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Factors influencing microbiological, physicochemical quality, and yield of dry-aging beef

[1,2,*]Hanagasaki, T.

[1]*Food Research Section of Okinawa Industrial Technology Center, Uruma, Okinawa, Japan*
[2]*Okinawa Agricultural Research Center, Miyako Branch, Miyakojima, Okinawa 906-0013, Japan*

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Abstract

The dry-aging business is widely popular across Japan. In Okinawa, most dry-aged beef is usually produced using imported beef. Microorganisms are likely to thrive on the surface of imported beef during transport to Japan, despite storage at chilled temperatures. To prevent the growth of microorganisms in such cases, accurate control of aging is critical. A fundamental understanding on a scientific level with regard to the maintenance of hygiene is essential for developing a method for dry-aged products. In this study, changes were analyzed in the water activity and the microorganisms on beef meat, in addition to moisture and trim losses (aging loss), during aging under varying levels of relative humidity and temperature, with or without airflow, and mold treatment. As the relative humidity during the aging process was lower, the number of microorganisms was lower but the aging loss was higher. Similarly, as the temperature during the aging process was lower, the number of microorganisms was lower, and the aging loss remained almost unchanged. Hence, according to the results, it is essential to lower water activity as quickly as possible to 0.94 by keeping relative humidity below 75% as a guide and freezing temperatures below 4°C but above −2 to −3°C for meat is strongly recommended. In addition, direct airflow to the beef during the aging process prevented the growth of microorganisms. In particular, it decreased *Escherichia* spp. but increase aging loss. As a result, air circulation via moderate airflow is recommended during the aging process. Besides, it became apparent that mold treatment did not prevent the growth of microorganisms.

1. Introduction

Recently, restaurants serving dry-aging beef have dramatically increased in Japan. Now, it has become popular in Western countries and Asian countries such as Japan, South Korea, Taiwan, Hong Kong and Singapore (Dashmaa *et al*., 2016; Hanagasaki and Asato, 2018a). Also, the business related to dry-aging beef has already been opened in Okinawa, the southernmost prefecture of Japan. Lots of steak houses serving beef steaks since long ago have been found, mainly because Okinawa has been influenced by American culture since World War II. Beef served at steak houses in Okinawa relies mostly on imported beef from New Zealand, Australia, and the US. Imported beef mainly consists of lean meat different from Japanese "Wagyu," marbled meat (Gotoh and Joo, 2016). Generally, lean meat is suitable for dry-aging products.

Imported beef is often used for producing dry-aging products in Japan. During the dry-aging of beef, amino acids increased in normal- and mold-aging beef, drip and cooking losses decreased in normal- and mold-aging beef, and the hardness of mold-aging beef gradually decreased (Hanagasaki and Asato, 2018a; 2022). Microorganisms are likely to increase on the surface of imported beef during transport to Japan, despite a chilled storage temperature. Thus, to prevent the growth of microorganisms when producing dry-aging products using imported beef, handling beef meats with special care is required. Okinawa belongs to the subtropical oceanic climate with high temperature and relative humidity throughout the year, different from Western countries with lower relative humidity. Thus, dry-aging products strongly require accurate control of aging and manipulating beef meats, especially in Okinawa. The primary factors that determine the quality of dry-aging products include the length of aging, storage temperature, relative humidity, airflow (Savell, 2008; Dashmaa *et al*., 2016), and mold inoculation (Hanagasaki and Asato, 2018a). Hence, each company

