**ARTICLE** 

# Changes in Meat Quality Characteristics of the Sous-vide Cooked Chicken Breast during Refrigerated Storage

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#### **Abstract**

This study was performed to investigate the changes in meat quality characteristics of the sous vide cooked chicken breast during refrigerated storage at 4°C for 14 d between before and after sous-vide cooking. Cooking loss and shear force were significantly increased, whereas expressible drip was significantly decreased along with reduction in the water holding capacity in both of two groups. Redness of meat juice was significantly (p<0.05) increased during storage, and considerably increased in the refrigerated samples after sous-vide cooked at the 7 to 10 d. The thiobarbituric acid reactive substances (TBARS) was significantly increased and was higher in the refrigerator stored chicken breast samples after sous-vide cooking. The volatile basic nitrogen (VBN) value was significantly increased in both groups, but the VBN value of the stored raw meat sample before sous-vide cooking was increased at an early storage, while the VBN value of the stored sample after sous-vide cooking was increased gradually in this study. Total viable counts and coliform counts were significantly decreased during storage, and coliforms were not detected after 7 d of storage in both groups. Salmonella spp. was not detected during the whole studied period. The outcome of this research can provide preliminary data that could be used to apply for further study of chicken breast using sous-vide cooking method that could be attractive to consumers.

Keywords: sous vide, molecular gastronomy, chicken breast, meat characteristic, poultry meat

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# Introduction

Sous vide technique, which is a method of cooking under vacuum packaging, with application of mild heat treatment and long cooking times, followed by rapidly cooling and chilled storage, has been used in restaurants, catering industry, and ready-to-eat industry (Creed, 1998). Sous vide cooking method came into the limelight because it provides a high nutritional value, improved texture and tenderness, maintains the juiciness as a result of lowtemperature cooking (Church and Parsons, 2000; Schafheitle, 1990), also reduces lipid oxidation for an extended shelf life and prevents loss of volatile flavors because of vacuum packaging (Vaudagna et al., 2002; Wang et al., 2004). Although these advantages of sous-vide technology for food, it was remain problems for solution to apply to poultry meat as low temperature cooked poultry meats have often pink color defection for affecting appearance

and causing consumer's complains such as uncooked or blood (Kieffer et al., 2000). These phenomena have been led to the limited study for sous-vide cooked chicken breast compared to other sous-vide cooked vegetables, fish, and meat product.

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In many Western and European countries and Asian countries included Korea, Sous vide cooking process has been researched for several years (Church and Parsons, 1993; Church and Parsons, 2000; Creed, 1995). They have reported that the sous-vide and cook-chill method ensures the microbiological safety of foods, improves meat quality, and provides prolonged storage of vegetables or meat products (Juneja, 2006; Oh et al., 2006; Renna et al., 2014). The Korean poultry industry, especially the chicken meat industry, has been preferred thighs and wings rather than chicken breast for the past years (Jin et al., 2007). As the economic development and an increasing interest in health, meat products, especially red meat products with a high fat content, have been considered as the primary cause for obesity and hypercholesterolemia (Wang and Beydoun, 2009). Consequently, the interest and consumption of poultry meat products including chicken breast with a low fat content but high protein content has inc-

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reased (Jayasena *et al.*, 2013). The smoked chicken breast with vacuum packaged, and marinated canned chicken breast are usually consumed. In some partially developed markets, the sale of sous-vide cooked chicken breast has been gradually increased. As the muscle fibers begin to shrink at 35-40°C and continue to shrink up to 80°C (Baldwin, 2012), low-temperature, heat treatment Sous vide cooking technique results in soft and moist chicken breast. Thus, Sous vide cooking method may improve the sensory quality of chicken breast and prevent the large disadvantage of obtaining dry and crumbled cooked chicken breast.

Although Sous vide cooked chicken breast business is an early planning phase, there are limited studies on the Sous vide cooked chicken breast. Therefore, this study was performed to investigate the changes in meat characteristics of sous-vide cooked chicken breast during refrigerated storage for 14 d that could be utilized as the preliminary data of this study for applying sous-vide cooking method in the poultry industry.

#### **Materials and Methods**

# **Experimental design**

In each of three independent replicate trials, 210 boneless and skinless raw chicken breasts were purchased from FarmsVill Co. (Korea) within 2 d after slaughter, and were used immediately. Each raw chicken breast (110±10 g) was placed in a vacuum bag and packed under 80% vacuum in a vacuum chamber (FJ-500XL, Fujee, Korea). Sous-vide cooking was performed in a circulating water bath (Diamond M, Julabo, Germany) that maintained a temperature of 61°C for 35 min after the core temperature of chicken breast reached 61°C using food thermometer (Testo 108, Testo Inc., USA) and then the chicken breast was quickly cooled in ice water. R group was 4°C chilling storage with raw-meat condition for 0, 3, 5, 7, 10, and 14 d and then cooked sous-vide on that sampling day, respectively. S group was cooked sous vide at initial and stored at 4°C for 0, 3, 5, 7, 10 and 14 d until on the scheduled sampling day.

