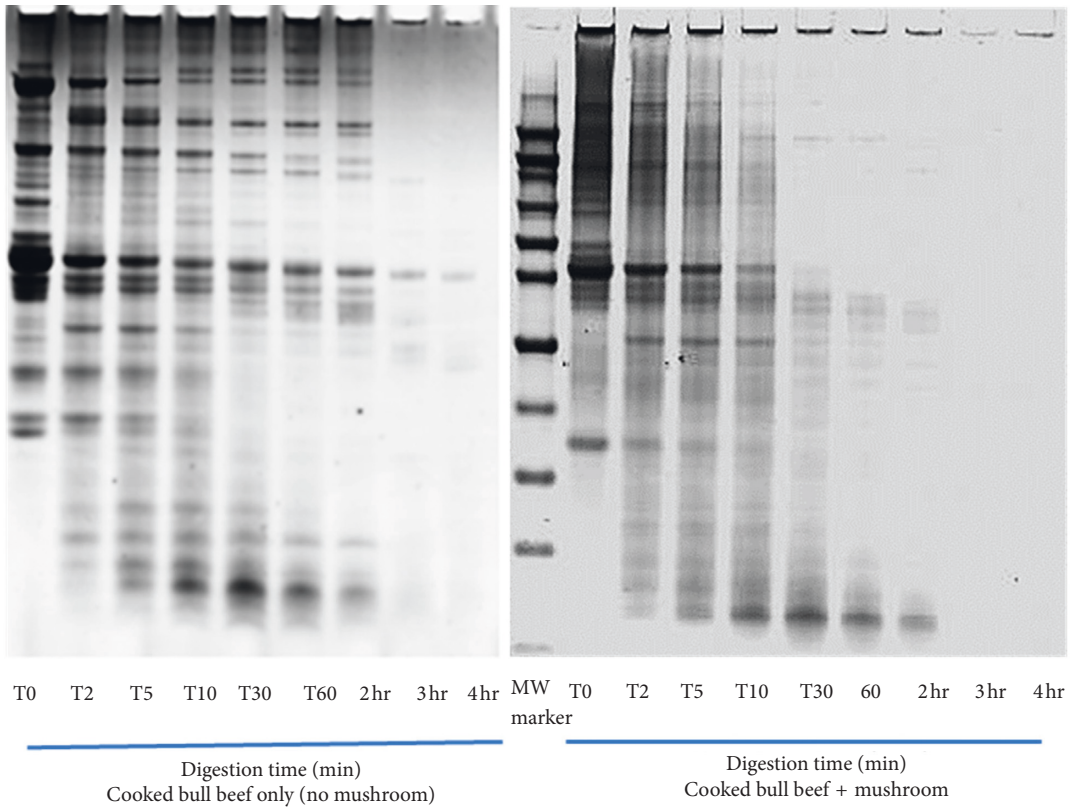

Figure 8: Experiment 5: SDS PAGE showing the effect of vegetable accompaniments in a cooked "meal" containing semimembranosus from cow, bull, or calf on the digestibility of total proteins. Results of gastric and intestinal phases for the meal containing pooled bull meat from Experiment 1 with and without mushroom are presented as examples.

proteolytic enzymes in these vegetables that were not present in the other accompaniments as observed in the results (zymograms bands visible on gels but too faint when photographed, thus not included) of the zymogram gel separation of enzymes extracted from the six accompaniments which showed faint protease activity seen for pumpkin and mushroom and not for the other accompaniments.

4. Discussion

Meat is usually considered to be the skeletal muscle of animals, along with any attached fat, connective tissue, blood and blood vessels, and may also include organ tissues such as the liver, heart, kidney, and intestines [11]. It makes important contributions to a balanced diet because it is dense with essential nutrients [12–14]. While the gross composition of organ meat varies by tissue, its protein content is always different from skeletal muscle, with less of the myofibrillar fraction comprising structural proteins. The supply chain of meat from the time the animal is selected, slaughtered, sold, and prepared varies around the world in some respects. One major difference is that in developing countries where cold storage facilities are limited, meat is often cooked and consumed in a prerigor state, when it is still muscle before it is converted to meat. Another difference is that in the cuisine of many cultures, meat is cooked together with its starch and vegetable accompaniments, in contrast to Western style meal preparation where meat is often roasted and eaten separately as intact cuts. In addition, animal organ meat, sometimes referred to as offal or the fifth quarter of a carcass, is only a minor contribution to typical Western diets for a variety of reasons, thus missing out on its potential culinary and

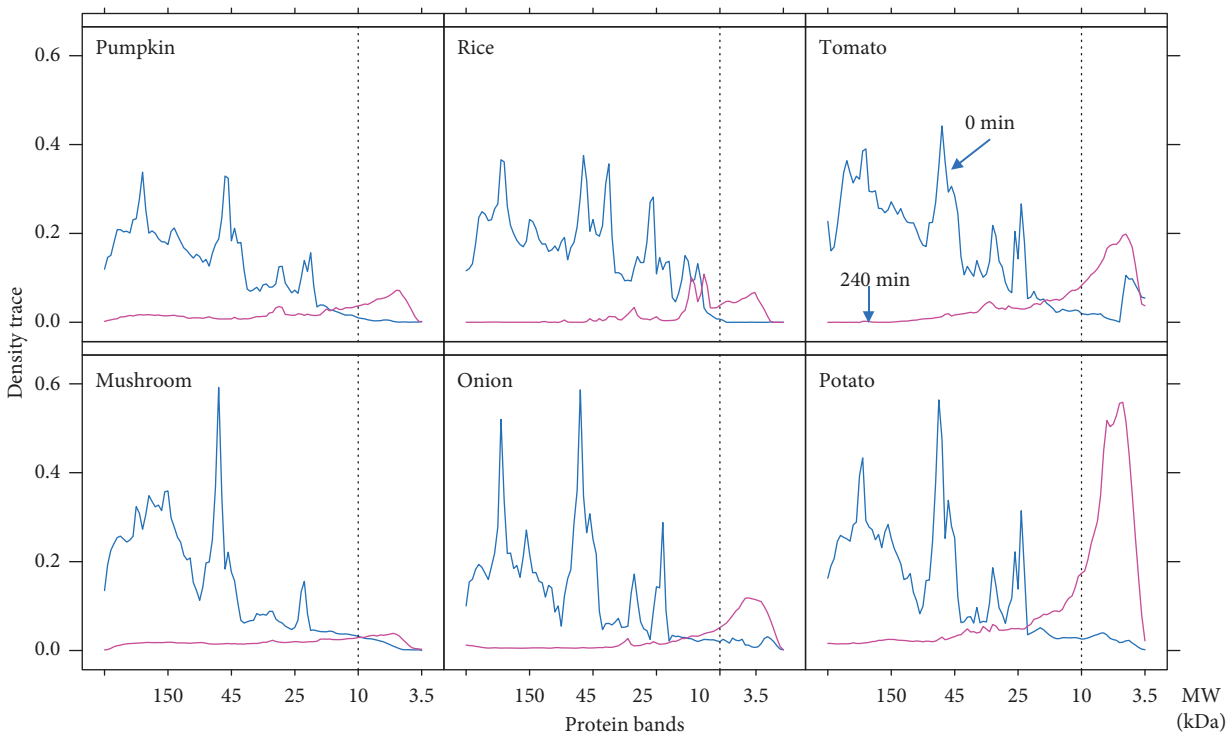

Figure 9: Experiment 5: density tracings of SDS PAGE showing the effect of vegetable accompaniments in a cooked "meal" containing semimembranosus from cow, bull, or calf on the digestibility of total proteins. Results at T0 of gastric phase and T240 of intestinal phase for meals containing pooled bull meat from Experiment 1 with six accompaniments are presented as examples.

nutritional values. Regardless of how meat is presented and consumed, the goal for the consumer is biologically available nutrition through the gateway of digestion. The rate and extent of meat digestibility and the factors affecting those processes are important determinants of the utilisation, differentiability, and value of meat [6].

We previously determined the effects of ultimate pH, ageing, and cooking on beef digestibility and suggested that chefs could exploit the attributes to tailor the choice of muscle and preparation to the requirements of customers [6]. In the current study, we extend these factors to include animal age, muscle state of rigor, cut, particle size, organ meats, and meal accompaniments.

Age of livestock at slaughter is a significant determinant of meat functionality and eating quality, and it may also affect digestibility. Experiment 1 with three ages of beef demonstrated that when the relative digestibility of all the proteins resolved in Figure 2 including the resistant stromal proteins (collagen-related) were considered, it would appear digestibility increased with animal age (calf < bull < cow) (see T5 and T60 in Figure 3). This counterintuitive observation could be due to the high molecular weight connective tissue proteins that are more soluble in young tissue than old and consequently more extractable when the meat is prepared for assay [15]. Although soluble, the connective tissue substances are resistant to *in vitro* digestion with pepsin and so lowered the total relative peptic digestibility of calf beef compared to the older cattle when assessed using gel electrophoresis. Experiments 2 and 3 demonstrated that beef digestibility was not substantially affected by muscle state of rigor, or the type of cut, or how fine the meat was ground at the time of cooking. All three factors did have a minor influence on the proteolysis of resistant proteins near 42–40 kDa, previously identified to be fragments of isoforms of myosin (myosin-1, myosin-2, and myosin-7). They were more digested (less intense) in prerigor bull beef than in 48 h postrigor, in less collagenous cuts compared to higher-containing cuts, and in finely ground milled meat compared to coarsely smashed meat. If those proteins were yardsticks or proxies for assessing beef digestibility, then prerigor, low-collagen supraspinatus muscle finely ground prior to cooking would be judged more digestible than the alternatives in these experiments.

Sustainable production of animals as a source of food demands that we make full use of every carcass. Unlocking the potential of the less familiar cuts and promoting their inherent benefits is an important role for nutritional research. Experiment 4 demonstrated that beef organ meats/offals such as liver and kidney were more digestible than muscle meat from the same carcass (Table 1). This suggests new opportunities for organ meat as a versatile ingredient, perhaps by formulating highly digestible animal protein foods for infants with less developed GIT or for elder consumers with compromised GIT function. The soft texture and minimal myofibril content of the liver and kidney also offer functionality. These could be a valuable resource for the 1st and 3rd age consumer groups who struggle with chewing and swallowing muscle meat [16].

When meat is served at a meal, its accompaniments are usually chosen to provide a balance of nutrition or for

culinary and gustatory purposes. Well-informed combining can also produce beneficial biochemical synergies. For instance, consuming orange juice that contains ascorbic and citric acids will enhance the bioavailability of ferric iron in plant foods [17]. It is possible that some accompaniments affect the digestion of food and so might be chosen to optimise benefits for a particular consumer or to better suit an occasion.

For Experiment 5, we decided that the "best" accompaniments to study were those in wide and common use. A survey of meal designs in restaurants provided insight and objective measures, although perhaps biased towards luxury and indulgence eating. The top accompaniments were potato, onion, mushroom, tomato, and rice (Figure 1), with 32% of the accompaniments cooked with meat and 68% served alongside.

Although the present study was not designed to determine the effect of cooking *per se*, it is important to note that cooking meat on its own has variable effects on meat digestibility depending on both temperature and time. For instance, peptic digestibility of beef is lowered, and pancreatic digestibility is enhanced when meat is cooked quickly to 100°C, with longer cooking at the same temperature reducing overall susceptibility of meat proteins to proteolytic enzymes; cooking pork mildly at 70°C enhanced peptic digestion, while at 100°C slowed peptic digestion [3–5]. In the present study, the combined meat and accompaniments were cooked at 100°C; cooking at this temperature with some of the accompaniments improved the digestibility of muscle meat from animals of all ages; Figure 8 shows how mushroom affected the bull beef. Note that even the resistant proteins near 42–40 kDa were digested by T30. A zymogram of the enzymes in extracts of accompaniments revealed proteolytic enzymes in mushroom and pumpkin. These enzymes may be contributing to digestibility. Mushroom and pumpkin are known to contain proteolytic enzymes [18, 19], but their effects on whole-tissue digestion had not been demonstrated.

5. Conclusions and Implications

Within the parameters of the present study, beef was observed to be more digestible or digested faster when it came from an older animal, at prerigor, and when it had high ultimate pH or contained less collagen content. Some beef organ meats were more digestible than beef muscle. Digestibility improved when meat was cooked with vegetables that contain proteolytic enzymes and diminished slightly with carbohydrate-rich or starchy foods such as rice and potatoes.

These results help to support a rational basis for the design of prepared meals where meat protein is a central ingredient, such as ready meals, institutional catering, and novel product categories of foods. Formulating around digestion functionality creates opportunities for speciality foods suited to infants and elder consumers. These are novel, value-adding ways to make the nutrition of meat more widely available and to stimulate sustainable utilisation of the entire carcass.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Quality and Acceptability of Fresh and Long-Term Frozen In-Bag Dry-Aged Lean Bull Beef

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In-bag dry-aged lean beef was produced using a stepwise ageing process. Lean bull beef striploins were dry-aged at 2°C, 75% RH under three different air velocities: 0.5, 1.5, and 2.5 m·s^{-1} for 7 days followed by wet-ageing for 14 days. The quality and acceptability of the dry-aged beef were compared with equivalent beef dry-aged for 21 days at 0.5 m·s^{-1} which served as a control. Two portions of the dry-aged beef (7/21 days) were randomly selected and held frozen at −18°C for 12 months. Shear force, drip, and cook loss decreased significantly ($p < 0.05$) with dry-ageing time. Increased air velocities accelerated dehydration process with no negative impact on the meat quality, microbiological safety, and consumer acceptability compared to the control ($p > 0.05$). Frozen storage for 12 months had little or no effect on the quality and acceptability of the dry-aged lean beef ($p > 0.05$). Dry-aged lean beef of equivalent quality and palatability, with a lower level of surface microorganisms and higher yield compared to the control, could be produced using the stepwise ageing process.

1. Introduction

Postmortem ageing of fresh beef for retail and foodservice is essential in meeting the high demands and expectations of discerning consumers seeking exceptional eating experience. Ageing improves tenderness [1, 2] and flavour [3]. Ageing of beef up to 14 days has been reported to increase the fatty and distinct aged flavours (beefy, brothy, sweet, and browned caramel), and these contribute positively towards the consumers' liking of premium beef cuts from the loin and rib [4]. The most widely used ageing practice is wet-ageing, which refers to ageing of a piece of meat in a moisture and air impermeable vacuum package bag under chilled storage conditions. Traditional dry-ageing involves ageing of the primal and subprimal cuts without the use of any packaging or ageing bags to produce meat considered superior to wet-aged meat by purveyors due to the intense beefy and roasted flavour from the process [5]. Dry-aged beef is typically described as having a buttery, rich, nutty, and/or earthy flavour profile [6].

Dry-ageing requires critical control of processing parameters including temperature, air velocity, and relative humidity, to prevent excessive weight loss and growth of microorganisms. A new method called "in-bag dry-ageing" has emerged over the last decade to address the concerns associated with the traditional dry-ageing process [7, 8]. The dry-ageing bags allow loss of moisture from the meat in a similar way to that of the traditional dry-ageing method. The dry-ageing bags act as an oxygen barrier to reduce oxygen exchange with the surrounding air, thereby limiting oxidation and its associated consequences, which include oxidative deterioration which produces rancid or off-flavour [9]. The bags also act as protective barriers to prevent contamination from the surroundings and reduce the proliferation of spoilage microorganisms during the ageing process [10], which in turn reduces the need of excessive trimming. Excessive trimming cannot be avoided with the traditional dry-ageing method and often causes a loss to the meat purveyors. A novel dry-ageing strategy called "smart ageing" proposed by Kim et al. [11] has shown improvement

in meat quality and value through modification of specific postmortem ageing conditions. Stepwise ageing is one of the smart-ageing strategies which combine different ageing methods to produce dry-aged beef of equivalent quality compared with those using dry-ageing only [12].

Most dry-aged beef is produced from well-marbled premium beef cuts from prime steers or heifers with high intramuscular fat (IMF) and consumed locally rather than exported and fresh rather than thawed. Lean bull beef, on the contrary, is characterised as low-value beef with fat content of 1-2%, reduced juiciness, and tough texture. As a result, it is usually processed to sausages, patties, and other further processed meat products and hardly used for premium products such as the dry-aged products. Recently, dry-aged *Longissimus thoracis et lumborum* from young bull (IMF around 2%) was produced and rated to be preferred over the wet-aged counterparts from the consumers [13], suggesting the potential to produce dry-aged beef products from lean cuts. The use of lean beef may offer further advantages over the prime fatty beef for storage stability and reduced off-flavours associated with high fat content and the interaction of the latter with storage time and temperature.

Long-term storage of dry-aged beef may need to be considered if the meat industry was to produce dry-aged meat for commercial export as it currently does for chilled and frozen wet-aged meat. Freezing of meat at −18°C during storage and distribution is a common practice in the meat industry, particularly for the export market. A processing strategy called "aged and then frozen" was of great interest over the last decade. This strategy refers to applying a certain period of wet-ageing (2–4 wks) prior to the frozen storage. It has been proven to improve the colour stability, tenderness, and water-holding capacity without negative impact on the meat quality [14–18]. Kim et al. [12] reported on the improvements of water-holding capacity and shear force tenderness with no impact on colour and sensory quality of stepwise aged beef (dry-aged 10 d + wet-aged 7 d) followed by frozen storage for 6 months. However, whether dry-aged lean beef can be frozen without deterioration in quality or not and how long the frozen storage can be continued without deterioration in quality remain unknown. Answers to these questions are required if dry-aged meat was to be commercially traded globally and particularly to growing markets in countries like China where frozen rather than chilled meat imports is the norm.

This study aimed to investigate the effects and interactions of air velocities, ageing time, and long-term frozen storage on the meat quality and acceptability of in-bag dry-aged striploins from lean bull beef using the stepwise ageing regime. The current study was carried out to test the hypothesis that the combination of in-bag dry-ageing at higher air velocities for shorter ageing time followed by wet-ageing in vacuum barrier bags would produce dry-aged meat of equivalent quality, long-term frozen stability, and acceptability to in-bag dry-ageing meat produced using longer dry-ageing time and with no wet-ageing involved.

2. Materials and Methods

2.1. Sample Collection and Dry-Ageing Procedure. A total of 15 pairs ($n = 30$) of beef striploins (*longissimus lumborum*) from bull (≈2-year-old, boneless) beef carcasses were obtained on the slaughter day from a local meat processing plant. All the loins were held at 12°C for 12 hrs until they entered rigor and then randomly assigned to four treatments (Figure 1): T1: in-bag dry-ageing for 21 d (control, $n = 6$) at an air velocity of 0.5 m·s^{-1}; T2 – T4: in-bag dry-ageing for 7 d + wet-aged for 14 d at an air velocity of 0.5 m·s^{-1} (T2), 1.5 m·s^{-1} (T3), and 2.5 m·s^{-1} (T4) ($n = 8$ for each stepwise ageing treatment). All the loins were vacuum packaged in dry-ageing bags (TUBLIN® 10 and 50 μm thick, polyamide mix with a water vapour transmission rate of 2.5 kg/50 μ/m^2/24 h at 38°C, 50% RH, TUB-EX ApS, Denmark) and laid out on wire racks inside a dry-ageing chamber set at 2 ± 0.5°C and relative humidity of 75 ± 5%. Samples were weighed during the ageing process (0 d, 3 d, 5 d, 7 d, 13 d, 16 d, 18 d, and 21 d), and the weights were used to calculate the % weight loss: % weight loss = [(initial weight of the sample before ageing – weight at a given time point)/initial weight before ageing] × 100.

A thin layer of dried and discoloured surface (including subcutaneous fat) was trimmed off from the striploins aged for 7 d and 21 d and then fabricated into 2 cm thick steaks. Minimum three steaks were taken from each loin of each treatment (T1–T4) and at different ageing time points (0, 7, and 21 d) for further analysis of fresh beef. No subsample was taken at 7 d of ageing time for the control (T1). Another three fresh steaks (minimum) were obtained from each loin of each treatment and ageing time point, as described above, vacuum packed immediately after ageing, and stored frozen at −18°C for 12 months to determine the effect of long-term frozen storage on the quality of dry-aged lean beef.

2.2. Surface Microbial Growth and Water Activity (A_w). Microorganisms from the untrimmed surface of fresh (unfrozen) beef samples were enumerated before (0 d) and after the ageing process (21 d) for all four treatments using standard methods in the Compendium of Methods of Microbiological Examination of Foods [19] for *Escherichia coli* (*E. coli*, Chapter 8.91), aerobic bacteria (Chapter 7.62), lactic acid bacteria (Chapter 19.522), Enterobacteriaceae (Chapter 8.63), and moulds and yeast (Chapter 20.51).

A_w of the fresh beef samples (one steak per loin) collected on 0 d and 21 d of ageing from all four treatments was measured in duplicate at 20°C using a water activity meter (Aqua lab CX-2, Decagon Devices, Inc., Washington, US). The water activity meter was calibrated using a saturated potassium sulphate solution and water at 20°C.

2.3. pH and Proximate Content. The pH of fresh and frozen (thawed at 4°C overnight) in-bag dry-aged beef samples (T1–T4 at all ageing time points, one steak per loin) obtained from *longissimus lumborum* was measured by inserting a calibrated pH probe (Hanna 99,163 pH meter with a FC232D combined temperature and pH insertion probe,

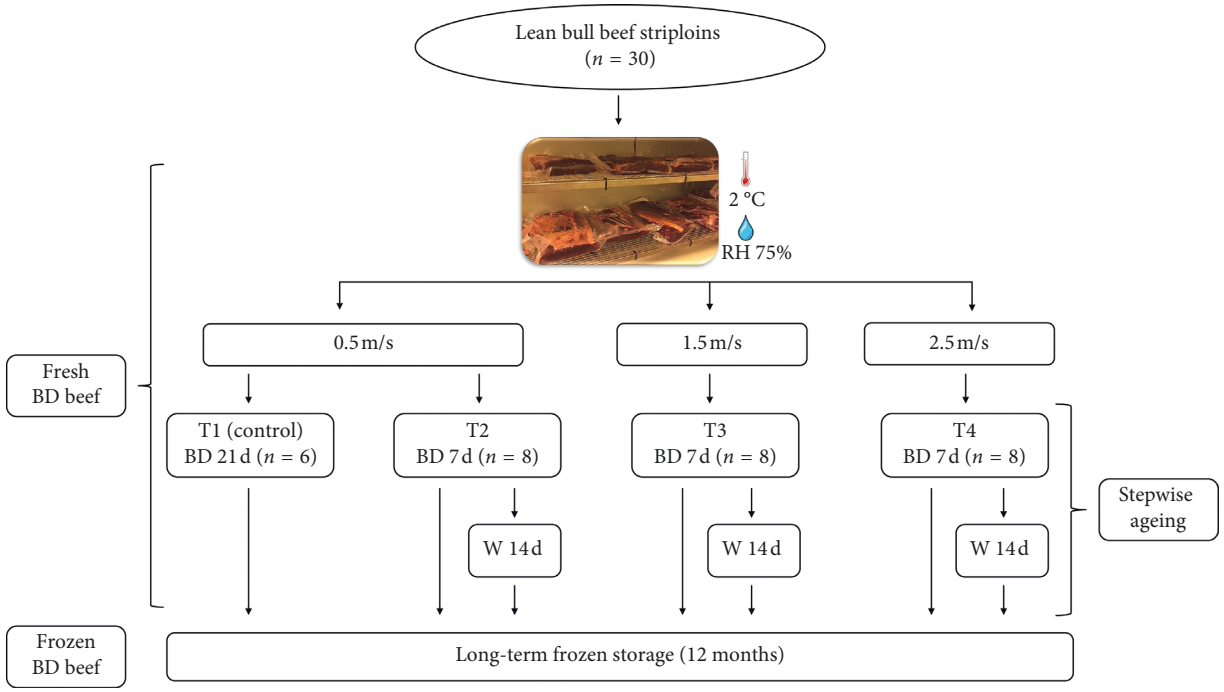

Figure 1: Schematic illustration of the ageing process and treatment combinations in the current study. BD: in-bag dry-ageing; W: wet-ageing; T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d.

Rhode Island, USA) directly into the beef samples. Triplicate measurements were taken and averaged.

Beef steaks from pH measurement were minced individually after trimmed off intramuscular fat and subsamples were collected for proximate analysis. Moisture content was measured using the oven-drying method described in AOAC 950.46 [20]. Crude fat content was measured using the Soxhlet extraction method of AOAC 960.39 [20]. The extraction of total muscle protein was as described in Lomiwes et al. [21] using an extraction buffer consisting of 50 mM Tris-HCl (pH = 5.8), 10% glycerol, 2% SDS, and 2% β-mercaptoethanol. Protein content of total muscle extracts was determined using a RC-DC protein assay kit (Bio-Rad® Laboratories, Hercules, CA, USA) based on Lowry assay [22]. Muscle protein concentrations were determined from the standard curve prepared with bovine serum albumin of concentrations from 0 to 2.0 mg·mL^{-1}.

2.4. Instrumental Colour. The steaks (one steak per loin) from fresh (0, 7, and 21 d) and frozen (7 and 21 d, thawed at 4°C overnight) in-bag dry-aged beef loins (T1–T4) were placed on a polypropylene foam tray lined with moisture absorbent pads and then overwrapped with the polyvinyl chloride (PVC) film and allowed to bloom for 30~60 min under simulated retail display light at 4°C. Surface colour was measured using a Minolta Chroma Meter (CR-400; Konica Minolta Photo Imaging Inc., Mahwah, NJ, USA) that had been calibrated using a standard white tile. CIE (L* (lightness), a* (redness), and b* (yellowness)) values were measured (Illuminant D65, 8 mm diameter aperture, 10 standard observers) through the PVC film at three random locations on each steak.

2.5. Water-Holding Capacity. The water-holding capacity was evaluated in the form of % drip loss for the fresh beef (7 and 21 d of in-bag dry-aged), % thaw + drip loss for the frozen beef (7 and 21 d of in-bag dry-aged), and % cook loss for both the fresh and frozen samples (one steak per loin).

2.5.1. Drip Loss. The bag drip method by Honikel [23] with some minor changes proposed by Kim et al. [24] was used on the collected samples from 7 d to 21 d of ageing time for both the fresh and frozen in-bag dry-aged beef samples. The % drip loss was calculated as follows: % drip loss = [(initial weight − weight after hanging for 48 hrs)/initial weight] × 100. The drip loss of frozen storage samples was measured from the frozen state. Therefore, the drip loss was expressed as the total loss from thawing and suspension in drip bags which was calculated as % thaw + drip loss = [(initial weight at frozen − weight after suspension for 48 hrs)/initial weight at frozen] × 100.

2.5.2. Cook Loss. Fresh beef portions (one portion per loin, 6 cm thickness) with weight of approximately 400 g from 7 d to 21 d of ageing were cooked in a boiling water bath (99°C) to the internal temperature of 70°C. Frozen steaks (one steak per loin, thaw at 4°C overnight) of 2 cm thickness from 7 d to 21 d of ageing time were cooked sous vide at 70°C for 1 hr.

Immediately after cooking, the cooked samples were transferred into an ice bath for 30 min to prevent further cooking, blotted dry, and weighed. The % cook loss was calculated as follows: % cook loss = [(initial weight − cooked weight)/initial weight] × 100.

2.6. Instrumental Texture. Cooked steaks from Section 2.5.2 were further analysed for instrumental texture. Tenderness of cooked fresh beef was measured with a MIRINZ tenderometer [25]. A 10 mm × 10 mm cross section was cut from each cooked steak to measure the force required to shear through the sample at a right angle to the fibre axis. The results were expressed as shear force (N) from the average shear force values of ten replicates for each of the cooked sample. A tender meat is defined as the meat of the shear force value ≤88 N (9 kgF) measured by the MIRINZ tenderometer [25].

The texture profile of long-term frozen in-bag dry-aged lean beef was analysed using texture profile analysis (TPA) according the procedure described by Zhang et al. [26]. In brief, a cube (1 cm^3) was measured using Stable Micro Systems TA.HD Plus texture analyser (Surry, UK) with a 50 mm cylinder probe at 50% strain setting using a test speed of 5.0 $m \cdot s^{-1}$. Maximum load force of 50 kg was used with trigger force of 5 g at the auto mode. A minimum of ten replicates from each steak were measured and averaged.

2.7. Consumer Sensory Testing

2.7.1. Cooking and Sample Preparation. Fresh in-bag dry-aged steaks (21 d, minimum three steaks per loin) were cooked in a conventional oven at 170°C until the core temperature reached 70°C. Frozen in-bag dry-aged steaks (21 d, minimum three steaks per loin) were thawed at 4°C chiller overnight and precooked sous vide at 70°C for 1 h and reheated on a grill set at 230°C for 90 s on each side. Once cooked, each steak was cut across the grain into a 1.3 × 1.3 × 2.0 cm piece and randomly assigned to a plastic cup. All the cups were prelabelled with unique codes made of panelist numbers (1, 2, 3, etc.) × sample ID (A, B, C, and D). Each panellist was asked to taste one sample each time in the order from A to D. Each sample ID (A–D) of the same panellist corresponded to one of the ageing treatments (T1–T4). The panellists may taste the same sample from the same steak more than once due to the randomised design model. Water and water crackers were provided as palate cleansers. Consumers were asked to take a bite of cracker, rinsed their mouths, and rest for 30 s between the samples. Consumers have been informed that swallowing was allowed but not compulsory.

2.7.2. Sensory Evaluation of Fresh and Long-Term Frozen Beef

(1) Fresh In-Bag Dry-Aged Beef. The aim of the first sensory session was to determine the effect of stepwise ageing and ageing chamber air velocity on the palatability of fresh in-bag dry-aged lean bull beef. A total of 44 untrained panellists (20 to 65 years old) who are frequent meat consumers and familiar with sensory evaluation of various meat products have participated in the study. Each consumer was provided with a computer to accomplish the webpage-based questionnaire. Consumer panels were asked to evaluate the acceptance and liking of the steak samples in terms of aroma, tenderness, juiciness, flavour, and overall liking on a scale of 1–100, where 1 represented "extremely dislike," and 100 represented "extremely like." Consumers were also asked to rate the degree of off-flavours where 1 represented "not detected" and 100 represented "detected very strongly." After tasting every two samples (A and B or C and D), panellists were also asked to rank for a preferred sample.

(2) Frozen In-Bag Dry-Aged Beef. The aim of the second sensory session was to determine the acceptability of long-term frozen stored (for 12 months) in-bag dry-aged lean bull beef. A total of 72 panellists (20 to 65 years old), consisting of 40% female and 60% male, participated in this study. Each panellist evaluated four samples from four different ageing treatments in a random order. Panellists were asked to rate their acceptance on a 9-point hedonic scale using a printed questionnaire where 1 represented "extremely dislike" and 9 represented "extremely like." Detection of the off-flavour was evaluated on a 5-point hedonic scale where 1 represented "not detected" and 5 represented "detected very strongly."

2.8. Statistical Analysis. A randomised trial was designed with 30 striploins from 15 beef carcasses ($n = 30$) which were unevenly assigned to four different treatment combinations (1 control dry-ageing; T1: $n = 6$ and 3 stepwise ageing regimes; T2–T4: $n = 8$ for each). Linear mixed effect regression analyses were performed on the data using R (version 3.4.1), with the "lme4" package to determine the difference between four treatment combinations across the ageing time. The ageing treatments (T1–T4) and ageing time (0, 7, and 21 d) were included as fixed effects, where the sample ID (loin number) was included as a random effect to account for the uneven number of samples between the control and other treatments. One-way analysis of variance (ANOVA) was performed to determine the effect of air velocity, stepwise ageing, and ageing time on the fresh and frozen in-bag dry-aged beef. The effect of air velocity was determined by the comparison between T2, T3, and T4 at 7 d of ageing time. The effect of stepwise ageing was determined by the comparison between T1 and T2 at 21 d of ageing time. The interaction of stepwise ageing and air velocity was determined by the comparison between all four treatments at the 21 d of ageing time. The effect of ageing time on each treatment was analysed separately by the comparing across the ageing time from 0 to 21 d. The effect of frozen storage on the proximate content (0, 7, and 21 d), pH (7 and 21 d), and instrumental colour (7 and 21 d) of in-bag dry-aged beef was analysed by comparing fresh and frozen beef samples aged for the same ageing time individually. Post hoc comparison of means was performed using Fisher's least significant differences (LSD) and Tukey's (HSD) test at the 5% significance level.

3. Results and Discussion

3.1. Effect of Dry-Ageing Chamber Air Velocity on the Physicochemical Properties of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.1.1. Fresh In-Bag Dry-Aged Beef. Weight loss of lean beef during in-bag dry-ageing increased ($p < 0.05$) with the increase in air velocity over the first 7 d of ageing time, as shown in Figure 2. The highest weight loss was associated with the highest air velocity of 2.5 m·s^{-1} (T4, 11.19%) compared with those of medium (T3, 10.57%) and low velocity (T2, 9.37%). Significantly higher weight loss with increased velocity from 0.2 to 0.5 m·s^{-1} after 21 d of ageing time has been reported for dry-aged prime steer beef loins [24].