*Corresponding author.
Email: *hangskit@yahoo.co.jp*

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has its own method of dry-aging beef (Hanagasaki and Asato, 2018a; Mikami *et al*., 2021; Mikami *et al.*, 2022). Although rough guides are recommended for such factors (Savell, 2008), no definitive rules refer to "dry-aged" beef from a performance standpoint (Savell, 2008). It is critical to clarify the issue of meat hygiene in producing dry-aging products. Healthy muscle tissue contains few, if any, bacteria, but cut and exposed surfaces become easily contaminated after slaughter, as well as during dressing and butchering (Harrigan, 1998). For example, dressed carcasses are directly dry-aged in factories in US cities, such as New York, but not in Japan as Japanese factories tend to be small and use block meats for dry-aging beef. Factory workers might produce dry-aging beef using their own methods based on their experiences. Dry-aging beef produced under the wrong conditions, could be of low quality and cause food poisoning. A study of hygiene issues relevant to the dry-aging of beef products is essential for establishing a method for aging beef products. Currently, little is known about the microbiological aspects of dry-aging beef hygiene. The present study analyzed changes in the water activity and the microorganisms on beef meat, in addition to moisture and trim losses (aging yield), during aging under varying levels of relative humidity and temperature, with or without airflow, and mold treatment.

2. Materials and methods

2.1 Beef material

The meat used for the experiments was beef loin imported from New Zealand, from a crossbreed between Angus and Hereford, raised in pastures. Each meat block (approximately 500 g) for experiments was cut in the grocery store factory.

2.2 Aging environment

The aging environment was established in a refrigerator (Showa Denko K.K., Tokyo, Japan) in Okinawa Industrial Technology Center.

2.3 Conditions of different relative humidity

Each dry box was placed in the refrigerator at a temperature of 2°C. Depending on the concentration of glycerol solution, each dry box maintained a different relative humidity. A meat block was placed on the metallic net laying on the container with glycerol solutions in the dry box to control relative humidity levels (from 52% to 98%).

2.4 Conditions of different temperature

Each dry box was placed in the refrigerator at 2°C, 6°C and 10°C at different times. A meat block was placed in the dry box under maintained conditions of approximately 73% relative humidity controlled by glycerol solutions described above.

2.5 Conditions of direct airflow to beef

Dry boxes were placed in the refrigerator at a temperature of 2°C. A meat block was placed in the dry box under maintained conditions of approximately 75% relative humidity controlled by the glycerol solution described above. A small fan provided airflow (San Ace 120, MODEL109S085, Sanyo Denki Co. Ltd., Tokyo, Japan) in the dry box. Its wind velocity was approximately 2.0 m/s measured by an anemometer. For comparison, a meat block was in the same condition of relative humidity and temperature without air. The experiment under the conditions of 92% relative humidity was done by putting a meat block directly into the refrigerator at 2°C with the fan blowing on the meat block (KGS-1845 45 cm, Shimabukuro Ltd., Okinawa, Japan). The average wind velocity was approximately 2.0 m/s measured by an anemometer. For comparison, a block of meat was in the refrigerator without air at a different time.

2.6 Mold treatment

Dry boxes were placed in the refrigerator at a temperature of 2°C. A meat block was placed in the dry box under maintained condition of approximately 75% relative humidity controlled by the glycerol solution described above. As for the mold aging experiments, the mold strain identified as *Mucor flavus* (Hanagasaki and Asato, 2018a) was cultured on potato dextrose agar (PDA) plates (Merck Ltd., Tokyo, Japan) for a week, and then its spore and mycelium were allowed to contact a meat block. For comparison, a meat block was in the same condition of relative humidity and temperature without mold treatment.

2.7 Measurement of relative humidity and temperature

Relative humidity and temperature were measured every 5 mins during the aging process using DL171 (AS ONE Corp., Osaka, Japan).

2.8 Water activity measurement

Three samples were collected at an appropriate interval (over 5 cm apart) from each other in one meat block. The sampling was performed approximately 5 cm^2 × 1 mm thick from the surface of the beef meat (the surface of the trimming part) during the aging process. Water activity was measured using a water activity meter CX-2 (Japan general appliance corp., Tokyo, Japan).

