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Relationship of Specific Microbial Growth and TBARS Value in Radiation-Sterilized Raw Ground Pork

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Abstract

Sterilized raw ground pork was inoculated with *Pseudomonas aeruginosa* (PA) and *Lactobacillus casei* (LC) to investigate the relationship between microbial growth and 2-thiobarbituric acid reactive substances (TBARS) values. The analyses including microbial growth, pH, and TBARS values were performed during 3 weeks of storage at room temperature (20°C). The radiation-sterilized control sample did not show any microbial growth, but the samples inoculated at different levels (diluted twice vs non-diluted) exhibited differences until 1 week. However, the difference disappeared at weeks 2 and 3. The pH of raw ground pork inoculated with PA increased, but that of LC decreased. The pH of non-inoculated samples increased slightly after storage. The TBARS values in non-inoculated and LC inoculated with pork increased, but TBARS remained unchanged in samples inoculated with PA after 1 week. Results indicated that the microbial growth level and strains can influence the TBARS value of raw ground pork. Thus, it is important to use samples exposed to the same microbial conditions to compare the oxidation of lipids in meat samples.

Key words: raw ground pork, microbial growth, pH, TBARS

INTRODUCTION

The 2-thiobarbituric acid (TBA) test (1) is the most widely used method for quantifying lipid oxidation development in meat and meat products (2). The TBA test determines the amount of malondialdehyde (MDA), a major secondary byproduct of lipid oxidation, in an oxidized lipid. Lipid oxidation has been reported to be a primary cause of off-odor in refrigerated raw beef (3) and significant correlations between the TBA values and sensory scores of poultry meat have been reported (4). Compared with the chromatographic method (5), TBA reactive substances (TBARS) method is a fast, sensitive, and cost-effective way of investigating the extent of lipid oxidation in a variety of systems (6).

The influence of microbial growth on the accumulation of lipid oxidation products has been reported for lard and ground pork (7,8). Moerck and Ball (9) compared bacterial growth in mechanically deboned chicken meat treated with 1% chlortetracycline (CTC) to untreated control samples. The authors reported that TBARS values were higher in treated samples, suggesting that microorganisms in the untreated meat may have removed malonaldehyde and other TBARS. In another study, peroxides and carbonyls were compared in chicken tissues (muscle and fresh or rancid adipose tissue) inoculated with bacteria and yeasts with uninoculated tissues. Many of the tested microorganisms decreased the concentration of aldehydes in chicken tissue during storage (10). Branen (11) suggested that the removal of TBARS due to microbial growth was plausible. The amine compounds produced by bacterial metabolism of proteins could directly react with aldehydes as in nonenzymatic browning reactions. In addition, Rhee et al. (12) reported that ground beef muscle pre-treated (PT) with 60 ppm chlortetracycline/0.2% potassium sorbate had a lower pH than that of a control during storage at 4°C which was not pre-treated (NPT) by the additives. The lower pH of PT may have contributed to higher TBARS compared to NPT (12). However, other studies have suggested that microbial growth may have little effect on TBARS, thus the influence of microbial growth on TBARS is still controversial (13,14).

The objective of this research was to study the effect

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of microbial growth on TBARS and pH for improving the accuracy of using the TBARS test method for accessing oxidative stability of meats. *Pseudomonas aeruginosa* and *Lactobacillus casei*, which are the most common bacteria in chilled meat (15), were inoculated in ground raw pork which was then stored for 3 weeks at room temperature and evaluated for bacterial growth, pH, and TBARS.

MATERIALS AND METHODS

Raw ground pork preparation

Raw ground pork (30 g) was purchased from a local market, and vacuum packed into oxygen-impermeable polyethylene bags (2 mL O_2/m^2/24 hr at 0°C; 20 cm × 30 cm; Sunkyung Co. Ltd., Korea) using a packaging machine (Leepack, Hanguk Electronic, Gyeonggi, Korea).

