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Effect of Water Activity, Yuza (*Citrus junos* Sieb. ex Tanaka) Powder and the Mixture of Sodium Lactate and Sodium Acetate on Quality and Safety in Beef Jerky

Yeon Ho Kim, Ju Young Lim, Ki Sun Yoon*

Department of Food and Nutrition, Kyung Hee University, Seoul, Republic of Korea
Email: *ksyoon@khu.ac.kr

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Abstract

Despite its low water activity (A_w), food poisoning accidents associated with beef jerky consumption and problems due to mold growth have been reported. The objects of this study were to investigate the effect of yuza powder and the mixture of sodium lactate (SL) and sodium acetate (SA) to prevent the growth of *S. aureus* and *A. flavus*, as well as aflatoxin production in beef jerky. Different concentrations of the SL and SA (0, 0.75, and 1%) mixture were added to sliced beef and the A_ws were adjusted to 0.70, 0.75, and 0.80. The effect of yuza powder and the SL and SA mixture on *S. aureus* in beef jerky (A_w 0.80) was also investigated at 10˚C and 25˚C. A rapid decline is that *S. aureus* population was observed in beef jerky containing yuza powder and mixtures of SL and SA. Antifungal effects against *A. flavus* were observed in beef jerky containing the SL and SA mixture at A_w below 0.75. The growth of *A. flavus* and aflatoxin production were prevented in beef jerky containing the yuza powder and a 1% mixture of SL and SA at 10˚C and 0.80 A_w. The addition of yuza powder increased the hardness score of the beef jerky. Under current A_w regulations (0.80), beef jerky may pose a public health risk due to the growth of *A. flavus* and the presence of aflatoxin.

Keywords

Beef Jerky, Yuza, *Staphylococcus aureus*, *Aspergillus flavus*, Aflatoxin

1. Introduction

Beef jerky is a popular snack in many countries. Recently, various jerky products with different types of meats and seasonings have been introduced in on- and

off-line markets and they have become popular snack items in Korea. Beef jerky is one of the oldest traditional foods and is prepared by simple processing, such as drying and salting [1]. However, no regulations govern the safety of beef jerky in Korea because it has been considered to be a safe food. The water activity ($A_{w)}$ of jerky products in Korea ranges from 0.70 to 0.88 [2], while the US Department of Agriculture (USDA) regulates maximum water activity of jerky products at 0.80 [3]. Despite the low A_w, over 250 cases of accidental food poisoning associated with the consumption of jerky were reported between 1966 and 2003 [4]. *E. coli* O157: H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and several Salmonella strains [5] [6] were implicated as causative bacteria. A recent study reported the growth of *Staphylococcus aureus* on beef jerky (A_w 0.78) at temperatures above 21˚C [7]. Jerky products are associated; not only foodborne outbreaks but also with spoilage problems resulting from mold growth [8].

In the jerky industry, mold is a more serious problem than bacteria (personal communication). *Aspergillus flavus* was reported in Spanish dried-cured ham and, thus, produced aflatoxin [9]. In order to control the mold, beef jerky was exposed to 0.47 kGy radiation [10]. Nitrites and sodium chloride are commonly used to prepare beef jerky. However, nitrites do not control the growth of fungi [11] and the addition of sodium chloride may cause many health problems, so low-sodium preparation methods have been explored.

Yuza (*Citrus junos* Sieb. ex Tanaka) is mainly cultivated in Korea, Japan, and China and is a citrus fruit [12]. Unlike oranges, grapefruit, and lemons, the peel, as well as the flesh of yuza, can be used as food ingredients. Yuza is consumed with tea, where the sliced yuza peel and flesh are sugared and made into a marmalade-like syrup sweetener. Currently, yuza has received attention as a healthy food ingredient from natural plants. Yuza fruit contains vitamin C, dietary fiber, beta-carotene, flavonoids, and limonoids, which cause a bitter taste. New product development efforts using yuza's peel and flesh residue have been reported [13]. The antimicrobial effects of other citrus fruits, such as grapefruit [14] and oranges [15] have also been reported. But yuza's antimicrobial and antifungal effects have not yet been investigated.

Opti.Form Powder Ace S50 contains a 5:5 mixture of sodium lactate (SL) and sodium acetate (SA), produced by fermentation from sugar. It is also known to extend the shelf life of meat products [16]. Various concentrations of sodium (di) acetate and sodium lactate mixtures have been used to prevent spoilage and the growth of pathogenic bacteria in meat products [17] [18]. Lactate alone (1.5% to 3%) or in a mixture of 0.125% to 0.25% diacetate (wt/wt) has been used in ready-to-eat (RTE) meats as antimicrobial additives [19].

In this work, we investigated the effects yuza powder and the mixture of sodium lactate and sodium acetate on the physicochemical, antimicrobial, antifungal, and aflatoxin-inhibiting properties of beef jerky as functions of A_w and temperature.

2. Materials and Methods

2.1. Preparation of Dried Yuza Powder

Yuza was purchased in Corporation StoreFarm (Goheung, Korea) and washed with tap water. The washed yuza was cut in half and was squeezed to exclude juice. Then, the squeezed yuza halves were dried in a dried oven (HFD-6000HL, Hanil, Seoul, Korea) at 55˚C for 24 h. Dried yuza was grounded in a blender (HR2870/00, Philips, Armsterdam, Netherland) as a powder, which was stored at −20˚C until it is being used.