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2.9 Microbiological analysis

Samples for water activity measurement described above were also used for microbiological analysis. The numbers of microorganisms were measured by the modified plate count method (Yamazato *et al*., 1986). Samples (0.5 g) were blended with 4.5 mL of sterilized distilled water (10 times dilution), and 10^{-1} to 10^{-8} serial dilutions were made in sterilized distilled water. Three samples were used as replications per each test. From each dilution, 0.1 mL of suspension was spread on agar plates. General bacteria (the total viable count of bacteria) and *Escherichia* spp. were counted on nutrient agar and blue light agar plates (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), respectively, after incubating at 30°C for a day. Yeast and mold were counted on PDA plates (Merck Co. Ltd., Tokyo, Japan) with 100 mg/L chloramphenicol after incubating at 30°C for 2 days. Molds were distinguished from yeast by colony appearance and cell morphological observation. *Salmonella* spp. and *Staphylococcus* spp. were respectively counted on Sanitakun for Salmonella and Staphylococcus (Chisso Corp., Tokyo, Japan) after incubating at 35°C for a day. Colonies were counted as viable numbers of microorganisms [log colony-forming units (log CFU)/g].

2.10 Measurement of moisture and trim losses

Moisture loss was the weight of water lost from meat and was determined by measuring the difference in meat weight between before and after it had been subjected to aging. The trim loss was the weight of the trimming part of the discolored and dehydrated meat (Hanagasaki and Asato, 2018a).

2.11 The growth limit of water activity for the mold

Water activity level was controlled by each different glucose concentration contained in PDA plates. Using these PDA plates having different water activities, it was confirmed whether the growth of the mold was observed or not. In the same way, PDA plates with different water activities controlled by maltose, sorbitol, and sodium chloride were used to confirm the mold growth (data not shown).

3. Results

Water activity on the beef surface was lower after 21 days of aging as the relative humidity level during the aging process was lower (Figure 1). Equally, the number of general bacteria, *Escherichia* spp. and yeast were lower. The sum of moisture and trim losses was higher after aging as the relative humidity level during the aging process was lower (Figure 2). As for the experiments under maintained conditions of different temperatures, as the temperature level during the aging process was lower, water activity was higher after aging, but general bacteria, *Escherichia* spp., and yeast grew less and resulting in their number being lower after aging (Figures 3 and 4). The sum of moisture and trim losses was constant even though the temperature level during the aging process differed (Figure 5). As for the experiments under maintained conditions with direct airflow to beef, water activity decreased at an early stage of aging within a week (Figures 6 and 7). Especially, it dramatically decreased in maintained conditions of 75% relative humidity. And airflow prevented the increase of microorganisms through 3 weeks of aging. Specifically, *Escherichia* spp. apparently decreased in 2 weeks in the condition of 75% relative humidity with an airflow whereas it increased without air. The sum of moisture and trimming losses was higher in both conditions of 75% or 92% relative humidity with an air flow than in the conditions without air (Figure 8). As for the experiments with mold treatment or not, water activity decreased as the number of days of aging increased in both experiments (Figure 9). However, both experiments increased general bacteria, *Escherichia* spp., and yeast. The growth rate of general bacteria and *Escherichia* spp. was almost the same in both experiments, but the growth

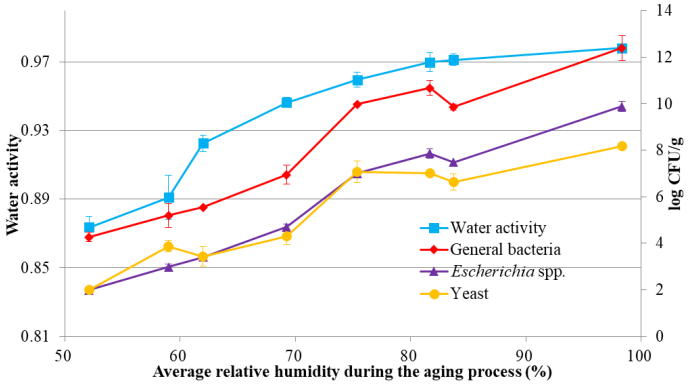

Figure 1. Water activity and the number of microorganisms after 21 days of aging under different relative humidities. The average of temperature during the aging process was 2°C.