A significantly lower ($p < 0.05$) moisture content was found in beef that was in-bag dry-aged at medium velocity of 1.5 m·s^{-1} (T3, Table 1). However, the % moisture differences were less than 1% between different air velocities. Therefore, the effect of ageing chamber air velocity on the moisture content of in-bag dry-aged beef was minimum. This could be explained by the moisture loss during dry-ageing being mainly on the surface and the outer section of the beef at higher velocities forming "crust-like" dry skin faster compared to lower velocities, which might have reduced moisture loss. Hence, the higher loss at medium velocity was observed as compared to the lower or higher velocities. The significant difference ($p < 0.05$) detected in the crude fat content was likely to be caused by the variations of moisture content.

Meat colour is widely used by consumers to determine the freshness of the meat products [27]. Colour is closely associated with pH values of the meat. As shown in Tables 2 and 3, increase of air velocity had no impact on the pH and instrumental colour of in-bag dry-aged beef except for the lightness (L*). L* decreased significantly ($p < 0.05$) with the increase of velocity to 2.5 m·s^{-1}. Similar findings have been reported with the increase in air velocity from 0.2 to 0.5 m·s^{-1} in dry-aged beef [24]. The reduced lightness could be attributed to the moisture loss on the surface of the meat, resulting in more light absorption and darker colour [28].

Water-holding capacity (Table 4) and shear force tenderness (Table 5) of fresh in-bag dry-aged samples were not influenced by the increase of air velocity ($p > 0.05$) which agreed with the findings reported by Kim et al. [24] on dry-aged beef.

3.1.2. Frozen In-Bag Dry-Aged Beef. There was no interaction ($p > 0.05$) between air velocity and frozen storage on the quality and physicochemical properties of in-bag dry-aged lean bull beef. Air velocity had no effect on the proximate content, pH, water-holding capacity, instrumental colour, and instrumental texture of in-bag dry-aged beef (7 d of ageing, T2–T4) stored frozen at −18°C for 12 months. This suggests that the use of higher air velocity in the dry-ageing of lean bull beef would not negatively influence the meat quality of in-bag dry-aged lean beef frozen for 12 months.

3.2. Effect of Stepwise Ageing on the Physicochemical Properties and Acceptability of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.2.1. Effect of Stepwise Ageing on the Physicochemical Properties. Stepwise ageing involving the in-bag dry-ageing of lean bull beef for 7 d followed by 14 d of wet-ageing (T2) significantly ($p < 0.0001$) reduced % weight loss of the dry-aged meat compared to the control (T1, in-bag dry-aged straight for 21 d). In this study, stepwise ageing had no effect on the pH, proximate content, and instrumental colour of fresh in-bag dry-aged beef ($p > 0.05$, Table 1) as was previously reported [29–32]. The water-holding capacity (measured as drip and cook losses) of fresh in-bag dry-aged lean beef (21 d, T1 and T2) were not affected by the stepwise ageing regimes, as shown in Table 4. Conflicting findings reported by Kim et al. were [12] that stepwise ageing (dry-aged 10 d + wet-aged 7 d) significantly decreased the drip loss but did not differ in cook loss as compared to dry-aged only. They attributed the higher drip loss from the dry-aged-only beef loins to the possible higher protein oxidation in the samples which resulted in the decrease of water-holding capacity. The lack of difference in cook loss between ageing regimes observed in the current study was in line with the outcomes of most of the previous studies on dry-aged beef [13, 24, 33, 34].

The instrumental tenderness (shear force) of dry-aged lean beef produced using stepwise ageing did not differ with its control counterpart produced by dry-ageing of lean beef for 21 d straight (Table 5). This result contradicts the findings reported Kim et al. [12] that stepwise ageing produced significantly lower ($p < 0.05$) shear force compared with traditional dry-ageing. The difference between our finding and that of [12] could be the type of beef used in the studies particularly the difference in the fat content of our lean bull beef and the beef (USDA low choice) used in [12].

There was no effect of stepwise ageing on the measured quality parameters of frozen in-bag dry-aged lean beef (21 d, T1 and T2). Therefore, the use of stepwise ageing produced in-bag dry-aged lean beef of equivalent quality to those of in-bag dry-ageing only without an adverse effect, even after a long-term frozen storage.

3.2.2. Interaction between Air Velocity and Stepwise Ageing. A significantly higher ($p < 0.05$) average % weight loss (20.48%) was found in the control (in-bag dry-ageing only, T1) at low velocity of 0.5 m·s^{-1} compared to the other treatments (T2 = 9.80%, T3 = 10.92%, and T4 = 11.54%), as shown in Figure 2. There was no significant ($p > 0.05$) weight loss arising from the extended wet-ageing process for T2, T3, and T4 regardless of the velocity as expected. The pH, b*, and chroma of stepwise ageing were similar to those of dry-ageing-only beef samples (Tables 2 and 3). Although the values slightly ($p < 0.05$) decreased at the high velocity (T4), the difference was negligible and should not impact meat quality. The other colour attributes, proximate content and water-holding capacity of fresh in-bag dry-aged beef (21 d, T1–T4), did not differ between the treatment combinations

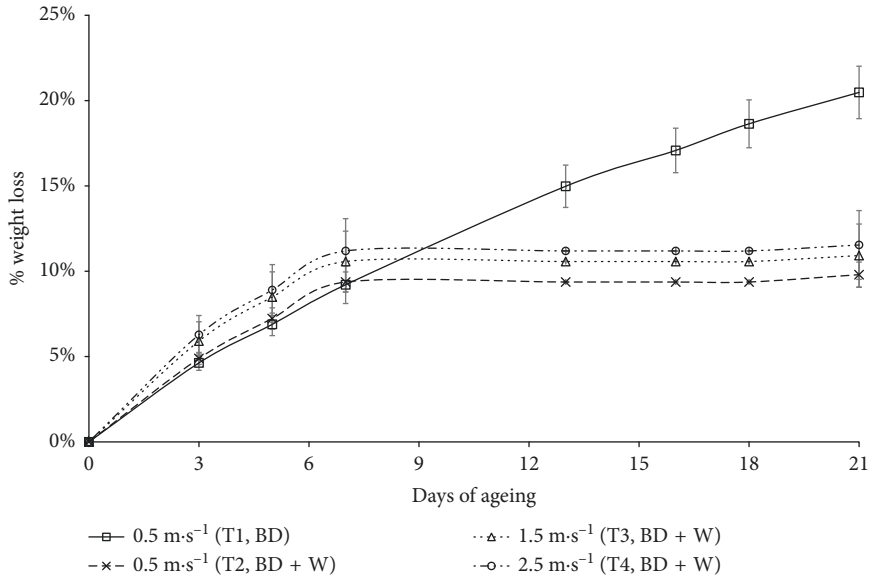

Figure 2: Average % weight loss of lean beef striploins of four different ageing treatments across different ageing times (days). BD: in-bag dry-ageing; W: wet-ageing. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d.

Table 1: Effect of ageing treatments, ageing time, and frozen storage on the proximate content of in-bag dry-aged lean bull beef.

Attributes/ storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (unaged)	p storage (7 d ageing)	p storage (21 d ageing)
% moisture												
Fresh	0 d	76.22x	76.29x	75.84x	75.81x	0.43	0.029	0.532	0.450	0.561	0.727	0.810
	7 d		75.83ay	74.77by	75.84ay							
	21 d	74.93y	75.19z	74.67z	74.33z							
	p ageing time	0.001	***	***	***							
Frozen	0 d	76.27	76.37	76.55	75.69	0.82	0.002	0.824	0.494			
	7 d		76.22a	74.83b	75.69a							
	21 d	74.93	75.09	74.57	74.08							
	p ageing time	0.241	0.145	0.068	0.075							
% crude fat												
Fresh	0 d	0.69	0.64	1.10	1.17	0.26	0.018	0.796	0.163	0.201	0.370	0.832
	7 d		0.63a	1.21b	0.62a							
	21 d	0.66	0.69	1.05	1.25							
	p ageing time	0.799	0.654	0.298	0.146							
Frozen	0 d	1.09	0.99	0.88	2.00	0.33	0.056	0.394	0.222			
	7 d		0.73a	1.28b	0.85a							
	21 d	0.68	0.78	1.13	1.08							
	p ageing time	0.232	0.541	0.600	0.116							
% muscle protein												
Fresh	0 d	22.31x	22.17x	22.26x	22.26x	0.29	0.948	0.709	0.236	***	***	***
	7 d		22.65y	23.21y	22.77y							
	21 d	23.48y	23.33z	23.70z	23.77z							
	p ageing time	0.003	<0.001	***	***							
Frozen	0 d	19.01x	18.25x	18.65	20.34	1.04	0.198	0.874	0.432			
	7 d		19.78xy	21.37	20.12							
	21 d	21.35y	21.23y	21.11	22.11							
	p ageing time	0.043	0.044	0.125	0.058							

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 2: Effect of ageing treatments, ageing time, and frozen storage on pH of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (7 d ageing)	p storage (21 d ageing)
pH											
Fresh	0 d	5.34x	5.34x	5.36x	5.32x	0.04	0.082	0.232	0.035	0.226	0.654
	7 d		5.74y	5.60y	5.69y						
	21 d	5.66ay	5.62abz	5.64ay	5.58bz						
	p ageing time	***	***	***	***						
Frozen	7 d		5.66	5.65	5.62	0.03	0.420	0.644	0.525		
	21 d	5.63	5.62	5.61	5.6						
	p ageing time		0.068	0.299	0.588						

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 3: Effect of ageing treatments, ageing time, and frozen storage on the instrumental colour of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments T1	T2	T3	T4	SED	p AV	p SA	p SA × AV	p storage (7 d ageing)	p storage (21 d ageing)
L *											
Fresh	0 d	33.51x	33.34x	33.05x	32.65x	0.62	0.022	0.588	0.411	***	***
	7 d		40.78ay	41.28ay	38.54by						
	21 d	39.80y	39.46z	39.46z	38.85y						
	p ageing time	<0.001	***	***	***						
Frozen	7 d		35.99	36.52	35.44	0.97	0.492	0.712	0.926		
	21 d	36.22	35.86	36.08	36.48						
	p ageing time		0.897	0.678	0.232						
a *											
Fresh	0 d	18.05	18.21x	17.44x	17.47x	0.90	0.797	0.927	0.084	0.004	***
	7 d		15.25y	15.29y	14.62y						
	21 d	18.45a	18.55ax	17.06abx	16.38bz						
	p ageing time	0.232	<0.001	0.004	0.001						
Frozen	7 d		13.37	11.79	13.82	0.81	0.075	0.312	0.742		
	21 d	13.77	13.02	13.34	13.04						
	p ageing time		0.686	0.303	0.157						
b *											
Fresh	0 d	11.84x	11.74x	11.44	11.31x	0.49	0.059	0.53	0.018	***	***
	7 d		10.81y	11.39	10.23y						
	21 d	12.93ay	12.69az	11.98ab	11.44bx						
	p ageing time	0.005	0.001	0.298	0.058						
Frozen	7 d		6.56	5.53	6.87	0.58	0.116	0.53	0.471		
	21 d	7.43	6.93	7.17	6.55						
	p ageing time		0.547	0.154	0.355						
Chroma											
Fresh	0 d	21.59x	21.68x	20.87x	20.82x	0.99	0.585	0.347	0.047	***	***
	7 d		18.70y	19.08y	17.84y						
	21 d	22.54ay	22.48ax	20.86abx	19.98bx						
	p ageing time	0.034	<0.001	0.048	0.005						
Frozen	7 d		14.9	13.03	15.44	0.94	0.078	0.994	0.653		
	21 d	15.66	14.00	15.15	14.6						
	p ageing time		0.888	0.236	0.169						
Hue											
Fresh	0 d	33.36x	32.85x	33.34x	32.93x	0.75	0.267	0.885	0.775	***	***
	7 d		35.42y	36.79y	35.01y						
	21 d	35.25y	34.42z	35.14y	34.95y						
	p ageing time	0.01	<0.001	0.001	***						
Frozen	7 d		26.04	24.89	26.38	1.22	0.401	0.442	0.544		
	21 d	28.34	27.92	28.2	26.67						
	p ageing time		0.061	0.158	0.755						

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

Table 4: Effect of ageing treatments, ageing time, and frozen storage on water-holding capacity of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments				SED	p AV	p SA	p SA × AV
		T1	T2	T3	T4				
% drip loss									
	7 d		3.86x	3.35x	3.26x	0.29	0.226	0.152	0.257
Fresh	21 d	1.35	1.56y	1.58y	1.92y				
	p ageing time		<0.001	0.001	0.001				
% thaw + drip loss									
	7 d		12.38x	10.36x	11.75x	1.41	0.335	0.322	0.443
Frozen	21 d	5.79	7.49y	5.86y	7.33y				
	p ageing time		0.009	0.013	0.003				
% cook loss									
	7 d		30.22	29.46	32.18x	1.69	0.519	0.979	0.204
Fresh	21 d	27.77	27.74	25.98	28.69y				
	p ageing time		0.240	0.120	0.020				
	7 d		31.81	31.81	33.04x	1.04	0.174	0.498	0.644
Frozen	21 d	29.60	30.29	30.59	29.40y				
	p ageing time		0.103	0.371	0.003				

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$).

Table 5: Effect of ageing treatments, ageing time, and frozen storage on instrumental texture of in-bag dry-aged lean bull beef.

Attributes/storage type	Ageing time	Treatments				SED	p AV	p SA	p SA × AV
		T1	T2	T3	T4				
Shear force (N)									
	0 d	132.00x	116.70x	131.81x	114.25x	12.45	0.071	0.932	0.919
Fresh	7 d		89.34xy	69.24y	83.95y				
	21 d	72.67y	71.98y	68.26y	70.61y				
	p ageing time	0.001	0.011	***	0.007				
Texture profile analysis									
Frozen									
	7 d		2.49	2.81	2.72	0.37	0.348	0.601	0.920
Hardness (kg)	21 d	3.02	2.88	3.11	3.10				
	p ageing time		0.404	0.362	0.193				
	7 d		0.53	0.51	0.54	0.02	0.678	0.634	0.969
Springiness	21 d	0.53	0.53	0.53	0.52				
	p ageing time		0.778	0.051	0.582				
	7 d		0.55	0.53	0.55	0.41	0.313	0.609	0.410
Cohesiveness	21 d	0.55	0.54	0.54	0.54				
	p ageing time		0.380	0.388	0.537				
	7 d		0.72	0.75	0.81	0.12	0.394	0.547	0.944
Chewiness (kg)	21 d	0.89	0.83	0.89	0.89				
	p ageing time		0.440	0.159	0.509				
	7 d		0.23	0.22	0.22	0.01	0.522	0.909	0.276
Resilience	21 d	0.23	0.22	0.22	0.22				
	p ageing time		0.181	0.473	0.773				

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$).

of air velocity and stepwise ageing ($p > 0.05$). As discussed above, air velocity and stepwise ageing process mainly influenced the water fraction on the surface of the samples.

A significantly ($p < 0.05$) higher amount of yeast was detected in the control (in-bag dry-ageing only) compared to those from stepwise ageing although the growth of surface microorganisms in all samples was low (Table 6). The results are supported by the outcomes of other studies that reported higher yeast counts in the beef samples of in-bag dry-ageing as compared to the wet-aged counterparts [8, 10, 29, 34]. The lactic acid bacteria, Enterobacteriaceae, *E coli*, and moulds did not differ between the treatment combinations of air velocity and stepwise ageing because the proliferation of these microorganisms was low and below the detection limit (Table 6). The use of different ageing regimes (dry/wet-ageing) had no impact on growth of Enterobacteriaceae and

Table 6: Effect of ageing treatments and ageing time on A_w and surface microbial growth of fresh-and-never frozen in-bag dry-aged lean bull beef.

Attributes	Ageing time	Treatments				SED	p SA × AV
		T1	T2	T3	T4		
A_w	0 d	0.992x	0.993x	0.994x	0.992x	0.001	0.325
	21 d	0.987y	0.987y	0.988y	0.986y		
	p ageing time	0.005	***	0.003	***		
Microbial load (log cfu/g)							
APC	0 d	4.21x	2.62	3.09	2.74x	0.61	0.281
	21 d	2.50y	3.29	2.56	2.00y		
	p ageing time	0.038	0.459	0.494	***		
LAB	0 d	1.15	ND	1.50	ND	0.07	—
	21 d	ND	ND	ND	ND		
	p ageing time	—	—	—	—		
Mould	0 d	1.39	ND	ND	ND	0.05	—
	21 d	ND	1.00	ND	ND		
	p ageing time	—	—	—	—		
Yeast	0 d	2.21x	ND	1.48	1.39	0.40	0.003
	21 d	4.06ay	1.57b	2.12b	2.41b		
	p ageing time	0.007	—	0.237	0.076		
Enterobacteriaceae	0 d	3.16	1.84	1.38	1.71	0.71	—
	21 d	ND	2.69	ND	1.35		
	p ageing time	—	—	—	—		
E. coli (MPN/g)	0 d/21 d	ND	ND	ND	ND	—	—

ND: not detected; BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "x, y, or z" within the same column mean results are significantly different from each other ($p < 0.05$). Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

moulds [8, 10] and *E. coli* [9]. Some studies have suggested that wet-ageing may contribute to a higher amount of lactic acid bacteria and lower aerobic bacteria count [8–10, 34, 35] postageing compared with (in-bag) dry-ageing due to the anaerobic ageing condition favouring the proliferation of lactic acid bacteria and suppressing that of aerobic bacteria. The aerobic bacteria counts tended to decrease ($p < 0.05$) with the air velocity in stepwise aged beef samples (T2–T4, Table 6). Current findings of lactic acid bacteria and aerobic bacteria could be explained by the faster dehydration on the meat surface at higher air velocities playing a major role in creating an adverse environment for the growth of both bacteria.

The quality parameters of a long-term frozen-stored in-bag dry-aged lean beef (21 d, T1–T4) were not affected by the treatment combinations of air velocity and stepwise ageing. The differences in colour (a*, b*, and chroma) observed in the fresh in-bag dry-aged beef due to ageing treatments disappeared after long-term frozen storage (Table 3). This could be due to the biochemical changes of muscle cells and myoglobin during the frozen storage.

3.2.3. Sensory Quality of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

(1) Acceptability of Fresh In-Bag Dry-Aged Beef. The consumer panel could not detect any difference ($p > 0.05$) between the samples produced using the four ageing treatment combinations in terms of aroma, texture, tenderness, juiciness, flavour, and overall liking (Table 7). The lack of differences in the consumer acceptability of the tenderness and texture of the in-bag dry-aged samples (21 d) from the four ageing treatments agreed with the instrumental shear force measurement of tenderness (Table 5). Kim et al. [12] also found no difference in the sensory acceptability of dry-aged beef loins when comparing the stepwise ageing regimes with those traditional dry-aged only.

Debate on the consumer preference and acceptability of dry-aged beef over the equivalent wet-aged beef is ongoing. Some of the studies found no significant difference in the consumer acceptability of the tenderness and juiciness of dry-aged as compared to the wet-aged beef [13, 24, 33, 36, 37]; others reported higher preference of (in-bag) dry-aged beef compared to the wet-aged in terms of tenderness and juiciness [1, 8, 10, 13, 38]; while others suggested the tenderness of wet-aged beef was more acceptable/preferred than the dry-aged counterparts [4, 32, 39]. The differences arising from ageing time, ageing conditions, and muscle types across the studies of dry-ageing may contribute to the inconsistent findings in consumer acceptability.

For the overall liking and the flavour liking, findings from previous studies were also controversial. Thus, the conclusion over the most effective ageing method to maximise palatability cannot be easily drawn. In the current study, consumers gave similar ratings of overall liking to all four samples; however, different findings were observed when they were asked to express their preference between samples. As shown in Table 7, about 94% of the panellists were able to discriminate the difference between four treatment combinations. The control (in-bag dry-aged straight for 21 d) was the most preferred by the consumers

Table 7: Effect of ageing treatment combinations and frozen storage on sensory acceptability of in-bag dry-aged lean beef for 21 days.

Attributes	Storage type	Treatments				SED	p SA × AV
		T1	T2	T3	T4		
	Fresh						
Aroma		56.53	55.58	55.78	56.60	2.81	0.977
Tenderness		53.62	48.73	45.84	55.01	4.88	0.224
Juiciness		54.39	53.64	48.35	47.80	4.12	0.244
Flavour		44.85	44.76	41.87	42.95	4.35	0.881
Off-flavour		20.68	16.73	19.16	17.79	3.66	0.727
Overall liking		59.77	58.20	52.88	57.17	3.33	0.218
Preference ranking (%)		28.41	22.73	22.73	20.45		
	Frozen						
Aroma		5.75	5.81	5.81	6.00	0.19	0.548
Tenderness		5.74	5.46	5.75	6.02	0.30	0.397
Juiciness		5.86a	4.55b	5.24c	5.91a	0.26	***
Flavour		6.12	5.76	5.96	6.33	0.23	0.126
Off-flavour		1.71	1.89	1.85	1.78	0.12	0.401
Overall liking		6.03a	5.37b	5.89a	6.25a	0.24	0.006

BD: in-bag dry-ageing; W: wet-ageing; SA: stepwise ageing; AV: air velocity. T1: BD at 0.5 m·s^{-1} for 21 d; T2: BD at 0.5 m·s^{-1} 7 d + W for 14 d; T3: BD at 1.5 m·s^{-1} for 7 d + W for 14 d; T4: BD at 2.5 m·s^{-1} for 7 d + W for 14 d. $p < 0.0001$ presented as *** for level of significance. Different letters of "a, b, or c" within the same row mean results are significantly different from each other ($p < 0.05$).

(28.41%) in the current study compared to other ageing treatments which were equally preferred (20–22%).

(2) Acceptability of Frozen Dry-Aged Beef. For the first time, we report the sensory quality of dry-aged beef that were frozen for 12 months. Despite the negative impression over the frozen storage of beef, the sensory quality of the in-bag dry-aged beef (21 d) of all the ageing treatments, including aroma, tenderness, flavour, and overall liking, was rated to be higher than that of the fresh (never frozen) in-bag dry-aged counterparts (Table 7). No significant difference ($p > 0.05$) was found in tenderness, between the four ageing treatments, which is in agreement with the instrumental texture profile analysis of the corresponding frozen in-bag dry-aged samples. Panellists failed to differentiate the control in-bag dry-aged beef (T1) and stepwise aged samples except for those aged at low ageing chamber air velocity (T2), and this was the least preferred by the consumers at a significant level ($p = 0.006$). This may have been caused by significantly ($p < 0.05$) lower rating of juiciness, which is further supported by higher % thaw + drip loss and % cook loss compared to the other ageing treatments (Table 4).

Rancidity, noted as the off-flavour, is another important indicator for the consumers to determine the freshness of cooked meat. Low mean values of the off-flavour were found in the cooked steaks of all the treatments, suggesting the difficulty in recognising the rancid flavour from the frozen in-bag dry-aged (21 d) lean beef samples. The rancidity note generated from the deteriorated meat is mainly caused by the oxidation and hydrolysis of the fat in meat. The meat samples containing higher fat (such as prime cuts and wagyu meat) were more susceptible to oxidation and consequently give off the rancid flavour. It is worth noting that the beef samples used in this study were lean bull beef, which only contained approximately 1% of IMF (Table 1). Therefore, the low level of rancidity in the beef samples after long-term frozen storage was expected. Kim et al. [12] recently reported no effect of short-term (1 month) freezing on the sensory quality of beef loins (USDA low choice) dry-aged by the stepwise ageing process. However, how the sensory quality would change if the storage time was extended (e.g., ≥6–12 months) and how the long-term frozen storage would affect the sensory quality of dry-aged beef with different IMF contents have not been explored. Answers to these questions would be of great significance for the export of frozen dry-aged beef.

3.3. Effect of Dry-Ageing Time on the Physicochemical Properties of Fresh and Long-Term Frozen In-Bag Dry-Aged Beef

3.3.1. Fresh In-Bag Dry-Aged Beef. The length of dry-ageing time had a significant ($p < 0.05$) impact on the % weight loss observed in the present study as expected (Figure 2). There was a significant ($p < 0.05$) decrease in moisture content with the increased ageing time (Table 1). This finding is consistent with the outcomes of some previous studies [29, 40] and contradicted others [9]. The variation in the outcomes reported in the literature may have arisen from the different sampling methods used in the studies though often these were not clearly stated in the studies. The slight increase in the protein content ($p < 0.05$) with ageing time observed in the current study (Table 1) was more likely to be due to the decrease in moisture content ($p < 0.05$) in the in-bag dry-aged samples (T1–T4) over the ageing time.

Overall, the pH of in-bag dry-aged striploins increased significantly ($p < 0.05$) after 21 d of ageing (Table 2) as compared to unaged counterparts which was in agreement with other studies on dry-aged beef [24, 29, 39]. Within the first 7 d of in-bag dry-ageing, the pH increased from an average of 5.34 to 5.74 (T2), 5.60 (T3), and 5.69 (T4). Although a slight but significant decrease ($p < 0.05$) of pH values during the extended 14 d of wet-ageing was detected

in T2 and T4 samples, the actual difference was minimal (0.1 units). The increase of pH values after dry-ageing could be associated with the generation of nitrogenous compounds caused by proteolysis as suggested by Aksu et al. [41].

After in-bag dry-ageing for 21 d (T1, Table 3), all the colour parameters (L*, b*, chroma, and hue) measured in this study increased significantly ($p < 0.05$) except for a* which did not change. However, a* has been reported in other studies to increase with ageing time on dry-aged beef using ageing bags [8] and traditional (no bag) dry-ageing [34]. The stepwise ageing process affected the colour properties differently from the control across the ageing time. The first 7 d of the in-bag dry-ageing process significantly ($p < 0.05$) increased the L* and hue angle, decreased a* and chroma compared with unaged (0 d) counterparts (T2–T4, Table 3). The extended wet-ageing from 7 d to 21 d of ageing slightly decreased L* (T2 and T3) and hue (T2) but significantly increased ($p < 0.05$) a* and chroma (T2–T4) to similar levels as unaged counterparts. The inconsistent changes of a* and chroma over the stepwise ageing process is unclear. It could be associated with the difference of metmyoglobin reducing ability and stability of myoglobin at different ageing times. The increase of L* has been linked with the myofibrillar protein denaturation which consequently gave rise to a tighter and more opaque structure [42]. The lack of change in L*, a*, and b* values over the dry-ageing process was also reported in previous studies [8]. The stepwise ageing has been reported to have no negative effect on the instrumental colour quality [12]. However, to the best of our knowledge, the effect of stepwise ageing time on the instrumental colour of in-bag dry-aged beef has not been explored in previous studies.

Drip loss significantly decreased ($p < 0.05$) in in-bag dry-aged beef at 21 d compared to 7 d regardless of the ageing treatment combinations (Table 4). This could be due to the significant amount of moisture lost by evaporation after 21 d of ageing thereby reducing the amount of moisture that could be lost as drip compared to 7 d, as discussed above. The reduced drip loss at 21 d could also be attributed to the higher muscle protein breakdown with 21 d of ageing compared to 7 d resulting in the "sponge effect" proposed by Farouk et al. [43], which physically entrap the water and improve the water-holding capacity by lowering the water loss by gravity. The water-holding capacity of lean beef increased with ageing time, particularly in terms of the decrease in drip loss. The cook loss, on the contrary, could be another indicator of the water-holding capacity under extreme conditions, i.e., heating. The cook loss of in-bag dry-aged lean beef decreased with the ageing time from 7 d to 21 d in general, but the significant decrease was only seen in the beef aged at highest air velocity (T4). Other studies also did not find any difference in cook loss of dry-aged beef from 14 to 35 d [9, 29, 44].

Shear force values decreased significantly ($p < 0.05$) with dry-ageing time at the first 7 d from an average of approximately 120 N (untender) to 80 N (tender) and then further decreased slightly ($p > 0.05$) during the extended 14 d of the wet-ageing period to a similar level (approx. 70 N), regardless of the ageing treatments (Table 5). The majority of studies on dry-aged beef reported no difference of shear force between wet-ageing, traditional dry-ageing, and in-bag dry-ageing of beef [2, 4, 9, 10, 36], which further support the findings of the current study. Therefore, it was the ageing time rather than the ageing methods that played the key role in tenderisation of beef. A significant decrease of shear force occurring within the first 14 d of ageing time has been observed by Gudjónsdóttir et al. [34]. Extension of ageing time beyond 4 weeks showed a minor effect on the improvement of tenderness [29, 36, 39, 44]. Therefore, the most rapid improvement of in-bag dry-aged beef tenderness occurs within the first 7 to 14 d of ageing time. This could be explained by the activity of endogenous enzymes (mainly μ-calpain) which plays a significant role in the tenderisation through proteolysis [45]. The activity is decreased significantly after 7 d of ageing time [46]. A more recent study by Velotto et al. [37] observed slightly faster decline of μ-calpain activity in dry-aged beef compared to the wet-aged over 15 d of ageing time. Further tenderisation during extended ageing time was mainly affected by more stable lysosomal proteases (mainly cathepsin B and B + L), though the rate of tenderisation decreased. Measurement of shear force at 0 d and/or 7 d of ageing time is absent in many of the studies to date, which may have contributed to overlooking of the fact that the longer ageing time is not necessary for the improvement of tenderness. Though tenderisation is well known to improve the eating quality of meat, it should be critically controlled to avoid overtenderisation and loss of texture and mouth appeal.

A_w is one of the most important parameters to indicate the shelf life of a food product. A_w of beef samples from all four treatments decreased significantly ($p < 0.05$) from an average value of 0.993 to a similar level of 0.987 after 21 d of ageing time, as shown in Table 6. In general, a low level of growth of surface microorganisms was found before and after ageing. The significant decrease ($p < 0.05$) of the aerobic bacteria counts in the in-bag dry-aged beef of T1 and T4 may be associated with the decline of A_w because of the surface being dried. The proliferation of aerobic bacteria with the increase of dry-ageing time has been reported [1, 7, 9]. However, contradictory findings were reported in Ahnström et al. [29] that no difference of aerobic bacteria counts on dry-aged beef regardless of ageing methods and ageing time. Yeast in the present study increased with ageing time ($p < 0.05$) in the control in-bag dry-aged beef (T1) which is in line with other studies on (in-bag) dry-aged beef reported by Degeer et al. [9]. The increase of yeast could be attributed to yeast species being able to grow on the dry meat surface with low moisture content compared to other microorganisms.

There was no difference in lactic acid bacteria, Enterobacteriaceae, and moulds across ageing time in the current study. *E. coli* was not detected in all the samples. Inconsistent results have been reported that Enterobacteriaceae [8], *E. coli* [9] and moulds [34] increased with ageing time. The decreased [9, 29] or unchanged [1] counts of lactic acid bacteria were also observed on dry-aged beef across the ageing. The proliferation of microorganisms has been observed in the traditional dry-ageing process which could cause the

spoilage of meat, and some toxin-producing pathogens may lead to serious food poisoning and even death [47]. On the contrary, microorganisms could facilitate the deterioration of meat quality and generation of off-flavours such as cheesy and dairy [48] and discolouration [49]. Therefore, the strict control of the processing hygiene and monitoring of the level of microorganism contamination are extremely important in terms of meat quality and food safety assurance. Current dry-ageing treatment combinations were able to produce microbiologically safe dry-aged lean beef products after 21 d of ageing time. This enables the meat industry to produce dry-aged products that satisfy the food safety standard for both local and export markets.

3.3.2. Frozen In-Bag Dry-Aged Beef. After long-term frozen storage, the proximate content of in-bag dry-aged beef from all four ageing treatments did not differ across the ageing time except for the muscle protein content which increased with ageing (Table 1). Significant ($p < 0.05$) increase of muscle protein content was seen in T1 and T2 which was likely to be attributed to the decrease of moisture content with ageing time. The water-holding capacity of frozen in-bag dry-aged samples increased significantly ($p < 0.05$) with ageing time in terms of % thaw + drip loss which was also evident in the fresh counterparts (Table 4). In general, the cook loss of frozen in-bag dry-aged samples decreased with ageing though the significant ($p < 0.05$) decrease was only found in the samples aged at highest air velocity (T4). Therefore, the water-holding capacity improved by ageing time was stable over the long-term frozen storage which was in agreement with Farouk et al. [50].

The pH, colour, and texture profile of in-bag dry-aged lean beef was not affected by ageing time ($p > 0.05$) after long-term frozen storage. The differences of pH, instrumental colour, and texture detected in fresh in-bag dry-aged samples between 7 d and 21 d of ageing were not observed after long-term storage. The changes in texture could be explained by two theories suggested in Dransfield [51]: (1) proteolysis during long-term frozen storage although the rate was slow because of calpain activity being suppressed; (2) a more rapid proteolysis occurred when thawing prior to freezing due to the reactivation of calcium-depend proteases. Another possible explanation could be the interaction between ageing time and frozen storage as suggested by Vieira et al. [52] that the beef aged for 3 d showed significant decrease on shear force during longer frozen storage time as compared to those aged for 7 d which did not change. Therefore, understanding the texture profile of in-bag dry-aged lean beef which has been long-term frozen stored is more important than the shear force tenderness because the frozen storage could further tenderise the meat as discussed above. The changes of other texture properties are not able to determine by single shear force measurement.