# Proximate analysis

R and S samples were analyzed in triplicate for the moisture, crude fat, crude protein and ash composition as per the AOAC procedures (AOAC, 2005). Moisture content was determined by the air oven drying method at 105°C for 20-24 h. Crude protein content was measured by the Kjeldahl method using a conversion factor, 6.25. Crude fat content was measured by soxhlet method using

auto soxhlet fat extractor (FE-6SN, Mitamura Riken Kogyo Inc., Japan). Ash content was analyzed by the burning method at 550°C for 4 h. These results were presented in weight percentage samples.

# Measurement of the physical characteristics of chicken breast samples

All of the chicken breast samples were measured for cooking loss, shear force, expressible drip, and redness of the drip. Cooking loss was measured by dividing the percentage of weight loss in the meat sample by the difference between raw weight and cooked weight. Chicken breast meat was cut into a square pillar shape  $(150 \times 150)$ × 300 mm thickness) and shear force was measured by using TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). The conditions for measuring shear force using the texture analyzer were as follows; test speed was 2.0 mm/s, and trigger type was automatic 10 g. Expressible drip was measured according to the method of Ng (1987). As per this method, 0.3 g of chicken breast sample was placed between two Whatman No.1 filter papers and pressed by applying a 9.9 kg/cm<sup>2</sup> force for 2 min (IF 32B-S50, Ilshin Tech. Co. Ltd., Korea). The pressed chicken breast sample was weighed after removing the filter papers. Expressible drip was expressed as the difference in weight before and after pressing. Redness of meat juice was measured using the modified method described by Maeng et al. (2007) and Snyder (2006). Chicken breast samples were plated on the scheduled day and the drip containing myoglobin was collected. Drip was filtered using the Whatman No.1 filter paper and redness was measured using a spectrophotometer (OPTIZEN UV2120, Mecasys Co. Ltd., Korea) at 550 nm.

# Measurement of TBARS/VBN values

Thiobarbituric acid reactive substances were measured according to the modified method described by Witte *et al.* (1970), using a UV/VIS spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Korea). Briefly, 10 g of chicken breast sample was homogenized 1 min in 10 mL of 10% trichloroacetic acid, and the sample volume was adjusted to 50 mL by adding distilled water. The suspension was filtered using Whatman No. 1 filter paper, and 5 mL of the supernatant was added to 5 mL of 2-thiobarbituric acid (2.88 g/L). Chicken breast samples were then mixed slightly, heated at 95°C in a water bath for 10 min, and absorbance was measured at 532 nm. Results were expressed as mg malonaldehyde (MDA) equivalents/kg sample. Standard curve was prepared using 1, 1, 3, 3,-tet-

ramethoxypropane as a standard malonaldehyde.

Volatile basic nitrogen content was determined according to Conway's microdiffusion method (Conway, 1950). Briefly, 5 g of chicken breast sample was homogenized at 1,000 rpm for 1 min after adding 15 mL of distilled water and adjusting the sample volume to 50 mL by adding distilled water, and then filtered using Whatman No. 1 filter paper. Then, 1 mL of the filtrate was added to the outer circular wall and 1 mL of 0.01N H<sub>3</sub>BO<sub>3</sub> was added to the inner wall of Conway dish. Afterwards, 100 µL of Conway sol- ution was added to the inner wall, the cover was opened slightly, and then 1 mL of 50% K<sub>2</sub>CO<sub>3</sub> was added to the outer diffusion chamber. The Conway dish was placed in an incubator at 37°C for 2 h. After removing the lid, boric acid was titrated to the end point, which was when the solution turned pink in color, with 0.02 N H<sub>2</sub>SO<sub>4</sub>. Blank test was conducted following the same process without adding 1 mL of 50% K<sub>2</sub>CO<sub>3</sub>.

# Microbiological measurements

Microbiological analysis of sous-vide cooking chicken breast meat was conducted for determining the total cell count (TVC), Salmonella sp., and coliform count. Briefly, 18 mL of distilled 0.89% saline solution was added to 2 g of chicken breast sample in the side-filter bag and homogenized for 1 min (Bagmixer 400W, Interscience, USA). For measuring the total cell count, the supernatant was inoculated onto the plate count agar (Difco, USA) using decimal dilutions and incubated at 37°C for 48 h. *Salmonella* spp. growth was measured using MacConkey agar plate at 37°C for 48 h. Coliform count was investigated using 3M Petrifilm coliform plate (3M, USA) at 37°C for 48 h. Results were presented as colony forming units (CFU/g).