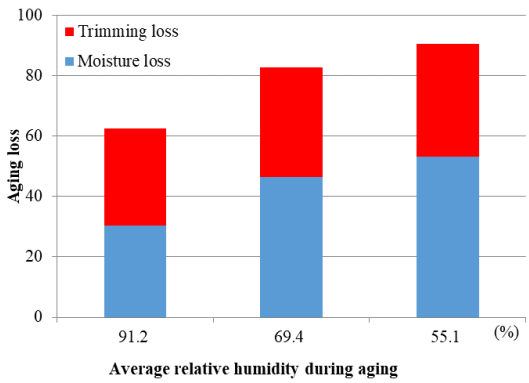

Figure 2. Moisture and trimming losses after 21 days of aging under different relative humidities. The average temperature during the aging process was 2°C.

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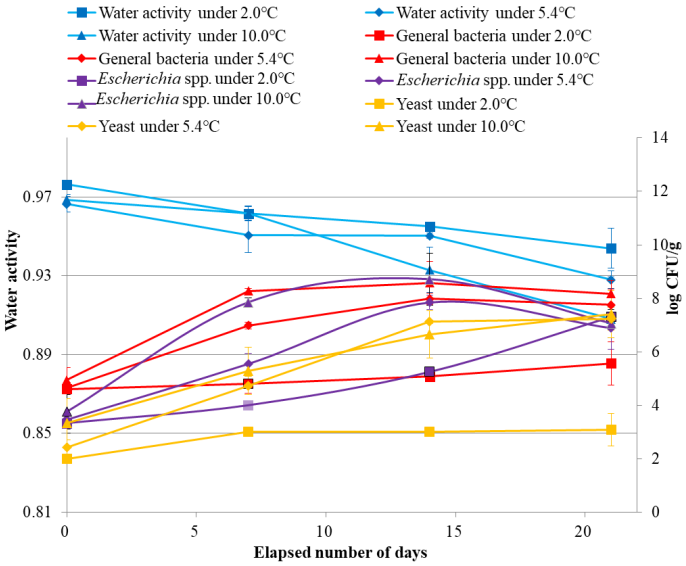

Figure 3. Water activity and the number of microorganisms during aging under different temperatures. The average relative humidity during the aging process was 73%. The dilute color indicates an undetected maximum value, which means a real value is under the point. The point outlined black line indicates the maximum value of three means.

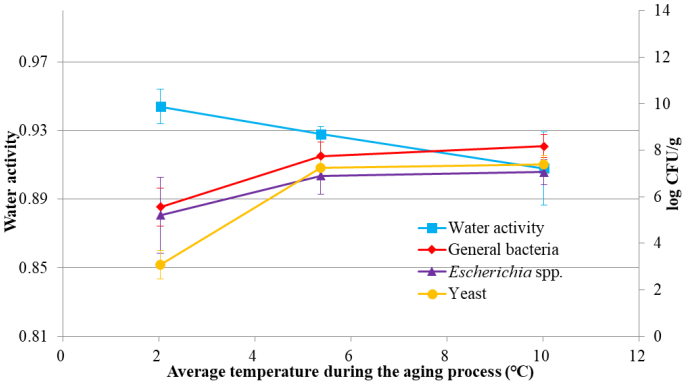

Figure 4. Water activity and the number of microorganisms after 21 days of aging under different temperatures. The average of relative humidity during the aging process was 73%.

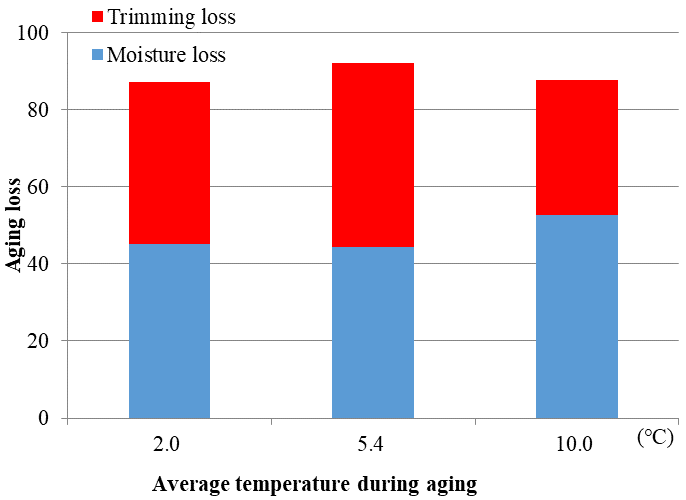

Figure 5. Moisture and trimming losses after 42 days of aging under different temperatures. The average of relative humidity during the aging process was 73%.