3.4. Effect of Frozen Storage on Lean Dry-Aged Beef for Different Ageing Times. The pH of in-bag dry-aged beef was not affected by long-term frozen storage ($p > 0.05$). This supports the outcomes of the 6 months frozen storage of stepwise dry-aged beef reported by Kim et al. [12]. Proximate content of in-bag dry-aged beef at all three time points (0, 7, and 21 d) was not affected by the frozen storage except for the muscle protein content which decreased significantly ($p < 0.0001$) after frozen storage. A consistent moisture and fat content over the frozen storage of up to 52 weeks was also reported by Holman et al. [53] on beef, with wet-ageing for up to 5 weeks prior to freezing. The muscle protein content in this study was measured from the extracted muscle protein solution. The decrease of muscle protein content was more likely to be attributed to the decrease of protein solubility in the extraction buffer due to protein denaturation after long-term frozen storage which has also been reported by Farouk et al. [50].

Frozen storage had the major effect on the instrumental colour of in-bag dry-aged beef (Table 3). All colour attributes have significantly ($p < 0.05$) decreased for in-bag dry-aged samples (7 and 21 d) after the frozen storage. Thawed in-bag dry-aged beef in the current study became darker but still within the consumer acceptable range (approx. 35–40 of L^*) [54]. a^* declined to around 13.0 which was slightly below the threshold of 14.5, according to their study. However, a lesser brown colour (lower hue) as compared to the fresh counterparts was detected. Decrease of hue in the current study was attributed to the significant decrease in b^*. The change in colour observed in this study due to long-term frozen storage could be partially explained by the damage of muscle cells which altered the optical properties of the meat [55]. This may support the loss of lightness and redness of meat colour. The decreases in b^* [52] and hue [50] of beef over the frozen storage have also been reported previously. The decrease in b^* may have been caused by the migration of the oxygenated layer to a deeper position due to the reduced oxygen consumption rate over long-term frozen storage, and this may have resulted in a delay of the oxidation of myoglobin to metmyoglobin. Another possible reason may be that, due to the low fat content of the lean bull beef used in this study, a lower level of lipid oxidation could contribute lesser to the generation of metmyoglobin and yellowness of beef [56]. A significant decrease of L^* and a^* and increase of b^* and hue have also been reported by Kim et al. [12] on dry-ageing/stepwise aged-then-frozen beef loins.

The water-holding capacity of frozen in-bag dry-aged beef decreased due to the extrafluid loss upon thawing (Table 4). Cook loss also tended to increase after frozen storage which may be associated with the decrease of juiciness [57]. However, there was no clear decline in the juiciness rating of frozen in-bag dry-aged samples in this study (Table 7), which was also observed on beef [52] and lamb of other studies [58].

4. Conclusion

The increase of dry-ageing chamber air velocity accelerated the weight loss of in-bag dry-aged lean bull beef but had no other negative effects on meat quality, microbiological safety, and consumer palatability. Ageing time, on the contrary, played a more important role in improving the quality of the dry-aged products. Combining in-bag dry-

ageing with traditional wet-ageing as a stepwise ageing strategy was able to produce dry-aged lean beef of equivalent quality compared to those of dry-ageing only for the same period of ageing time but with lower weight loss/higher yield. In-bag dry-aged lean bull beef products could be long-term frozen stored for up to 12 months and still be acceptable to consumers.

5. Implications of the Study

The following are some of the implications particularly the stepwise ageing process used in the present study:

(i) The process can be applied by the meat industry to shorten the turnover time of the dry-ageing chamber because the wet-ageing component can be accomplished during chill chain distribution without any loss in quality.

(ii) The process produced microbiologically safe dry-aged products with improved ease of handling and potentially free of trimming and increased yield. This enables the meat industry to produce dry-aged products easier, safer, and cheaper for both local and export markets.

(iii) Long-term frozen storage of in-bag dry-aged lean beef produced using the process had no effect on the quality of the thawed product except for the minor discolouration. Thus, postthawing display may not be recommended for long-term frozen in-bag dry-aged lean beef. Exporting the product frozen in vacuum packages or supplying the product pre-cooked in sous vide for local and international restaurants and markets is suggested to retain the value of the in-bag dry-aged products.

(iv) In-bag dry-aged lean beef from the process have potential as a value-added product for the low marbled fresh and frozen beef market locally and globally. Future work regarding the oxidative changes of lipids and proteins, the changes of flavour precursors from the ageing treatments and frozen storage, and their impact on the shelf life and functionality of the products need to be explored.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Review Article

Mechanical and Biochemical Methods for Rigor Measurement: Relationship with Eating Quality

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Meat quality parameters are affected by a complex series of interacting chemical, biochemical, physical, and physiological components that determine not only the suitability for consumption and the conditions for further processing and storage but also consumer acceptability. Deep understanding and careful manipulation of these intrinsic and extrinsic factors have to be taken in account to ensure high quality of meat, with better technological properties and increased safety for consumers. Among meat quality characteristics, meat tenderness has been perceived as the most important factor governing consumer acceptability. Therefore, being able to early predict meat texture and other related parameters in order to guarantee consistent eating quality to the final consumer is one of the most sought-after goals in the meat industry. Accurate measurements of both the biochemical and mechanical characteristics that underpin muscle and its transformation into meat are key factors to an improved understanding of meat quality, but also this early-stage measurements may be useful to develop methods to predict final meat texture. It is the goal of this review to present the available research literature on the historical and contemporary analyses that could be applied in early postmortem stages (pre-rigor and rigor) to determine the biochemical and physical characteristics of the meat that can potentially impact the eating quality.

1. Introduction

Since the 1970s, there has been an academic interest in the management of meat quality with the goal of reducing the inherent variability of the product at retail [1–3]. Consumer perspectives on meat quality are complex, and meat quality is a continuously evolving concept [4]; however, for decades, meat tenderness has been perceived as the most important factor governing consumer acceptability [5], although there is a growing acceptance that other factors, specially flavour, have greater influence than initially thought [6, 7]. More recently, other factors are known to play a role in this arena, such as sustainability [8], animal welfare [9, 10], or healthy lifestyle [11].

Despite breakthrough research and the implementation of the findings to the management of meat quality, surveys from 2017 indicated that considerable variation in the tenderness of beef cuts was still present and the same range of variation was found than in the previous surveys carried out in the past 15 years [12–16]. Reducing this variation is paramount in order to win consumers' confidence [6]. Meat quality in general, particularly textural characteristics, has become so important for the consumers that several countries have included a quality classification in their grading systems: Australia, Canada, Japan, and USA [17]. The quality assessment is usually done on the ribeye by means of marbling determination, but the Australian system (Meat Standard Australia) has also included a cook x cut

based model, taking into account the consumer perception on tenderness, juiciness, flavour liking, and overall liking [18].

There are many pre- and postslaughter factors affecting eating quality attributes of meat. Intrinsic preslaughter factors include genetics, breed, gender, type of muscle, and age of the animal, while the extrinsic encompasses nutrition/dietary management, animal health, animal welfare, stress management, and climate [19–21]. Postslaughter extrinsic factors include slaughter protocol, postslaughter interventions (e.g., temperature control, electrical stimulation, hanging method, or ageing), storage, conservation and display (e.g., packaging), and cooking time/type [22–24],which are some of the main factors.

In this regard, after slaughtering, the most important processes affecting the development of meat texture begin when the blood irrigation stops, triggering the development of the conversion process from muscle to meat [20]. This process has been divided into three main stages: (a) pre-rigor phase; (b) rigor phase; and (c) tenderisation phase (proteolysis by enzymes, such as calpains and cathepsins) [25]; however, the contribution of a fourth step (apoptosis, taking place before the rigor step) has been proposed by Ouali [26].

Briefly, during the pre-rigor and rigor phases, several changes occur within the muscle. Firstly, a depletion of the total available energy takes place in muscle, followed by a shift from aerobic to anaerobic metabolism, which, in turn, leads to the production of lactic acid. Subsequently, there is a rise in the ionic strength of the muscle, due, in part, to the lack of adenosine triphosphate (ATP) which prevents the work of the ion ATP-dependent pumps (calcium, sodium, and potassium) [27]. Finally, the cell is not able to maintain reducing conditions and calcium pumps stop working, inactivating the relaxation capacity of the muscle and establishing rigor mortis [28]. Regarding the tenderisation phase, two main theories have been proposed to explain the changes observed in meat structure and tenderness. The most widely accepted theory is based on the presence of endogenous calcium-activated proteinases (such as calpain and its regulator calpastatin, and cathepsins) which are able to hydrolyse the myofibrillar proteins, giving as result a loss of structure and further tenderisation [29]. To date, the proposed mechanisms of proteolysis have been comprehensively reviewed by others [29–31]. The other theory suggests that the accumulation of calcium ions in the sarcoplasm—due to the lack of activity of ATP-dependent pumps—increases the ionic strength, leading to a solubilisation of the myofibrillar structural elements which will be further degraded [32]. However, none of the theories is currently fully supported by empiric evidence. In any case, it seems clear that proteolysis is, at least in part, one of the main players for postmortem tenderisation. Recent research has demonstrated that degradation of structural proteins such as desmin, nebulin, or titin (which are the substrates of calpains) is highly correlated with the final tenderness [33, 34]. Finally, the role of apoptotic routes has been recently highlighted [35]. Briefly, apoptotic factors such as cytochrome C when overexpressed promote calpain activity, thus improving tenderisation on the aging phase. On the other hand, heat shock proteins (HSP) related to stress have been found to inhibit calpain activity when overexpressed, leading to lesser degree of tenderisation.

In order to measure the meat tenderness of the final cut, several methods have been developed. The most common include both objective (TPA and shear force) and subjective (sensory panel) measurements. However, an early prediction of eating quality during the pre-rigor and rigor phase will have a clear impact on processing, research, and production. In this regard, the present paper wishes to review the methods that have been employed or can be employed during the pre-rigor and rigor phase to evaluate meat characteristics and how they relate to the final meat eating quality, with main focus on textural parameters. We assessed how these methods could be potentiality used as early meat quality/tenderness markers.

These methods can be grouped into biochemical methods, which aim to determine the concentration and activity of molecules and enzymes involved in the glycolysis process during rigor mortis; physical/mechanical methods measuring the textural and mechanical properties of the muscle during this early stage; and finally, rapid non-destructive techniques performed online usually based on image analysis or spectroscopy.

2. Muscle Metabolism, Postmortem Glycolysis, and Meat Quality

In order to fully understand the principles and mechanisms underpinning these methods, it is important to review the biochemical processes at play in the early postmortem stage and the subsequent rigor mortis onset.

Despite the fact that carbohydrates make up a relatively small percentage of muscle tissue (0.5–1.5% of total muscle weight [36]), they play a major role in the process of transforming muscle into meat [37, 38]. Glucose and glycogen are the preferred molecules employed in muscle metabolism and can be both used in either an aerobic (oxidative phosphorylation) or an anaerobic (anaerobic glycolysis) environment [39]. By far, the most important one of the two energy sources is glycogen as its preslaughter concentration (500 mmol/kg dry muscle) is related with pH decline during anaerobic metabolism (due to the accumulation of lactic acid during the transformation of glycogen to ATP). It has been reported that about 45 mmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5 [40]. This value is quite constant, and this process stops when the pH of meat is 5.6 to 5.3 [40], due to the inactivation of involved enzymes; therefore, when the amount of residual glycogen after pH 5.5 has been reached, it can be used to determine the preslaughter glycogen concentration [41].

Glycogen can be found in two forms: (1) as proglycogen (PG), which is smaller (up to 400 kDa) and not soluble in perchloric acid ($HClO_4$) and (2) as macroglycogen (MG), which is larger (approx. 107 kDa) and is $HClO_4$-soluble [42]. The PG degradation rate is higher at normal or high glycogen levels compared to MG [43]. Other sources of energy are phosphocreatine, lactate, fatty acids, and triglycerides. However, lipids and lactate can be employed as source of

energy only under aerobic conditions. In the case of lipids, the efficiency of adenosine triphosphate (ATP) generation is very high, but the rate of conversion into ATP is twice as slow as compared to ATP synthesis from glycogen in aerobic conditions (1.5 vs 3 mmol/kg·s, respectively) [44]. When glycogen is used in anaerobic conditions, the conversion rate increases up to 5 mmol/kg·s [44].

The glycogen content of animals at rest, or just after slaughter, has mostly been estimated by the glycolytic potential (GP). The GP is defined as the ability of the muscle to generate postmortem energy and can be determined by measuring the content of glycogen, glucose, glucose-6-P, and lactate expressed as lactate equivalents as follows [45]:

$$[\mathrm{GP}] = [\mathrm{lactate}] + 2\,([\mathrm{glycogen}] + [\mathrm{glycogen}-6-\mathrm{P}] + [\mathrm{glycogen}]). \quad (1)$$

During muscle function in vivo, myoglobin is responsible for oxygen transportation within the muscle cell. However, the oxygen supply ceases after slaughter and the remaining oxygen is rapidly consumed, resulting in extreme hypoxia conditions. The lack of oxygen activates the anaerobic pathways. Immediately after the oxygen supply has ceased, the muscle goal is to keep the homeostasis and maintain ATP levels. The initial system of ATP production is via the phosphagen system [46], in which a phosphate is transferred from phosphocreatine (PCr) to ADP by the action of creatine kinase; as a result creatine and ATP are generated, but supplies deplete quickly [47]. As the ATP is consumed, the PCr is depleted, while ADP and phosphate are accumulated. As additional source of ATP, it can be generated by the myokinase reaction ($2\mathrm{ADP} \leftrightarrow \mathrm{AMP} + \mathrm{ATP}$). After that, the AMP is rapidly deaminated to generate inosine monophosphate (IMP) by means of AMP deaminase.

Glycogen is then metabolised via anaerobic pathways with the aim of generating the needed ATP but is considerably less efficient compared to aerobic conditions: 38 mol of ATP against only 3 mol of ATP from mol of glucose-1-P from glycogen. Nonetheless, as mentioned before, the conversion rate is better under anaerobic conditions. Glycogen phosphorylase seems to be the rate-limiting enzyme in glycolysis; glycogen concentration, glycogen structure, and glycolytic rate might have a positive association [48]. Energy sources within the muscle (creatine phosphate, glycogen, glucose, ATP, ADP, and adenosine monophosphate (AMP)) are depleted, and lactic acid (from glycogen) and hypoxanthine (from ATP) are produced and accumulated [48]. The combined action of all these enzymes increases the glycogen degradation, yielding lactate and H^+. Finally, the ATP hydrolysis, which yields ADP, phosphate, and H^+, accelerates the pH drop in muscle. All these factors in combination exert an effect on the pH decline, thus affecting the meat quality development [49]. As lactate accumulates, a concomitant drop in muscle pH takes place. Any reaction that modulates the postmortem metabolism by enhancing the ATP production and altering the pH decline will impact on the meat quality development [50].

Once the pH drops to the isoelectric point (pI) of the main proteins, especially myosin (pI = 5.3), a loss in the ability of these proteins to retain the water takes place [51]. The repulsion between the proteins decreases resulting in a tighter packing of the muscle fibres, reducing the space that separates myofibrils. Consequently, fluids between myofilaments are forced into the extramyofibrillar space, which is held in place by capillary forces [52]. The ability of meat to retain this fluid when a force is applied is termed as the water holding capacity (WHC) of meat [53], being an important attribute from a meat quality standpoint as it is related with textural and sensory characteristics such as tenderness and juiciness, among others. Factors that alter the gap between the fibres can affect the amount of water that is immobilised. The main extrinsic and intrinsic factors affecting the spatial order of the proteins include (1) pH changes; (2) the presence of divalent cations (Mg and Ca); (3) denaturing conditions (rapid pH declines while temperature is still high); and (4) the presence of plasticising agents such as ATP, enzymes as ATPase, and necessary cofactors that prevents myofibrillar protein crosslinking [28, 54]. Immediately after slaughter, meat has excellent WHC due to the combination of high pH and high levels of ATP. However, over the subsequent 12–24 h, WHC decreases because of the enzymatic breaking down of ATP and the muscle acidification due to lactic acid accumulation. Rapid postmortem glycolysis is led by alterations in carcass temperature (which can reach values of 42°C in the first 45–65 minutes after slaughter) and is related with high drip loss, poor WHC, and pale colour. These conditions together generate what is known as pale, soft, and exudative (PSE) meat. Even if the final pH is within the normal range, WHC is too low in these cases. It has been reported [55] that high stress conditions before slaughter decreased muscle glycolytic potential, leading to increases in plasma lactate, muscle temperature, cortisol, rate of pH decline, ultimate pH, and yellowness of meat [55].

With ATP becoming depleted, the ionic equilibrium in the muscle cell is disrupted due to Na/K being unbalanced and Ca pumps interrupting the cellular activity. A relaxed animal contains approximately 4–10 mmol ATP per kg of muscle [48]. After slaughter, the subsequent anaerobic glycogenolysis keeps the ATP concentration constant for a short time. When glycogen reaches around 50% of its original value, the ATP level starts to fall along with the pH [48]. When the temperature drops below 15°C and the ATP concentration is under a certain level, the calcium pump is unable to remove the calcium from the sarcoplasmatic reticulum, resulting in a build-up of calcium. This prevents proteins related with muscle movement—actin and troponin—from interacting, since the myosin will not be able to release from actin, impeding muscle relaxation, and therefore, the fibres will remain contracted. When the pH of meat drops to 5.9 and ATP is lower than 1 μmol/g, rigor mortis commences.

It is then clear how the conversion of muscle to meat is a key process that affects several meat eating quality attributes. A successful determination of the compounds involved in this conversion can lead to a better understanding of not

only the factors affecting the process per se but also the relationship with endpoint eating quality attributes such as juiciness, tenderness, and chewiness. In the next section, we review how these metabolites can be determined.

3. Biochemical Methods: Quantification of Molecules and Metabolites from the Glycolytic Pathway

In order to early predict meat quality characteristics such as texture, numerous methods have been developed with the aim of determining the concentration and activity of the molecules and enzymes involved in the glycolysis process. A scheme showing the relationships between the different metabolic pathways; analytes and enzymes involved; pH values; ATP, inosine, glycogen, glucose, and lactate levels; and meat defects is presented in Figure 1. The analysis of metabolites as a predictive tool to estimate the final tenderness is still not having high correlation values, as stated by Muchenje et al. [56] and Lahucky et al. [57]. For instance, these authors found poor correlation between objective texture measurements (Warner–Bratzler shear force (WBSF)) and glycogen amount premortem and at 1 h, 3 h, and 48 h postmortem. However, the molecules and metabolites from the glycolytic pathway involved in the rigor and pre-rigor are clearly related with meat quality as they have good correlation with both PSE and DFD (dark, firm, and dry) meats; they can be useful as meat quality defect biomarkers. The presence of meat defects causes important financial loss to the meat industry [13–16]; in this regard, DFD defects have been reported in the main meat-production species with exception of horse [58], while PSE meat has been reported in pig, cattle [59], turkey [60], chicken [61], and ostrich [62].

Numerous biochemical measurements have been developed with the aim of determining the concentration and activity of the molecules and enzymes involved in the glycolysis process, and these methods could be deemed indirect measures of meat quality, as they involve analysing biochemical markers of the ongoing muscle-to-meat conversion. Even though the analysis of these metabolites has not showed a significant correlation with objective tenderness, their involvement in the muscle-to-meat conversion cannot be neglected. While correlations of single metabolites have not been successful, better results are to be expected from a more holistic approach. Furthermore, in addition to their ability to predict meat quality defects, there is still room for research on their capacities to predict subjective eating quality measurements. Objective texture measurements do not explain full meat eating quality characteristics.

The most common methods to determine the glycolytic potential and the activity of the most important enzymes involved in the process of glycogen metabolism under anaerobic conditions are listed below. The methods are divided into three groups: (1) quantification of molecules and metabolites; (2) enzymatic activity assays (Tables 1 and 2, respectively); and finally (3) new online methods.

3.1. Quantification of Molecules and Metabolites

3.1.1. Glycogen, Reducing and Phosphorylated Sugars, and Lactate. Table 1 gives a general overview of many of the methods currently used for analysing the products of postmortem glycolysis. As a general rule, samples must be immediately frozen using liquid nitrogen and then stored at temperatures around −80°C. This is to halt enzymatic reactions and to try to preserve the actual amount of glycogen, glucose, and other compounds of interest. Then, depending on the analyte, an extraction process must be carried out which typically involves a deproteinisation step using $HClO_4$. This is the underlying approach carried out by Choe et al. [63] modified from Dreiling et al. [64], in their determination of glycogen, who describe their extracted supernatants (0.4 mL) being combined with iodine solutions (2.6 mL) before determining concentrations from a glycogen standard curve. Alternatively, Chan and Exton [65] describe the measurement of initial glycogen through stoichiometry by determining both glycogen and glucose contents. In this two-stage approach, glycogen was extracted after washing in cold ethanol in stage one, while an enzyme digestion was employed to extract the glucose before being determined by Glucose PAP kit (spectrophotometric assay based on glucose oxidase and Trinder reaction where the end product is quinoneimine dye [66]). The determination of the two forms of glycogen (PG and MG) was described by Ferguson et al. [43]. After the extraction, the supernatant (100 μl) was used for the determination of MG and the pellet was used for PG. Further incubation, neutralization, and centrifugation steps occurred before the total glucose concentration of the two fractions was determined. Choe et al. [63] and Apaoblaza et al. [67] described the determination of lactate using commercial kits (Boeringer-Mannheim, Germany, and liquiform lactate, Labtest Ref. 116, respectively), while Henckel et al. [68] terminated the extraction prior to centrifugation by adding 1 ml of 2M $KHCO_3$, before measuring the lactate content by the method of Passonneau and Lowry [69]. For the determination of reducing and phosphorylated sugars, Aliani et al. [70] described the combination of two experiments to facilitate a common extraction procedure for sugars and nucleotides. This enabled one extract to be used for all these analytes, economising on chemicals and time. Aliquots of extracted chicken muscle filtrate (Table 1) were resin-treated to remove any interfering compounds and analysed (as described by Aliani and Farmer [76] using a modified resin, i.e., Marathon WBA ion-exchange resin). A secondary aliquot (1.5 ml) was used to analyse phosphorylated sugars by enzymatic treatment using sodium carbonate buffer (50 mM containing MgCl2 0.5 mM at pH 9.2) to improve peak separation.

3.1.2. Nucleotides, Glycolytic Rate, and Minerals. The nucleotide ATP is the main source of energy for the muscle and the driver of the Na/K and calcium pumps, powering contraction and relaxation of the muscle. In the very early postmortem stage, ATP concentration is apparently stable due to its formation from the creatine phosphate through

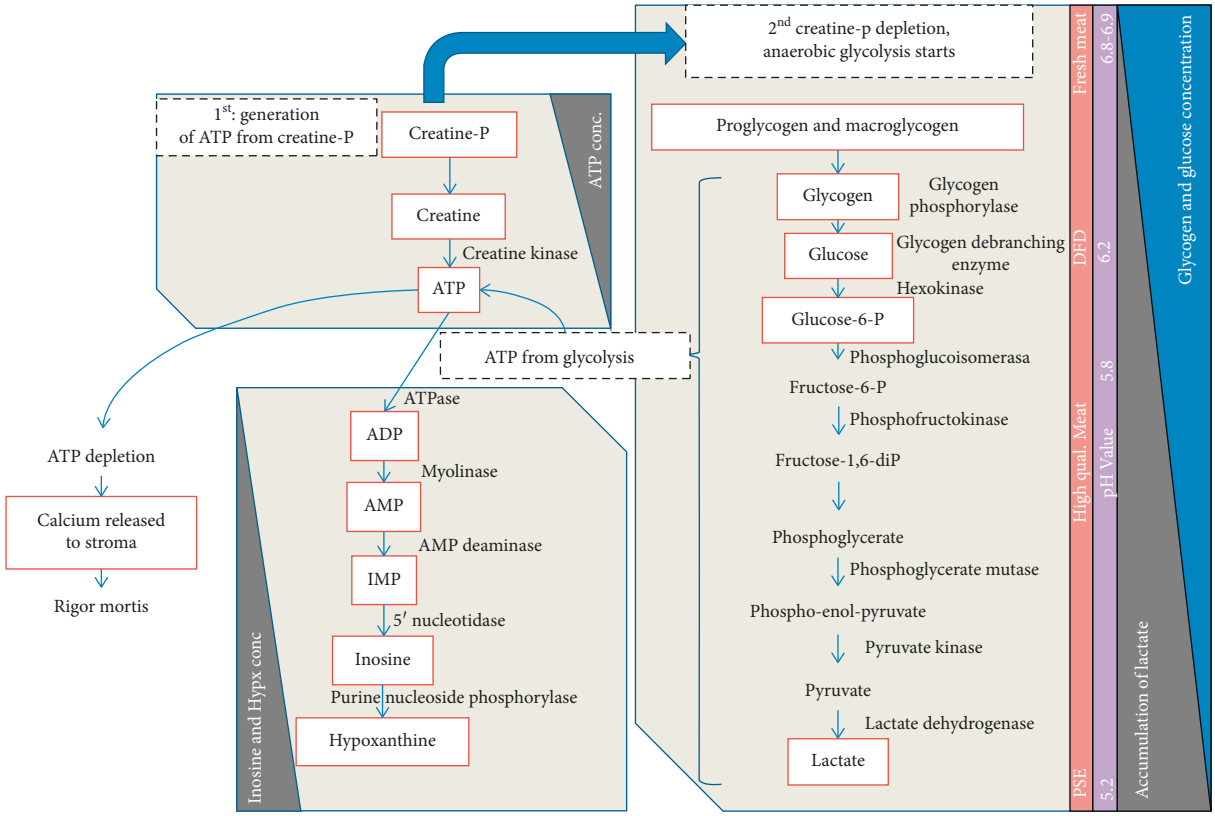

Figure 1: Compounds, enzymes, and metabolic pathways involved in the rigor mortis process. Enzyme activity currently assayed is given in blue letters and compounds currently quantified are in red border.

creatine kinase and anaerobic glycolysis routes. Once these sources are exhausted, ATP concentration starts to drop by converting to other compounds such as ADP, AMP, IMP, inosine, and hypoxanthine. Whereas ADP, AMP, and IDP are intermediates and their concentrations are negligible after 24–48 h and inosine and hypoxanthine experience a substantial increase as muscle is aged. Depletion of ATP in PSE muscle is very rapid, and as commented before, the rate is even faster at high temperatures. England et al. [75] reported that a decreased activity in AMP deaminase could extend the postmortem glycolytic process, which results in a lower pH. Initially, the measurement of pH at 45 min postmortem was considered as a valid detector for PSE meat but, it is now clear, that using this measurement alone leads to gross classification errors. As a result, several methods based on the analysis of nucleotides and nucleosides have been developed in order to predict meat quality. Traditionally, ion-exchange chromatography was used to analyse ribonucleotides and related compounds (e.g., ATP, ADP, AMP, IMP, inosine, hypoxanthine, and creatine), but this was time-consuming and the resolution was poor. Currently, HPLC coupled to reverse-phase columns have given good analytical separations and recoveries [68, 71].Common to these studies was the use of low molarity phosphate buffers and methanol as solvents and similar column lengths (150–250 mm). Henckel et al. [77] described the use of an isocratic method using a flow gradient (0.5–1.5 mL/min), while Battle et al. [71] and Aliani et al. [70] used solvent gradients for their separations. It has been shown that the ATP level in PSE meat at 4 h postmortem is almost ten times lower than that of normal meat; as a consequence, IMP, inosine, and hypoxanthine are found in higher quantities in PSE meat. Rapid methods based on the measurement of the *R* value have been developed (Table 1). These methods analyse the ratio between inosine-related and adenosine-related compounds, which can be easily measured in meat extracts at 250 and 260 nm [63]. Furthermore, they have shown good correlations with meat quality when meat is analysed within the first 8 hours postmortem. In the absence of ATP, the cessation of the calcium pumps results in a build-up of calcium in the sarcoplasmic reticulum, making muscle relaxation impossible, i.e., onset of rigor mortis. Young & Lyon [32] discussed the extraction and determination of calcium ions using atomic absorption spectroscopy.

3.2. Enzymatic Activity Assay. As it has been stated, besides analysing the content of metabolites which have a role in the postmortem process, determining the activity of the enzymes involved in the transformation of such molecules is essential to understand what the real state of the muscle is, during its transformation into meat. Not all the enzymes involved in the process can be currently analysed; however, available methods for some of them have been reported, for example, glycogen debranching enzyme [67, 74], glycogen

Table 1: Direct chemical measurements of molecules and metabolites involved in post-mortem glycolysis.

Molecule	Physical treatment	Solvent	Centrifuge	Neutralised	Detection (nm)	Ref.
Glycogen	Homogenised: 30–45 s	10 mL $HClO_4$ (9%)	15,000 g 4°C		460	[63, 64]
	Incubated: 100°C–15 min	400 μl KOH (30%)	—			
	Digested: amyloglucosidase (1 mL) Incubated: 37°C–2 h	$C_2H_3NaO_2$ buffer pH 4.8	—		505	[65, 66]
Proglycogen and macroglycogen	Incubated: on ice 25 min	3M $HClO_4$	1900 g 20 min 4°C	2 M Trizma base		[43]
Lactate	Homogenised: 30 s	2 ml of 1 M $HClO_4$		KOH (2 M)	340	[63]
	Homogenised	20 mM citrate, 50 mM phosphate buffer, 2.5 mg/ml EDTA, pH 6.8.				[67]
	Incubated: on ice (30 min)	600 μl of 3M $HClO_4$	1500 g 10 min 4°C			[68, 69]
Reducing and phosphorylated sugars	Homogenised: 2 min Ultra Turrax	6 ml of $HClO_4$ (0.6 M), deionised water (1.0 ml); 0.5 ml 20 mM rhamnose and 10 mM cytidine as IS for sugar and ribonucleotide	3000 g 15 min 4°C	KOH (~0.8 ml of 4 M) (supernatant)		[70]
Ribonucleotides: ATP, ADP, AMP, IMP	Homogenised: 4 min, 4°C masticator	15 mL cold 0.6 M $HClO_4$	15000 g 20 min 4°C	Solid potassium carbonate	254, 280	[71]
	Incubated: on ice (15 min)	600 μl of ice-cold 0.6 M $HClO_4$ containing bromthymol blue and phenolphthalein 0.004%	10,600 g 5 min 4°C	540 μl of ice-cold 0.8 M KOH and 25 ml ice-cold 25% KH_2PO_4 buffer	210	[68]
	Homogenised: 2 min full-speed Ultra Turrax	6 ml $HClO_4$ (0.6 M) deionised water (1.0 ml)	3000 g 15 min 4°C	KOH (~0.8 ml of 4 M) (supernatant)	254	[70]
	Homogenised: 1 min full-speed Virtis Macro 45	20 ml $HClO_4$ 0.9 M	3000 g 10 min	2M potassium hydroxide	Several wavelengths	[72, 73]
Glycolytic rate	Homogenised: 5000 rpm for 90 s (Ace homogeniser AM-8)	6% $HClO_4$	3000 g 10 min 4°C		Ratio of absorbance: 250/260	[63, 73]
Creatine, creatine phosphate	Incubated: on ice (15 min)	600 μl of ice-cold 0.6 M $HClO_4$ containing bromthymolblue and phenolphthalein 0.004%	10,600 g 5 min, 4°C	540 μl 0.8 M KOH + 25 ml 25% KH_2PO^4 buffer ice-cold	210	[68]
Calcium	Ashed: furnace 450°C	HNO_3 and $HClO_4$ digestion			Atomic absorption spectrometry	[32]

phosphorylase [67, 75], AMP kinase (AMPK) [67], phosphofructokinase, pyruvate kinase, AMP deaminase [75], or phosphoglucoisomerase [78]. These methods are summarised in Table 2. Most of these methods are based on the extraction of the active enzyme in an appropriate buffer providing the optimum medium (co-factors and pH stability) to observe the activity of extracted enzymes. A measurement of the "in vitro" activity of these enzymes is then performed using specific subtracts from each of the particular enzymes under study. Subsequently, either the substrate depletion or product formation can be monitored by means of colorimetric methods or by HPLC analysis.

3.3. Online Techniques of Metabolic Pathways Analytes Involved in Pre-Rigor and Rigor Stages. The main problem with

Table 2: Methods employed for enzymatic activity assays.