Gamma irradiation

The samples were irradiated in a cobalt-60 irradiator (Nordion International, Ottawa, Ontario, Canada) at the Korea Atomic Energy Research Institute (Daejeon, Korea). The source strength was about 100 kCi with a dose rate of 5 kGy/h at 11±0.5°C. Dosimetry was performed using 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The absorbed doses in this study were 30 kGy for sterilizing samples and the actual doses were within 2% of the target dose.

Inoculation

After irradiation, the samples were inoculated with *Pseudomonas aeruginosa* KCTC 1636 or *Lactobacillus casei* KCTC 3100 which were either non-diluted (ND; 8.7 and 9.2 log CFU/g, respectively), diluted (D; 5.7 and 6.2 log CFU/g, respectively), or control (no inoculation). The inoculated samples were sealed after pouring an air into the package. The samples were prepared in duplicate and stored at room temperature (20°C) for 3 weeks.

Microbial analysis

Meat samples (5 g) and 45 mL of sterile 0.1% peptone water (Difco Labs., Detroit, MI, USA) were homogenized using a stomacher lab blender (Model 400, Tekmar Co., Cincinnati, Ohio, USA). A series of decimal dilutions were prepared with sterile peptone water. Nutrient agar (Difco Labs., Detroit, MI, USA), incubated at 37°C for 2 days, was used for determination of *Pseudomonas aeruginosa* and MRS agar (Difco) for *Lactobacillus casei*. Microbial counts were expressed as log CFU/g.

pH

Samples (2 g) and distilled water (18 mL) were homogenized with a homogenizer (DIAX 900, Heidolph, Schwabach, Germany). The pH of the homogenate was measured using a pH meter (Orion 520A, Orion Research Inc., Boston, MA, USA).

2-Thiobarbituric acid reactive substances (TBARS) values

Samples (5 g) were homogenized in 15 mL of distilled water and 50 μL of butylated hydroxytoluene (BHT) for about 5 seconds. The homogenate (1 mL) was transferred into a disposable test tube (10 mL), and 2 mL of a thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA) was added. The mixture was vortexed, heated in a 90°C waterbath for 15 min, cooled in a tap water for 10 min, and centrifuged at 600×g for 20 min using a centrifuge (VS-5500, Vision Scientific Co. Ltd., Seoul, Korea). The absorbance of the supernatant was measured at 532 nm with a spectrophotometer (UV 1600 PC Shimadzu, Tokyo, Japan). TBARS were expressed as mg malondialdehyde/kg sample.

Statistical analysis

The experimental design used was a 2 (inoculation) ×4 (storage) factorial. The entire experiment was conducted twice. One-Way Analysis of the Variance was performed using SAS software (16) and Duncan's multiple range test was used to compare the differences among the mean values. Mean values and pooled standard errors of the mean (SEM) were reported with significance defined as $p<0.05$.

RESULTS AND DISCUSSION

Microbial analysis

Microbial growth in raw ground pork samples treated with both non-diluted (ND) and diluted (D) *Pseudomonas aeruginosa* (PA) and *Lactobacillus casei* (LC) and in control was monitored for 3 weeks (Table 1). Immediately after inoculation, bacteria counts in the D sample were 4 log CFU/g, and in ND 78 log CFU/g. After 1 week of storage, both samples increased to 7 log CFU/g in D and 8 log CFU/g in ND. However, after 2 weeks of storage, the number of viable cells in the samples inoculated with PA and LC exceeded 8 log CFU/g. No microorganisms were detected in radiation-sterilized controls during storage.

pH

The pH was monitored in raw ground pork inoculated with different levels of microorganisms during storage at 20°C (Fig. 1). During storage for 3 weeks, the pH of control samples was not changed. However, the pH of the samples inoculated with PA increased during

Table 1. Microbial growth (log CFU/g) of the radiation-sterilized raw ground pork inoculated with microorganisms during storage at room temperature (20°C)