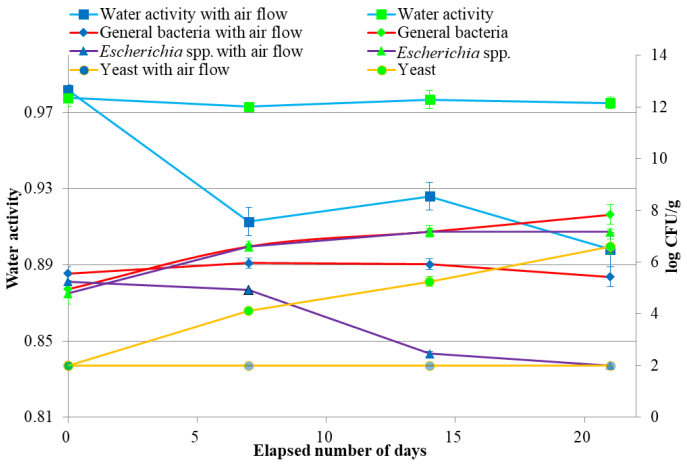

Figure 6. Water activity and the number of microorganisms during aging with air flow or without. The relative humidity and temperature average during the aging process was 2°C and 75%. The dilute color indicates an undetected maximum value, which means a real value is under the point. The point outlined black line indicates the maximum value of three means.

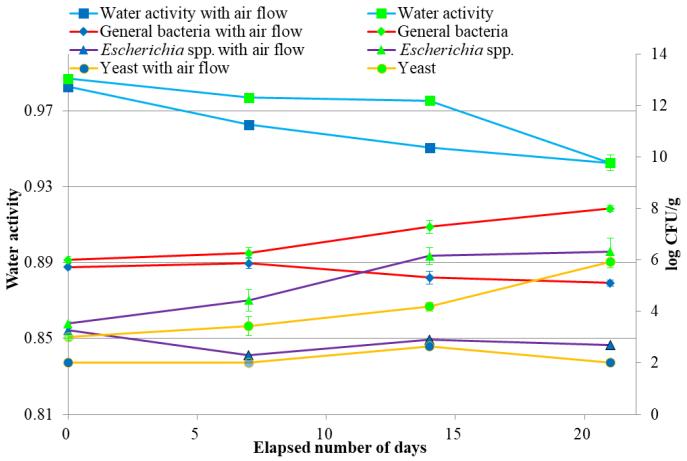

Figure 7. Water activity and the number of microorganisms during aging with airflow or without. The relative humidity and temperature average during the aging process was 2°C and 92%. The dilute color indicates an undetected maximum value, which means a real value is under the point. The point outlined black line indicates the maximum value of three means.

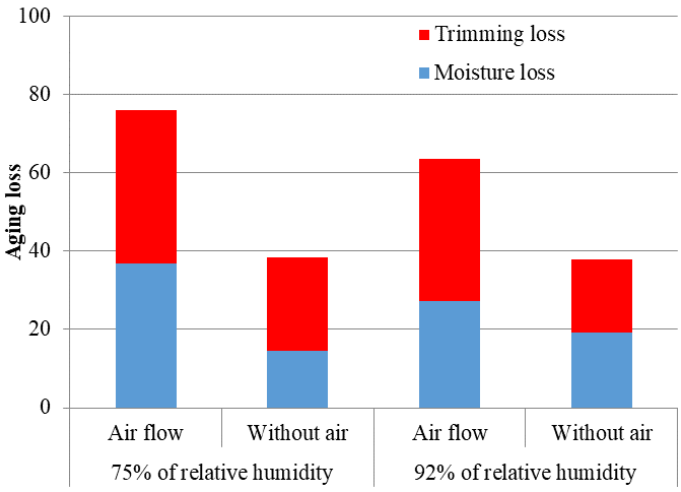

Figure 8. Moisture and trimming losses after 21 days of aging with airflow or without. The average of temperature during the aging process was 2°C.