2.2. Inoculum Preparation

The *Staphylococcus aureus* (ATCC 13565) strain used was purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and kept at −80˚C in tryptic soy broth (TSB, Difco, Becton Dickinson, Sparks, MD, USA) containing 20% glycerol. For the experiments, 10 μL of thawed stock culture was inoculated into 10 mL of sterilized TSB and was incubated at 35˚C for 24 h on a rotary shaker (VS-8480SR, Vision, Seoul, Korea) at 140 rpm. One milliliter of stationary phase *S. aureus* was diluted into 9 mL of 0.1% sterilized peptone water (Difco) before inoculation onto beef jerky.

Aspergillus flavus (KCCM 11453) was also purchased from KCCM. The strain was stored at −20˚C in a mixed solution of sterilized distilled water containing 10% glycerol. The culture was placed in 45 mL of potato dextrose broth (PDB, Difco) at 25˚C for seven days on a rotary shaker (VS-8480SR, Vision, Seoul, Korea) at 140 rpm. Culture suspension (100 μL) in PDB was inoculated on potato dextrose agar (PDA, Difco) and stored in an incubator (VS-1203P4S, Vision, Seoul, Korea) at 25˚C for seven days.

2.3. Preparation of Beef Jerky

Sliced beef (0.7 cm thickness) was purchased from a local market (MangWon market, Seoul, Korea) and marinated with 15% sugar and 10% soy sauce with 0 (control) or 1.5% yuza powder. Then, 0.75 or 1% of the 5:5 mixture of sodium lactate and sodium acetate (Opmti. Form Powder Ace S50) (Corbion Purac, Amsterdam, Netherlands) was added to the marinated sliced beef with 0% yuza powder (BJ), 1.5% yuza powder (YBJ), 0.75% SL + SA with 0% yuza powder (0.75 BJ), 1% SL + SA with 0% yuza powder (1 BJ), 0.75% SL + SA with 1.5% yuza powder (0.75 YBJ), or 1% SL + SA with 1.5% yuza powder (1 YBJ). All samples were stored overnight in the refrigerator. In order to investigate the effect of A_w on the safety of beef jerky, 0.75 BJ and 1 BJ were placed in hot air dryer (HFD-6000HL, Hanil, Seoul, Korea) to adjust A_w into 0.80, 0.75, and 0.70, respectively. On the other hand, A_w of beef jerky marinated with 1.5% yuza powder was adjusted to 0.80. Then, the beef jerky was cooled to room temperature. The preparation step for beef jerky is summarized in Figure 1.

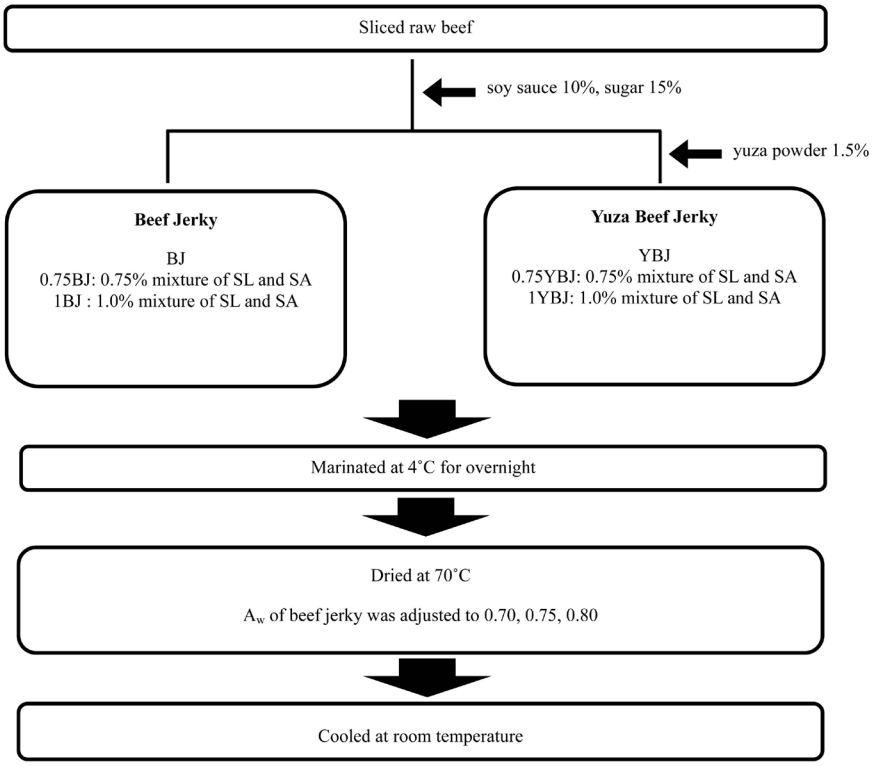

Figure 1. Flow chart of process for beef jerky containing yuza powder and the mixture of sodium lactate (SL) and sodium acetate (SA).

2.4. Inoculation of *S. aureus* and *A. flavus* onto Beef Jerky

Beef jerky was cut into 5 g pieces using sterilized scissors and forceps and put into sterilized Petri dishes (60 × 15 mm). Diluted *S. aureus* culture (25 μL) was evenly inoculated twice onto the surface of the beef jerky using an electronic pipet (MPA-200, A & D Company, Tokyo, Japan). The initial level of *S. aureus* in the beef jerky was approximately 4 log CFU/g. The inoculated beef jerky was stored at 10˚C and 25˚C.

A mixture of 1 mL 0.1% Tween 80 solution and 5 mL sterilized distilled water was added to fully grown *A. flavus* on PDA media. Then, the spores were scraped and washed using a sterilized loop and the numbers of spores per mL were counted using YM 3M Petrifilm (3M Corporation, St Paul, MN, USA). The concentration of the spores was approximately 6 - 7 log CFU spores/mL. The diluted *A. flavus* spore solution was inoculated onto the surface of the beef jerky evenly using an electronic pipet at a final concentration of 4 - 5 log CFU/g and the beef jerky was stored at 10˚C and 25˚C for eight weeks and four weeks, respectively. The spores were counted every week using YM 3M Petrifilm.