Treatment[1)]	*Pseudomonas aeruginosa*				*Lactobacillus casei*			
	Storage				Storage			
	0	1	2	SEM[2)]	0	1	2	SEM[2)]
Control	-[3)z]	-[z]	-[z]	-	-[z]	-[z]	-[z]	-
D	4.97^{cy}	7.00^{by}	8.26^{ay}	0.010	4.60^{cy}	7.06^{by}	8.53^{ay}	0.025
ND	8.25^{cx}	8.79^{ax}	8.51^{bx}	0.007	7.85^{cx}	8.82^{bx}	8.92^{ax}	0.013
SEM[2)]	0.004	0.009	0.006		0.013	0.025	0.004	

[1)]Control, radiation-sterilized raw ground pork; D, the microorganisms was diluted twice using sterilized pepton water and inoculated; ND, the microorganisms were inoculated as is.
[2)]Standard error of the mean (n = 9).
[3)]Not detected.
[a-c]Means within a row with no common superscript differ significantly ($p<0.05$).
[x-z]Means within a column with no common superscript differ significantly ($p<0.05$).

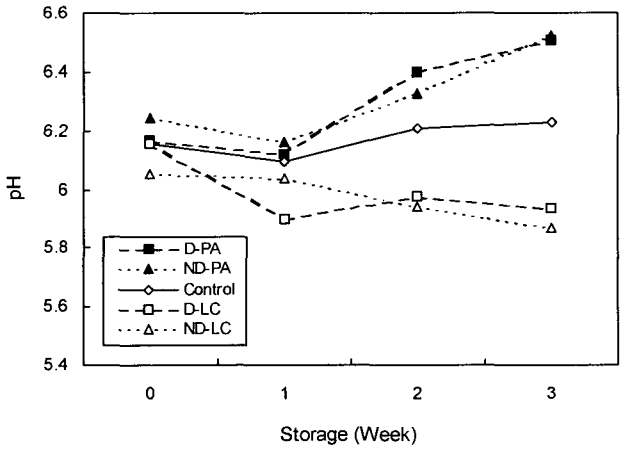

Fig. 1. Changes of pH of the radiation-sterilized raw ground pork inoculated with microorganisms during storage at room temperature (20°C). Abbreviation: Control, radiation-sterilized raw ground pork; D-PA, the *Pseudomonas aeruginosa* inoculum was diluted in half with sterilized peptone water; ND-PA, the *Pseudomonas aeruginosa* was used undiluted; D-LC, the *Lactobacillus casei* inoculum was diluted in half with sterilized peptone water; ND-LC, the *Lactobacillus casei* was used undiluted.

storage, especially after 1 week. Species of *Pseudomonas* have been identified as the major amine-forming bacteria and amine compounds (basic) from bacterial spoilage of meat can increase pH (17,18). Gram-negative bacteria such as *Pseudomonas* produce ammonia and amines from urea and amino acids. These products provoke an increase in the pH of fresh meat such as ground pork or beef (19). Rhee et al. (12) also reported that the increased pH was pronounced after 12 days of storage because of rapid growth of microorganisms. In contrast, the pH of the radiation-sterilized raw ground pork inoculated with LC decreased significantly. Production of lactic and acetic acids from lactic acid bacteria is responsible for this pH decrease (20). Lefebvre et al. (19) reported that organic acid, produced by Gram-positive bacteria, decreased the pH of fresh ground beef. Yoo et al. (21) also reported that the pH was decreased by lactic acid, which is a secondary metabolite in batch cultures of LC. Minor-Pérez et al. (22) studied pork inoculated with biopreservative strains (*Lactobacillus alimentarius* and *Staphylococcus carnosus*) and stored at 4 and 20°C. Both tested strains produced more than 6 mg lactic acid/g tissue. Therefore, growth of LC might be responsible for pH decrease.