#### Statistical analysis

A 2-treatment and 7-storage-period one-way factorial design was carried out with three replications. Data were analyzed by analysis of variance (ANOVA). All statistical data were analyzed using the General Linear Model (GLM) procedure of SPSS 19.0 (SPSS Inc., USA). The means were compared for significance (p<0.05) using the Tukey test between periods. The Student's t-test was performed for assessing significant (p<0.05, p<0.01, p<0.001) differences between the two experimental groups.

## **Results and Discussion**

### Proximate analysis

The moisture and crude protein, crude fat and ash contents of chicken breast meat were measured during the storage period. The moisture and crude protein contents were shown in Table 1. There was no significant difference in the moisture content of the R and S group during the entire period. In addition, there was no significantly difference between R and S groups. Moisture contents ranged from 70.36% to 73.84%, were higher than other study that presented 60-67% in cooked chicken breast (Khan and Van Den Berg, 2006; Sampaio et al., 2012). This higher moisture value might be caused sous-vide cooking method. Sous vide technique is typically used to prevent loss of water from the meat, especially by using vacuum packing. This is in agreement with the technique suggested by Vaudagna et al. (2002), vacuum cook-in-bag container technology, which reduces the loss of flavor, aroma compounds, and water from food. Crude protein content in the both groups were not significantly different (p>0.05) during the storage period. Crude fat content range in the R and S groups were 0.80-1.11% and 0.90-1.11%, respectively and both groups had not significantly

Table 1. Changes in the moisture and crude protein contents in raw meat storage samples (R) and Sous-Vide meat storage samples (S) at 4°C

Storage period	Moisture	e content	Crude protein content		
(d)	R group	S group	R group	S group	
0	$71.43\pm1.778^{NS}$	$71.43\pm1.778^{NS}$	$26.69\pm0.317^{NS}$	26.69±0.317 <sup>NS</sup>	
3	$71.16 \pm 1.593$	$70.81 \pm 0.582$	$26.73 \pm 1.256$	28.56±1.272	
5	$72.59 \pm 1.340$	$72.35 \pm 0.483$	$27.03 \pm 1.076$	$26.77 \pm 0.449$	
7	$73.84 \pm 1.149$	$72.18 \pm 0.357$	$26.32 \pm 0.631$	27.66±1.006	
10	$70.36 \pm 0.702$	$71.71 \pm 0.745$	$27.90\pm0.953$	27.42±0.595	
14	$72.23 \pm 1.635$	$71.80 \pm 0.217$	$28.38 \pm 1.001$	$26.51 \pm 0.563$	
SEM	1.15	0.70	0.76	0.63	
<i>p</i> -value	0.116	0.355	0.119	0.055	

All values are presented as means±SD (n=15).

<sup>&</sup>lt;sup>NS</sup>Not statistically significant.

change (p>0.05) during 14 storage days. Ash content in the R and S group was not significantly different (p>0.05) during storage, ranged from 1.38 to 1.61 (data not shown).

#### Physicochemical analysis

The changes in cooking loss (%), shear force (kg/cm²), and expressible drip are shown in Table 2. Cooking loss and shear force were significantly (p<0.05) increased in the R and S groups during storage. This result was caused due to the decrease in the water holding capacity (WHC). Aaslying *et al.* (2003) reported that low WHC of the cooked camel meat resulted in high cooking loss. Also, other study noted that WHC values significantly decreased during storage time that caused an increase in drip loss in the packaged meat (Jouki and Khazaei, 2011).

Shear force is one of the most important attributes for chewing meat products and is highly related to preference (Jouki and Khazaei, 2011). Meat tenderness was measured by the shear force in this study. The shear force of all the chicken breast samples was significantly (p<0.05) increased during storage (Table 2). Also, shear force in both groups was significantly (p<0.05) increased from 5 to 10 d of storage. This increase in the shear force during storage may occur due to the decrease in the WHC, which caused an increase in cooking loss and shear force (Aaslying  $et\ al.$ , 2003; Jouki and Khazaei, 2011).