2.5. Survival Model

The survival curves were generated and iteratively fitted to the Weibull equation using a GinaFiT V 1.5 program (Geeraerd and Van Impe Inactivation Model Fitting Tool).

Weibull model:

$$\log_{10}(N) = \log_{10}(N_0) - (t/delta)^p \quad (1)$$

N_0: log initial number of cells, t: time

The Weibull model provides two parameters: delta which is referred to as a characteristic of time parameter and p is the shape parameter. The delta value is the time of the first decimal reduction concentration for part of the population. A p value < 1 indicates a concave upward survival curve and $p > 1$ is a concave downward survival curve. If $p = 1$, the decrease is log-linear, which corresponds to a first-order decay reaction [20].

2.6. Effect of Yuza Powder on the Inhibition of Aflatoxin Production

A. flavus solution (100 μL) at a concentration of 10^6 - 10^7 spores/mL was inoculated into beef jerky samples, which were either stored at 10˚C for eight weeks or at 25˚C for four weeks. At the end of each storage period, 25 mL of 70% methanol was added to 5 g of each sample, which was shaken on a rotary shaker at 140 rpm during 15 minutes. Then, the total aflatoxin quantity was estimated using the AgraQuant Aflatoxin Test Kit (Romer Labs, Getzersdorf, Austria).

2.7. Water Activity (A_w)

The water activity (A_w) of beef jerky was measured using a waster activity meter (AwTherm Rotronic, Hauppaugue, NY, USA) at 23˚C.

2.8. Instrumental Color Analysis

For color analysis of the beef jerky, the values of a^* (redness), b^* (yellowness), and L^* (lightness) were measured using a colorimeter (Minolta CR-400, Osaka, Japan). Before sample measurements, the colorimeter was calibrated with a white standardization plate. All samples were cut into rectangular shapes (5 × 5 cm). The color of beef jerky was measured at five different parts of the sample.

2.9. Texture Profile Analysis

The beef jerky was cut into pieces (10 × 10 × 3 mm) and the texture was measured using a texture analyzer (CT-10K, Brookfield, Middleboro, MA, USA). A TA7 knife edge (60 mm) was used as a probe. Conditions for the texture profile analysis (TPA) of beef jerky were 2.0 mm/s for pre-test speed, 1.0 mm/s for test speed, and 5.0 mm/s for post-test speed with 70% deformation of the test target type and 10 g of trigger load. Texture profile analysis values, such as hardness, adhesiveness, cohesiveness, springiness, chewiness, and gumminess, were measured as described by the Bourne method [21].

2.10. Sensory Evaluation

In order to study the effect of yuza powder and the mixture of SL and SA on beef jerky, four kinds of beef jerky were prepared for sensory evaluation: 0.75 BJ

(0.75% SL + SA with 0% yuza powder), 1 BJ (1% SL + SA with 0% yuza powder), 0.75 YBJ (0.75% SL + SA with 1.5% yuza powder), and 1 YBJ (1% SL + SA with 1.5% yuza powder). Untrained panelists were recruited via on and off-line advertisement and were asked to indicate their degree of liking, preference or acceptance of a product directly. The samples were served in random order to seventy untrained panelists to evaluate the sensory properties of color, smell, taste, and texture, as well as their overall preference for beef jerky containing yuza powder and the SL and SA mixtures. A 7-point hedonic scale was used to evaluate color, smell, texture, and overall preference ranging from 1, indicating extremely disliked to 7, indicating extremely liked. In addition, taste (bitterness and sweetness) was also evaluated on a 7-point scale, where 1 indicated no taste at all and 7 indicated a strong taste.

2.11. Statistical Analyses

All experiments were performed at least twice. The significance of the differences between the sample treatments was measured by one-way ANOVA, followed by Duncan's multiple range test ($p < 0.05$) using the SAS program (version 9.3, SAS Institute, Inc., Cary, NC, USA).

3. Result and Discussion

3.1. Effect of Water Activity and the Mixture of Sodium Lactate and Sodium Acetate on the Fate of *S. aureus* in Beef Jerky

Water activity affects the growth of bacteria. *S. aureus* may grow at A_w values near 0.86 [22]. In order to investigate the effect of water activity and the SL and SA mixture on the behavior of *S. aureus* in beef jerky, 0.75 or 1% of the SL and SA mixture was added and the water activity of beef jerky was adjusted to 0.70, 0.75, and 0.80 and it was stored at 25˚C. The *S. aureus* population decreased in the original beef jerky, regardless of the A_w values, and the reduction rate was affected by the water activity and the concentration of the SL and SA mixture (**Figure 2**).

The delta value of *S. aureus* was the lowest at A_w 0.75. The shape parameters (p) of all samples at 25˚C were $p > 1$ (data not shown), which corresponded to downward concavity (**Figure 2**). This indicated that *S. aureus* in beef jerky displayed a long survival lag phase in the beginning but a rapid death at the end of 13 days of storage at 25˚C. Regardless of the presence of the SL and SA mixture, *S. aureus* in beef jerky at A_w 0.70 survived longer than that at A_w 0.75. According to the work by Ingham and others [23], *S. aureus* and *L. monocytogenes* in vacuum packaged beef jerky rapidly decreased at A_w 0.87 compared to A_w 0.63, suggesting that other factors, such as seasoning, smoke compounds, besides A_w affected *S. aureus* behavior. A recent study reported that *S. aureus* grew in commercial aerobically packaged beef jerky with an A_w of 0.78 at temperatures above 21˚C [7].