Enzyme	Homogenization/other physical treatements	Buffer	Assay	Detection (nm)	Ref.
Glycogen debranching enzyme	20 min, 18,000 rpm	137 mM NaCl, 1 mM $MgCl_2$, 1% NP^{40}, 10% glycerol, 2 mM PMSF, 10 mM $Na_4P_2O_7$, 2.5 mM EDTA, 10 μg/ml Aprotinin, 10 μg g/ml Leupotinin, 100 nM·NaF	Glucose release	PAP kit	[67]
	Ultra tTurrax + centrifugation	0.05% $KHCO_3$ + 0.004 M EDTA (pH 7.8 at 25 °C)	Glucose release	Iodine reagent, Abs. 525	[74]
Glycogen phosphorylase	50 mM Tris–HCl buffer, (pH 7.6).	50 mM Tris (pH 7.6), 5 mM imidazole, 2 mM EDTA, 10 mM KH_2PO_4/Na_2HPO_4 (pH 7.6), 1.4 mM mercaptoethanol, 0.6 mM NADP, 0.05 mM glucose-1,6-diphosphate, 2 mM AMP, 0.65 U phosphoglucomutase, 0.28 U G-6-P dehydrogenase	Abs. increase	Abs. 340	[67]
	100 mM K_2HPO_4 ice-cold, pH 7.4	50 mM K_2HPO4, 2 mg/mL glycogen, 1.3 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM NADP, 200 mM MES (pH 6.8), 1 U/mL phosphoglucomutase and 1 U/mL G-6-P dehydrogenase	Abs. increase	Abs. 340	[75]
AMP- kinase					
Phosphofructokinase	100 mM K_2HPO_4 ice-cold, pH 7.4	120 mM MES (pH 6.5), 3.2 mM MgSO4, 2 mM ATP, 0.15 mM AMP, 1 mM NADH, 3 mM fructose 6-phosphate, 2 U/mL triosephosphate isomerase, 1 U/mL glycerol-3-P dehydrogenase and 1 U/mL aldolase		Abs. 340	[75]
Pyruvate kinase	100 mM K_2HPO_4 ice-cold, pH 7.4	120 mM MES (pH 6.5), 100 mM KCl, 10 mM $MgCl_2$, 1.25 mM ADP, 1 mM NADH, 0.5 mM PEP and 2 U/mL lactate dehydrogenase	PEP conc. decrease.	Abs 340	[75]
AMP deaminase	Ffrozen powdered muscle + 90 mM K_2HPO_4 pH 6.5, 180 mM KCl, and 0.1 mM dithiothreitol	200 mM 2-(*N*-morpholino) ethanesulfonic acid 150 mMKCl, and 10 mMAMP at pH 6.5	AMP level	HPLC	[75]
Phosphogluco isomerase	100 mM K_2HPO_4 pH 7.4	200 mM MES, 10 mM Na_2HPO_4, 5 mM MgCl2, 60 mM KCl, 0.5 mM NAD^+, 25 mM glycogen, 25 mM carnosine, 30 mM creatine, 10 mM gG-6-P and 10 mM $C_2H_3NaO_2$ at pH 5.5–6.0	F-6-P level		[75]

the traditional biochemical methods discussed above are that they are time-consuming, and biomarkers should be easy and fast to determine and preferably online. In this regard, later studies based on spectroscopy indicate the possibility to determine early postmortem metabolic analytes using Raman spectroscopy. The research of Scheier et al. [79] on porcine semimembranosus muscle indicates a good agreement between measured biochemical parameters and simulated Raman. In this regard, in the pre-rigor and rigor frame, they were able to find clear signals of phosphocreatine, ATP, IMP, and α-helical proteins (correlated with glycogen to lactate). Sheier et al. [79] provided a first semiquantitative description of the early postmortem Raman spectra of meat which show potential for fast detection of the metabolic state of meat and hence for the identification of meat quality defects.

4. Mechanical Methods

As the muscle enters rigor mortis, it loses its ability to relax (until further enzyme activity degrades the complex), which is of critical importance for meat quality since it increases the toughness of the meat. It is clear that the tenderness and other palatability characteristics depend directly on the rigor mortis state. For this reason, an early mechanical measurement of this phenomenon will help to predict the final meat characteristics, in this case mainly texture-related characteristics. Contrary to biochemical methods, mechanical methods provide a direct

measurement of the meat toughness "at rigor" that is directly related with the final texture and palatability of the steak [80, 81]. Physical methods have been used or have the potential to be used to determine the texture during onset and progress of rigor mortis include elasticity, ultramicroscopic observation, tensile and adhesive properties, myotonometry, isometric tension, NMR, NIR, and sonoelasticity.

These studies have given us a profound understanding of rigor mortis in skeletal muscles, especially "red" muscles, and a technological guidance to control meat quality [82]. However, these studies are usually done on single muscle fibres or single muscle bundles, and thus, it is difficult to give an accurate depiction of rigor mortis for an intact muscle. The advantage of many of the techniques discussed here is that they are not destructive. Damez and Clerjon [83] published a review gathering available biophysical methods related to meat structure. In the present article, we expand the array of mechanical methodologies used for meat quality prediction during rigor mortis.

4.1. Elasticity. Measuring the elastic properties of the muscle was firstly investigated in the 1930s, culminating with the development of a "rigor-meter" instrument. The method is based on the accurate measurement of muscle length extension when a fixed amount of force is applied to the muscle and the extent to which the original length is recovered after the force is removed. Meat rich in ATP is able to endure significant stretching, while maintaining its ability to recover its original shape. However, when ATP becomes depleted, the muscle is only very slightly extensible. Muscle strips are maintained in an anaerobic atmosphere to prevent aerobic surface resynthesis of ATP [84]. Despite this method being created to concretely to evaluate the rigor phase, there have been not studies relating rigor elasticity with the final meat texture.

4.2. Isometric Tension. The principle of isometric tension is the application of a load to a muscle that is too great to be moved, and in response, the muscle generates increasing tension as it attempts to shorten. The isometric tension (IT) is expressed as force per unit area and the muscle shortening (SH) as percentage decrease in the length of the muscle. The isometric tension test was developed by Rheologica Instruments (Lund, Sweden) in conjunction with the Swedish Meat Research Institute. It is a unique analytical instrument (RigoTech Muscle Texture Analyser) with the aim of determining the optimum cooling conditions for meat tenderness. Tornberg et al. [85] describe the collection of data with strips (35 mm; 1.5 ± 2 g) of muscle tissue (M. *longissimus dorsi*). The isometric and isotonic recording components record the readings every 5 min in a closed chamber at a controlled temperature. Regarding the potential of isometric tension measurements as predictive method for final meat quality, a good correlation has been established between isometric measurements and other parameters such as myofibrillar and sarcomere length, a good predictor of meat tenderness [86]. On the other hand, trials comparing muscles with different shortening levels due to differences in temperature found a good correlation between isometric measurement and toughness even after 14 days of storage on some muscles [87]. On the contrary, Devine et al. [88] found that meat going into rigor at around 15°C is equally tender at 14 of ageing, independently of isometric measurements (restrained and unrestrained muscles) implying higher enzymatic importance at these temperatures.

4.3. Myotonometry. Vain et al. [89] describe the use of a myotonometer, a handheld instrument used to grade rigor mortis. The instrument delivers a mechanical impact against the muscle to which there is a damped vibratory response. This information is received and treated mathematically, i.e., the period and logarithmic decrease/decay of the vibrations are found to change with the development of rigor mortis, yielding quantitative information on muscular stiffness. It is particularly useful in determining the time of death. To-date, there is no study relating this technique with final meat quality.

4.4. Ultrasound Technologies. Ultrasonic properties have been used to assess physicochemical properties of many foods including meat. For the quality evaluation of muscle foods, mainly two methods have been employed: ultrasonic spectral analysis [90] and ultrasonic elastography [91]. The former is based on the differences in some ultrasonic parameters, such as velocity, attenuation, and backscatter intensity, due to differences in muscle composition and structure which generate the differences in wave propagation. Ultrasound has been applied to determine meat quality of different individual muscles [92–94], but it has also been used for predicting meat quality in live animals [95, 96] and carcasses [97, 98]. The ultrasound probe placed in the inside of the carcass has proved to reveal promising results about intramuscular fat determination in pork carcasses [98].

Ultrasound elastography originates from the medical diagnostic field; however, it is beginning to emerge as a diagnostic tool for evaluation of the skeletal muscle mechanical properties. Shear wave elastography (SWE) is an ultrasound-based technique that uses the propagation of remotely induced shear waves to characterise the mechanical properties of the tissue. Using the shear wave propagation and the tissue density, the shear modulus is calculated and can be correlated with tensile load. The main drawback of this promising technology is the cost, which at this point is too high for industry application. Eby et al. [99] described an experimental setup combining traditional materials testing techniques with SWE. The tensile test was applied to porcine *brachialis* whole muscle tissue at 1.15% of the initial length per second with simultaneous ultrasound measurements. The orientation of the ultrasound transducer to the muscle fibre direction played a key role in the importance of the obtained data; at 45° and perpendicular to the fibres, the shear waves did not propagate properly, whereas when parallel there was a positive correlation between SWE and the tensile load.

4.5. Sonoelasticity. Sonoelasticity is a nondestructive method that combines ultrasonic waves with Doppler imaging to indicate differences in biological tissues. Ayadi et al.

[100] used this technique on *biceps femoris* muscle of beef to monitor changes during rigor and ageing. The authors applied perturbation (80 and 100 Hz) and ultrasound waves (5 MHz) and recorded different sonoelastic parameters, including velocity and attenuation. In addition, the authors also took linear compression measurements and hourly pH determinations. When these were compared with sonoelasticity parameters, significant and higher correlations were obtained (coefficient of correlation up to 0.97). In the same study, the authors were also able to detect the mechanical textural changes during ageing with the sonoelasticity parameters. To our knowledge, there are no studies relating the results obtained by sonoelasticity in pre-rigor and rigor with final muscle tenderness or other meat quality characteristics.

4.6. Laser Air-Puff Systems. This technique has been adopted from the fruit processing industry, where it is applied for measuring the firmness of peaches or kiwis [101]. The potential application of a laser pressurised air system to assess poultry meat tenderness was investigated by Lee et al. [102]. Lee et al. [102] carried out experiments where raw chicken breasts (deboned at 1.25, 4, or 24 h postmortem) were longitudinally scanned on a conveyor belt by a laser distance sensor in order to obtain overall shape profiles; subsequently, they were scanned again with a pressurised source of air (206.8 kPa). The two resulting profiles were superimposed to quantify the amount of deformation caused by the application of pressurised air. Five parameters including the height and length of each fillet were calculated and used to establish a model to predict tenderness. Even though this technique has only been applied to chicken fillets, there is potential for developing it for implementation in the abattoir line.

4.7. Texturometer Analysis. The use of texture analysers is well documented for meat texture determination in lab [103–105]. However, Li et al. [106] used a texture analyser (TAXT2i, Stable Micro Systems Ltd., Godalming, UK) to monitor changes during rigor mortis or thaw rigor in chicken breast muscle. Plastic film-wrapped samples were penetrated by a cylindrical probe to a distance of 2 mm at a velocity of 1 mm/s and a trigger force of 0.02 N. The probe was held for 48 h, and data were acquired at 0.1 points per second. Representations of the force as function of time provided information of the differences in rigor mortis and thaw rigor at two different temperatures (4°C and 15°C). It was found that, even at lower temperatures where the process of rigor mortis takes longer times, the final contracture reached the same extent than at the higher temperature. When the rigor commences, the sarcomere shortens and the muscle fibre diameter increases and hence the force is higher. When the rigor finishes, the sarcomere length increases and the diameter decreases producing a decrease in the force. Texturometer analyses at pre-rigor and rigor phases have been used exclusively to monitor the process of rigor, and no experiments have been found relating these early measurements with final meat quality parameters.

5. Rapid Nondestructive Techniques Performed Online

The use of rapid nondestructive technologies can be applied to early predict meat quality parameters. They offer the ability to increase the control checks during meat processing and retailing but can also present an opportunity to evaluate meat quality right after slaughter. Apart from the use of Raman spectroscopy (section 3.3.) to determine biochemical parameters, there are a few other technologies based on imaging and spectroscopy that have being recently implemented or are under investigation for the evaluation/prediction of final texture.

5.1. X-Ray Analysis. Two main technologies based on X-ray have been used for meat quality analysis: computed tomography (CT) and dual X-ray absorptiometry (DXA). CT is widely used in the medical field, but it has also been used as a measuring tool of animal body composition (lean, fat, and bone) since the early 80s [107]. It has also been employed for meat quality determination in pigs and lambs by means of intramuscular fat [108, 109]. The main drawbacks of this technique are the generated radiation and the small aperture of the actual devices that make it impossible to scan full bovine carcasses.

DXA couples the information acquired at two energy levels (high and low) and was initially designed for the measurement of bone mineral density but later on was used for body composition. Kröger et al. [110] used DXA to predict tenderness in steaks obtaining a correlation of 0.69. A few years later, a patent was filed regarding the use of X-rays to evaluate meat tenderness in meat products [111]. According to the author of the invention, the meat sample is analysed through an X-ray beam and the amount of radiation transmitted can be related to a characteristic of the shear force measured by Warner Bratzler. Even though this technique has been only applied to meat products, it could be potentially used on carcasses to have an earlier prediction of meat tenderness.

5.2. Video Image Analysis (VIA). VIA systems are based on the differences in light intensity received by a video camera and were developed specifically for beef carcass evaluation. Nonetheless, some specific systems have been also utilised to predict tenderness and marbling in beef [112] and pigs carcasses [113]. Usually in these systems, the camera is located at some distance from the rib eye section.

5.3. NIR and Vis-NIR Spectroscopy. Spectrophotometric methods use probes in contact with or penetrating into the meat [114]. Earlier pre-rigor analysis was applied between 2 and 4 hours postmortem by Rodotten et al. [115] using near-infrared spectroscopy (NIR), and their results showed a poor correlation coefficient with the final texture after 7 days ageing (0.47–0.55). Hoving-Bolink et al. [116] investigated the ability of visible-NIR spectroscopy (1 h postmortem in pork) to predict meat

quality. Their results indicate a poor correlation between NIR and meat quality measurements. Vis-NIR spectroscopy has been also used to predict ultimate pH in beef [117], obtaining a prediction model able to correctly segregate 90 % of the carcasses with high (>5.8) or normal pH (<5.8).

5.4. Hyperspectral Imaging (HIS). HIS is a relatively modern technique that combines imaging and spectroscopic data. Applied on the ribeye surface of a hanging carcass, it was able to predict 14-day aged cooked beef tenderness with an accuracy of 86.7% [118]. pH and drip loss have been also assessed in different muscles and species [119, 120]. As this technique stores enormous amount of data, the processing is paramount. With new data processing techniques and hardware and software improvements, this technique could be a potential technique for meat quality determination at an early stage; however, few experiments have been done during the onset and rigor stages. A thorough review of the application of HIS in red meat has been published by Xiong et al. [121].

6. Conclusion

It is long established that proteolysis is responsible for a considerable portion of postmortem tenderisation. However, of great importance are the stages that precede, namely, pre-rigor and rigor mortis stages. Increasing accuracy and sensitivity of chromatography systems have led to more accurate measurements of the biochemical compounds that underpin the transformation of muscle into meat, leading to a better understanding of their connection with the final meat eating quality attributes, namely, tenderness. Furthermore, the use of increasingly nondestructive mechanical or spectroscopic methods to monitor the physical changes brought on by rigor mortis could be used to evaluate and predict a different array of meat quality attributes. However, despite the high number of techniques reviewed in the present manuscript, there is no particular method (chemical or physical) that can accurately predict the final meat eating quality characteristics in the early postmortem period. Therefore, further research is needed where combinations of the methods here described, the incorporation of emerging tools as proteomics and metabolomics, or their correlation with sensory properties will be of benefit to accurately predict meat eating quality attributes.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Effects of Marinating Breast Muscles of Slaughter Pheasants with Acid Whey, Buttermilk, and Lemon Juice on Quality Parameters and Product Safety

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Along with the growth of the group of consumers paying attention to the relationship between diet and health, there is a trend of interest in natural products and the possibility of their use in meat processing. Raw material used for the study was the breast muscles of pheasants (*Phasianus colchicus*), which were marinated for 24 hours with acid whey, buttermilk, and lemon juice. Physical parameters (marinade absorption, pH, WHC, colour, shear force, texture profile analysis (TPA), and thermal losses) and microbiological parameters (the number of mesophilic aerobic bacteria, Enterobacteriaceae, *Pseudomonas* spp., and lactic acid bacteria) of the nonmarinated and marinated muscles (raw and roasted) were evaluated, and sensory analysis was made. The studies have shown that whey and buttermilk can be used as a natural marinade for marinating pheasant meat, as it ensures microbiological safety of the product and has a positive effect on tenderness (measured by shear force) and chewiness. In the sensory evaluation, it improves the juiciness and tenderness of the roast product and has obtained general acceptability.

1. Introduction

The growing interest in high-quality food makes consumers increasingly pay attention to pheasant meat. The meat of these birds has valuable nutritional, dietary, and sensory values. It is a rich source of protein and micronutrients (especially selenium and phosphorus) and contains small amount of fat with a low content of saturated fatty acids [1, 2]. Due to high levels of vitamins B_6 and B_{12} and niacin in pheasant meat, the meat of these birds can be similar to the meat of chickens, geese, or ducks [3, 4]. However, it still remains a niche product. The factors limiting the production and demand for pheasant meat are the seasonality of production and a small group of farms dealing with their breeding [5]. Consumers are increasingly looking for meat with good and health benefits and are willing to pay a higher price for unique high-quality products.

Meat marinating has been known for many years. The selection of marinade ingredients and marinating techniques are constantly improved so that the product obtained meets the expectations of consumers. Consumers are increasingly aware of diet-related health problems and

therefore demanding natural ingredients which are expected to be safe and health-promoting [6].

Juice of lemon (*Citrus limon*) is commonly used for culinary purposes around the world: used as a food preservative and often used for marinating meat to improve the juiciness and tenderness of meat [7, 8]. Lemon juice contains citric acid, L-ascorbic acid, sugars, proteins, and fibres, as well as B-group vitamins, beta-carotene, macro- and micronutrients, and biologically active ingredients, such as essential oils (mainly limonene), bioflavonoids, phytoncides, and pectins [9].

Acid whey and buttermilk are by-products of the dairy industry which are produced in large quantities during the production of cottage cheese and the production of butter using appropriate cultures of lactic acid bacteria. These products are a source of many valuable components: lactose, calcium and phosphorus compounds, organic acids (including high content of lactic acid), and vitamins [10]. Acid whey has a very high biological value and contains peptides and proteins (α-lactalbumin, β-lactoglobulin, immunoglobulin, bovine serum albumin (BSA), and lactoferrin), of which the most important are α-lactalbumin and β-lactoglobulin. They are characterized by a high nutritional value and have a strong antimicrobial and antioxidant effect [11]. The health and technological properties of whey proteins make it a unique raw material of the food industry. Whey contains high amounts of B-group vitamins (especially B_2 vitamin) and vitamin A and significant amounts of tryptophan and cysteine and mineral compounds [12]. Buttermilk is a dietetic product (fat content does not exceed 1%), distinguished by a high content of lecithin and B-group vitamins. Buttermilk is well tolerated in people with lactose intolerance. The protein content in the product is low but has a high biological value. By-products of the dairy industry, such as whey and buttermilk, are currently used in the production of functional foods and enjoy the interest of the meat industry and consumers in households. The results of the latest scientific research indicate the possibility of using acid whey for marinating beef and pork [12–18] to improve the sensory characteristics and tenderness and inhibit oxidative changes in the meat products. Vlahova-Vangelova et al. [19] showed the beneficial effect of marinating on organoleptic characteristics (smell and consistency) of broiler chicken meat. The lack of information in the scientific literature presenting the use of acid whey and buttermilk for marinating pheasant meat prompted the authors to undertake research in this field.

The aim of this study was to evaluate the use of acid whey, buttermilk, and lemon juice to marinate pheasant breast muscles and the effect on the quality parameters and product safety.

1.1. Practical Applications. Meat of pheasants has valuable nutritional values. The growing interest in high-quality food makes consumers pay attention to the meat of pheasants. The use of whey and buttermilk for marinating pheasant meat had a positive effect on the quality of the product, ensuring full health safety and consumer acceptance. Based on the research results, it can be concluded that acid whey and buttermilk can be used as a marinade for pheasant meat.

2. Materials and Methods

Research material for the study consisted of breast muscles obtained from 16-week-old pheasants (*Phasianus colchicus*). The pheasant carcasses were bought from local farms dealing with the rearing of pheasants. The birds were kept in partially roofed aviaries on a sand-gravel substrate. They were fed *ad libitum*, using in the first 4 weeks of life complete commercial feed mixtures containing 28.0% of total protein and 11.6 MJ of metabolic energy and up to 10 weeks mixtures containing 21.5% of protein and 12 MJ of metabolic energy. Above that age till the end of rearing, farm fodder was fed, i.e., wheat grains, maize, green forage of grasses and alfalfa, and crumbled carrot and pumpkin. 24 hours after slaughter, skinless breast muscles were manually trimmed from chilled carcasses [20]. Single breast muscles were used in the study. The control group (C) consisted of nonmarinated breast muscles ($n = 40$ left). Before marinating, the muscles were weighed with an accuracy of 0.01 g (balance type ED 423S-0CE, Sartorius Mechatronics, Poland) and individually labelled. The samples were stored at 4°C.

Three acidic marinades were used in the study, which were based on acid whey (W group), buttermilk (B group), and lemon juice (LJ group). The marinades were supplemented with sea salt (1.0%), cane sugar (1.0%), and, in the LJ group, distilled water. The marinades were prepared for 1 hour before using for the study and stored at 4°C. The acid whey and buttermilk came from a local producer of dairy products that were obtained directly from the quark and organic butter production line. The by-products of the dairy industry were subjected to the microbiological control at the manufacturer in accordance with the following standards: PN-EN 12322 : 2005 [21] and PN-EN ISO 11133 : 2014-07 [22], on the mediums *Salmonella-Shigella* Lab-Agar, Violet Red Bile with Lactose Lab-Agar, and Tryptocasein Soy Lab-Agar. The products have a quality control certificate and are on the market. Analysis of the chemical composition of whey and buttermilk was performed with the analyzer of the chemical composition of milk and products, Bentley B-150 (Bentley, USA). The whey contained 0.59% protein, 0.21% fat, 4.65% lactose, and 6.56% dry matter. Buttermilk contained 3.95% protein, 1.87% fat, 4.97% lactose, and 11.96% dry matter. The active acidity in the products was determined by the pH meter FiveEasy Plus FP20 (Mettler Toledo, Switzerland) equipped with an LE438 electrode with an integrated temperature sensor; the pH of the products was similar: whey 4.53 and buttermilk 4.51. The total acidity was determined in accordance with the guidelines of Jemaa et al. [23]: for whey, it was 0.49 (g of lactic acid/l), and for buttermilk, it was 0.87 (g of lactic acid/l). Fresh bio-lemon juice (pH 2.46 ± 0.32) was used which was squeezed by hand. The concentration of lemon juice was designed to correspond to the average pH (4.52) of whey and buttermilk. For this purpose, the fruits were scalded (3 minutes in boiling water), cut with a sterile knife, and filtered. Sea salt and cane sugar were purchased at the organic food store.

The process of marinating consisted in immersion of breast muscles—W group ($n = 40$), B group ($n = 40$), and LJ group ($n = 40$)—in the prepared marinade. The ratio of meat to marinade was set at 1 : 1 (meat : marinade). Samples from the groups were immersed in the marinade inside plastic containers. Breast meat was stored in a refrigerator for 24 hours at 4°C ± 1°C in atmospheric conditions. After the fixed marinating period, the samples were weighed again with an accuracy of 0.01 g.

Nonmarinated (C) and marinated (W and LJ) breast muscles were weighed with an accuracy of 0.1 g and processed using an electric oven at 180°C to achieve a temperature of 80°C ± 2°C inside the muscle sample. The temperature inside the muscles was measured with a digital thermometer with an external K-type thermocouple probe (Therma Plus, England).

To determine the marinade absorption, the control and marinated meat samples were weighed before and after marinating. The marinade absorption was calculated using the following formula: marinade absorption (%) = weight of sample after marinating (g) − weight of sample before marinating (g) × 100/weight of sample before marinating (g). The pH measurements of nonmarinated and marinated breast muscles were made using a dagger electrode, fitted with a pH meter (HI-99163, Hanna, Germany). The sample's water-holding capacity (WHC) determined using the Grau and Hamm [24] method was based on the amount of juice squeezed from it. The colour assessment of the cross-sectional surface of nonmarinated and marinated breast muscles was performed, based on the reflection method, using the Chroma Meter colorimeter (Konica Minolta, Osaka, Japan), fitted with a CR-400 head ($\varnothing = 11$ mm), calibrated with a Konica Minolta calibration plate (observer 2°, illuminant D_{65}, $Y = 93.5$, $x = 0.3160$, and $y = 0.3324$). The reading of the measurement results was achieved in a CIELAB colorimetric system [25], with L^* (lightness), a^* (redness), and b^* (yellowness). Brittleness analysis and texture profile analysis (TPA) were performed on roast control and marinated meat samples. Brittleness was measured based on the shear force (F_{max}), using a Zwick/Roell testing machine BT1-FR1.OTH.D14 (from Zwick GmbH & Co. KG, Ulm, Germany), applying a wide-width Warner–Bratzler V-blade with a head speed of 100 mm·min^{-1} and a 0.2 N precut force. The cutting was carried out on cubes of control and marinated breast muscles with a cross section of 100 mm^2 and a length of 50 mm. Texture profile analysis (TPA) was performed with a CT3 25 texture analyzer (Brookfield, USA) equipped with a cylindrical probe with a diameter of 38.1 mm and a length of 20 mm. A test of double compression of samples to 50% of their height was performed [26]. The texture was determined on nonmarinated and marinated breast muscle samples in the form of 10 mm cubes. The speed of the roll during the test was 2 mm/s, while the gap between pressures was 2 s. The TPA parameters hardness (N), springiness (mm), gumminess (N), and chewiness (MJ) were calculated from the force-time curves recorded for each sample using Texture Pro CT [27]. To assess the post-heat treatment leakage, 30 g of meat samples was weighed, placed, and kneaded in 150 cm^3 beakers and weighed with an accuracy of 0.01 g (balance type ED 423S-0CE, Sartorius Mechatronics, Poland). The samples thus prepared were covered with polyethylene foil and heated in a water bath at 72 ± 2°C for 30 minutes and subsequently cooled [28]. The amount of post-heat treatment leakage is expressed based on the following formula: $W = [(m_1 - m_2) : (m_1 - m_0)] \times 100\%$, where W is the amount of postthermal leakage (%), m_0 is the mass of the empty beaker (g), m_1 is the mass of the beaker with meat before thermal treatment (g), and m_2 is the mass of the beaker with meat after pouring out the leaked meat juice (g). Weight loss (%) was calculated based on the weight difference before and after heat treatment.

An amount of 10 g (10 cm^2) of the breast muscles was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 90 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a stomacher at room temperature. Microbiological analyses were conducted by using standard microbiological methods. Anaerobic plate count (AC) was determined using Tryptocasein Soy Lab-Agar (TSA, Biocorp) after incubation for 48 h at 35°C under aerobic conditions. For *Pseudomonas* spp., 0.1 ml from serial dilutions of meat sample homogenates was spread onto the surface of *Pseudomonas* Isolation Agar (PIA, Oxoid). *Pseudomonas* spp. were counted after incubation for 48 h at 25°C. For lactic acid bacteria, Rogosa and Sharpe Agar (MRS, Oxoid, UK) was inoculated with a 1.0 ml of sample suspension. Inoculated plates were incubated for 48–78 h at 37°C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For Enterobacteriaceae, a 1.0 ml of sample was transferred into 10 ml of molten (45°C) Violet Red Bile Glucose Agar (VRBL, Biocorp). Inoculated plates were incubated at 37°C for 24 h. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation. All tested groups of bacteria were counted in triplicate. Sampling for microbiological assessment after heat treatment was carried out after 24 hours of cold storage in a cooling cabinet (FKv 36110, Liebherr, Germany) at 4°C ± 1°C.

The sensory quality of marinated and nonmarinated pheasant breast muscles was scaled using the method according to Baryłko-Pikielna and Matuszewska [29]. In order to conduct the sensory assessment, the heat-treated samples were cooled to 20°C ± 2°C and cut into 1.5 cm thick slices, perpendicular to the run of meat fibres. They were placed in disposable plastic boxes that were covered with lids. All samples for evaluations were coded individually and given in random order. The sensory evaluation was carried out by a 6-person evaluation team tested in terms of sensitivity and sensory fitness according to ISO 8586-2:2008 [30] and ISO 8587:2006 [31]. The evaluating persons had experience in assessing meat and meat products. A 5-point evaluation was applied with a defined value limit, including the following qualitative indices: odour intensity (very negative (typical) and very strong), flavour intensity (very negative, very sour (typical), and very desirable), odour desirability (not desirable and highly desirable), flavour desirability (not desirable and highly desirable), juiciness

(very dry and very juicy), and tenderness (very hard and very tender). All the evaluations were performed at a sensory laboratory that conformed to all the requirements of the relevant standard [32]. Between each sample test, assessors took a break for 30 s and rinsed their mouths using mineral water.

Results obtained were statistically analysed with the analysis of variance (ANOVA) using the Statistica 13.1 software package [33]. The arithmetic mean ($\overline{x}$) and standard deviation (SD) were determined. To indicate the significance of differences between means in groups, Tukey's post hoc test with a level of significance $p < 0.05$ was applied.

3. Results and Discussion

The study showed that the percent marinade absorption significantly ($p < 0.05$) differed among treatments. Marinade absorption ranged from 3.26 to 7.32%. Pheasant breast muscles marinated in buttermilk showed the highest absorption, while those marinated in the lemon juice the lowest absorption (Table 1). Differences in marinade absorption could be due to the density of marinades, differences in the osmotic pressure exerted by different marinade solutions, and also the marinades' pH. Many authors [8, 14, 16, 34, 35] indicate that the acidity of marinated meat depends on the pH of the marinade, as confirmed in the present study. The pH value of raw pheasant breast muscles marinated with acid whey and buttermilk was significantly ($p < 0.05$) lower than that of those marinated with lemon juice. This result was unexpected because the pH of the marinades used was the same. The difference in pH may have resulted from the microbial load of the dairy industry by-products that could produce lactic acid. Water-holding capacity of meat is the ability to maintain its own and added water. In the case of marinated meat, water absorption depends on the composition of the marinade [36]. Meat with higher water-holding capacity loses less juice during thermal processing, which may affect its juiciness. In the present study, it was shown that pheasant breast muscles marinated in buttermilk and whey were characterized by a higher ($p < 0.05$) water absorption, while those marinated in lemon juice by a lower absorption. Increase in the moisture content might be attributed to marinade absorption [37]. In the present study, it was shown that marinating significantly affected ($p < 0.05$) the brightening of colour of raw and heat-treated pheasant breast muscles (increase in parameter L^*) in comparison to nonmarinated muscles (Table 1). On the contrary, no significant differences ($p > 0.05$) of the brightness parameter L^* between the marinades used were found. The brightening of colour of the pheasant breast muscles marinated in acidic marinades could be due to decrease in its pH and a higher amount of extracellular water introduced into the meat during marinating. Serdaroğlu et al. [35] reported that L^* values increased when turkey meat samples were marinated in citric acid. According to these researchers, one possible reason for increased L^* values is that muscle proteins swell and light reflection alters at low pH and ionic strength, resulting in lighter colour. In the study by Vlahova-Vangelova et al. [19], the effect of whey marinade used on the surface colour of raw and grilled breast muscles of broiler chickens was not demonstrated. Kim [14], on the contrary, noted the effect of acid whey on the reduction of the L^* brightness parameter in a raw and heat-treated bovine marinated product. In the present study, it was found that marinating with whey and buttermilk caused a significant increase in yellow colour ($p < 0.05$) of raw pheasant muscles. This tendency was preserved after heat treatment with marinating in whey, while marinating in buttermilk caused an increase in the colour saturation towards red (Table 1). The reduction of the L^* brightness parameter could result from the participation of mineral compounds and sugars present in whey and buttermilk, possibly by alteration of the oxidation state of myoglobin. In the present study, it was shown that marinating had an effect ($p < 0.05$) on the amount of thermal drip, whereas no effect of the marinades used on this characteristic was noted ($p < 0.05$).

Health safety in microbiological terms can be defined as the absence of pathogenic organisms and toxins of microbiological origin in a specified quantity of food [17]. It should be strictly ensured for meat products marinated using natural marinades. In the conducted research, it was found that the use of acid whey and buttermilk for marinating significantly ($p < 0.05$) affected the inhibition of the number of mesophilic aerobic bacteria and *Pseudomonas* spp. in a raw marinated pheasant breast muscle (Table 2). It was also shown that marinating with whey and buttermilk effectively inhibited the growth of bacteria from the family Enterobacteriaceae. The pH reduction caused by organic acid (lactic) was the primary factor that affected the reduction of microorganisms. In an environment with acidic pH, the processes of multiplication of most microorganisms are slowed down [38]. From the microbiological perspective, the antimicrobial substances contained in acid whey and buttermilk, such as organic acids or whey proteins, were responsible for the inhibition of microbial growth. Moreover, it was shown that marinating raw pheasant muscles with the use of whey and buttermilk significantly ($p < 0.05$) contributed to an increase in the number of lactic acid bacteria compared to the control and marinating with the use of lemon juice (Table 2). The growth of lactic acid bacteria in the conducted research, their ability to control the environment and to compete with other microorganisms for amino acids or easily fermentable saccharides, could limit the possibilities for the development of saprophytic and pathogenic bacteria [10]. Vlahova-Vangelova et al. [19] proved that the concentration of acid whey and the time of marinating affected the increase in the population of lactic acid bacteria in the raw breast muscles of broiler chickens. The growth of lactic acid bacteria was noted by Wójciak et al. [15], Wójciak et al. [16], and Wójciak and Dolatowski [39] in a pork product marinated in whey and a model beef product with the addition of sea salt. In the microbiological assessment of pheasant meat after heat treatment and cold storage for 24 hours, no lactic acid bacteria and bacteria from the family Enterobacteriaceae were found (Table 2). It was shown, however, that marinating with whey and buttermilk inhibited the growth of aerobic bacteria and *Pseudomonas*

Table 1: Physical composition of nonmarinated and marinated slaughter pheasants.