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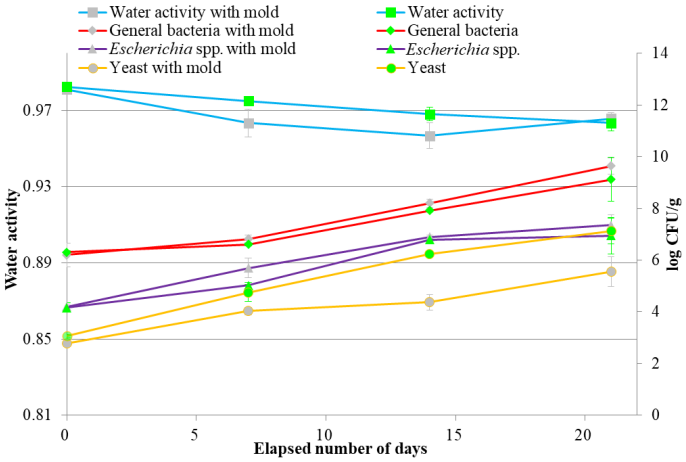

Figure 9. Water activity and the number of microorganisms during aging with mold or not. The relative humidity and temperature average during the aging process was 2°C and 75%.

rate of yeast was lower in the experiment with mold compared to the experiment without mold. Incidentally, the growth limit of water activity for the mold was 0.94 (Figure 10).

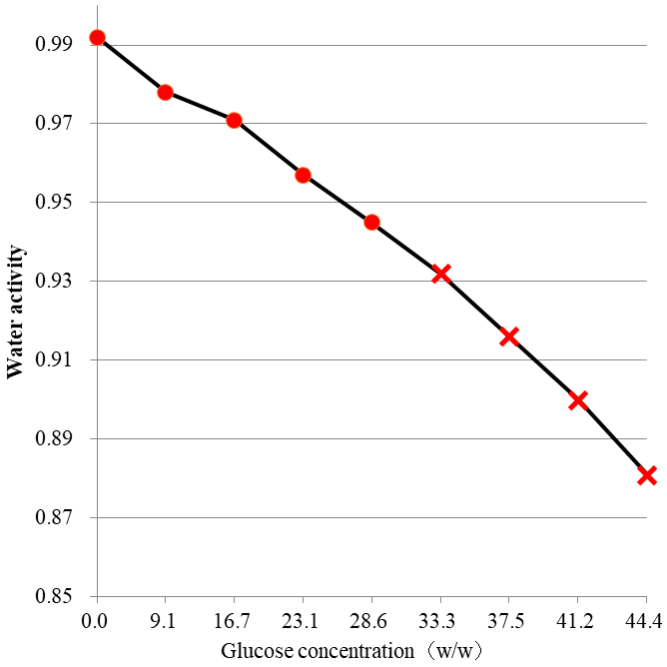

Figure 10. Growth limit of water activity for the mold on plates with different glucose concentrations. ○, growth observed; ×, growth not observed.