In addition, the effect of the 5:5 mixture of SL and SA on *S. aureus* in beef

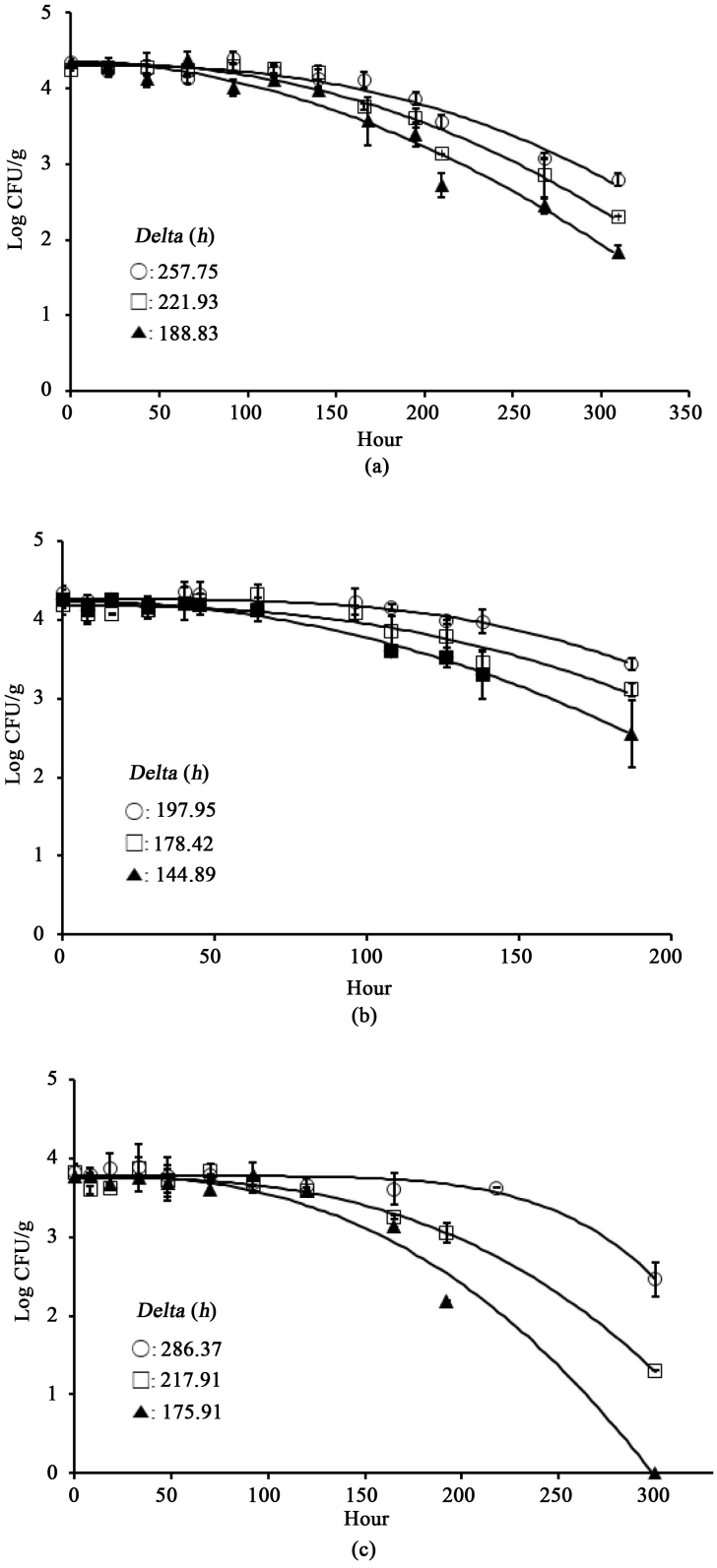

Figure 2. Effect of A_w and the mixture of sodium lactate (SL) and sodium acetate (SA) on *S. aureus* survival in beef jerky at 25˚C. (a) A_w 0.70; (b) A_w 0.75; (c) A_w 0.80; ○: control beef jerky without the mixture of SL and SA □: beef jerky containing 0.75% mixture of SL and SA ▲: beef jerky containing 1.0% mixture of SL and SA.

jerky was investigated at 25˚C. Regardless of the water activity, the delta and *p* values were reduced as the concentration of the SL and SA mixture increased from 0.75 to 1%, indicating that the SL and SA mixture had an antimicrobial effect on *S. aureus* in beef jerky. The SL and SA mixture prevented the growth of *Listeria monocytogenes* in ready-to-eat meat products at 4˚C for three months [24]. Most previous studies on the antimicrobial effect of lactate and diacetate have concentrated on *L. monocytogenes* in meat products. Since beef jerky is manufactured by a manual process, the risk of *S. aureus* due to cross-contamination during manufacturing should not be ignored and control measures for the safety of beef jerky must be implemented during the manufacturing process.