Parameter	Nonmarinated	Marinated			SEM
	Group C	Group W	Group B	Group LJ	
Raw marinated					
Marinade absorption (%)	—	$5.07^{b} \pm 0.62$	$7.32^{a} \pm 0.78$	$3.26^{c} \pm 0.51$	0.03
pH	$6.11^{a} \pm 0.03$	$5.49^{c} \pm 0.04$	$5.47^{c} \pm 0.03$	$5.59^{b} \pm 0.08$	0.01
Water-holding capacity (%)	$22.59^{c} \pm 2.56$	$28.73^{a} \pm 2.86$	$30.33^{a} \pm 3.12$	$25.67^{b} \pm 3.56$	0.12
Colour					
L^* (lightness)	$52.10^{b} \pm 3.94$	$53.18^{a} \pm 3.16$	$53.84^{a} \pm 3.10$	$58.89^{a} \pm 2.92$	0.50
a* (redness)	11.20 ± 1.20	11.86 ± 1.65	13.05 ± 1.05	10.86 ± 1.40	0.15
b* (yellowness)	$4.68^{a} \pm 1.25$	$6.11^{b} \pm 1.52$	$5.92^{b} \pm 1.2$	$3.16^{c} \pm 0.92$	0.09
Marinated and roasting					
pH	$6.24^{a} \pm 0.03$	$5.81^{b} \pm 0.03$	$5.83^{b} \pm 0.02$	$5.86^{b} \pm 0.04$	0.08
Colour					
L^* (lightness)	$76.82^{a} \pm 3.79$	$80.04^{b} \pm 3.51$	$79.35^{b} \pm 1.86$	$80.42^{b} \pm 3.82$	0.42
a* (redness)	10.82 ± 1.58	9.58 ± 2.10	10.86 ± 1.80	9.10 ± 1.45	0.12
b* (yellowness)	$10.89^{b} \pm 2.02$	$12.10^{a} \pm 2.05$	$11.48^{b} \pm 1.89$	$9.68^{b} \pm 2.50$	0.28
Treatment leakage (%)	$16.50^{b} \pm 2.60$	$22.29^{a} \pm 2.86$	$23.51^{a} \pm 2.88$	$21.94^{a} \pm 3.85$	0.26

Data are expressed as arithmetic mean ± standard deviation ($\overline{x} \pm s$). Group C: control group, nonmarinated; group W: marinated in acid whey; group B: marinated in butter milk; group LJ: marinated in lemon juice. [a,b,c]Values in rows with different letters differ significantly ($p < 0.05$).

Table 2: Microbiologial composition of nonmarinated and marinated breast muscles of slaughter pheasants.

Parameter (log cfu.g^{-1})	Nonmarinated	Marinated			SEM
	Group C	Group W	Group B	Group LJ	
Raw marinated					
Mesophilic aerobic bacteria	$5.21^{a} \pm 0.04$	$3.46^{b} \pm 0.22$	$3.27^{b} \pm 0.33$	$5.24^{a} \pm 0.17$	0.06
Enterobacteriaceae	4.73 ± 0.01	—	—	4.80 ± 0.02	0.08
Pseudomonas spp.	$5.22^{a} \pm 0.03$	$3.72^{b} \pm 0.09$	$3.94^{b} \pm 0.01$	$5.32^{a} \pm 0.06$	0.09
Lactic acid bacteria	$3.04^{b} \pm 0.10$	$3.72^{a} \pm 0.15$	$3.69^{a} \pm 0.15$	$3.00^{b} \pm 0.12$	0.08
Marinated and roasting					
Mesophilic aerobic bacteria	$2.19^{a} \pm 0.16$	$1.42^{c} \pm 0.18$	$1.65^{b} \pm 0.49$	$1.77^{b} \pm 0.32$	0.03
Enterobacteriaceae	—	—	—	—	—
Pseudomonas spp.	$1.89^{a} \pm 0.58$	$1.35^{b} \pm 0.46$	$1.30^{b} \pm 0.26$	$1.62^{a} \pm 0.32$	0.02
Lactic acid bacteria	—	—	—	—	—

Data are expressed as arithmetic mean ± standard deviation ($\overline{x} \pm s$). Group C: control group, nonmarinated; group W: marinated in acid whey; group B: marinated in butter milk; group LJ: marinated in lemon juice. [a,b,c]Values in rows with different letters differ significantly ($p < 0.05$).

spp., increasing the microbiological safety of the product subjected to heat treatment. In the study by Wójciak et al. [16], it was proved that acid whey can be used effectively to improve microbiological quality without adversely affecting organic sausage quality.

Texture is an important sensory characteristic of muscle foods, such as pheasant and beef. Many studies [8, 14, 16, 17, 35, 40, 41] have indicated that the low meat pH after marinating has positive effects on the texture, which was confirmed in the present study (Table 3). In the instrumental evaluation of the texture of meat, the most commonly used parameter, interdependent with tenderness, is the value of the maximum shear force obtained by the Warner–Bratzler test. The analysis of measurements of the maximum shear force showed a significant ($p < 0.05$) effect of marinating using acidic marinades on the change of mechanical properties of raw and heat-treated meat. However, it was shown that the breast muscles marinated using whey and buttermilk were characterized by a smaller ($p < 0.05$) shear force as compared to those marinated with lemon juice. The results obtained by Ergezer and Gokce [40] showed that the use of lactic acid for marinating turkey breast muscles decreased the value of shear force compared to the control. Kim [14] did not confirm the effect of the use of acid whey in the process of marinating beef on the tenderness of meat measured by shear force. Texture profile analysis (TPA) takes into account the multiparameter properties of the product and mimics the conditions to which the material is subjected throughout the mastication process [27]. Meat texture analysis was based on the measurement of strains occurring during sample compression (Table 3). In the present study, it was shown that marinating had a beneficial effect ($p < 0.05$) on reduction of the hardness of roasted breast muscles of slaughter pheasants compared to the control. Significant statistical differences ($p > 0.05$) were not found between used marinades. The hardness results obtained for the control group coincide with those reported by Kotowicz et al. [2] for slaughter pheasants from aviary breeding. According to Kumar et al. [8], the acid breaks the transversal bounds of collagen, leading to the unstable structure loss of this connective tissue protein. According to Berge et al. [42], the mechanism of the

Table 3: Texture parameters (Warner–Bratzler test and texture profile analysis) of nonmarinated and marinated roasting breast muscles of slaughter pheasants.

Parameter	Nonmarinated	Marinated			SEM
	Group C	Group W	Group B	Group LJ	
Warner–Bratzler shear force (N)	$25.86^{a} \pm 2.86$	$18.26^{c} \pm 2.34$	$19.85^{c} \pm 1.98$	$21.48^{b} \pm 2.02$	0.42
Texture profile analysis (TPA)					
Hardness (N)	$24.48^{a} \pm 3.80$	$17.18^{b}c \pm 2.80$	$16.82^{b} \pm 2.56$	$18.29^{b} \pm 3.20$	0.20
Resilience	0.20 ± 0.05	0.18 ± 0.04	0.17 ± 0.06	0.19 ± 0.04	0.01
Cohesiveness	0.30 ± 0.06	0.23 ± 0.05	0.22 ± 0.05	0.29 ± 0.04	0.02
Springiness (mm)	$2.29^{a} \pm 0.30$	$1.41^{c} \pm 0.28$	$1.47^{c} \pm 0.32$	$1.76^{b} \pm 0.42$	0.08
Chewiness (MJ)	$18.02^{a} \pm 2.56$	$5.40^{c} \pm 1.86$	$5.11^{c} \pm 1.90$	$9.53^{b} \pm 2.56$	0.26

Data are expressed as arithmetic mean ± standard deviation ($\overline{x} \pm s$). Group C: control group, nonmarinated; group W: marinated in acid whey; group B: marinated in butter milk; group LJ: marinated in lemon juice. ^{a,b,c}Values in rows with different letters differ significantly ($p < 0.05$).

Table 4: Sensory properties of nonmarinated and marinated roasting breast muscles of slaughter pheasants.

Parameter (pt)	Nonmarinated	Marinated			SEM
	Group C	Group W	Group B	Group LJ	
Odour intensity	$4.20c \pm 0.42$	$4.48^{b} \pm 0.32$	$4.82^{a} \pm 0.48$	$4.59^{b} \pm 0.36$	0.06
Flavour intensity	$4.00^{c} \pm 0.50$	$4.82^{a} \pm 0.36$	$4.85^{a} \pm 0.40$	$4.64^{b} \pm 0.44$	0.08
Odour desirability	$4.34^{c} \pm 0.76$	$4.52^{b} \pm 0.46$	$4.58^{b} \pm 0.38$	$4.72^{a} \pm 0.50$	0.05
Flavour desirability	$4.10^{c} \pm 0.34$	$4.16^{c} \pm 0.40$	$4.88^{a} \pm 0.36$	$4.56^{b} \pm 0.51$	0.06
Juiciness	$4.12^{c} \pm 0.40$	$4.73^{a} \pm 0.38$	$4.73^{a} \pm 0.44$	$4.46^{b} \pm 0.32$	0.06
Tenderness	$3.80^{c} \pm 0.34$	$4.84^{a} \pm 0.32$	$4.80^{a} \pm 0.48$	$4.48^{b} \pm 0.40$	0.09
General acceptability	$4.10^{c} \pm 0.46$	$4.60^{b} \pm 0.38$	$4.80^{a} \pm 0.42$	$4.56^{b} \pm 0.48$	0.08

Data are expressed as arithmetic mean ± standard deviation ($\overline{x} \pm s$). Group C: control group, nonmarinated; group W: marinated in acid whey; group B: marinated in butter milk; group LJ: marinated in lemon juice. ^{a,b,c}Values in rows with different letters differ significantly ($p < 0.05$). Sensory scale: Odour and flavour intensity: 1, changed; 2: moderately changed; 3, typical, weak; 4, typical, strong; 5, typical, very strong. Odour and flavour desirability: 1, not desirable; 2, fairly desirable; 3, desirable; 4, very desirable; 5, highly desirable. Juiciness: 1, very dry; 2, dry; 3, slightly juicy; 4, juicy; 5, very juicy. Tenderness: 1, very hard; 2, hard; 3, slightly tender; 4, tender; 5, very tender.

tenderising action of acidic marinades is revealed to involve in the weakening of structures due to swelling of the meat and increased conversion of collagen to gelatine at low pH during cooking. In the present study, it was shown that marinating with the use of acid whey and buttermilk caused a significant ($p < 0.05$) decrease in springiness and chewiness compared to the muscles marinated with lemon juice and the control group. Ferysiuk et al. [12] showed that the combination of acid whey and sea salt also had a positive effect on the springiness parameter of the marinated pork product.

The results of the sensory evaluation (Table 4) indicate that the use of marinade has improved the sensory characteristics of pheasant breast muscles compared to the control group. It was shown that the muscles marinated with whey and buttermilk were characterized by significantly higher juiciness and tenderness and lower odour desirability as compared to the muscles marinated using lemon juice. Kim [14] also stated that the use of acid whey for marinating beef improved the tenderness and juiciness of the product compared to the control group. Vlahova-Vangelova et al. [19] showed a beneficial effect of whey marinating (50% whey and 50% water) on the hardness of broiler chicken meat subjected to grilling. In the present study, the buttermilk used for marinating had a beneficial effect on the desirability of flavour of the product that was highly rated by the evaluation panel, which indicates that the buttermilk ingredients had a positive effect on the flavour. On the contrary, the intensity of the flavour was rated the lowest in the product marinated using whey, which did not lower the acceptability of this characteristic in comparison to the nonmarinated pheasant muscles. In the study by Wójciak et al. [16], sensory evaluation revealed that the application of acid whey or set milk as a marinade in production of organic ripening beef enhances the feeling of good smell and sour taste which result from marinating and correctly conducted fermentation and ripening processes. Wójciak et al. [17] proved that the whey used for marinating maturing sausage caused a higher intensity of bitter taste, while the remaining sensory characteristics were rated high. Wójciak et al. [15], using whey and mustard seeds, gained the acidic smell of boiled sausage.

The lack of literature data on the use of buttermilk for marinating meat and the few studies related to the use of whey in marinating meat and products indicate the need to continue research in this area.

4. Conclusions

Based on the obtained results, it can be concluded that acid whey and buttermilk can be used as a pheasant meat marinade to obtain a high-quality product. In the sensory evaluation, marinating with whey and buttermilk increased the juiciness and tenderness of the product compared to those marinated in lemon juice and to the control group. The flavour desirability was rated the highest for the product marinated in buttermilk.

Pheasant meat is not an easy kind of meat for cooking or processing, but it was found that marinating with whey and buttermilk gives positive effect on tenderness as well as chewiness and springiness in the roast product. Marinating also ensured high microbiological quality of the raw and heat-treated product. Both acid whey and buttermilk can be used as a fully biologically safe ingredient in marinades for the meat of pheasant, providing a basis for further research on its use in marinating this kind of meat.

Data Availability

All the numerical data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Research Article

Investigation on Texture Changes and Classification between Cold-Fresh and Freeze-Thawed Tan Mutton

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To study the texture, microstructural changes, and classification of cold-fresh (C-F), freeze-thawed once (F-T_0), and freeze-thawed twice Tan mutton (F-T_t), the aforementioned three types of Tan mutton were subjected to near-infrared hyperspectrum scanning, scanning electron microscopy, and TPA testing. The original spectrum of Tan mutton was obtained at a wavelength range of 900~1,700 nm after hyperspectrum scanning; a spectrum fragment ranging from 918 nm to 1,008 nm was intercepted, and the remaining original spectrum was used as a studied spectrum ("full spectrum" hereafter). The full spectrum was pretreated by SNV (standard normal variate), MSC (multiple scattering correction), and SNV + MSC and then extracted feature wavelengths by SPA (successive projections algorithm) and CARS (competitive adaptive reweighted sampling) algorithm, and 25 feature wavelengths were obtained. By combining these feature wavelengths with classified variables, the SNV + MSC–CARS–PLS-DA (partial least squares-discriminate analysis, PLS-DA) and SNV + MSC–SPA–PLS-DA models for classification of C-F and F-T Tan mutton were established. In contrast, SNV + MSC–CARS–PLS-DA yielded the highest classification rate of 98% and 100% for calibration set and validation set, respectively. The results indicated that the texture and surface microstructure of F-T Tan mutton deteriorated, and more worsely with F-T time. SNV+MSC-CARS-PLS-DA could be well used to classify C-F, F-T0, and F-Tt Tan mutton.

1. Introduction

Tan sheep (Yanchi, Ningxia, China) is a representative meat sheep breed with Chinese regional characteristics, even fat distribution, minimal mutton taste, tender meat, and reasonable nutritional composition [1]. However, due to deep processing and low level of conversion technology, more than 90% of Tan mutton is sold as cold-fresh (C-F) and frozen Tan mutton [2].

Freezing is the most effective means of preservation in the circulation and sale of Tan mutton products [3]. In addition to effectively inhibiting microbial growth and prolonging shelf life, it can easily lead to some unexpected changes in Tan mutton products such as the destruction of microstructure, loss of cell sap, and deterioration of texture. Moreover, the taste, flavor, and nutrient levels decrease after freeze-thaw (F-T) treatment [4]. Criminals use F-T Tan mutton instead of C-F Tan mutton in selling for illegal profit [5].

Therefore, it is critical to develop a feasible and reliable technique that can be used for discrimination between C-F and F-T Tan mutton. In general, it is difficult to differentiate between C-F and F-T Tan mutton due to their similar appearance in shape, color, and texture [6]. To date, correlational analyses have been conducted to investigate the differences between C-F and F-T Tan meat using different approaches, including in enzymatic [7, 8], physiological [9], physical [6, 10], chemical [6, 10, 11, 12], and microbiological [11, 13] methods.

NIRS is a well-consolidated analytical technology that can fuse spectral information with component and surface sample information, thus precisely reflecting the "inside and outside" comprehensive changes [4]. Several hyperspectral techniques have been applied to the field of component analysis and F-T meat classification [14–16].

The hyperspectral imaging technique was investigated for nondestructive determination of refrigeration time and

moisture content in chilled Tan mutton within the wavelength range of 400–1,000 nm [17]. Jin [18] proposed that the BP-ANN (back propagation-artificial neural network) quantitative prediction model for fat and protein content of fresh Tan mutton using the hyperspectral technique (wavelength: 900–1,700 nm), which showed that the PSO-BP-ANN (established based on feature wavelengths extracted by PSO; particle swarm optimization, PSO) allows better prediction for fat and protein content in fresh Tan mutton. Furthermore, PLSR and LDA (linear discriminant analysis) classification models based on nine spectrum input variables (nine feature wavelengths extracted using the PLSR weight method) were applied to roughly discriminate tenderness grades of fresh Tan lamb [19] and achieve the desired discriminant results.

However, despite these investigations, no relevant study on the texture, microstructural changes, and classification of F-T Tan mutton has been conducted, to date. The specific aims of this study were (1) to perform scanning electron microscope (SEM) scanning to analyze the surface microstructural changes of C-F, F-T_0 (freeze-thawed once), and F-T_t (freeze-thawed twice) Tan mutton; (2) to measure the hardness, gumminess, cohesiveness, springiness, chewiness, and resilience of above three types of Tan mutton to analyze their internal textural changes; (3) to collect spectral information and extract feature wavelengths of C-F, F-T_0, and F-T_t Tan mutton; and (4) to establish and select classified models based on feature wavelengths.

2. Materials and Methods

2.1. Sample Preparation. Samples were derived from six-month-old Yanchi (Ningxia, China) Tan mutton. The Tan sheep were slaughtered, and their hindquarters were eviscerated and packed with clean, transparent polyethylene film, and then transported (−4°C, constant temperature) to the laboratory within six hours.

Each eviscerated hindquarter was cut along the vertical muscle fiber direction into 213 pieces of 5 cm × 5 cm × 1 cm (length × width × height) and used as testing samples at room temperature (25°C). The average weight of the sample was approximately 49.50 g, and the weight ranges from 40.68 g to 59.21 g (AB104-N, Mettler Toledo, Shanghai, China).

The above 213 samples were randomly divided into the C-F Tan mutton group (rapid cooling treatment, with center temperature of the sample dropping to 0–4°C within 24 h), F-T_o group (rapid cooling treatment; samples were frozen at −23°C for 36 h and thawed at 25°C for 2 h), and F-T_t group (rapid cooling treatment; F-T_o reprocessed twice, samples were frozen at −23°C for 36 h and thawed at 25°C for 2 h, and then frozen at −23°C for 36 h and thawed at 25°C for 2 h). A total of 210 samples were used for spectral image acquisition and texture measurement, and the remaining three samples were used for scanning electron microscopy.

To avoid the influence of thawing moisture on the absorption efficiency of the sample, filter paper was used to absorb the surface moisture of the sample before the spectral imaging.

2.2. Spectral Image Acquisition. The hyperspectral images of the Tan mutton were acquired using a hyperspectral collecting system (wavelength range: 900–1,700 nm, spectral resolution: 5 nm, 256 bands). The system consisted of a CCD camera (1,004 pixels × 668 pixels, Zelos-285 GV, Kappa Optronics GmbH, Gleichen, Germany), Inspector (N17 E Spectral Imaging, Ltd., Oulu. Finland), slit width: 30 μm, slit length: 9.6 mm, 35 W tungsten lamp light source (HSIA-LS-TDIF, Zolix Instruments Co., Ltd., Beijing, China), electronic control displacement platform (PSA200-11-X), and computer (Lenovo Intel® Core i7-2600CPU@3. 4 GHz, RAM4. 00G) equipped with data acquisition software (SpectrumSENS, Zolix Instruments).

The hyperspectral collecting system was precalibrated before capturing spectral images of the samples. The steps were as follows: all-black images were obtained by covering the camera lens to eliminate the effect of the dark current in the camera. All-white images were obtained by collecting standard whiteboard images to eliminate the influence of uneven distribution of indoor light [20].

Tan mutton samples were placed on a piece of black light-absorbing cloth, the reflection of which was nearly zero at every wavelength, thereby decreasing the influence of reflection of the background during the process of hyperspectral image acquisition. To collect a nondistortional hyperspectral image, the speed of the stepper motor was adjusted to synchronize with the scanning speed. Hence, each sample moved at a constant speed of 10 mm/s over the translation stage. The actual length of the scan line was 80 mm (clear sample images are available). The distance between the surface of the Tan mutton being imaged and the lens and the lamps were fixed at 385 mm and 300 mm, respectively. The exposure time of the CCD camera was set to 10 ms (allows the best definition of the image). Hyperspectral images were acquired within the spectral range of 900~1,700 nm, with 2.97 nm intervals between contiguous bands, with a total of 256 bands. The images were saved in a band-interleaved-by-line (BIL) format.

2.3. Measurement of Textural Indices. To better analyze the internal and external changes of the F-T Tan mutton, mechanical parameters, including hardness, gumminess, cohesiveness, springiness, chewiness, and resilience, were measured after acquiring hyperspectral images.

Fifteen samples were randomly selected from each of the three groups that were placed on the platform of the texture analyzer. The upper surface was a test contact plane, whose direction is parallel to the muscle fiber. Textural indices were measured by compressing four points on a cross equilateral line and one point in the middle position of the upper surface. The mean of five measurements was the final result.

Texture apparatus (TA.XT Plus, SMS Corp., England) equipped with a P/36R probe was implemented by means of a TPA mode. The other parameters were set as follows: falling speed: 1 mm·s^{-1} before trigger; loading rate: 1 mm·s^{-1}; rising speed: 5 mm·s^{-1} after the test; trigger force: 5 g; data acquisition rate: 200 pps; deformation: 30%; and measurement interval time: 5 s. Hardness, gumminess, cohesiveness,

springiness, chewiness, and resilience were obtained by analyzing the strength-time curve.

2.4. Scanning Electron Microscopy. The formation and growth of ice crystals in the muscle cell has a destructive effect on the tissue structure, including the cell membrane [21]. Therefore, it is necessary to analyze the microcosmic structural differences of the F-T Tan mutton.

The three samples that are selected from 213 samples were cut into 3 mm × 1 mm × 1 mm (length × width × height) sample strips along the direction of muscle fiber, then the sample strips were immediately observed to obtain high-definition images via a field emission scanning electron microscope (15 kV : 1 nm: Jsm-7500f03040701, Japan Electronic Co., Ltd.).

The experimental method was based on Haga and Ohashi [22] with minor modification: 0.1 mol/L phosphate buffer (pH 7) was used to prepare the glutaraldehyde solution and formaldehyde solution, with a volume fraction of 2.5% and 4%, respectively. The two solutions were mixed at a 1 : 1 ratio and used in fixing the samples for 24 h at 4°C. After fixation, the samples were rinsed with 0.1 mol/L phosphate buffer (pH 7) for thrive, for 30 min each wash. The samples were then fixed in 1% osmium tetroxide solution (0.1 mol/L phosphate buffer, pH 7) at 4°C for 2 h, then rinsed with distilled water thrice, and then dehydrated across an ethanol gradient (30%, 70%, and 100%). The samples were observed and pasted on the SEM sample table after freeze-drying (ES-2030 HITACHI) and sprayed using an ion sputtering film apparatus (E-1010 Giko). The samples were observed and photographed under a SEM.

2.5. Spectral Image Preprocessing. A total of 210 hyperspectral cube images (including two-dimensional spatial and spectral information) were collected based on the parameters described in Section 2.2.

To eliminate the uneven intensity distribution of light sources in each band and the nonspectral information introduced by sensor responses, raw images (R_0) were corrected and images in reflectance mode (R_c) were obtained using the following formula:

$$R(\%) = \frac{R_0 - D}{W - D} \times 100, \quad (1)$$

where D is the dark reference image (~0% reflectance) obtained by covering the camera lens using the opaque cap and W is the white reference image (~100% reflectance) obtained by a standard whiteboard. This step is called image calibration. Here, the dark and white reference images were regularly updated during the experiments.

After image calibration, the hyperspectral images were resized to reduce their irrelevant information. Based on the actual size of the sample, the pixels of the hyperspectral cubic image of each sample were adjusted from 1,004 pixels × 668 pixels to 600 pixels × 480 pixels in ENVI 4.8 (Research System, Inc., Boulder, CO, USA) software, then the whole sample surface (600 pixels × 480 pixels) was selected as the region of interest (ROI), and the average reflection spectrum of ROI was extracted.

The average spectrum was pretreated using SNV, MSC, and SNV + MSC methods to eliminate irrelevant information and noise (e.g., stray light, sample background, and electrical noise) contained in the spectrum as much as possible.

2.6. Extraction of Feature Wavelengths. The pretreated spectrum still contains a lot of redundant information, and the difference in effective spectrum is small, and thus it takes time to establish a model and weakens the performance of the model due to the introduction of invalid information. The CARS and SPA algorithms have their own advantages in selecting feature wavelengths and reducing data collinearity.

The CARS algorithm combines exponentially decreasing function (EDP) with adaptive reweighted sampling (ARS) to select the PLS subset model with the minimum RMSECV (80% of the classified variables that were randomly selected during each selection process to establish the PLS model calibration set model) [23]. The selected PLS subset model consists of optimal classified variables. Thus, the CARS algorithm has a significant advantage in selecting the most effective wavelengths.

The SPA can calculate the projection of the remaining wavelengths of the preselected variable combination and select out the smallest collinearity combination, thereby eliminating multicollinear wavelengths to the maximum extent [24].

2.7. Establishment of the PLS-DA Model. PLS-DA based on partial least squares (PLS) algorithm is powerful in solving nonlinear classification problems, noise reduction, and variable selection [25].

In this study, PLS-DA was applied on a dataset that consists of X and Y matrices. The X matrix consisted of the spectral information [26], with n being the gross of the spectrum and p the quantity of classified variables. The Y matrix contains n rows and three columns that map three classes of Tan mutton, namely, C-F, F-T_o, and F-T_t of the dataset.

The sample set partitioning based on the joint X-Y distance (SPXY) method was used to partition the sample set before establishing PLS-DA, which can calculate each sample distance based on spectral and classified variables individually. The calculated sample distance was divided by its own maximum value so that it equals the weight of the two feature parameters when the sample is selected [27].

In this study, the sample ratio of calibration and validation set is 3 : 1. The distance between samples is defined by the following formula:

$$d_{xy}(i,j) = \frac{d_x(i,j)}{\max_{i,j\in(1,z)}\left[d_x(i,j)\right]} + \frac{d_y(i,j)}{\max_{i,j\in(1,z)}\left[d_y(i,j)\right]}, \quad i,\ j \in [1,z], \quad (2)$$

where $d_x(i, j)$ and $d_y(i, j)$ are the distance of each sample based on spectral and classified variables, respectively. A total of 160 samples were selected to set up the calibration model, and 50 samples were used to validate the prediction performance of the model.

3. Results and Discussion

3.1. Microstructural Analysis of C-F, F-T_o, and F-T_t Tan Mutton. Figure 1 represents the SEM images of C-F, F-T_o, and F-T_t Tan mutton. The muscle fiber surface of C-F Tan mutton is smooth and clear, and the arrangement of muscle fiber is neat and regular. The compact muscle bundle gap of muscle fiber is smaller (Figure 1(a)). After F-T treatment (Figure 1(b)), the structure of the muscle fibers showed slight "deterioration", which includes slight contraction, deformity, slight protrusion on the surface, a decrease in smoothness of the muscle fiber, an increase in the gap among bundles, and variable degrees shape of muscle bundle distortion. These features may be attributable to the elasticity of the connective tissue membrane in muscles, which plays a role in maintaining muscle integrity and preventing muscle fiber damage [28]. During the freezing process, the formation and growth of ice crystals in the muscle cells caused crush damage to the connective tissue membrane. The internal structure integrity of the muscle cells was destroyed [29]. The smaller gaps in the muscle bundles were due to thawing and stiffening, and the sarcomere contracts 36 h after slaughter, thereby resulting in a decrease of muscle fiber gap and an increase of muscle bundle space. In addition, these changes may also be due to cold contraction of the carcass as these were immediately placed in a frozen environment (low temperature stimulates the neural conditioned reflex or the calcium pump in the sarcoplasmic reticulum is sensitive to low temperatures, which results in stronger interactions between actin and myosin) [30].

After F-T_t treatment (Figure 1(c)), the muscle stiff mature tended to be completed and the muscles stiffen within 72 h of slaughter; the muscle fibers are severely deformed, broken, disorganized, and loosely structured, which have resulted from the destruction of myofibrillar skeleton and degradation of intermuscular line protein and troponin T [31].

Texture can directly reflect the mechanical properties and taste changes in C-F, F-T_o, and F-T_t Tan mutton. Table 1 illustrates that the hardness (253.12 N and 221.55 N) and chewiness (118.11 N and 116.33 N) of the F-T_o and F-T_t groups were significantly lower than those of the C-F group (323.70 N and 179.15 N) ($p < 0.05$). There was no significant difference ($p > 0.05$) in springiness between the F-T_t and C-F groups, but was significantly lower than that of the F-T_o group ($p < 0.05$). No significant difference ($p < 0.05$) in cohesiveness among the three groups was observed.

Hardness of Tan mutton relates to the content and distribution of protein and muscle fiber, which decreases with loss of muscle moisture [19]. After F-T treatment, cell structure was destroyed, cell sap was los,t and myofibrillar protein degraded, which resulted in a significant decrease in hardness.

The springiness of Tan mutton increases with moisture content to a certain extent [19]. It does not induce detectable damage to cell structure (compared with Figures 2(a) and 2(b)) and leads to slight exudation of muscle fiber cell sap after F-T_o treatment, which may be the reason why F-T_o Tan mutton's springiness was higher than that of C-F Tan mutton.

The chewiness of Tan mutton is a comprehensive reflection of hardness, cohesiveness, and springiness [32]. Theoretically, springiness and hardness also affect the chewiness of Tan mutton, so the chewiness of C-F Tan mutton was significantly higher than that of the other two groups.

3.2. Spectral Pretreatment. Spectral absorbability is closely related to sample composition [33]. Figure 3 plots the original near-infrared spectral curve of the 210 samples. Due to minimal differences in the main components of C-F, F-T_o, and F-T_t Tan mutton, the trend of the spectral curve of each sample was similar and no distinct abnormal samples were observed.

The spectral curve within the wavelength range of 918~1,008 nm was chaotic because of the low signal-noise ratio of the hyperspectral camera, and it was easy to amplify the noise after calibration [34]. Thus, the spectral fragment ranging from 918 nm to 1,008 nm was intercepted, and the original spectrum containing the 225 bands and ranging from 1,010 nm to 1,678 nm was used for further investigation.

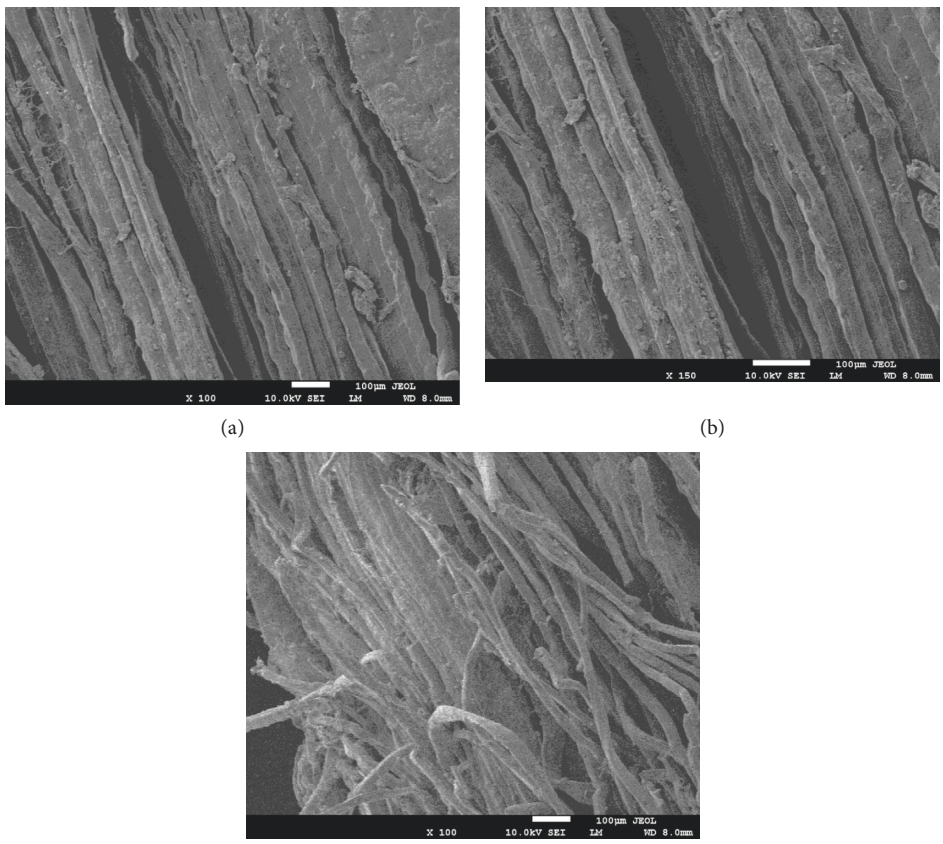
There were less different chemical components with samples from each group, and each chemical component showed relatively stable spectral absorption, which leads to the analogous ROI spectrum for each group of samples. Therefore, the average reflectance spectral curves of ROI ranging from 1,010 nm to 1,678 nm are shown in Figure 2(b), the increase and decrease trend is consistent, and the positions of their peaks and troughs are very close.

Figure 2(b) shows the following:

(1) The ROI spectral curves of C-F, F-T_o, and F-T_t Tan mutton are similar, their trends of increase and decrease are consistent, and the locations of the peaks and troughs are very close. The general trend of the ROI spectral curves shows an oblique "M" shape within 1,065 nm~1,415 nm. Each curve has one peak at 1,150 nm and 1,325 nm, a trough at 1,260 nm, and a gentle curve trend within the spectral range of 1,440 nm to 1,650 nm.