A significant (p<0.05 or p<0.01 or p<0.001) difference in the shear force was observed between the R group and the S group after 3 d of storage, and the shear force in the R group was much higher than that in the S group. Increased proteolysis during refrigerated storage might be occurred due to a looser structure and can affect the ability to retain water, and thereby result in high expressible drip (Rawdkuen *et al.*, 2010). In this study, expressible

drip was significantly (p<0.05) lower during refrigerated storage in both groups. This result may be caused by decline in the moisture content along with an increase in cooking loss and drip exudation in chicken breast meat during cooking and storage in vacuum packaging. Furthermore, similar to the result of cooking loss as mentioned above, WHC affected the shear force. That is, a decrease in the WHC might be caused by protein denaturation in cooked chicken breast during refrigerated storage and loose structure.

The result of pink color measurement by spectrometry in meat juice represented in Fig. 1. Pink color of the meat juice was caused by leaching out of myoglobin, pigment protein in the muscle of chicken breast meat, during refrigerated storage after cooking with Sous vide technique. Consumers may consider that this pink color is due to blood, the meat is inadequately cooked, or it is not even safe for consumption. Consumers do not like this pink color and it has resulted in consumers complaints. Maeng et al. (2007) studied for beef quality characteristics including meat color by using the spectroscopic method at 540-580 nm, especially at around 560 nm, which is related to the myoglobin content. Pink color of meat juice was significantly (p<0.05) stronger in both groups during storage because of increased myoglobin exudate due to reduction in the WHC as mentioned earlier. In addition, pink color of the meat juice was significantly (p<0.05) weaker in the S group than in the R group until 7 d of storage. However the pink color was significantly (p<0.05) increased in the S group between 7 and 10 d of storage, and the pink color in the S group was significantly (p<0.05) stronger than that in the R group. This result may explained by the stability of meat protein for the difference between before and after cooking during the storage period. It also

Table 2. Changes in cooking loss, shear force, and expressible drip in raw chicken breast storage samples (R) and Sous-Vide storage samples (S) stored at 4°C for 14 d

Storage period	Cooking loss (%)		Shear force (kg/§²)		Expressible drip (%)	
(d)	R group	S group	R group	S group	R group	S group
0	11.50±1.460 <sup>a</sup>	12.41±1.472 <sup>a</sup>	1.01±0.042 <sup>a</sup>	1.01±0.042 <sup>a</sup>	50.26±4.101 <sup>NS</sup>	51.06±2.718 <sup>b</sup>
3	$13.84\pm2.410^{ab}$	$15.06\pm1.073^{ab}$	$1.56\pm0.046^{b*}$	$1.19\pm0.106^{b}$	$45.87 \pm 4.944$	$46.48\pm2.671^{ab}$
5	15.55±1.217 <sup>abc*</sup>	$19.48 \pm 1.634^{bc}$	$1.66\pm0.017^{bc^{**}}$	$1.30\pm0.052^{bc}$	$43.19\pm0.854$	$44.79{\pm}1.079^a$
7	20.32±1.521°	$17.06\pm0.554^{bc}$	$1.87\pm0.100^{c^{**}}$	$1.37\pm0.059^{c}$	43.23±3.672	$42.62\pm1.319^a$
10	$20.01\pm2.635^{c}$	19.88±1.083°	$2.68\pm0.170^{d*}$	$2.22 \pm 0.067^d$	$45.09\pm0.416$	$43.16\pm1.074^a$
14	$17.57{\pm}2.843^{bc}$	$17.21\pm1.537^{c}$	$2.64\pm0.018^{d^{***}}$	$2.24{\pm}0.020^{d}$	$46.20 \pm 1.150$	$43.76{\pm}1.486^a$
SEM	1.725	1.045	0.069	0.052	2.516	1.520
<i>p</i> -value	0.001	< 0.001	< 0.001	< 0.001	0.131	0.001

All values are presented as means±SD (n=15).

a-dMean values with different superscripts within the same column differ significantly (p<0.05).

<sup>\*\*\*\*\*\*</sup>Mean values with an asterisk mark within a row during the same storage period differ significantly (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001).

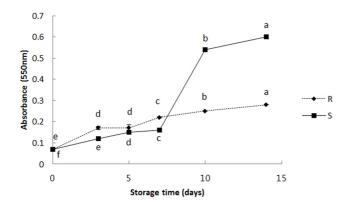


Fig. 1. The pink color of the drip in raw-meat storage samples (R) and Sous-Vide cooked storage samples (S) stored at 4°C was assessed using a spectrophotometer at 550 nm. <sup>a-f</sup>Means sharing different letters during the each storage period are significantly different (*p*<0.05). SEM: R=0.006, S=0.005, *p*-value: R<0.001, S<0.001.

indicates that heating cause denaturation of meat proteins, and increases connective tissue solubility and structural changes in the meat, as reported by Tornberg (2005). These results suggest that chicken breast meat storage for sous-vide cooking under raw meat conditions could be more effective than under sous-vide cooked condition.