3.2. Effect of Water Activity and The mixture of Sodium Lactate and Sodium Acetate on the Fate of *A. flavus* in Beef Jerky

In the commercial market, various beef jerkies are sold in aerobic or anaerobic packages and, thus, fungal growth occurs easily in aerobically packaged jerky [8]. Nitrite is added to jerky to control bacterial growth [25]. However, nitrites do not prevent the growth of fungi [11]. In this study, the ability of A_w and the SL and SA mixture to control the growth of *A. flavus* in beef jerky at 25˚C for four weeks was investigated (**Figure 3**). The growth of *A. flavus* in beef jerky containing the SL and SA mixture was inhibited at 25˚C for four weeks, regardless of the A_w, indicating that addition of the SL and SA mixture to beef jerky had an antifungal effect. Without the SL and SA mixture, at A_w above 0.75, the growth of *A. flavus* in beef jerky lagged for 2 weeks, then increased over 6 log CFU/g, while the growth of *A. flavus* was not observed for four weeks at A_w 0.70. The synergistic effect of a low A_w and the SL and SA mixture on *A. flavus* inhibition was stronger than when water activity alone was varied or the SL and SA mixture alone was used to control the *A. flavus* growth. The results of this study indicate that the growth of *A. flavus* cannot be controlled only by adjusting water activity and that various techniques, such as temperature control and antifungal agents, must be used. *A. flavus* in jerky has also been controlled by vacuum packaging [10], radiation [10] and flexible thin-layer plasma [4]. The addition of sodium lactate, followed by storage at 4˚C also inhibited the growth of mold and yeast in traditional meat products in Turkey [26]. The addition of 2% sodium lactate to vacuum packaged sausage inhibited the growth of mold and yeast for eight days at 8˚C [27]. These results indicate that sodium lactate had an antifungal effect in these meats.

Samapundo and others [28] developed a model to predict the effect of water activity and temperature on *A. flavus* in PDA media. At A_w 0.80, no growth of *A. flavus* was observed at all temperatures tested (16˚C, 22˚C, 25˚C, 30˚C and 37˚C). Although A_w 0.80 inhibited the growth of *A. flavus* in PDA in the previous work, the growth of *A. flavus* in beef jerky without the SL and SA mixture was observed at A_w 0.80 in the present study. These results indicate that the risk of *A. flavus* in beef jerky was high. Thus, additional control measures are needed to prevent the growth of *A. flavus* in beef jerky.

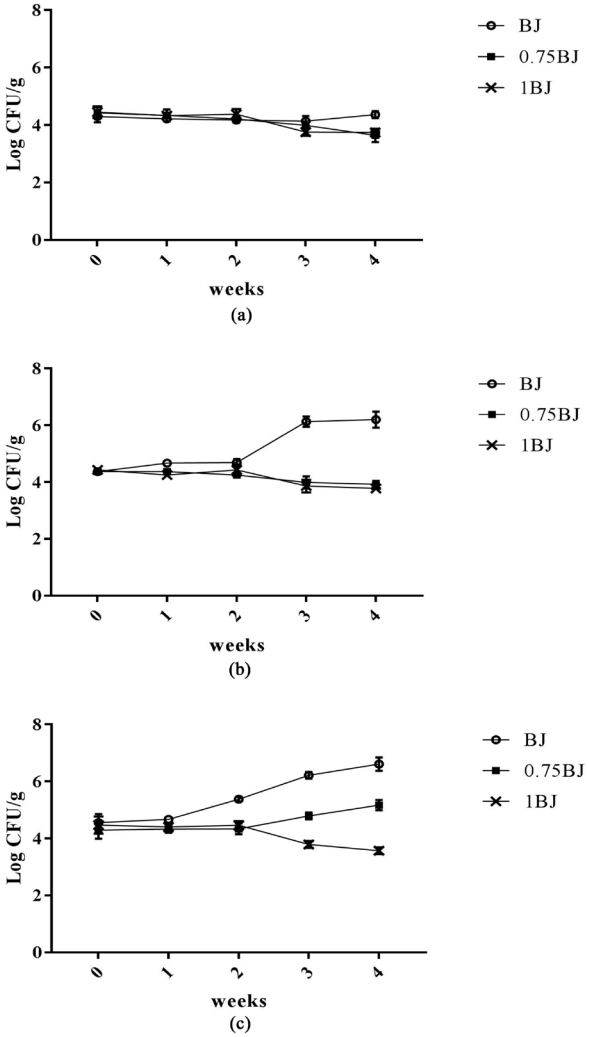

Figure 3. Effect of A_w and the mixture of sodium lactate (SL) and sodium acetate (SA) on *A. flavus* in beef jerky at 25˚C. (a) A_w 0.70; (b) A_w 0.75; (c) A_w 0.80 BJ: control beef jerky without mixture of SL and SA 0.75 BJ: beef jerky containing 0.75% mixture of SL and SA 1 BJ: beef jerky containing 1.0% mixture of SL and SA.

3.3. Effect of Yuza Powder and the Mixture of Sodium Lactate and Sodium Acetate on the Fate of *S. aureus* in Beef Jerky at 10˚C and 25˚C

Yuza's peel and flesh residue have been tested in new product development investigations because of its numerous health benefits. In this work, the synergetic antimicrobial activity of yuza powder and the SL and SA mixture on *S. aureus* in beef jerky was investigated. Sliced beef was marinated with 1.5% yuza powder with and without the SL and SA mixture and was dried to adjust the water activity to 0.80. The amount of *S. aureus* in prepared beef jerky was investigated at 10˚C and 25˚C (Table 1). The *S. aureus* population decreased more rapidly after the addition of the SL and SA mixture, regardless of the storage temperature. Without the SL and SA mixture, *S. aureus* in all beef jerky containing yuza powder survived well and had a long lag phase at both 10˚C and 25˚C. The

Table 1. Effect of yuza, temperature and the mixture of sodium lactate (SL) and sodium acetate (SA) on *S. aureus* survival in beef jerky at 10˚C and 25˚C

Temperature	10˚C		25˚C	
Parameter	Delta (h)	*p*	Delta (h)	*p*
YBJ	645.29	3.54	201.47	3.35
0.75 YBJ	279.41	1.29	186.55	2.95
1 YBJ	253.99	1.64	157.42	2.34