(2) The ROI average spectral reflectance of C-F Tan mutton at 1,010 nm~1,415 nm was higher than that of F-T Tan mutton. A significant difference in ROI average spectral reflectance was observed among the three groups at 1,010 nm~1,177 nm (C-F > F-T_o > F-T_t).

(3) The ROI average spectral reflectance of C-F, F-T_o, and F-T_t Tan mutton had the local minimum values at 1,065 nm, which showed strong absorbability (protein denaturation and hydrolysis due to the



(a) (b) (c)

Figure 1: Scanning electron microscopy of Tan mutton. (a) C-F Tan mutton. (b) F-T_o Tan mutton. (c) F-T_t Tan mutton.

Table 1: Textures of C-F, F-T_o, and F-T_t Tan mutton.

Category	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
C-F Tan mutton	323.7008 ± 39.3047a	0.8749 ± 0.0674b	0.6281 ± 0.0676a	203.8078 ± 36.2140a	179.1529 ± 38.0677a	0.2278 ± 0.0554a
F-T_o Tan mutton	253.1150 ± 49.2373b	1.5866 ± 0.3850a	0.6236 ± 0.0549a	158.6631 ± 38.4950b	118.1115 ± 39.1555b	0.2401 ± 0.0331a
F-T_t Tan mutton	221.5507 ± 54.7750b	0.8081 ± 0.0677b	0.6547 ± 0.0464a	144.4275 ± 32.4243b	116.3317 ± 26.1189b	0.2374 ± 0.0284a

Mean of three replicates ± standard deviation; means in the same column with different letters are significantly different ($p < 0.05$). Reversely no difference ($p > 0.05$).

destruction of cell structure by crystallization, an increase in nitrogen-containing exudates may be related to the third order frequency-doubling of N-H bond) and the local maximum values at 1,150 nm, which showed strong reflectivity (accounting for the water bands after F-T treatments, which was possibly due to the water loss after being thawed).

SNV, MSC, and SNV + MSC were applied to pretreat the original spectrum. Comparative analysis indicated that SNV + MSC is the best method, owing to the better performance of established PLS model based on spectral pretreatment by SNV + MSC. This may be due to an amount of spectrum noise being removed and increasing with the differences among original spectral variables after SNV + MSC pretreatment, which improves the robustness and performance of the PLS model. Therefore, the spectrum after SNV + MSC pretreatment (Figure 2(c)) was applied for further investigation.

3.3. Extraction of Feature Wavelengths. Before running CARS, the optimal principal component number of the PLS model was first determined. The maximum principal component number was set to 40, and the sampling number of Monte Carlo was 200. Three quarters of the total samples were used as the calibration set, and the RMSECV under different the principal component numbers were obtained (Figure 3). When the principal component number is 18, the minimum RMSECV is 0. 2674, so the optimal principal component number was 18.

Setting CARS running parameters: The Monte Carlo sampling number is 200, the principal component number was 18, and cross-validation group number was 10. The CARS running results are shown in Figures 4(a)–4(c), which represent the changes in the number of sampled variables, the RMSECV of 10-fold cross validation and regression coefficient with the increasing number of MC sampling runs.

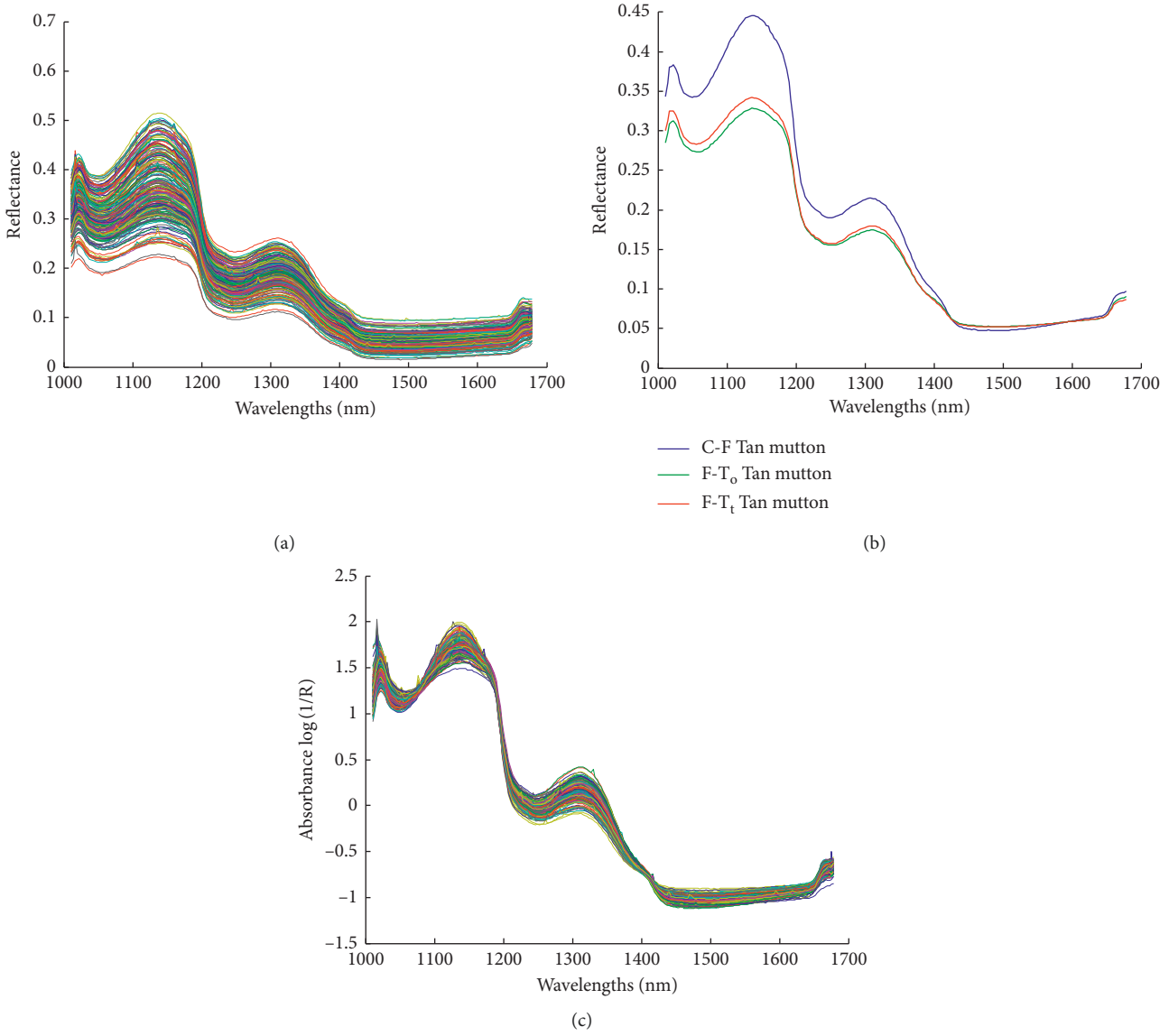


Figure 2: Spectral images. (a) Near-infrared original spectrum. (b) ROI average reflectance spectrum. (c) Spectrum preprocessed by SNV + MSC.

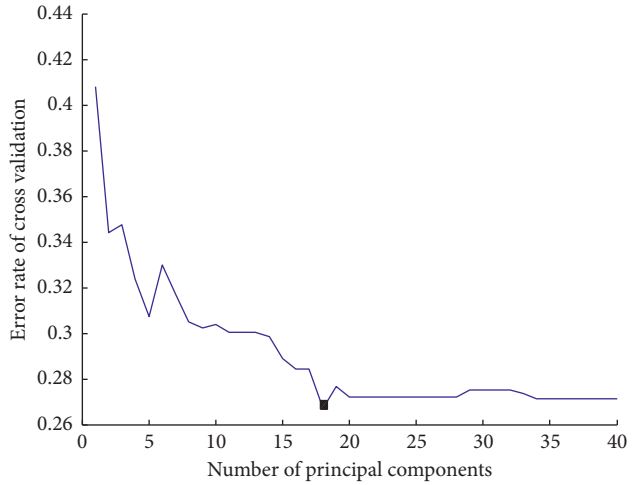


Figure 3: Variation in RMSECV values with principal numbers of the PLS model.

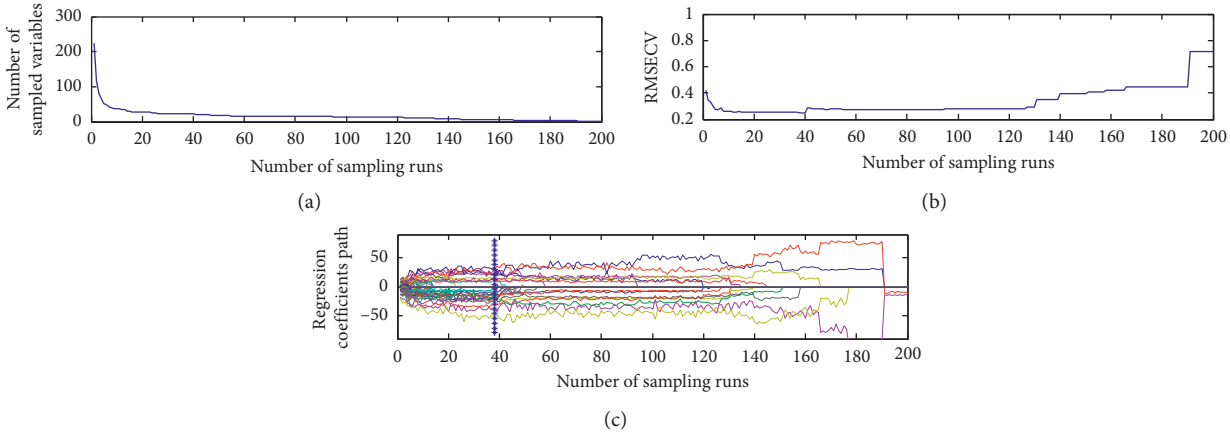


Figure 4: The result of feature wavelengths extracted by CARS.

Figure 4(a) shows that a rapid reduction in the number of sampled variables was observed in the first four MC sampling runs, and then gradually decreased, which shows the "rough" and "elaborate" selection process of key variables based on EDP [23].

Figure 4(b) shows that the RMSECV of the established PLS model that was based on selected variables becomes smaller with increasing MC sampling time in the first 38 MC sampling runs, which indicates that a large amount of irrelevant and collinear information was eliminated. When the sampling run is 38 (located in the asterisk vertical line in Figure 4(c)), the RMSECV reaches a minimum value 0.2490. After 38 MC sampling runs, the RMSECV gradually increased with more MC sampling runs, which may be due to the removal of some important information from classified variables. Therefore, 23 feature wavelengths were obtained as follows: 1,052 nm, 1,062 nm, 1,130 nm, 1,169 nm, 1,205 nm, 1,220 nm, 1,240 nm, 1,300 nm, 1,324 nm, 1,327 nm, 1,357 nm, 1,366 nm, 1,372 nm, 1,398 nm, 1,401 nm, 1,413 nm, 1,443 nm, 1,488 nm, 1,544 nm, 1,571 nm, 1,628 nm, 1,643 nm, and 1,655 nm.

To identify the optimal method for screening feature variables, the SPA is also used as a comparison algorithm to extract the feature wavelengths from 1,010 nm to 1,678 nm so that it can minimize the collinearity among selected variables. The SPA running parameters were set as follows: the selected number of feature wavelengths ranged from 1 to 35, and the step length was 1. The results are shown in Figure 5. Figure 5(a) shows that RMSE varies with the number of variables included in the model, which indicates the dynamically eliminated process of collinear variables. When the RMSE was 0.2560, the number of collinear variables included in model was lowest, and 23 feature wavelengths were selected from Figure 5(b): 1,011 nm, 1,017 nm, 1,032 nm, 1,053 nm, 1,056 nm, 1,076 nm, 1,080 nm, 1,083 nm, 1,094 nm, 1,106 nm, 1,136 nm, 1,160 nm, 1,181 nm, 1,190 nm, 1,226 nm, 1,243 nm, 1,261 nm, 1,401 nm, 1,410 nm, 1,479 nm, 1,595 nm, 1,643 nm, and 1,673 nm.

3.4. Establishment of the PLS-DA Classified Model. PLS-DA is a regression model based on PLS, which integrates classified variables with spectral variables. In this study, 23 feature wavelengths were, respectively, selected using the CARS and SPA algorithms as spectral variables to establish a PLS-DA classified model. The number of optimal principal components of the SNV + MSC–FS–PLS-DA, SNV + MSC–CARS–PLS-DA, and SNV + MSC–SPA–PLS-DA models were determined to be 14%, 13%, and 14%, respectively, based on the minimum RMSECV before modeling.

PLS-DA classified models based on different pretreatment methods of C-F, F-T_o, and F-T_t Tan mutton were established as shown in Table 2.

Table 2 shows that calibration and validation set accuracy of SNV + MSC–FS–PLS-DA based on the full spectrum removal of the noise band, which ranges from 1,010 nm to 1,678 nm, can be up to 99% and 100%, respectively, which shows that the full spectrum containing the 225 wavelength variables well preserves the effective information that was used in the model. The cross-validation error rate (7%) of the model was lower than that of SNV + MSC–CARS–PLS-DA, which indicates the existence of invalid spectral variables among the 225 wavelengths. The variables selected using the CARS and SPA method only account for 10.2% of the full spectral variables, which indicates that these two methods can reduce the dimensions of the spectrum. The cross-validation error rate of SNV + MSC–CARS–PLS-DA was only 3% (well below 7%), and the calibration and validation set accuracy was 98% and 100%, respectively. Its stability was better than SNV + MSC–FS–PLS-DA and SNV + MSC–SPA–PLS-DA as fewer optimal principal components were required for the former, which illustrates that the CARS algorithm can dispel redundant and irrelevant variables on the premise of maintaining better performance of the model. With fewer variables needed for modeling, the calculated speed of the SNV + MSC–CARS–PLS-DA model also increased. In contrast, the cross-validation error rate of SNV + MSC–SPA–PLS-DA, respectively, decreased by 5% and 4%, and the error rate of cross validation was higher than that of the other models.

Therefore, we concluded that CARS is a better method for extracting feature variables than SPA, with SNV + MSC–CARS–PLS-DA better than SNV + MSC–FS–PLS-DA and SNV + MSC–SPA–PLS-DA. In addition, as

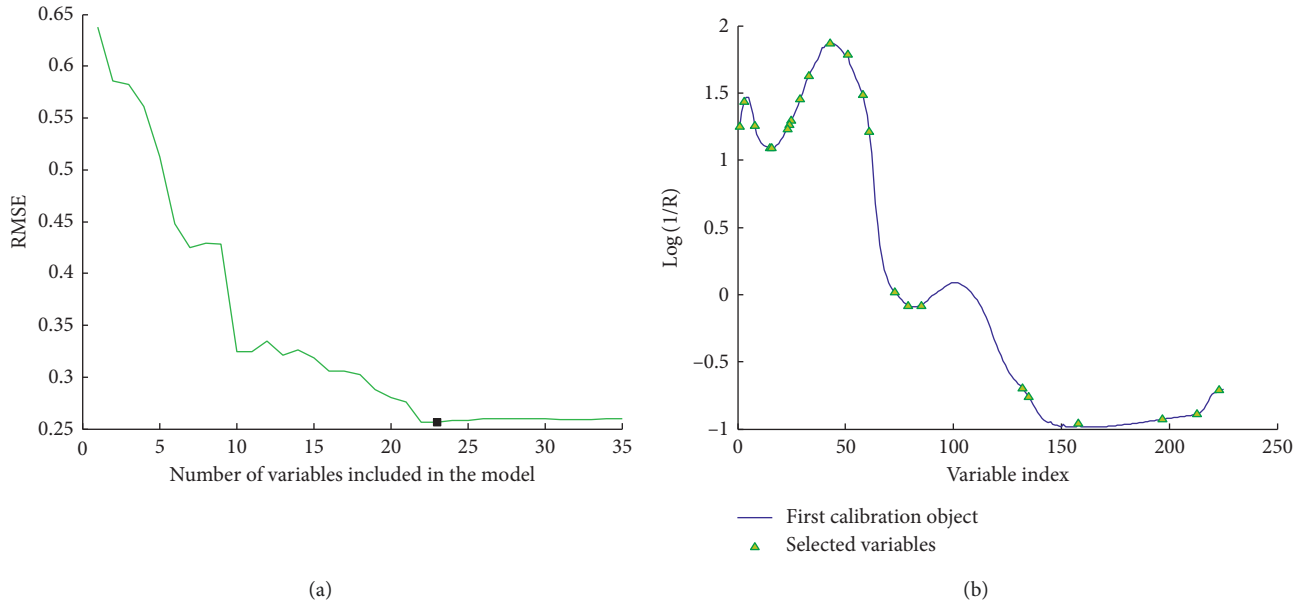


Figure 5: Feature wavelength extraction by SPA. (a) RMSE varies with the number of variables included in the model. (b) Feature wavelength extraction by SPA.

Table 2: Classified models of C-F, F-T_o, and F-T_t Tan mutton.

Modeling method	Wavelength number	Number of principal components	Cross-validation error rate	Calibration set accuracy	Validation set accuracy
SNV + MSC–FS–PLS-DA	225	14	0.07	0.99	1.00
SNV + MSC–CARS–PLS-DA	23	13	0.03	0.98	1.00
SNV + MSC–SPA–PLS-DA	23	14	0.09	0.94	0.96

shown in Figure 6, C-F, F-T_o, and F-T_t Tan mutton can be well classified in the calibration and validation sets of SNV + MSC–CARS–PLS-DA.

4. Conclusions

Freezing is currently one of the most widely used methods to extend the shelf life and maintain the quality of Tan mutton. This study explicitly showed that the surface microstructure, texture (including hardness, gumminess, springiness, and chewiness) of C-F Tan mutton extensively deteriorated after F-T treatment, and further degradation occurred with increasing F-T time. The above changes during F-T storage results in a substantial decrease in the quality, taste, and commodity value of C-F Tan mutton.

To classify C-F and F-T Tan mutton and scientifically guide its storage and sale in the market, this study conducted a preliminary investigation on the internal and external changes in Tan mutton after F-T treatment. The hyperspectral data of C-F, F-T_o, and F-T_t Tan mutton were collected, pretreated by SNV, MSC, and SNV + MSC, and then feature spectral variables were extracted by CARS and SPA algorithms at 1,010~1,678 nm. SNV + MSC–CARS–PLS-DA based on 23 feature wavelengths was established and has superiorly classified performance (calibration set accuracy: 98%) and the fastest computation speed.

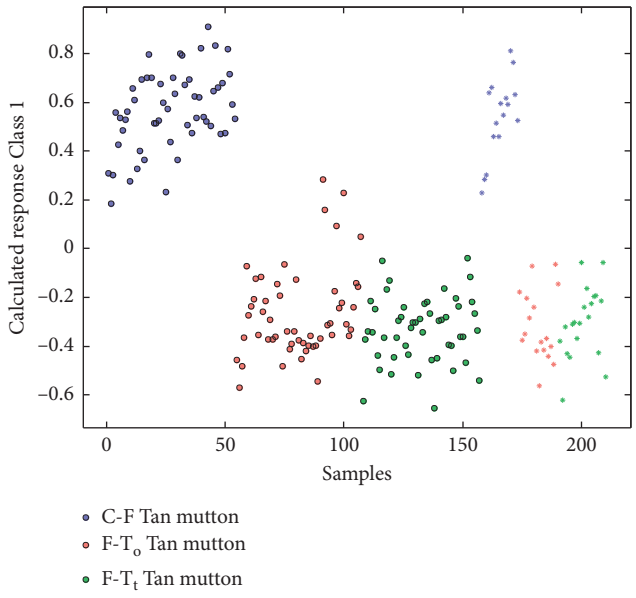


Figure 6: SNV + MSC–CARS–PLS-DA classification model.

There are also other changes in the F-T process such as fat degradation, protein denaturation, soluble nutrient loss, and surface chroma variations (including L^*, a^*, and b^*). Additional comprehensive studies on the above changes in

F-T mutton are warranted and, in combination with the results of this study, may accomplish quality classification of Tan mutton based on different F-T times.

Data Availability

The authors and affiliated agency allow disclosing the data to the public. The primary data used to support the findings of this study are available from the corresponding author or co-first author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Review of Biological and Chemical Health Risks Associated with Pork Consumption in Vietnam: Major Pathogens and Hazards Identified in Southeast Asia

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Foodborne illness is a difficult public health burden to measure, with accurate incidence data usually evading disease surveillance systems. Yet, the global scope of foodborne disease and its impacts on socioeconomic development make it an important health risk to address, particularly in low- and middle-income countries. In Vietnam, rapid development has seen large-scale commercial operations rise to coexist amongst traditional value chains in the food landscape, most of which operates outside of a domestic food safety network. Rapid socioeconomic development has also seen an increase in meat consumption, with pork being the most consumed meat product nationally. Expanding pork value chains, and the increasing diversity of actors within them, facilitates the growth and propagation of hazards which are passed onto Vietnamese consumers. In order to guide illness prevention and governance efforts, this review was conducted to examine health risks associated with pork consumption in Vietnam. Synthesis of the available literature provided evidence that *Salmonella* spp. bacteria are a major cause of foodborne illness from Vietnamese pork products. However, contaminants of global concern, including *Salmonella* spp. and *Trichinella spiralis*, occur alongside those considered neglected tropical diseases, such as *Taenia solium*. Infections and complications associated with ingestion of *Streptococcus suis* bacteria are also an issue, with *Streptococcus suis* infections usually limited to occupational infections amongst meat handlers in modernised value chains. A risk factor underscoring transmission of *Trichinella spiralis*, *Taenia solium*, and *Streptococcus suis* in Vietnam that emerges from the literature is the consumption of dishes containing raw or undercooked pork. Available data indicates that infections associated with raw pork consumption disproportionately affect men and people in regional mountainous areas of northwest Vietnam, where many of Vietnam's ethnic minority communities reside. In addition, epidemiological data from recorded disease outbreaks that result from raw pork consumption demonstrate that these outbreaks usually follow major sociocultural events such as weddings, funerals, and Lunar New Year celebrations. Potential health impacts resulting from residues of antibiotics and heavy metals are also cause for concern, though the direct links between chemical contaminants in food and the development of disease are difficult to conclusively deduce.

1. Introduction

The World Health Organization (WHO) estimates that the global burden of foodborne illness is at a magnitude comparable to other major global health issues including HIV/AIDS, malaria, and tuberculosis [1]. Representing a multifaceted threat to public health across society, food contamination and foodborne illness stems from a myriad of underlying chemical and biological agents, with the adverse effects of unsafe food disproportionally affecting children. Specifically, the WHO estimates suggest that approximately 40% of the global foodborne illness burden is borne by children under five years of age [1, 2]. Recurrent bouts of foodborne illness in children can be particularly severe and have prolonged health impacts, as nutrients crucial to childhood growth and development processes are redirected to resource-intensive immune responses [3]. Resulting symptoms of gastrointestinal disease and infection have also been demonstrated to interfere with other outcomes such as education, with mass de-worming programs significantly

increasing school attendance rates [4]. Decreased rates of participation in education and employment similarly impact working age adults, who may experience foodborne illness themselves or take leave to care for others. Monetary or material loss from decreased work participation can exacerbate poverty by straining already limited resources for low-income families and individuals [2]. Culminating on a larger scale, these financial losses represent a significant burden borne by the macroeconomy of nation states. As such, food safety is being increasingly seen as a socioeconomic and development issue, particularly in low- and middle-income countries [1, 2].

Prevalence rates of foodborne illness are a difficult public health concern to measure, particularly in countries with emerging health surveillance systems. At present, robust figures to compare levels of foodborne disease and subsequent impacts between different states remain largely unavailable [1]. Accurate data on foodborne illness is difficult to ascertain, in part, because most people who suffer from foodborne illness will do so privately [2]. Even in countries with advanced surveillance systems to monitor notifiable diseases, only a small percentage of those suffering from foodborne illness will seek medical care [2]. Thus, acquiring accurate data on foodborne illness is particularly challenging for developing countries where surveillance systems are less established, as cases in patients who do seek medical care are less likely to be recorded or reported to wider health authorities [2]. In order to define the scope of foodborne illness rates, the WHO leveraged existing data and expertise to publish the first global estimates of foodborne disease by the region in 2015. The report acknowledged data-related challenges and advised that the resulting estimates were conservative, although it was a rare quantification of an often-neglected health issue. The results highlighted a considerable disparity in health outcomes related to food safety between high- and low-income countries [1], which mirror existing inequalities in other facets of health. However, while available research reveals that issues surrounding food safety disproportionally affect the world's poor, lower levels of foodborne disease in developed countries substantiate that much of the global foodborne illness burden is preventable [1].

In Vietnam, local food systems are experiencing a period of rapid change, mirroring the fast-paced development occurring across wider Southeast Asia. At present, Vietnam's food industry is a mixture of traditional smallholders and emerging enterprises, though there are regional variations. Traditional food systems and value chains dominate the market in the north, with northern Vietnamese consumers typically preferring to purchase fresh produce at traditional markets and wet markets over chain stores [5]. Food in the markets is generally locally sourced, and associated value chains are dominated by small-scale producers [6–8]. For instance, it is estimated that smallholders are responsible for 83% of domestically produced pork meat in Vietnam [5, 8]. However, in line with Vietnam's transition from a low- to lower middle-income country, larger-scale commercial operations have emerged and started integrating into the local food systems [7]. In Ho Chi Minh City, in particular, larger-scale wholesalers have started to become more prominent market players [5]. Supermarkets have also started to emerge as authorities have pushed for the development of a food landscape which imitates those found in developed nations [9]. However, across the country, demand for traditional markets remains significant, leaving supermarkets in Vietnam with one of the lowest market shares in all of Southeast Asia [5, 9].

The current food landscape in Vietnam makes it a particularly difficult sector over which to govern safety standards. In 2010, the Vietnamese government introduced world standard food safety protocols, with responsibilities for oversight shared across three major government ministries at central and provincial levels [5, 9]. This oversight is primarily risk-based and utilises a model centred around inspection and testing [5]. However, government efforts to develop and regulate the food sector, including the introduction of good practice accreditation schemes such as "VietGAP," have had limited impact [10–12]. With so many smallholders and limited resources, an effective domestic surveillance system to monitor food production and safety has not been established [5, 8]. For instance, despite government guidelines around the administration of antimicrobials to livestock, a significant amount of unsupervised antimicrobial use occurs in Vietnam's agricultural sector [13–15]. In addition to concerns around fostering antibiotic resistance (AMR) in zoonotic bacteria, unsupervised administration of antibiotics can leave antibiotic residues in meat products destined for human consumption. These and other chemical contaminants present potential health risks to consumers. However, the limited amount of testing that has been undertaken by the Vietnamese Food Authority (VFA) indicates that biological hazards are the most frequently recorded health risk in domestically produced food [5].

The real incidence of foodborne illness in Vietnam is largely unknown, though concern over food safety is salient in the community. Registered cases of foodborne illness and associated deaths are recorded by the VFA, yet the level of data that is captured is low [5]. For instance, in the first half of 2014, only 21 outbreaks of foodborne illness were documented, affecting a recorded 714 individuals in a population of over 90 million people [16, 17]. Further, in approximately a quarter of cases registered, the pathogen or source of illness is classed as "unknown" [5]. Nevertheless, the available public health data reflects VFA estimates, which suggest that biological hazards represent the largest source of foodborne illness nationally [5]. Consumer concerns, however, are more focused on chemical hazards; specifically, chemical additives and pesticides that might facilitate the development of cancer [5, 11]. While there are chemical hazards present in domestically produced food, the link between chemical residues and illness is not as robust or conclusive as in the case of biological hazards. Part of this difference in perceptions of risk between public health bodies and the public lies in the approach to risk communication adopted by the national media [11]. Though foodborne hazards have garnered widespread attention in Vietnam, biological hazards in foods remain

underreported, whereas chemical hazards are more likely to attract headlines and speculation [11].

This review will examine pathogens and hazards that have the ability to cause illness following the ingestion of pork products in Vietnam. Economic development has driven demand for animal products, with pork meat becoming particularly prominent in Vietnamese diets [5, 6]. Accounting for 70% of all meat consumed nationally, pork consumption per capita in Vietnam is the third highest in the OECD [18]. However, as is the case in other developing countries, raw food handling rarely meets good practice standards [15]. Pushes to transform the pork value chain, and the wider food industry, have looked to normalise Western-style paradigms with little consideration for local contexts and practices [9]. To date, these efforts have not achieved sustainable and equitable improvements in pork safety. Thus, there is an urgent need to develop context-appropriate, effective, and low-cost solutions [1, 9, 11]. By reviewing the scope and burden of different pork-borne health risks in the literature, this paper looks to identify high-value targets for future food safety objectives, highlighting relevant pathogens and hazards, as well as gaps in the current research.

2. Methods

A pilot search of the literature was undertaken to identify key terms and inform subsequent research, with a second search conducted using the PubMed database. English terminology and phrases used during the search included "pork AND illness AND Vietnam," "pork AND disease AND Vietnam," and "pork AND health risk AND Vietnam." However, due to a limited number of search results, the terminology was widened to "pork AND illness AND Southeast Asia," "pork AND disease AND Southeast Asia," "pork AND health risk AND Southeast Asia," and "pork meat AND infection AND Southeast Asia." Pathogens known to infect humans through pork consumption were also used as part of pathogen-specific search phrases, including "*Salmonella* AND Vietnam AND pork meat" and "*Escherichia coli* AND Vietnam AND pork."

As Vietnam transitioned from a low-income country to a lower middle-income country in 2008, only articles published between 2008 and 2018 were considered as relevant for inclusion. With relevant articles identified, aggregate search results were collated for analysis, and duplicate articles removed. The final list of articles was screened for eligibility by the main author (MC), and data pertaining to, or extracted from, the sources were managed using an evidence table. When articles were excluded after only the abstract had been read, this was recorded. However, articles read in full had major data points extracted including the objective of the study, study location, population, sample size, testing method undertaken, and outcome. The literature considered for inclusion in the review comprised both qualitative and quantitative studies, including clinical studies following the diagnosis and treatment of a cohort of patients. However, studies written exclusively on subjective perceptions of risk held by consumers or other actors in the pork value chain were excluded from the study. Pearling methods were also utilised to identify articles for inclusion from the reference list of relevant articles.

As the search had been extended to include studies from wider Southeast Asia, articles were screened to ensure the context in which the study was undertaken was comparable to Vietnam. As such, studies from Hong Kong and Singapore were excluded from the review, as local pork value chains do not mirror the pork value chains in Vietnam. However, studies from southern China, Thailand, Lao People's Democratic Republic (PDR), and Cambodia were considered for inclusion. These geographic regions were considered comparable to Vietnam given their geographic proximity, shared climate, level of development, the nature of their pork production industries, and some shared culinary customs. Regions of Lao PDR and China that share a border with Vietnam were considered particularly relevant, as they share some ethnic minority populations, and some cross-border pig trade occurs.

Research studies selected for inclusion demonstrated a direct health risk to humans through pork consumption, as a result of conditions in which pigs were raised and slaughtered. Here, direct health risks were considered to be risks that had the potential to bring on acute illness, either shortly after consumption or through long-term toxic or accumulative effects. As such, articles discussing health risks pertaining to the nutrient profile of pork or the role of diet in the development of noncommunicable or chronic disease were excluded from the study. Faecal coliforms or other pathogens that can be introduced to pork products by human handling only were also considered beyond the scope of this review. As customers regularly touch pork meat at markets and in retail outlets in order to evaluate potential purchases, it is difficult to deduce whether pathogens that can only be introduced through human handing were done so by actors in the value chain or by end-stage customers. Instead, included articles detailed pathogens that colonise pigs and pork meat directly from rearing and slaughter practices, sometimes with reference to how transport and aspects of the retail environment can facilitate cross-contamination and higher loads of these pathogens. Also beyond the scope of the review were articles detailing health risks that are only amplified by proximity to live pig production (i.e., Japanese encephalitis). The literature examining pathogens and contaminants posing a threat to the health of pigs only were also excluded.

Articles discussing pathogens that can be contracted directly from pork consumption were excluded if consumption of pork meat was not explored as a potential route of infection. Hence, articles detailing occupational exposure only to relevant pathogens were excluded from the study (Figure 1).