4. Discussion

The increase of microorganisms on the meat surface is affected by many factors. First, until imported beef was delivered to Okinawa, several factors were considered, conditions of slaughter, cuts, packing, vacuum, storage environment during import, and the length of import. Next, are the conditions of cutting and packing to make final products after delivery to grocery stores and restaurants. Last, the situations and conditions of each meat block, such as where the beef body part is, the shape of the meat block, the unevenness of the meat surface, and the distribution of fat on meat are other influencing factors. As per a grocery worker, although it was difficult to determine the accurate length of beef transport to Okinawa from foreign countries, it was believed to be 3 or 4 weeks. Above all, the importation process was considered the most influential because it took a relatively long time. In fact, The number of microorganisms was occasionally different depending on the meat lot obtained. The proof of this is that beef grown and sold at a grocery store in Okinawa has 2 log CFU/g of general bacteria at most, compared to 4–6 log CFU/g of imported beef. Accordingly, it was impossible to simply compare the number of microorganisms on aging imported beef because they were already different at the start of aging. Therefore, the present study did not adopt a statistical analysis of imported beef. The number of microorganisms on a meat surface was sometimes scattered, although three pieces of samples on a meat surface were obtained from one meat block. By contrast, their water activity was relatively less scattered. There were often differences among the number of microorganisms on beef meats affected by the processing and preservation process. This should be considered when the aging business begins. It is critical to take measures to produce aging products, such as searching the details of the import process and inspecting the microorganisms in imported beef. Especially, bacterial tests are essential and should be conducted periodically even after aging. In addition, it has to be continued as long as aging products are sold. It is the key to minimizing the growth of undesirable microorganisms to produce quality aging beef (Savell, 2008; Australian Meat Processor Corporation and Meat and Livestock Australia (AMPC and MLA), 2010). Generally, the growth of microorganisms is more prevented as water activity on the growing point is lower (Japan Food Research Laboratories, 2003). In the present study, as the relative humidity level during the aging process was low, water activity on the meat surface was lower after aging and resulted in fewer microorganisms. In the first place, it was almost impossible to kill all general bacteria only by aging. The number of microorganisms increased in aging beef even though water activity gradually decreased during the aging process. In the case of aging beef produced in 52.1% relative humidity for 21 days, there was 4.27±0.2 log CFU/g of general bacteria. This value was the least of all aging beefs produced for 21 days and almost the same as before aging. It means that decreasing the number of microorganisms existing before aging is difficult. However, in this case, it may change the composition of microflora. Actually, on this aging beef, water activity was under 0.90. Gram-positive bacteria, yeast, or mold could have generally survived in this environment (Japan Food Research Laboratories,

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2003). One of the Gram-positive bacteria, lactic acid bacteria, was believed to be the major flora because *Staphylococci*, yeast, and mold were undetected (data not shown). Besides, this aging beef contained an odor of lactic acid. Around 0.94 of water activity indicates the limit growth of many kinds of food-poisoning bacteria such as *Salmonella* spp. and *E. coli* (International Commission on Microbiological Specifications for Foods (ICMSF), 1996: Lake *et al*., 2002). Fortunately, *Salmonella* spp. was undetected even if water activity on the meat surface was over 0.94 after aging in the present study (data not shown). Nevertheless, it is essential to lower water activity as quickly as possible to 0.94 by keeping relative humidity below 75%, considered a target from a hygiene standpoint.

The generation time of all microorganisms is longer as the temperature is lower (Yotis and Teodoro, 1957). It means keeping a lower temperature can prevent the microorganism growth more, such as the result in the present study despite the fact the beef surface had higher water activity as the temperature was lower. It was noteworthy that *Escherichia* spp. increased by 10^4 times during the aging process at 10°C just for one week. *Escherichia* spp. existed over 7 log CFU/g level after 3 weeks of aging at 10°C even though water activity was below 0.94. Moreover, water activity was 0.85 after 6 weeks of aging but *Escherichia* spp. existed at 6 log CFU/g level (data not shown). Accordingly, it is too difficult to destroy *Escherichia* spp. once it increases. The temperature range for growth of *Salmonella* spp. is 5.2–46.2°C (ICMSF, 1996) and maintaining fresh-cut products at 5°C or below is critical for reducing the food safety risks from *E. coli* O157:H7 (Luo *et al.,* 2010). Thus, considering the possibility of the growth of these foodborne pathogens, aging at 10°C is believed to be risky. Accordingly, aging at 4°C or below but above −2 to −3°C of freezing temperatures for meat is strongly recommended.