YBJ: control beef jerky containing yuza powder without the mixture of SL and SA, 0.75 YBJ: beef jerky containing yuza pwder and 0.75% mixture of SL and SA, 1 YBJ: beef jerky containing yuza powder and 1.0% mixture of SL and SA.

shortest delta values of *S. aureus* were observed in the 1.0% SL and SA mixture in beef jerky, which were accompanied by a long lag phase in the beginning, followed by a rapid death at the end of storage with a downward concave shape ($p > 1$). Overall, the populations of *S aureus* in beef jerky decreased faster at 25˚C compared to 10˚C, regardless of the presence of yuza powder or the SL and SA mixture. The worst condition for the survival of *S. aureus* was observed in beef jerky containing yuza powder and 1% SL and SA mixture at 25˚C. These results indicate that yuza powder had a synergetic antimicrobial effect with the SL and SA mixture. In commercial settings, beef jerky is sold at refrigerated or ambient temperatures. The settings at retail markets must be verified for the safety of beef jerky. The results of the present study showed that the addition of yuza powder and the SL and SA mixture to beef jerky decreased the populations of *S. aureus* at both 10˚C and 25˚C and, thus, reduced the risk of food poisoning that occurs from enterotoxin produced by *S. aureus*. Yuza peel consists of many compounds, such as limonene, hesperidin, and naringin [29]. Limonene has been shown to have an antimicrobial effect [30]. Although yuza contains these antimicrobial substances, studies on the antimicrobial properties of yuza have not been researched, unlike other citrus fruits. The results of this study showed that yuza can be used, not only as a flavor enhancer but also as an antimicrobial in processed meat products.

3.4. Effect of Yuza Powder and the Mixture of Sodium Lactate and Sodium Acetate on *A. flavus* and Total Aflatoxin Production in Beef Jerky at 10˚C and 25˚C

In order to investigate to antifungal activity of yuza powder and the SL and SA mixture on *A. flavus* and aflatoxin production in beef jerky, *A. flavus* was inoculated into beef jerky containing 1.5% yuza powder and 0.75% or 1% SL and SA mixture. The jerky was stored for eight and four weeks at 10˚C and 25˚C, respectively (**Figure 4**). No growth of *A. flavus* in the beef jerky containing yuza powder and 1% SL and SA mixture was observed at 10˚C for eight weeks, while the growth of *A. flavus* was not prevented at 25˚C. The growth of *A. flavus* was also prevented in beef jerky containing 1% SL and SA mixture but the growth of *A. flavus* was not prevented with just yuza powder at A_w 0.80 (**Figure 3**). The

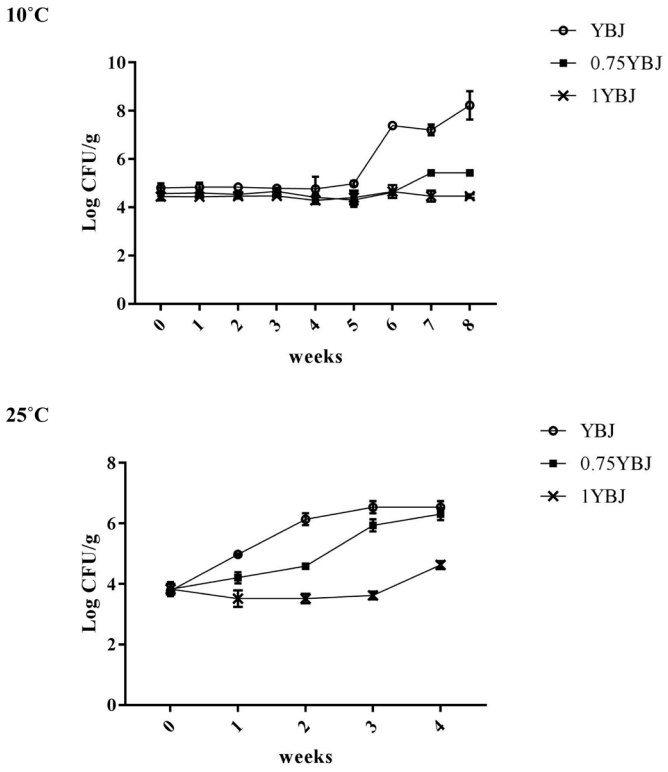

Figure 4. Effect of yuza and the mixture of sodium lactate (SL) and sodium acetate (SA) on *A. flavus* in beef jerky at 10˚C and 25˚C. YBJ: control beef jerky containing yuza powder without the mixture of SL and SA, 0.75 YBJ: beef jerky containing yuza pwder and 0.75% mixture of SL and SA, 1 YBJ: beef jerky containing yuza powder and 1.0% mixture of SL and SA.

most common cause of citrus fruit decay is mold [31]. In this work, the lag time of *A. flavus* in beef jerky containing just yuza powder without the SL and SA mixture was extended up to five weeks at 10˚C, indicating that storage at low temperature may decrease the risk of beef jerky due to the growth of *A. flavus*.

Aflatoxin production is affected by several environmental conditions, such as A_w, CO_2 levels, temperatures, and incubation times [32] [33]. These results indicate that the optimum conditions for aflatoxin production varied depending on the environment. **Figure 5** shows the effects of A_w, yuza powder and the mixture of SL and SA on total aflatoxin production in beef jerky measured at 10˚C for eight weeks and 25˚C for four weeks. The water activity was the most important factor controlling aflatoxin production in beef jerky. Aflatoxin was not detected in any samples with an A_w less than 0.75 (data not shown). However, the maximum accumulation of total aflatoxin (7.31 ppb) was observed in beef jerky without the SL and SA mixture at A_w 0.80. Although aflatoxin accumulation in original beef jerky significantly declined with increasing amounts of the SL and SA mixture (0.75% and 1.0%) at A_w 0.80, the addition of the SL and SA mixture did not completely prevent aflatoxin production in beef jerky. These results suggest that the A_w of beef jerky should be maintained at less than 0.75 to avoid aflatoxin accumulation.