3. Results and Discussion

A variety of risks to the health of Vietnamese pork consumers were identified through the literature. Broadly, these risks fall into two categories: biological pathogens and chemical risks. Major risks detailed in the literature are

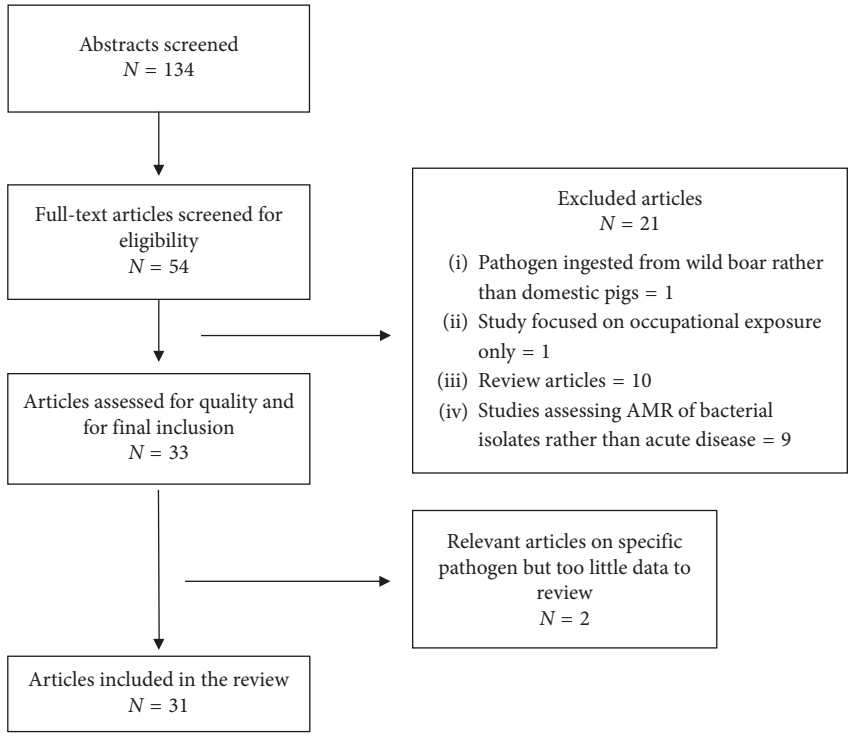

Figure 1: Flow diagram displaying systematic search results and reasons for exclusion.

discussed below according to type of hazard and phylogenic kingdoms where appropriate.

3.1. Bacteria

3.1.1. Salmonella spp. *Salmonella* spp. are zoonotic bacterial pathogens that can colonise the gastrointestinal tract of pigs and cause disease in humans. The two species, *Salmonella bongori* and *Salmonella enterica*, have many subspecies and serovars to group and delineate different characteristics. One major delineation is between "typhoidal" and "nontyphoidal" *Salmonella* [1, 2]; "typhoidal" *Salmonella* bacteria can cause typhoid fever and are adapted to be transmitted horizontally between humans, whereas "nontyphoidal" *Salmonella* can colonise humans and animals [1]. In pigs, most serovars of nontyphoidal *Salmonella* are commonly carried asymptomatically in the gastrointestinal tract. As such, good slaughtering and processing practices must be enacted to prevent contamination of the pig carcass with the contents of the bowel [19]. Human ingestion of food contaminated with a sufficient load of nontyphoidal *Salmonella enterica* can result in "salmonellosis," a gastrointestinal infection with symptoms including abdominal cramps, fever, vomiting, and diarrhoea [1, 2]. While cases of salmonellosis are often self-limiting, severe cases can result in death, with global estimates suggesting that nontyphoidal *Salmonella enterica* is the leading cause of death from a diarrheal disease agent [1].

The literature exploring *Salmonella* spp. on pork products in Vietnam is typically concerned with testing pork at various stages throughout the value chain to establish levels of *Salmonella* spp. contamination. In Vietnam, there are many small-scale pig producers generating independent value-chain linkages. As such, levels of *Salmonella* spp. contamination can vary widely according to the different practices adopted by independent actors within that chain. Results from studies exploring levels of *Salmonella* spp. on pork products vary accordingly. Studies of pork products conducted across Vietnam have found *Salmonella* spp. prevalence rates ranging from 25.0% to 72.7% [7, 19–21]. A major source of variation between studies is the level of cross-contamination that occurs between pork product slaughtered or sold in the same facility. A study in Hung Yen province established that 41.7% of carcasses in slaughterhouses were positive for *Salmonella* spp., though the presence of *Salmonella* spp. other surfaces provided a significant risk for further cross-contamination [19].

How the prevalence of *Salmonella* spp. on pork translates into actual cases of salmonellosis in Vietnam is difficult to infer. As previously mentioned, the WHO report detailing the first estimates of global and regional foodborne illness burdens acknowledged that data on foodborne illness are often underestimated [2]. Many individuals who experience symptoms will choose seeking treatment and recovery in private, resulting in the majority of cases going unrecorded by medical practitioners, even where good surveillance systems exist [2]. Still, the resulting WHO report estimated that, in the Western Pacific Region containing Vietnam, the annual incidence of foodborne salmonellosis was 1% (*R*: 0.2–7%) [2]. However, other research has estimated that the *Salmonella* spp. prevalence rates on raw pork, in combination with reported preparation methods (such as using the same knife and chopping board for raw and cooked pork),

put the annual risk of Hung Yen consumers contracting salmonellosis from boiled pork at 17.7% [19, 20]. The marked gap between these two estimates highlights the difficulties in enumerating the burden of foodborne illness, particularly if attempting to capture the impact of diverse actors and handling practices.

Transportation and handling of pork meat can further compound the risk of salmonellosis. Typically, pork is brought to traditional markets straight from the slaughterhouse, whereas supermarket pork usually requires additional time and added handling before reaching the consumer. However, given that pork is generally transported to traditional markets in bamboo baskets on motorbikes, and markets operate in ambient temperatures, cross-contamination and bacterial growth is easily facilitated [7]. Supermarkets, on the other hand, utilise cold chain transport and packaging in an effort to inhibit bacterial growth and limit further contamination.

3.1.2. Streptococcus suis. *Streptococcus suis* is a zoonotic pathogen that is typically spread between pigs via vertical or horizontal transmission [22]. Thirty-three serotypes of *S. suis* have been identified, with some that cause disease in young or embryonic piglets carried asymptomatically by adult pigs in the upper respiratory, gastrointestinal, and genital tracts [22, 23]. Of the 33 serotypes identified, serotype 2 (*S. suis* 2) is the most frequently involved in human disease [23, 24]. *S. suis* is primarily considered an occupational pathogen, and infections are common amongst those who work in pig rearing or slaughter [25]. However, *S. suis* can also infect humans through the consumption of raw, undercooked and fermented pork dishes [24, 25]. People with *S. suis* infections typically display symptoms such as fever, fatigue, neck stiffness, and acute meningitis [25]. Auditory effects such as hearing loss and tinnitus are also common amongst those with acute meningitis [25, 26]. While symptoms of tinnitus usually subside, attributed hearing loss is often permanent [27]. Complications in more severe cases frequently include sepsis, septic arthritis, and endocarditis, which can result in death [25]. The mortality rate as a result of *S. suis* infections in Southeast Asia varies significantly amongst clinical studies, though the development of sepsis amongst participants positively correlates with death [25–27].

S. suis is the most common cause of bacterial meningitis in Vietnam, and a leading cause of meningitis in Southeast Asia, where infection through consumption of raw pork is more common than in other regions [23, 26, 28]. Epidemiological studies in Southeast Asia have demonstrated that some *S. suis* infections can be attributed to occupational exposure, though occupational exposure alone cannot explain a significant proportion of observed cases. In Ho Chi Minh City, one study following 101 confirmed *S. suis* infections found that, in 25.7% of cases, eating high-risk raw pork dishes was the only risk factor reported by the patient [23]. In addition, while research on *S. suis* cases in rural Vietnam is slim, research from rural Thailand proposed that approximately 70% of *S. suis* infections investigated were linked to the consumption of raw pork [24, 29]. The occurrence of ritualised consumption in Thailand has been suggested, with incidences of *S. suis* food poisoning peaking over months where crop harvesting is celebrated with food [24].

Conclusions from epidemiological studies which implicate raw pork consumption as a route of transmission have been supported by genetic research. A study examining pigs slaughtered in southern Vietnam determined 41% of sample pigs harboured *S. suis* in their tonsils, with 8% of pigs harbouring pathogenic serotype 2 specifically [22]. The *S. suis* 2 isolated from the pigs were compared to isolates from patients with bacterial meningitis. It was found that virulence markers present in the pig isolates mirrored those in samples isolated from patients where consumption of pork was suspected to be the route of infection [22]. The *S. suis* 2 isolated from the carcasses and their genetic similarity to isolates from patients indicate that raw pork consumption may be a common route of infection in Vietnam. However with the ongoing development of health services and surveillance systems in the region, particularly in rural areas where raw pork consumption is more common, it is likely that the prevalence of *S. suis* infection remains significantly underestimated [24].

Epidemiological studies point to additional risk factors that may affect the risk of *S. suis* infection. Takeuchi et al. [29] traced confirmed *Streptococcus suis* cases in regional northern Thailand back to sites of exposure and determined that infection only occurred sporadically amongst people who would all share the same pork product. As such, Takeuchi et al. [29] concluded that infection with *S. suis* via the oral route may require predisposing factors or health comorbidities. Other studies documenting *S. suis* cases have confirmed that unfavourable health indicators, particularly alcoholism and diabetes, are relatively high amongst participants [25, 27, 28]. In addition, *S. suis* infections are consistently shown to occur at a higher incidence amongst males than females [23, 25, 28]. Age is another correlate, with recorded cases usually occurring in adults over approximately 50 years of age [23, 25]. Weather may be an additional influence over the number of infections observed, with two Hanoi-based studies seeing an increase in infections over the summer months [23, 28]. However, in Ho Chi Minh City, where seasonal weather is less varied, little disparity in case numbers occurs between seasons [23, 28].

In an effort to reduce the illness burden associated with *S. suis* infection in regional northern Thailand, Takeuchi et al. [29] launched a public health campaign in 2011 to highlight the risks associated with consumption of raw pork. The campaign achieved early success, and for the first two years, recorded cases of *S. suis* infection dropped to approximately a third of 2010 levels. However, by 2013, *S. suis* infections began increasing, reaching approximately half of 2010 levels before the study was terminated in September of that year [29].

3.2. Protozoa

3.2.1. Taenia spp. *Taenia* species, commonly known as "tapeworm," are zoonotic parasites whose intermediate

hosts include domestic animals such as pigs, cattle, and dogs [30, 31]. Two species present in Southeast Asian pig populations and known to colonise humans are *Taenia solium* and *Taenia asiatica* [30–33]. The lifecycle starts with pigs inadvertently ingest fertilised *Taenia* spp. eggs or proglottids (sexually reproductive segments of the tapeworm which contain eggs) present in their surrounding environment. Upon ingestion, the eggs will develop into larvae in the porcine gastrointestinal tract. The larvae will then penetrate through the wall of the digestive tract and encyst in other tissues of the body, a condition known as "cysticercosis." Subsequent human consumption of cystic pork meat sees the larvae establish in the human intestinal system and mature into an adult, referred to as "taeniasis" [30, 31]. Considered a neglected tropical disease, taeniasis can be asymptomatic or induce mild symptoms including nausea, abdominal discomfort, and diarrhoea [31]. A key sign of infection is passing proglottids and eggs through the faeces, which facilitates transmission to new hosts and completes the lifecycle of the parasite.

A variety of factors and behaviours facilitate the transmission of *Taenia* spp. and increases the risk of human taeniasis. Human taeniasis usually occurs in areas with low access to hygienic sanitation and where the practise of open defecation is common [30–32, 34]. In areas that practise open defecation, contaminated human faeces allows *Taenia* eggs to disperse throughout the environment where pigs are more likely to come into contact with them; the risk of transmission is further amplified in areas where pigs are not penned but allowed to roam and graze freely [30–32, 34]. Adequate application of heat during the cooking process can inactivate larvae in pork tissue, thus consumption of meat that is undercooked or raw also significantly increases risk of contracting taeniasis [30, 32]. Research in the Phongsaly Province of Lao PDR has demonstrated that local consumption of raw and undercooked pork dishes, typically eaten around rituals and milestone events, has facilitated a hyperendemic region of *T. solium* infection [32, 33]. In one hyperendemic village studied, the taeniasis prevalence was recorded at 26.1% (95% CI = 18.2–35.9) where other research has put the baseline taeniasis prevalence across northern Lao PDR at 8.4% (95% CI = 6.9–9.9%) [30, 32, 33]. Food insecurity can also further exacerbate risk of infection, as pigs known to be infected are sold at reduced prices, and cystic meat may not be discarded by a household under food pressures [31, 32]. In addition, Bardosh et al. [32] demonstrated through Laos-based ethnography and participant observation that cystic pork was sometimes consumed unintentionally, as intermittent access to sources of electricity made it difficult to identify and adequately cook cystic meat during food preparation.

Fertilised *T. solium* eggs can result in further disease if ingested by humans, resulting in human cysticercosis [31]. Human ingestion of *T. solium* eggs occurs via the faecal to oral route, either through environmental exposure to eggs or as a result of autoinfection. Once the outer layers of egg have been digested, the larvae of *T. solium* can bore through the human gut and encyst in the bodily tissues, as occurs in pigs [30]. *T. solium* larvae can invade a variety of human tissues, commonly including the nervous system, a condition known as "neurocysticercosis" [30, 32]. Neurocysticercosis following ingestion of *T. solium* eggs is one of the most severe clinical outcomes of *T. solium* infection and is a leading cause of preventable epilepsy in developing contexts [30–32]. In the case of *T. asiatica*, while there is some speculation in the literature, it has not been definitively proven to lead to cysticercosis [30, 31].

Although Southeast Asia is considered endemic for porcine cysticercosis, taeniasis and human cysticercosis may not be endemic to Vietnam specifically [31, 35]. Few recent studies have assessed the illness burden of human taeniasis and cysticercosis in Vietnam, and there is no wholly reliable estimate of its prevalence [36]. Given the role of inadequate sanitation in the transmission of *Taenia* spp., it would be less likely for cysticercosis to occur in developed areas with adequate sanitation. Rather, it is reasonable to suggest that *T. solium* may be concentrated in regional and underdeveloped areas. Northern provinces of Vietnam that border established hyperendemic areas of Lao PDR would be particularly at risk, as people either side of the border share similar environments, trade pigs, and engage in raw pork consumption [32]. Infection rates may also be gendered, with research from Lao PDR, indicating that cysticercosis is more frequently observed in men than in women [30]. This gendered distribution may be a reflection of socialised taste preferences, with Bardosh et al. [32] reporting that male interviewees were more likely to consume raw meat in line with cultural connotations of masculinity and strength.

3.2.2. Trichinella spp. *Trichinella* species are intracellular intestinal parasites that can infect domestic pigs and wild animals. Ingested larvae mature and mate in the lumen of the gastrointestinal tract, with females then invading the epithelial cells of the host's intestinal lining to produce larvae [37]. Larvae travel from the epithelial cells of the small intestine via the host lymphatic to the circulatory system, where they are then deposited into striated muscle cells [38]. Once in the striated muscle, the larvae develop a cystic capsule and redirect host resources the capsule in order to facilitate survival [38]. Adult worms are excreted by the host when they die, thus consumption of larvae in the host tissue by a new host is required to complete the lifecycle [38]. There are three species of *Trichinella* that are pathogenic to humans, though *Trichinella spiralis* is the most commonly found in domesticated pigs and the species most associated with foodborne disease [37].

Clinical symptoms of "trichinellosis" vary across the different stages of the parasites' lifecycle. As females invade the epithelial cells of the small intestine, human hosts may be asymptomatic or present with mild gastrointestinal symptoms such as diarrhea and abdominal pain [37, 38]. As trichinellosis progresses from the intestinal phase through to the muscular phase, larvae that encyst in striated muscle elicit symptoms including muscle pain and inflammation, fatigue, fever, facial oedema, and nausea [38]. A heavy parasitic load, or resulting secondary infections, can result in severe conditions including pneumonia, myocarditis,

encephalitis, and adrenal gland failure [34, 38]. Respiratory failure can also occur if the larvae invade the diaphragm or intercostal muscles [38]. As such, timely administration of antihelminthics is crucial for patients with a high parasitic load in order to prevent death [37].

Epidemiological data on trichinellosis cases in Vietnam is limited, and it is likely that many cases go undiagnosed or misdiagnosed [38]. Five outbreaks infecting between 20 and 36 people each were documented in Vietnam between 1970 and 2012, all of which followed large social gatherings including weddings and Lunar New Year celebrations [37, 39]. In the most recently recorded outbreak, occurring in 2012, patients experienced symptoms including fever, muscle tenderness, oedema, difficulty swallowing, laboured breathing, and difficulty moving within 5–8 days of raw pork consumption [39]. During documented outbreaks, multiple patients with trichinellosis had also been misdiagnosed as having leptospirosis in the initial stages, a bacterial infection common in Vietnam with similar clinical presentation [37]. These cases of misdiagnosis were corrected upon transfer to urban or speciality hospitals. Yet, these cases indicate that a number of trichinellosis illnesses or deaths in Vietnam may be misattributed to leptospirosis. It is reasonable to suggest that other outbreaks likely occur in Vietnam, though outbreaks with fewer patients or milder symptoms may escape the attention of health authorities altogether [37].

The existing literature indicates a number of risk factors that underscore the epidemiology of trichinellosis in Vietnam. In Vietnam, trichinellosis predominantly affects people in the northern mountainous regions that border Lao PDR and China [37, 38]. These regions are at increased risk of *T. spiralis* infection as domestic pigs commonly roam freely, where they may encounter and consume the carcass of other *T. spiralis* hosts including rodents, dogs, cats, and other pigs [37]. However, human infection is also strongly associated with consumption of raw, undercooked, or fermented meat products [34, 37, 38]. Compared to other outbreaks documented around the world, documented outbreaks in Vietnam are relatively severe with a higher mortality rate, indicating a large number of viable larvae consumed [37]. With estimates that up to 85% of trichinellosis cases in Vietnam occur in men, the illness burden is also gendered [38].

3.3. Antibiotic Residues. In addition to promoting antibiotic resistance in zoonotic pathogens, which makes human infections more difficult to treat, widespread antibiotic use in livestock also impacts human health through the oral consumption of residues in meat products. In Vietnam, many farmers have turned to antibiotic use in order to mitigate epidemics, minimise stock loss, and maximise profits [40]. As such, alongside administration for their acute therapeutic value, antibiotics are also used as a prophylactic measure and for growth-promotion effects [41]. Often, antibiotics are administered through feed and without veterinary supervision, so the dosage of antibiotic administered may not align with manufacturers' recommendations [41]. When a high dose is delivered to livestock, residues of the antibiotic can be detected in meat being sold for human consumption. This is particularly the case when withdrawal periods, a waiting period after antibiotic use to ensure it is eliminated from animal before slaughter, are not observed [42]. The threat that antibiotic residues pose to human health is twofold, with the more acute risk being that residues above certain levels can be toxic to humans [42]. However, even at low levels that are non-toxic, there is concern that long-term, low-level antibiotic exposure may alter the human microbiota and contribute to the development of chronic health conditions [42].

The Vietnamese government has taken steps to curb potentially harmful use of antibiotics in agriculture though regulation and surveillance remain difficult to enforce. In 2007, Vietnam joined the World Trade Organization and henceforth was obliged to adhere to its list antibiotics that are banned for use in livestock production [40]. Since joining, the Ministry of Agriculture and Rural Development has updated guidelines pertaining to the sale and use of prohibited drugs [40]. However, the proportion of smallholder pig producers in Vietnam makes monitoring the use of antibiotics in livestock difficult to achieve, particularly as most meat is sold at informal or traditional markets [40]. Further barriers to domestic testing and surveillance systems in Vietnam include the costs associated with testing the amount of pork produced nationally, as well as for the number of different antibiotics utilised by agriculture [7]. As a result, surveillance efforts have been more successful at maintaining the quality of products destined export, which are sold through formal channels and must meet safety regulations imposed by importers [5].

Available research has demonstrated that both legal and illegal antibiotic residues remain a problem for Vietnamese pork products. Two legal and widely used antibiotics used in pig production, tetracycline and fluoroquinolones, have had contrary results in the research. One major study found virtually no tetracycline and fluoroquinolone residue in pork meat products [41]. However, a 2013 study from the Red River Delta region found that 39% of pork samples collected from markets were positive for tetracycline or quinoline residues, or both [40]. Testing has also uncovered the use of banned drugs including chloramphenicol in pork production [41]. Low levels of beta-agonists, antibiotics classed as illegal growth promoters, have also been found [41].

Of the many antibiotic residues tested for across Vietnamese studies, residues from legal sulphonamides appear to be the most commonly found in pork. In one study of meat samples from central and southern Vietnam, which tested for residues of 21 different antimicrobials, 8.8% of pork meat samples were positive for sulfamethazine [8]. In this study, none of the other 20 antimicrobials being tested for were detected [8]. Another study testing pork meat from Ho Chi Minh City had similar results, with sulfamethazine residues (11–1600 μg/kg) found in 23% of samples, with none of the other 27 residues chosen for inclusion in the study detected [43]. Further, a quantitative and qualitative study of sulfamethazine levels in pork meat found that 11% of pork samples tested were positive with sulfamethazine above maximum residue limits [41]. However, more research will need to be undertaken to ascertain the full extent and impact of legal and illegal antibiotic use in livestock in Vietnam [41].

3.4. Heavy Metals. In the 2015 report on global foodborne illness, the WHO did not explore illness resulting from heavy metal exposure, citing that robust methods to estimate disease as a result of metals such as lead and cadmium do not currently exist [2]. To date, one study has examined heavy metals in pork liver, kidney, muscle, and pig feed in Vietnam. Lead was found in 11.1% of feed samples, 55.6% of liver samples, 38.9% of kidney samples, and 27.8% of muscle meat [41]. Cadmium was found in 94.4% of feed samples, 100% of liver samples, 100% of kidney samples, and 0% of muscle meat [41]. Arsenic was not found across any of the feed, liver, kidney, or muscle meat samples [41]. For both lead and cadmium, the levels detected were below maximum residue levels. However, further research will be required to adequately comment on the heavy metal contamination of pork products in Vietnam and any associated health consequences.

4. Conclusion

This review demonstrates the scope and complexity of risks that pork can present to consumers in Vietnam, though there are notable absences of some known risks in the published literature. Chemical risks in Vietnamese pork products are particularly hard to deduce, with only one major study assessing heavy metal contamination and few studies attempting to capture the mosaic of antibiotic residue contaminants. Biological hazards associated with Vietnamese pork products, specifically bacteria and parasites, have been more closely studied and definitively attributed to disease outcomes and mortality. Still, *Salmonella* spp., *S. suis*, *T. solium*, and *T. spiralis* do not represent the full scope of known pathogens associated with pork consumption. The omission of other appropriate pathogens from this review is a result of gaps in the available research specific to Vietnam and comparable countries of Southeast Asia. Other pathogens known to colonise pigs and infect humans following pork consumption (including Hepatitis E, *Staphylococcus aureus*, and *Campylobacter jejuni)* are virtually absent from relevant literature. Little research to assess the impacts of pathogenic *Escherichia coli* consumed through pork products results in another notable gap in the literature, with most studies focused on antibiotic resistance harboured by different pork-borne *E. coli* isolates. However, a limitation of this review is that only articles published in the English language were considered for inclusion. The aforementioned gaps may be less significant in Vietnamese language literature, or in literature written in other languages of Southeast Asia.

Pork-associated pathogens that are more comprehensively discussed by the literature and detailed in this review illustrate the presence and impacts of gradients of development in Vietnam. For instance, disease as a result of parasitic infection is more frequently documented in regional areas of Vietnam, where populations have lower relative levels of sanitation infrastructure, reduced access to healthcare, and where livestock may be permitted to roam in order to graze [37]. Falling under the umbrella of neglected tropical diseases, *T. solium* infection is associated with lower relative levels of socioeconomic development in affected areas. However, alongside pathogens that are more prevalent in low-income countries, Vietnam also has burgeoning food processing and chain retail sectors, where larger slaughterhouses and additional handling processing can facilitate cross-contamination of *Salmonella* bacteria between products. Ubiquitous in pig farming and slaughterhouse environments, high-income countries with developed food safety protocols, and surveillance systems have yet to eliminate foodborne salmonellosis [2]. Thus, any attempts to address food safety in Vietnam must capture this complex, yet rapidly evolving food landscape.

Review of the literature suggests that an effective way to curb the incidence of foodborne illness in Vietnam would be through public health interventions that successfully reduce the consumption of raw pork. Application of sufficient heat to pork products during the cooking process is crucial to reducing the load of viable organisms on or within the meat. A multitude of studies included in this review implicated raw pork consumption as a prominent risk factor in the development of disease from biological pathogens [23–25, 29, 30, 32–34, 37, 38]. However, epidemiological trends indicate that raw or undercooked pork dishes are part of prominent sociocultural events such as celebrations and funerals [24, 32, 33, 37, 39]. The cultural significance of raw pork consumption appears particularly prominent in mountainous areas of northwest Vietnam that border Lao PDR, which have been the site of multiple recorded outbreaks of pork-borne parasites [30, 32, 33, 39]. Thus, despite Takeuchi et al. [29] reporting some initial success using an educational intervention in Thailand, other authors have critiqued reliance on educational or biomedical paradigms in this respect [32]. Bardosh et al. [32] argue that transdisciplinary valuations of cultural and structural factors surrounding raw pork consumption are required to adequately engage with the issue.

Finally, the epidemiological data explored across the foodborne pathogens included in this study reflected relatively consistent patient demographics. Various studies have reported that males are affected by pork-related foodborne illness at a significantly higher rate than females across Southeast Asia [23–25, 30, 38]. Age was also positively associated with infection and disease following pork consumption in some studies [23, 25, 30]. Thus, if raw pork consumption is a result of socialised tastes, it may be changing with younger generations of Vietnamese consumers, predominantly remaining amongst older generations of Vietnamese men. However, trends in the gender and age of patients may be a reflection of other underlying risk factors, such as lifestyle behaviours, health comorbidities, or age-associated immunodeficiency. As such, further research to determine why these demographic patterns are repeatedly observed could help underscore disease prevention efforts.

Disclosure

This work was completed as part of ongoing research conducted by the Center for Public Health and Ecosystem Research, Hanoi University of Public Health.

Conflicts of Interest

The authors have no conflicts of interest or financial interest to disclose.

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Research Article

Influence of Different Production Systems on the Quality and Shelf Life of Poultry Meat: A Case Study in the German Sector

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Production-specific factors, such as breeding, diet, and stress, are known to influence meat quality, but the effect of different husbandry systems on the development of quality parameters and shelf life has hardly been investigated. Thus, the aim of the study was the investigation of an alternative production system based on a slow-growing, corn-fed, and antibiotics-free chicken line compared with conventional poultry production. Additionally, the effect on meat quality, microbiology, and spoilage was analyzed. In total, 221 breast filets from a German poultry meat producer were investigated. Nutritional, biochemical, and cooking loss analyses were conducted on a subset of samples 24 h after storage. The rest of the samples were stored aerobically at 4°C, and the spoilage process was characterized by investigating pH, color, lipid oxidation, microbiology, and sensory attributes subsequently every two days during storage. The alternative production line showed a significantly healthier nutritional profile with a higher protein and lower fat content. Additionally, the amount of L-lactic acid and D-glucose was significantly higher than in the conventional production line. The color values differed between both production lines, with the corn-fed line displaying more yellowish filets. The lipid oxidation and microbial spoilage were not affected by the production line. The shelf life did not differ between the investigation groups and was deemed 7 days in both cases. Despite the highest severity of white striping being observed most in the conventional production line, there was no overall difference in the incidence among groups. The purchase decision was affected by the occurrence of white striping and showed a tendency for a higher acceptance for the alternative production line.

1. Introduction

To meet the growing consumption and consumer demands, poultry production underwent a remarkable development of intensification. As a result of intense selection processes, poultry breeding lines were modified for shorter generation times, enhanced animal performance, and higher meat content [1]. The slaughter age was halved to five weeks, while the breast meat yield was significantly increased by 10% compared with poultry production 50 years ago [2, 3]. With the selection for growth velocity, an increase of muscle failures and health issues of the animals arose [4–6]. White striping (WS), for example, is a muscle myopathy correlated to heavy breast filets and fast muscle growth [7]. WS has a remarkable negative impact on consumer acceptance [7, 8]. Further undesirable characteristics caused by the selection for high production efficiency are immunological, behavioral, physiological, and stress-related problems [3].

Animal health issues were countered by increased application of antibiotics in industrial animal production,

which resulted in the proliferation of microorganisms with antibiotic resistance [9] with enormous impact on human health. In the context of these problems, the sustainability of poultry production is discussed increasingly, and consumer awareness is rising for animal health and welfare topics [10–13]. The increasing demand for extensive production systems which are vigilant for animal welfare resulted in a growing organic sector, local certified products, and the establishment of high-quality meat lines [10, 14]. One example for a high quality, local product is the French poultry line "Label Rouge," which was successfully introduced in the market in the 1960s and is widely accepted [15]. In Germany, the production of specialized corn-fed poultry lines is a similar attempt to launch high-quality meat in the market and experience a positive resonance with the customer. As the meat market reached the saturation point in Germany, meat quality as well as animal welfare and sustainability have an increasing impact on the purchase decision of the consumer. Thus, a production system was developed focusing on enhanced animal welfare, antibiotics-free, corn-based fattening, and a slow-growing breed. The use of more sustainable systems, such as the proposed one, may increase consumer acceptance and the willingness-to-pay higher prices; however, any modification of the production system may also cause differences in the meat quality, nutritional parameters, and the shelf life of the final product. Several studies are conducted under controlled laboratory conditions and not in commercial production systems and thus do not fully reflect practical conditions of meat production.

Thus, the focus of this case study was the comparison of two commercial production lines regarding typical meat nutritional and quality parameters, typical defects (such as WS), and the shelf life of the products.

2. Materials and Methods

2.1. Study Design. The investigation focused on the characterization of two different industrial production lines: conventional and alternative, of a German poultry producer. For the alternative production line, the producer recently changed breeds for a new slow-growing race showing optimized muscle growth within the prolonged production time. Additionally, detailed information on feedstuff ingredients is not provided due to confidentiality clauses.

Characteristics of the alternative production line were as follows: the used race was the slow-growing Ranger Classic at a maximal stocking rate of 32 kg/m^2 and a toy-enriched environment, such as bales of straw and boxes. The diet of the birds contained more than 50% corn. The fattening focused on a slower growth of the animals and was conducted without antibiotic medication. The birds were slaughtered after 42–45 d.

Characteristics of the conventional production line were as follows: the race Ross 308 was used at a stocking rate of 39 kg/m^2. Antibiotic medication was administered when required. The birds were fed with a grain-based diet and slaughtered after 30–35 d.

All animals were slaughtered and processed the same day and in the same industrial slaughterhouse. The breast filets were transported under temperature-controlled conditions to the laboratory of the University of Bonn.

To investigate the influence of the production system on the nutritional value (protein, intramuscular fat, collagen, and water content) and muscle characteristics (L-lactic acid and D-glucose), a subset of the samples ($n = 32$) was frozen directly after arrival at the laboratory.

A total of 221 filets were investigated in two repeated storage trials to assess the development of quality parameters and shelf life. After packaging aerobically in polypropylene trays with snap-on lids, the samples were stored in high-precision low-temperature incubators (Sanyo Mir 154-PE, Sanyo Electric Co., Ora-Gun, Gunma, Japan) at 4°C for 12 d. The investigations were conducted at six repeated investigation points during storage. For each investigation point, a total of 24 alternative and 13 conventional filets were investigated. The analyzed parameters comprised physicochemical parameters (pH, drip and thawing loss, and color measurements), microbial investigations (total viable count of *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae), and a sensory analysis including the assessment of WS and purchase decision (PD). The analyses were conducted on the complete filet with an excision only for microbial investigations. After all investigations were completed for an investigation point, samples were frozen at −18°C and stored for the measurement of thiobarbituric acid reactive substances. The first analyses started 24 h after slaughter (0 h of the experiment) to characterize the initial meat quality, WS, and microbial contamination of the poultry filets. Except for cooking and thawing loss, the development of quality and microbial parameters was investigated by six repeated measurements until the end of storage at 288 h (12 d).

2.2. Physicochemical Parameters

2.2.1. Analysis of Nutritional Value and Muscle Characteristics. To investigate the influence of the production system on the nutritional value of the meat and the susceptibility of the muscle for microbial spoilage, the main nutrients, D-glucose and L-lactic acid, were analyzed for a subset of the samples. The meat samples were frozen at a fresh condition 24 h after slaughter in a −18°C freezer. Before the analyses, the samples were thawed for 24 h at 4°C. The nutritional value of the poultry filets was analyzed with near-infrared spectroscopy on 32 filets. The whole filets were processed using a food processor (Moulinex DPA 141, Groupe SEB Deutschland GmbH, Offenbach, Germany). Afterwards, they were placed in the near-infrared spectrometer (NIRS DS2500, Foss, Rellingen, Germany) and analyzed automatically. The measurements comprised intramuscular fat, protein, water, and collagen content and are stated as percentages.

Two specific enzyme test kits were used to determine the content of L-lactic acid (Biopharm 1111281035, R-Biopharm AG) and D-glucose (Biopharm 10139106035, R-Biopharm AG) with a spectral photometer (Thermo Scientific™ GENESYS™, Fisher Scientific GmbH, Schwerte, Germany)

on 23 filets. Sample preparation was conducted following a modified protocol of [16]. A standardized sample size of $4 \times 8\,cm^2$ was extracted with a scalpel and processed using a food processor. 5 g of the meat paste was transferred to a beaker glass, dissolved in 35 ml Aqua Bidest, and homogenized for 5 min on a magnetic stirrer without heating. After Carrez clarification and adjusting the pH value to 7.5–8 (testo 206-pH1, Testo, Lenzkirch, Germany) with 0.5 mol sodium hydroxide solution, the solution was transferred to a graduated flask, filled with Aqua Bidest up to 100 ml, and swiveled slightly. The solution was filtered (Whatman Filter 595 1/2, GE Healthcare Europe GmbH, Freiburg, Germany) and further processed following the instructions of the test kit. Samples were measured in repeat determination at 340 nm.