TBARS values

The effect of microbial growth on TBARS values was monitored during 3 weeks of storage at 20°C (Fig. 2 and 3). All the TBARS values of the radiation-sterilized raw ground pork inoculated with PA were 0.70 at week 1. After 3 weeks of storage, the TBARS values of the control increased to 1.12 but those of ND and D samples decreased to 0.64 or remained stable at 0.70, respectively. This is in good agreement with the results of Branen (11), who found that the TBARS removal/losses

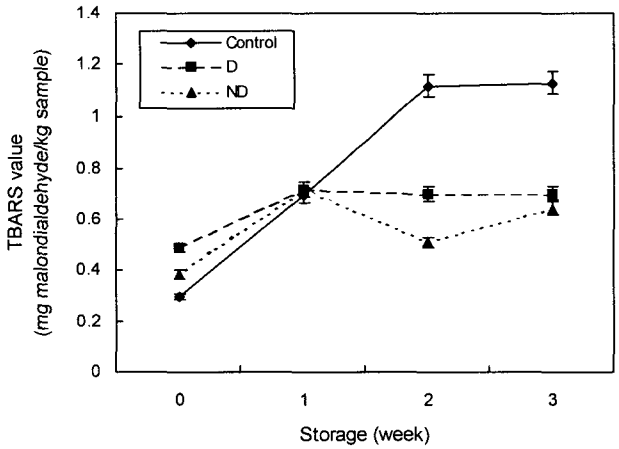

Fig. 2. TBARS value (mg malondialdehyde/kg raw ground pork) of the radiation-sterilized raw ground pork inoculated with *Pseudomonas aeruginosa* during storage at room temperature (20°C). Abbreviation: Control, radiation-sterilized raw ground pork; D, the *Pseudomonas aeruginosa* inoculum was diluted in half using sterilized peptone water; ND, the inoculum was not diluted.

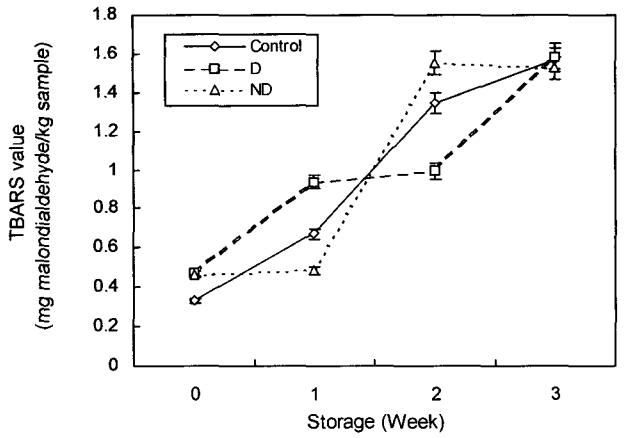

Fig. 3. TBARS value (mg malondialdehyde/kg raw ground pork) of the radiation-sterilized raw ground pork inoculated with *Lactobacillus casei* during storage at 20°C. Abbreviation: D, the *Lactobacillus casei* inoculum was diluted in half using sterilized peptone water; ND, the inoculum was not diluted.

might occur through direct microbial utilization of malonaldehyde and other TBARS or through reactions between these compounds and the amines produced by bacterial metabolism, or both (23). As mentioned previously, amines produced by PA increased the pH of raw ground pork inoculated with PA after 1 week storage. This result might provide evidence to support our TBARS values which decreased during storage because malondialdehyde and other TBARS reacted with amine. In contrast to PA, the TBARS values of the radiation-sterilized raw ground pork inoculated with LC increased in all samples during storage. Rhee (23) reported that lowering of meat pH can accelerate lipid oxidation in stored meat, resulting in increased TBARS values. In this study, the pH of raw ground pork inoculated with LC decreased because LC produced lactic acid during growth. It also supports that decreased pH contributed to the higher TBARS, and explains the higher TBARS value of raw ground pork inoculated with LC compared to PA.

In conclusion, the TBARS values were changed in response to the species and the growth of microorganisms in samples. Therefore, to obtain accurate data or make reliable comparisons of oxidative stability, microbial levels and species contaminating the samples should be identical or at least similar.

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