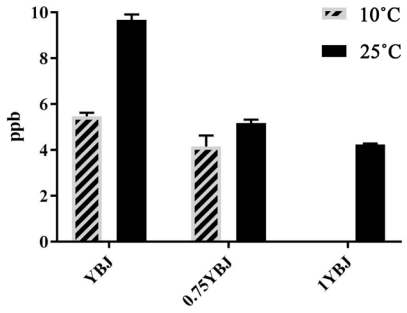

Figure 5. Effect of yuza powder and the mixture of sodium lactate (SL) and sodium acetate (SA) on aflatoxin production in beef jerky at 10˚C and 25˚C. YBJ: control beef jerky containing yuza powder without the mixture of SL and SA, 0.75 YBJ: beef jerky containing yuza pwder and 0.75% mixture of SL and SA, 1 YBJ: beef jerky containing yuza powder and 1.0% mixture of SL and SA.

Although there is risk of of aflatoxin in beef jerky, regulatory aflatoxin limits in many countries have not set. The regulatory limit of total aflatoxin in nuts is 20 ppb in the US and Korea [34] [35]. In the present study, the total amount of aflatoxin in all beef jerkies was lower than the present regulatory limits for nuts of Korea. The range of water activity of commercial beef jerky in Korea has been reported to range from 0.70 to 0.83 [2] and aflatoxin may be produced during the shelf-life of beef jerky at Aw 0.80 or higher. Thus, regulations for the safe limit of water activity for beef jerky are needed and temperature control must be implemented at retail markets.

Figure 5 shows the effect of yuza powder and the SL and SA mixture on total aflatoxin in beef jerky at A_w 0.80 at 10˚C and 25˚C. The growth of *A. flavus* in beef jerky proceeded for eight weeks at 10˚C and four weeks at 25˚C. Then, when the storage period was over, the amount of aflatoxin in beef jerky was measured using the AgraQuant Aflatoxin Test Kit. There were significant differences in the amount of aflatoxin in the beef jerky samples that varied according to the concentration of the SL and SA mixture at both 10˚C and 25˚C, regardless of the presence of yuza powder. No aflatoxin production was observed in beef jerky containing yuza powder and 1% SL and SA mixture after eight weeks of storage at 10˚C. However, aflatoxin production by *A. flavus* was not prevented in beef jerky with an A_w of 0.80 at 25˚C, regardless of the presence of yuza powder and the SL and SA mixture, indicating that these additives were not effective to prevent aflatoxin production at ambient temperatures. According to the results of the present study, the A_w of beef jerky must be adjusted to 0.75 to prevent aflatoxin production at ambient temperatures. Overall, the amount of aflatoxin produced in beef jerky was lower than that containing yuza powder at 25˚C, regardless of the addition of the sodium acetate and sodium lactate mixture of. Since more growth of *A. flavus* in beef jerky containing yuza powder was observed, more aflatoxin was detected. Beef jerky is commercially sold refrigerated or at ambient temperature, which is the optimal temperature for *A. flavus* growth and aflatoxin production [36]. Several studies have shown that the concentration of aflatoxin B_1 in sorghum seeds increased at 37˚C compared to that

at 25˚C [33] and that aflatoxin B_1 was produced by *A. flavus* on meat-based media at 25˚C and small amounts were produced at 10˚C, regardless of the A_w [37].

3.5. Effect of Yuza Powder and the Mixture of Sodium Lactate and Sodium Acetate on the Color and Texture of Beef Jerky

The effects of yuza powder and the SL and SA mixture on the color of beef jerky are shown in Table 2. Because brightness and redness of meat products are expected in meat products, the color of meat products is an important quality parameter. Addition of the SL and SA mixture significantly increased the lightness (L^*), redness (a^*), and yellowness (b^*) of beef jerky ($p < 0.05$), regardless of the addition of yuza powder. The lightness of meat products is affected by many factors, including the concentration of additives [38], fiber content and type of meat products [39].

Figure 6 shows the effect of yuza powder and the SL and SA mixture on the texture of beef jerky. The hardness and chewiness of the beef jerky were affected

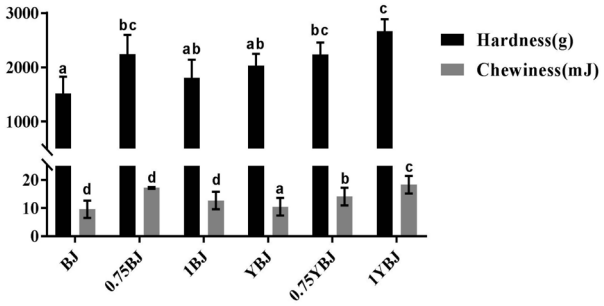

Figure 6. Effect of yuza powder on the texture characteristics of beef jerky at A_w 0.80. [a-e]Means values in a row within different letters are significantly different by Duncan's multiple range test at $p < 0.05$. BJ: control beef jerky without mixture of SL and SA, 0.75 BJ: beef jerky containing 0.75% mixture of SL and SA, 1 BJ: beef jerky containing 1.0% mixture of SL and SA, YBJ: control beef jerky containing yuza powder without the mixture of SL and SA, 0.75YBJ: beef jerky containing yuza pwder and 0.75% mixture of SL and SA, 1 YBJ: beef jerky containing yuza powder and 1.0% mixture of SL and SA.