2.2.2. pH Value. The surface pH of the filets was measured with a portable surface pH meter (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany). The pH meter works with a glass electrode, specifically developed for meat surface measurements. The pH meter is calibrated daily and checked regularly against penetration electrodes to justify correct measurements. Three measurements were performed for each meat sample by placing the electrode onto the meat surface. An average pH value was calculated for every sample ($n = 221$).

2.2.3. Cooking and Thawing Loss. As a measure for the water binding capacity of the breast filets, the cooking loss and thawing loss were analyzed. The cooking loss analysis was performed on the inner filet of the meat sample at the beginning of storage ($n = 36$). The inner filet was detached from the sample, weighed, transferred separately into an autoclave bag, and sealed. Samples were heated in an 80°C water bath (Memmert, Schwabach, Germany) until the core temperature reached 72°C. Temperature measurements were performed with a food core thermometer (Testo, Lenzkirch, Germany) in a reference sample. After cooking, the filets were dabbed and weighed again. The thawing loss was measured by weighing the whole filet before and after freezing in an −20°C freezing room. The thawing loss was determined at the beginning ($n = 22$) and end of storage ($n = 21$). Cooking loss and thawing loss, respectively, were calculated using the following equation:

$$W_L = \frac{m_1 - m_2}{m_1} \cdot 100\%, \quad (1)$$

where W_L is the water loss (%), m_1 is the weight before treatment (g), and m_2 is the weight after treatment (g).

2.2.4. Color Measurements. The color of the filets ($n = 196$) was measured using a large view spectrophotometer (ColorFlex EZ 4500L, HunterLab, Murnau). The color measurement was conducted at a wavelength between 400 nm and 700 nm and with a 45°/0° geometry. The CIE 1976 $L^*a^*b^*$ scale was used, measured with D65 illuminant (6500 K daylight). The filets were placed on the glass surface of the measurement device. The color was measured at three sample points for each filet to get a representative evaluation of the sample. Measurement values were averaged for each sample.

2.2.5. Measurement of Thiobarbituric Acid Reactive Substances. For the investigation of fat oxidation in the tissue, thiobarbituric acid reactive substances (TBARS) were determined by a quantitative assessment of malondialdehyde (MDA) via extraction with trichloroacetic acid (TCA) and a fluorometric measurement in a microplate reader (Synergy H1 Microplate Reader, BioTek Instruments Inc., Winooski, US). The measurement of TBARS was conducted during the second repetition of the trial on 131 breast filets ($n = 14$ alternative and $n = 8$ conventional, per investigation point). For the preparation of samples, poultry filets were thawed at 4°C for 24 h. A standardized surface of the meat tissue with a size of $4 \times 8\,cm^2$ and 0.5 cm thickness was punched and homogenized with a food processor. After transferring 7 g of the meat paste to a 50 ml tube, 15 ml TCA (7.5%) was added together with ethylenediaminetetraacetic acid (EDTA, 0.1%) and propyl gallate (0.1%). Each sample was homogenized with an Ultra Turrax (IKA Ultra-Turrax, Janke & Kunkel GmbH & Co KG, Staufen, Germany) for 60s, and a further 10 ml TCA was added. The samples were stored on ice to prevent heating during the homogenization process. The homogenized samples were centrifuged for 15 min at 2000 rpm and 4°C. Then, the homogenate was filtered through a Whatman No. 4 filter, aliquoted, and stored at −80°C until further processing. For the TBA reaction, 100 μl of the thawed homogenates was transferred to reaction tubes. After adding 200 μl TCA (10%), samples were incubated on ice for 5 min and then centrifuged for 6.5 min at 13.200 rpm and 4°C. The supernatant was taken and diluted in Aqua Bidest (1 : 2.5). TBA reagent was added to the samples and then incubated at 100.5°C for 60 min. The samples were then cooled in a 4°C centrifuge at 8000 rpm for 2 min. Samples were transferred to microplates and quantified in a microplate reader at excitation/emission 515/553 nm.

2.3. Microbiological Analyses. Microbiological analyses were conducted to assess the initial contamination of the meat samples and to investigate the proliferation of typical spoilage organisms. The focus of the analyses was on total viable count (TVC) which was analyzed for every sample ($n = 219$). *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae were analyzed for a subset of samples ($n = 119$). For microbial investigations, a standardized surface of meat tissue, with a size of $5\,cm^2$, was extracted aseptically using a sterile punch and a scalpel. The samples were transferred to a sterile, filtered stomacher bag. The ninefold amount of saline peptone diluent (0.85% NaCl with 0.1% peptone Saline tablets, Oxoid BR0053G, Cambridge, United Kingdom) was added with an accuracy of 0.1 g for the first dilution step. The samples were mixed with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden,

Germany) for 60 s. Tenfold dilutions of the homogenate were prepared in saline peptone diluents.

The total viable count (TVC) was determined by the pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany). The plates were incubated at 30°C for 72 h. *Pseudomonas* spp. (PSE) were detected by the spread plate technique on Pseudomonas agar with Cetrimide-Fucidin-cephalosporine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours. *Brochothrix thermosphacta* was determined by the drop-plate technique on SIN agar (Streptomycin Inosit Toluylene Red Agar, Oxoid Limited, Basingstoke, United Kingdom) and counted after incubation at 25°C for 48 h. Enterobacteriaceae were identified using the pour plate technique with overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany). VRBD plates were incubated 24 h at 37°C.

2.4. Sensory Investigations. Sensory investigations were conducted by a trained sensory panel (four panelists) for a total of 221 filets. The analyses comprised the PD, assessment of WS, and the characterization of the freshness loss via the sensory index (SI). The PD was assessed via dichotomic response options. In detail, the panelist had to decide whether they would buy the product in a closed package or not. WS was graded via a three-point scoring system from 0 to 2 (0, no WS; 1, medium WS; 2, severe WS). For assessing freshness, poultry filets were evaluated based on a graded five-point scoring system with five meaning highest quality and one meaning spoiled. The evaluation was performed for the parameters color, odor, texture, meat juice color, and meat juice quantity for each sample. The sensory index (SI) was calculated as a weighted average with the following equation:

$$\mathrm{SI} = \frac{2 \cdot \mathrm{O} + 2 \cdot \mathrm{C} + \mathrm{T} + \mathrm{JC} + \mathrm{JA}}{7}, \quad (2)$$

where O is the odor, C is the color, T is the texture, JC is the meat juice color, and JA is the meat juice.

According to the scheme, the product is spoiled when the SI reaches the level of 2.3. The SI was plotted as a function of time and fitted to a linear model. Thus, the shelf life of each sample was calculated following the procedure in [17].

2.5. Data Analysis and Statistics. Microbial data were $\log_{10}$ transformed and plotted as function of time. The data were fitted to a nonlinear model (Levenberg–Marquardt algorithm) using the software package OriginPro 8G (OriginLab Corporation, Northampton, MA, USA). To describe the microbial growth curve, the modified Gompertz model was used [18]:

$$N(t) = A + C \cdot e^{\left(-e^{(-B \cdot (t-M))}\right)}, \quad (3)$$

where $N(t)$ is the microbial count $\log_{10}$ (cfu/cm^2) at time t, A is the initial bacterial count (lower asymptotic line), C is the difference between upper asymptotic line of the growth curve (Nmax = maximum population level) and the lower asymptotic line, B is the relative growth rate at time M (1/h), M is the time at which the maximum growth rate is obtained (reversal point), and t is the time (h).

When TVC reached a level of $\log_{10}$ 7.5 cfu/cm^2, the product was considered spoiled. Microbial shelf life was calculated by transforming equation and including the calculated model parameters.

Since criteria for normal distribution and homoscedasticity were not met by most of the parameters, nonparametric testing was selected for all statistical analyses. Differences between both production lines were analyzed with the Mann–Whitney U test using SPSS Statistics 23 (IBM Corporation 1989, 2013, New York, USA). Spearman's rank correlation and correlation plots were performed using the package corrplot and the software *R* (*R* Development Core Team, http://r-project.org). Multivariate testing was discarded due to the sample size at the single investigation points.

3. Results

The analysis of the nutritional value and muscle characteristics revealed significant differences between both production lines (Table 1). The content of intramuscular fat and water is significantly lower for the alternative filets. Besides, the content of protein is significantly higher compared to the conventional poultry meat. There was no significant difference for collagen. The level of L-lactic acid and D-glucose was significantly increased for the alternative filets. Both parameters significantly affected the pH value (Figure 1). Higher amounts of L-lactic acid lowered the pH value (k: −0.806, $p < 0.001$, $n = 23$) and high amounts of D-glucose (k: −0.541, $p < 0.001$, $n = 23$).

The mean pH value of the filets was 6.25 for the alternative and 6.30 for the conventional production line at the beginning of storage (Table 2). The pH value remained stable during storage and showed an increase at 240 h. At the end of storage, the pH increased to 7.28 for alternative and 7.34 for conventional filets. The pH values for the alternative group were lower at every investigation point, but the difference is only significant for the investigation after 72 h and 168 h.

The measurements for cooking loss and thawing loss showed a high variation between the groups. The mean cooking loss was slightly lower for alternative (14.13%) than for conventional filets (16.54%), but the difference was not significant (Table 2). At the beginning of storage, the thawing loss was 5.05% for alternative and 4.89% for conventional filets. For both production lines, thawing loss declined until the end of storage. Altogether, no significant difference in the water binding capacity was detected between either production line. Both parameters showed a significant negative correlation to the pH value (cooking loss k: −0.616, $p < 0.001$, $n = 22$; thawing loss k: −0.599, $p < 0.001$, $n = 22$).

The color measurements of the filets revealed remarkable differences between both groups. The L^* value was lower for the alternative filets at most investigation points with significant differences after 120 h and 168 h of storage. For both

Table 1: Analysis of nutrients in the alternative and conventional production line, mean values, and standard deviations.

	Collagen (%)	Intramuscular fat (%)	Protein (%)	Water (%)	N	L-Lactic acid (g/100 g)]	D-Glucose (g/100 g)	N
Alternative	0.90	**1.01**	**23.68**	**74.25**	16	**0.952**	**0.056**	15
	±0.155	**±0.208**	**±0.534**	**±0.454**		**±0.0598**	**±0.0173**	
Conventional	0.85	**1.48**	**22.37**	**75.30**	16	**0.786**	**0.038**	8
	±0.179	**±0.473**	**±0.778**	**±0.562**		**±0.0173**	**±0.0161**	

Bold parameters indicate differences between the production systems are significant at the 0.05 level.

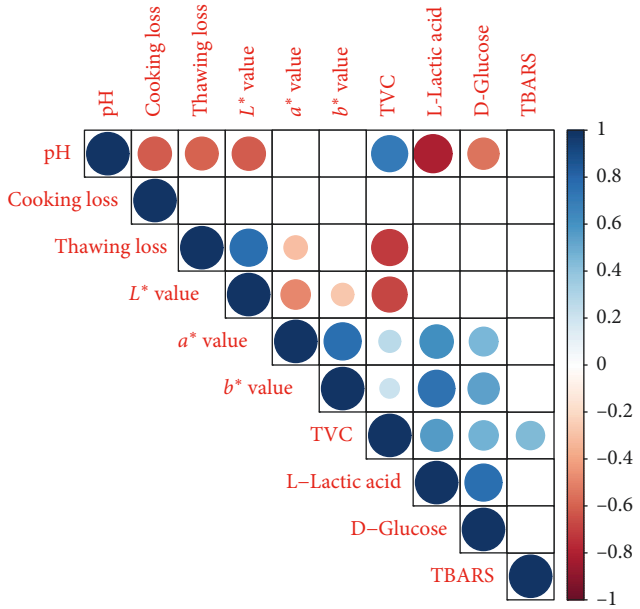

Figure 1: Correlation between physicochemical parameters. Only correlations significant at the 0.05 level are displayed.

production lines, the L^* value decreased significantly during storage with a mean ΔL^* of 5.30 for the alternative and ΔL^* of 4.38 for the conventional production line. The L^* value was negatively correlated to pH (k: −0.614, $p < 0.001$, $n = 196$) and positively correlated to the thawing loss (k: 0.762, $p < 0.001$, $n = 43$).

The a^* values were significantly higher for the alternative group at all investigation points, meaning a higher amount of red color in comparison to the conventionally produced filets. The a^* values showed no clear development during storage for both investigation groups. The b^* values were significantly higher for the alternative filets. As for the a^* values, also the b^* values showed no clear trend during storage. Altogether, the color values showed that the alternative filets display more yellowish filets with a darker color. In comparison, the conventional produced filets were paler and showed a light pink color.

The investigation of TBARS revealed no significant differences between the groups. At the beginning of storage, mean TBARSs were 0.120 mg MDA/kg meat (alternative) and 0.115 mg MDA/kg meat (conventional). TBARSs showed an increase during storage, but standard deviations were very high and showed a broad overlap between investigation points and investigated groups. After 240 h storage, mean TBARSs were 0.133 mg MDA/kg meat (alternative) and 0.144 mg MDA/kg meat (conventional). Regarding the overall storage time, TBARS was significantly correlated to TVC (k: 0.442, $p < 0.001$, $n = 131$). Additionally, TBARS was correlated significantly to all sensory parameters, especially odor (k: 0.499, $p < 0.001$, $n = 131$), color (k: 0.490), and the color of the meat juice (k: 0.487, $p < 0.001$, $n = 131$).

Regarding the microbial investigations, the initial TVC differed significantly and was higher for the alternative filets (2.44 $\log_{10}$ cfu/cm^2) than for the conventional filets (2.10 $\log_{10}$ cfu/cm^2). During storage, this difference in the initial contamination vanished with the proliferation of the microorganisms. The initial counts of *Pseudomonas* spp. were below the detection limit 24 h after slaughter. Pseudomonads grew progressively dominant during storage and remained at the same order of magnitude as TVC. Thus, *Pseudomonas* spp. can be confirmed as a specific spoilage organism (SSO) (Figure 2) for both groups. TVC reached a maximum of 9.43 $\log_{10}$ cfu/cm^2 and 9.34 $\log_{10}$ cfu/cm^2, respectively. The maximum of *Pseudomonas* spp. was at 9.35 $\log_{10}$ cfu/cm^2 for alternative and 9.30 $\log_{10}$ cfu/cm^2 for conventionally produced meat. No differences were observed in the progression of the growth curve of TVC and *Pseudomonas* spp. for both meat types.

For both investigation groups, no significant differences of the initial bacterial counts for *B. thermosphacta* and Enterobacteriaceae were detected since bacteria were under the detection limit 24 h after slaughter. The growth of *B. thermosphacta* reached a mean maximum of 6.72 $\log_{10}$ cfu/cm^2 (alternative) and 6.61 $\log_{10}$ cfu/cm^2 (conventional) after 288 h of storage. Enterobacteriaceae displayed mean maximum values of 5.86 $\log_{10}$ cfu/cm^2 (alternative) and 6.04 $\log_{10}$ cfu/cm^2 (conventional) after 288 h. Thus, the development of microbial growth was very similar for both investigation groups.

The shelf life also showed no differences between both investigation groups. The microbial spoilage level of 7.5 $\log_{10}$ cfu/cm^2 for TVC was reached by the alternative filets after 178 h and by the conventional produced filets after 175 h (Figure 3). The sensory shelf life was reached after 201 h (alternative) and 192 h (conventional), respectively. The alternative group achieved better scores at a few investigation points, mainly due to better evaluations for color or odor. However, these discrepancies did not lead to a significant difference in shelf life.

Severe WS was observed most in the conventional production line at every investigation point, but the difference is not significant. There was no clear tendency or pattern, indicating that the categories "no WS" or "medium WS" developed differently in either of the investigation group (Figure 4). Additionally, no effect of storage on the display of WS could be observed. Due to the strong

Table 2: Mean values and standard deviations of investigated meat quality parameters during storage (alternative: $n = 24$ per investigation point; conventional: $n = 13$ per investigation point).

	0 h	72 h	120 h	168 h	240 h	288 h
Weight (g)						
Alternative	277.44 ± 36.16					
Conventional	300.74 ± 28.19					
pH						
Alternative	6.25 ± 0.11	**6.15 ± 0.13**	6.24 ± 0.10	6.24 ± 0.13	**6.95 ± 0.49**	7.28 ± 0.35
Conventional	6.30 ± 0.23	**6.30 ± 0.18**	6.29 ± 0.16	6.35 ± 0.13	**7.24 ± 0.29**	7.34 ± 0.46
Cooking loss (%)						
Alternative	14.13 ± 1.63					
Conventional	16.54 ± 4.52					
Thawing loss (%)						
Alternative	5.05 ± 1.83					2.29 ± 0.79
Conventional	4.89 ± 1.24					3.12 ± 1.45
L^* value						
Alternative	57.82 ± 1.95	56.41 ± 1.97	**55.79 ± 2.45**	**55.60 ± 1.71**	55.59 ± 1.44	51.21 ± 2.49
Conventional	58.35 ± 0.96	57.43 ± 2.67	**57.80 ± 2.36**	**57.55 ± 2.35**	54.12 ± 2.29	52.85 ± 3.28
a^* value						
Alternative	**5.25 ± 0.64**	**5.89 ± 1.18**	**5.77 ± 1.46**	**5.75 ± 0.90**	**5.73 ± 0.80**	**6.70 ± 1.49**
Conventional	**4.07 ± 0.54**	**3.87 ± 0.53**	**4.15 ± 0.98**	**3.87 ± 0.79**	**4.15 ± 1.21**	**4.41 ± 1.21**
b^* value						
Alternative	**21.17 ± 1.67**	**24.86 ± 4.23**	**25.32 ± 3.67**	**25.14 ± 3.25**	**26.60 ± 3.91**	**24.95 ± 3.34**
Conventional	**16.45 ± 1.55**	**13.29 ± 0.81**	**14.93 ± 4.64**	**14.16 ± 1.16**	**18.07 ± 2.22**	**17.57 ± 2.37**
TBARS (mg MDA/kg)						
Alternative	0.120 ± 0.018	0.115 ± 0.013	0.121 ± 0.011	0.132 ± 0.017	0.152 ± 0.019	0.133 ± 0.028
Conventional	0.115 ± 0.014	0.111 ± 0.011	0.118 ± 0.13	0.119 ± 0.014	0.150 ± 0.017	0.144 ± 0.021

Bold parameters indicate differences between productions systems are significant at the 0.05 level.

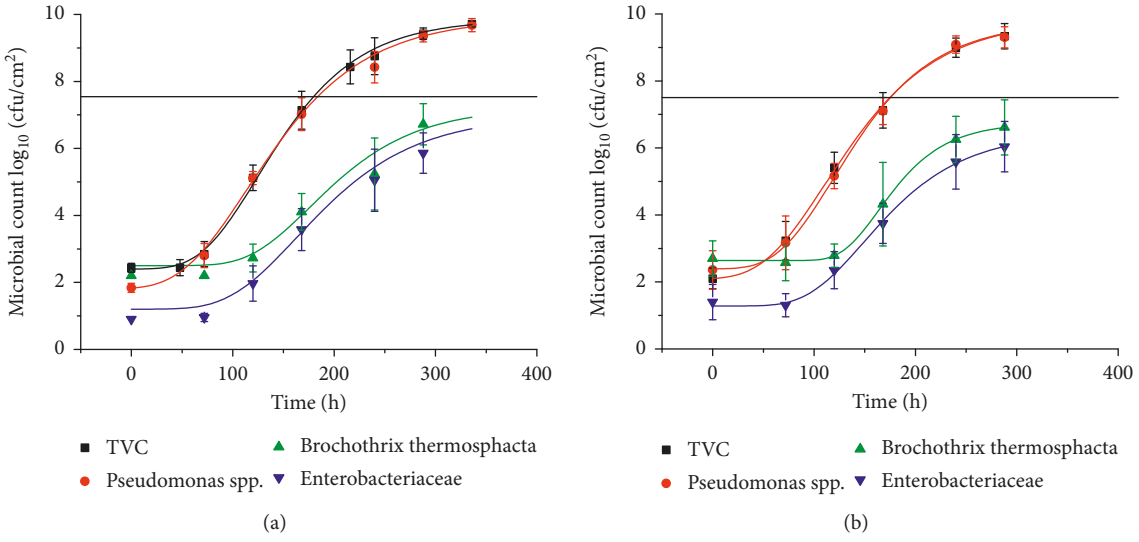

Figure 2: Evolution of microbiological contamination on filets of the alternative (a) and conventional (b) production line.

discoloration of the filets caused by spoilage, the incidence of WS is only displayed until 168 h of storage. WS showed a significant negative correlation to the b^* value (k: −0.426, $p = 0.048$, $n = 22$) at the first investigation point, meaning that more yellowish filets showed a less pronounced WS. Regarding the overall storage time, WS was significantly correlated to the pH (k: 0.377, $p < 0.001$) and significantly affected the PD negatively (k: −0.271, $p < 0.001$, $n = 221$). Moreover, WS was not correlated to filet weight or any other physicochemical parameters.

The PD was higher for alternative filets at every investigation point (Figure 5). This difference is significant for the first investigation point. The PD declined during storage, as the meat showed a loss of freshness. A high rejection rate of the samples was obvious after 120 h and less than 10% were accepted after 168 h of storage. All filets were rejected by the panel after 240 h, when the filets were spoiled. The PD was influenced significantly by the other parameters assessed by the sensory panel, especially color (k: 0.910, $p < 0.001$, $n = 221$) and WS. All other sensory parameters were also

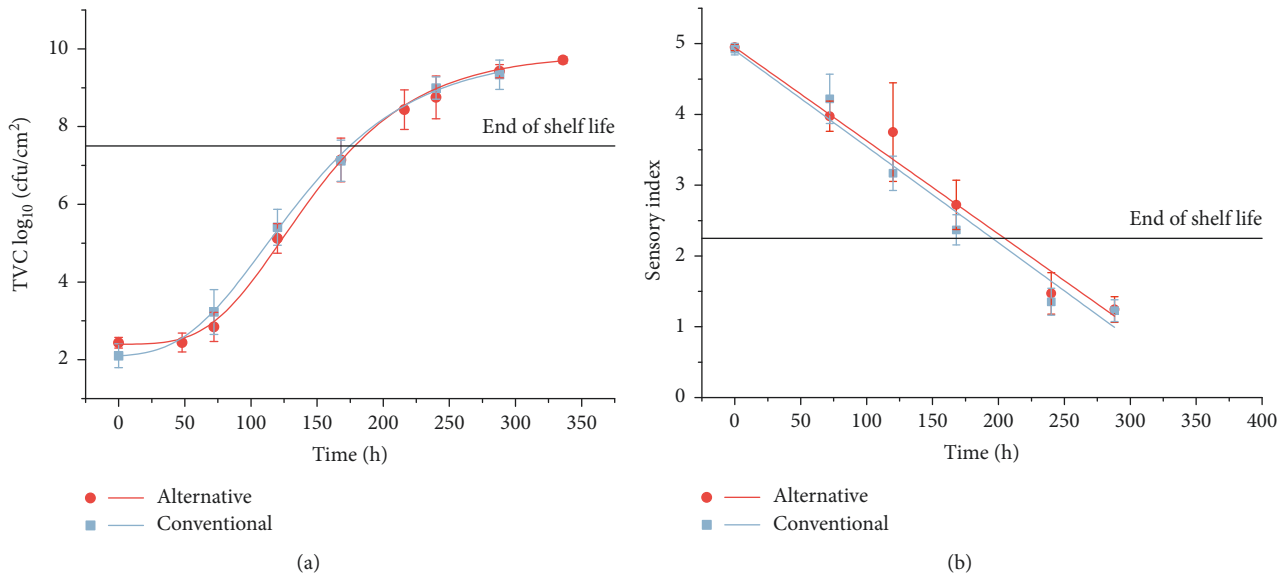

Figure 3: Shelf life of alternative and conventional produced poultry filets determined by microbiological contamination (a) and sensory index (b).

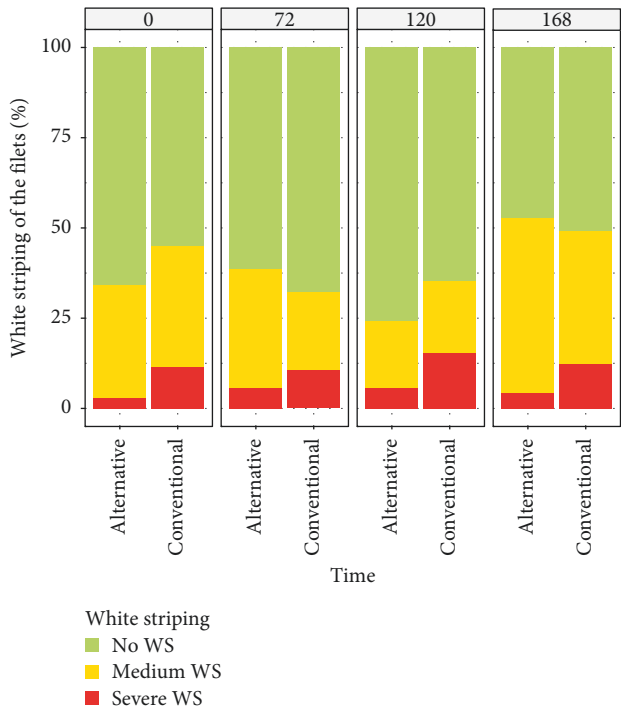

Figure 4: Occurrence and severity of WS for both production lines during storage.

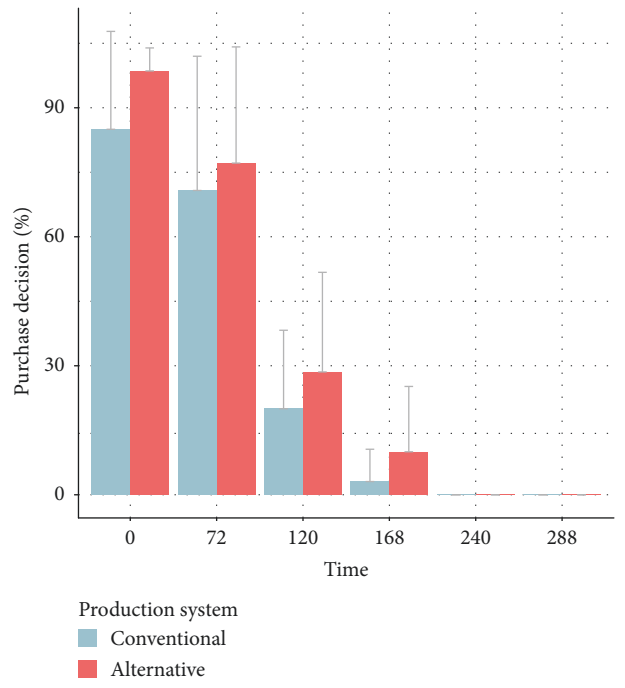

Figure 5: Purchase decision evolution during storage.

significantly correlated to the PD with correlation coefficients between 0.8 and 0.9. Additionally, PD was correlated to the L^* value (k: 0.461, $p < 0.001$, $n = 196$) with a higher rejection rate for darker filets.

4. Discussion

The results of this study showed that different production systems can have a significant influence on biochemical composition, nutritional value, and physicochemical characteristics of poultry meat. Generally, the nutritional values and muscle characteristics of the investigated poultry filets were comparable to former studies [19–21]. Filets of the alternative line had significantly higher protein and lower water and intramuscular fat content in comparison to the conventional production line. Different dietary strategies are known for their ability to modify the nutritional profile of poultry meat [22]. Maize-based diets provide an easily accessible source of energy leading to a higher protein conversion in comparison to wheat-based

feed [23]. Additionally, the race and the opportunity for a regular exercise can lower the fat and increase the protein content of poultry meat [24]. However, detailed analyses focusing on both investigated breeds are lacking. A higher motor activity favors myogenesis over lipogenesis as stated by Castellini et al [21]. Thus, a higher meat quality of the filets was observed in the alternative production line with a maize-based diet, lower stocking density, and enhanced motivation for physical activity by offering toys. The amount of L-lactic acid and D-glucose in the muscle was comparable to the results of Bruckner et al. [16]. The higher values of L-lactic acid and D-glucose can be explained by a higher glycolytic potential in the muscle of the alternative production line [25]. The glycolytic potential of the muscle antemortem has been related to the breeding and fattening of the animals, stress, exercise levels, or age [26–30]. Additionally, the selection for growth rate and age influences the glycolytic potential and the pH decline postmortem [31, 32]. The lower pH values observed for the alternative production line can be explained by the close relationship between L-lactic acid and pH value [16]. Thus, the production system and choice of a specific slower growing race showed implicit consequences for technological traits of the product. For fresh poultry meat, the pH value ranges between 5.8 and 6.2 [16, 26, 32, 33]. According to these studies, pH values observed in this investigation were comparatively high. However, higher pH values have been reported before for the Ross line and other modern poultry lines selected for fast growth and early slaughter age [31, 32, 34]. This is in agreement with the findings of this study in which the fast-growing Ross 308 had higher pH values than the slower growing line Ranger Classic. At the end of storage, the pH value shows a significant decrease which is typical for high bacterial cell counts. This is caused by the accumulation of ammonia as a result of amino acid degradation when glucose decreased to insufficient levels [29]. The pH value was closely related to the cooking loss, thawing loss, and color values of the poultry filets, which is in accordance with former studies [21, 35, 36]. Even though the dietary composition and breed were reported to have an impact on water holding capacity of the meat [27, 37–39], no differences in cooking and thawing loss could be detected between the production lines in this study.

The L^* values showed no significant difference between groups but were higher than the optimal range for poultry reported in former studies [36, 40–42]. Since cooking and thawing loss were in a normal range, a pale soft and exudative- (PSE-) like condition was not observed according to the criteria defined in former studies [40, 41]. Besides, a high variation in the L^* value of fresh poultry meat has been reported before and was explained by different preslaughter and processing conditions, resulting in varying L^* values [36]. The decrease of L^* values during storage for both investigation groups can be explained by the biochemical degradation of myoglobin and is typical for the spoilage of meat [43]. Differences in the a^* and b^* values between groups reflected the intense and more yellowish color of the filets of the alternative production. This effect was caused by the maize-based diet and higher amount of carotenoids [44]. During storage, a^* values and b^* values of the alternative line increased, while only b^* values of the conventional line showed a slight increase. Thus, the alternatively produced filets show no typical discoloration process during spoilage, characterized by a fading of the pink color typical for fresh meat. The filets rather displayed a change to a darker and more orange color.

The investigation of TBARS showed no significant difference between both production lines, even though an influence of animal diet on lipid oxidation has been reported before [45]. During storage, TBARS values showed a significant increase which is in accordance with former studies [46–48]. Thus, the differences in physicochemical properties measured between both groups did not result in a varying spoilage process. Significantly higher levels of glucose, which is a key substrate for microorganisms [49], could indicate an accelerated microbial growth on filets of the alternative group, but this was not observed. Since animals of both production lines were slaughtered and processed in the same production facility on the same day, the amount and diversity of contaminating and proliferating microorganisms showed no relevant difference. For both groups, the initial TVC contamination was low in comparison to other studies conducted under industrial slaughter conditions [16, 50–52]. The abandonment of antibiotics showed no impact on microbial contamination or growth in this study. For both groups, the microbial shelf life was in accordance with the sensory shelf life and is in the normal range for fresh, aerobically packaged poultry [37]. Shorter shelf lives for similar products were reported as well but could be related to higher initial microbial contamination [16, 52, 53]. Both production systems resulted in high quality poultry products with no implications for the spoilage process and shelf life. Since the usage of antibiotics in meat production has a high impact on environment, the increase of antibiotic resistances, and human health [9, 54], antibiotics-free systems reveal important opportunities towards a more sustainable poultry production. According to the results of this study, the realization of an alternative production system without antibiotics is possible without impacts on meat quality and shelf life. The decelerated growth of the animals and gentle fattening had no impact on the incidence of WS. Even though the conventional group displayed the highest occurrence of severe WS, no significant difference could be detected between the investigation groups. WS was significantly correlated to growth rate, genotype, slaughter age, and filet weight in former studies [2, 8, 55], but no significant correlation between the filet weight and WS was observed in this study. In contrast to former studies reporting an effect of WS on the water binding capacity [56–58], no effect of WS on drip or thawing loss was observed here, also stated by [59]. As a result of WS incidence, the PD was affected. A low consumer acceptance for filets displaying WS was also observed before [55]. The PD was mainly dominated by the color of the filets. A tendency for a preference for the alternative filets was observed, but the difference was only significant at the first investigation point.

5. Conclusion

The alternative line encompasses the opportunity towards a more sustainable poultry production due to an extensive husbandry system without antibiotics, a slower growth, and enhanced animal welfare. This investigation revealed a significant benefit for the biochemical composition and nutritional value of alternatively produced poultry meat. The poultry filets of both production lines showed an overall high quality, and no effect of the production system on the development of quality parameters and shelf life could be detected. The abandonment of antibiotics in the alternative line had no impact on the microbial quality, safety, or shelf life of the product. The decelerated growth of the animals did not lead to a significant improvement for the incidence of WS in comparison to conventional production. The PD was negatively influenced by WS and higher for breast filets from the alternative production system. A trial repetition to confirm the findings with a higher sample size is desired.

Data Availability

The authors declare that all results can be found in the Institute of Animal Science, University of Bonn, Germany.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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