Airflow allows temperature and relative humidity to be distributed evenly in the environment. In dry-aging beef, evaporated water remained around the beef surface and moved away by airflow directly hitting the beef surface. Moreover, this effect can make beef blocks more evaporated and promote aging (Savell, 2008). Air was continually blown over beef meat during the aging process in this study. Because of the limited number of fans working in limited space, this type of research is rarely conducted in factories. Nevertheless, the clear effect of airflow was obtained to prevent general bacteria from increasing and decrease *Escherichia* spp. This context demonstrated that airflow plays an important role for factories producing dry-aging products from a hygiene standpoint. As a result, air circulation via moderate airflow is recommended during the aging process.

The mold growth on the meat surface did not reduce other microorganisms. Mold treatment had no significant effect on reducing aerobic bacteria, coliforms or fecal streptococci at 4 and 8°C for 2 days, compared with uninoculated controls (Campano *et al*., 1985). Similarly, the present study showed that mold treatment did not reduce general bacteria, *Escherichia* spp., and yeast during 3 weeks of aging at 2°C. The mold needs 0.94 of water activity for its growth from the result but the value is also the threshold of the food poisoning bacteria growth (ICMFS, 1996; Lake, 2002). It is hard to strike a proper balance of water activity considering promoting the growth of the mold and preventing the growth of undesirable microorganisms. As the best way, It is suggested that the mold is to be inoculated as much as possible while contamination is avoided as much as possible. However, mold inoculation is believed to be unusual in production sites. It is said that 7–8 log CFU/g of general bacteria lead to initial decomposition. Even in the presence of over 12 log CFU/g of general bacteria, aging beef did not smell of decomposition in this study. The occurrence of spoilage odor must be avoided even if the beef surface is all trimmed. On the premise that the product will be grilled as steak, initial decomposition would not be a problem. However, in the case of providing dry-aging beef steaks with a degree of doneness, such as “Rare” and “Medium rare,” including raw meat, severe rules would need to be applied to dry-aging products.

In terms of aging yield, the sum of moisture and trim losses, there was a decreasing trend as the relative humidity level during the aging process was higher. As described above, lowering the relative humidity level is essential from a hygiene standpoint, but it is negative regarding aging productivity. Reducing the saleable yield of the product needs the ultimate price to recuperate this loss (Savell, 2008). Therefore, the relative humidity level needs to be well balanced, and that must be kept inside storage. 70% of relative humidity is thought to be the target in the case of no airflow, taking hygiene and productivity standpoint into account. The aging yield was lower as the number of days of dry aging increased (Hanagasaki and Asato, 2018a; 2018b). Interestingly, aging yield of mold aging beef was significantly higher than that of no mold aging beef in 4 weeks (Hanagasaki and Asato, 2018a). Also, smaller beef block was easier to evaporate, and its aging yield was lower (data not shown). As described in the Introduction, dry aging is generally conducted using the block part of the beef body in Japan. The size of beef block also must be considered to devise a strategy for producing dry aging

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beef. The following is a summary of the above. When dry aging is applied for imported beef, it is recommend that relative humidity is as low as possible to approximately 75% as a guide; temperature is necessarily below 4℃; air circulation via moderate airflow is recommended during the aging process, and mold is inoculated as much as possible while avoiding contamination as much as possible when treating mold.

4. Conclusion

As the relative humidity level during the aging process was lower, the number of microorganisms was lower but the aging loss was higher. As the temperature level during the aging process was lower, the number of microorganisms was lower, and aging loss was almost unchanged. Direct airflow to beef during the aging process prevented microorganism growth. Especially, it decreased *Escherichia* spp. but made aging loss high. Mold treatment had no effect of preventing the growth of the microorganisms. In conclusion, it is recommended that relative humidity is as low as possible to approximately 75% as a guide; temperature is necessarily below 4℃; air circulation via moderate airflow is recommended during the aging process.; and mold is inoculated as much as possible while avoiding contamination as much as possible when treating mold.

Conflict of interest

The author declares no conflict of interest.

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