Table 2. Effect of yuza powder and the mixture of sodium lactate (SL) and sodium acetate (SA) on the color (Hunter's L^*, a^* and b^*) values of beef jerky.

Color	Beef jerky[a]		
	0%	0.75%[1)]	1.0%
L^*	18.95 ± 2.20[a]	21.41 ± 1.39[b]	22.94 ± 2.46[b]
a^*	5.49 ± 1.23[a]	8.82 ± 1.65[b]	8.54 ± 1.17[b]
b^*	5.56 ± 1.07[a]	7.77 ± 1.28[b]	8.69 ± 2.44[c]
	Beef jerky with yuza powder[b]		
	0%	0.75%[1)]	1.0%
L^*	20.41 ± 2.03[a]	22.44 ± 2.14[b]	24.29 ± 1.98[b]
a^*	7.85 ± 2.53[a]	8.32 ± 1.94[b]	9.36 ± 1.74[c]
b^*	9.29 ± 3.12[a]	9.36 ± 2.45[a]	10.65 ± 1.58[b]

[1)]Concentration of mixture sodium lactate and sodium acetate. [a-c]Means values (n = 3) in a row within different letters are significantly different by Duncan's multiple range test at $p < 0.05$.

by yuza powder and the SL and SA mixture. Beef jerky containing yuza powder was harder than beef jerky without yuza powder, indicating that the addition of yuza powder in beef jerky affected the hardness. The addition of the SL and SA mixture to beef jerky containing yuza powder significantly increased the hardness and chewiness ($p < 0.05$). In the previous study, the addition of orange dietary fiber to sausage increased hardness by intensifying protein matrix formation during cooking [40]. Bologna sausage with orange, apple, and peach fiber have shown the same results [41].

3.6. Effect of Yuza Powder and the Mixture of Sodium Lactate and Sodium Acetate on the Sensory Scores of Beef Jerky

In order to test the taste preference for beef jerky containing yuza powder and the SL and SA mixture, color, flavor, sweetness, bitterness, texture, and overall acceptability were evaluated by 70 non-trained panelists (Table 3). Overall, no significant differences were observed in the beef jerky with or without yuza powder and the SL and SA mixture. However, the parameters of sweetness and bitterness were significantly affected by the yuza powder, regardless of the addition of the SL and SA mixture. The addition of yuza powder to beef jerky reduced sweetness and increased bitterness, indicating that sweetness and bitterness might be affected by the unique taste and flavor of yuza powder. However, the change was very minimal and other sensory characteristics were not affected.

4. Conclusion

The results from this study demonstrated the antimicrobial effect of yuza powder and the SL and SA mixture in beef jerky. However, the growth of *S. aureus* was not controlled in beef jerky with an A_w 0.80. In addition, yuza powder was not very effective in controlling the growth of *A. flavus* in beef jerky but the growth of *A. flavus* was controlled by the addition of 1% SL and SA mixture and

Table 3. The sensory scores of color, flavor, texture, overall acceptability and sweetness and bitterness of the beef jerkies.

Sensory parameters[1)]	Samples			
	BJ	1 BJ	YBJ	1 YBJ
Color	5.14 ± 1.13[a]	5.19 ± 1.31[a]	4.91 ± 1.40[a]	4.77 ± 1.37[a]
Flavor	4.80 ± 1.50[a]	4.71 ± 1.47[a]	4.60 ± 1.63[a]	5.06 ± 1.52[a]
Texture	4.04 ± 1.78[a]	4.11 ± 1.72[a]	4.64 ± 1.63[a]	4.34 ± 1.71[a]
Overall acceptability	4.86 ± 1.52[a]	5.09 ± 1.59[a]	4.69 ± 1.48[a]	4.80 ± 1.48[a]
Sweetness	5.20 ± 1.36[b]	5.13 ± 1.27[b]	4.61 ± 1.39[a]	4.54 ± 1.44[a]
Bitterness	2.50 ± 1.98[a]	2.46 ± 1.86[a]	2.96 ± 1.93[ab]	3.20 ± 1.93 [b]

[1)]Evaluated on a 7-point hedonic scale from 1 = disliked extremely to 7 = liked extremely. [a-b]Means values (n = 70) in a row within different letters are significantly different by Duncan's multiple range test at $p < 0.05$. BJ: control beef jerky without the mixture of SL and SA, 1 BJ: beef jerky containing 1.0% mixture of SL and SA. YBJ: control beef jerky containing yuza powder without the mixture of SL and SA, 1 YBJ: beef jerky containing yuza powder and 1.0% mixture of SL and SA.

storage at 10˚C. Water activity was the most important factor shown to control aflatoxin production in beef jerky. Since aflatoxin was produced in all beef jerky with an A_w of 0.80, the safe A_w level of jerky in Korea (0.70 - 0.83) must be reevaluated. The quality of beef jerky was also affected by the A_w, yuza powder, and the SL and SA mixture. Color parameters (L^*, a^*, b^*) were also enhanced by the addition of yuza powder and the SL and SA mixture. Hardness was increased by the addition of yuza powder. With the exception of sweetness and bitterness, no significant differences in sensory scores of the beef jerkies were observed between those with and without yuza powder. Scores for sweetness and bitterness of the beef jerkies containing yuza powder were higher than those of the other beef jerkies. The results of the present study indicate that yuza powder may be used as a new ingredient to create new tastes of beef jerky.

Conflicts of Interest

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

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