#### TBARS measurements and VBN analysis

The TBARS values in the R and S groups were expressed as mg MDA per kilogram of chicken breast meat and the results are shown in Table 3. TBARS values were significantly increased (p<0.05) in both groups on comparing the values between the initial and final storage periods. In addition, the TBARS values in the R group were significantly (p<0.05) or non-significantly lower than those in the S group over the storage period. A similar

result was observed in uncooked raw mutton having a lower TBARS value than that of cooked mutton over the storage period (Sen et al., 2014), and similar results was also found in the broiler chicken meat (Onibi and Osho, 2007). This result may be caused by acceleration of lipid oxidation during heating of meat (Beltran et al., 2003) and inactivation of antioxidant enzymes or compounds (Min et al., 2008). But TBA values of cooked chicken breast in this study was shown to lower value of 2.55 and 3.17 mg/kg of R and S group in 14-d storage, respectively, compared to other reports that 2 mg/kg at 4-d (Sampaio et al., 2012) and 3 mg/kg at 7-d (Min et al., 2008). This result suggest that sous-vide treatment with vacuum packaging could prevent lipid oxidation. This is in agreement with the study by Xiao et al. (2013) indicating that vacuum-packaging could delay lipid oxidation in chicken breast during refrigerated storage.

The VBN content has been used as a freshness indicator of meat, and it is known to be related to the sensory characteristics. The VBN content has a tendency to increase due to amino acid decarboxylase for microbiological effect along with an increase in the storage period (Jung et al., 2010). The VBN content in the R and S groups is shown in Table 3, and was found to be increased during the storage period in both groups. The VBN content in the R group during the initial storage phase, ranging 0 to 5 d of storage, was significantly (p<0.05)higher, while the VBN content in the S group was significantly (p<0.05) higher around 14 d of storage. From 5 to 10 d of storage, the VBN content in the R group was higher than that in the S group. This result suggested that refrigerated storage under raw meat conditions caused protein decomposition during the early storage period, as mentioned previously that the uncooked poultry meats

Table 3. Changes in 2-Thiobarbituric acid reactive substances (TBARS) and volatile basic nitrogen (VBN) values in raw meat samples (R) and Sous-Vide meat samples (S) during storage at 4°C

Storage period	TBARS	(mg/kg)	VBN (mg%)		
(d)	R group	S group	R group	S group	
0	1.30±0.24 <sup>a</sup>	1.14±0.39 <sup>a</sup>	19.42±1.71 <sup>a</sup>	19.04±1.12 <sup>a</sup>	
3	$1.25\pm0.16^{a^*}$	$1.72\pm0.16^{a}$	$22.96\pm0.56^{b}$	$22.97\pm0.56^{b}$	
5	$1.56\pm0.16^{a}$	$1.77\pm0.48^{a}$	25.19±0.56bc**	$22.95\pm0.56^{b}$	
7	$1.09\pm0.16^{a}$	$1.92\pm0.39^{ab}$	$25.40\pm1.41^{bc}$	$23.52\pm0.56^{b}$	
10	$1.66\pm0.45^{a^*}$	$2.81\pm0.16^{bc}$	$26.50\pm0.65^{c**}$	$23.31 \pm 0.85^{b}$	
14	$2.55\pm0.09^{b*}$	$3.17\pm0.24^{c}$	27.99±1.12°	$26.84 \pm 1.12^{c}$	
SEM	0.195	0.267	0.895	0.681	
<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	

All values are presented as means±SD (n=15).

NS Not statistically significant.

<sup>&</sup>lt;sup>a-c</sup>Mean values with different superscripts within the same column differ significantly (p<0.05).

<sup>\*\*\*</sup>Mean values with an asterisk mark within a row during the same storage period differ significantly (\*p<0.05, \*\*p<0.01).

have a shelf life of 7-8 d (Sawaya *et al.*, 1993), and thus they have much short shelf-life compared to cooked meats. The VBN values in these studied samples were within the acceptable range (19.04-27.99 mg%) according to the VBN value of 30 mg% is proposed as the limit of acceptance (Sikorski *et al.*, 1990).

# Microbial quality

The result of microbial measurements in the chicken breast meat samples of the R and S groups stored at 4°C for 14 d represented in Table 4. Total viable count and coliform counts in both groups were significantly (p< 0.05) decreased for 14 d, ranging from 4.38 to 1.63-3.01 Log CFU/g. Total viable count in sous-vide cooked chicken breast meat storage samples (S) were significantly (p <0.05) higher than those in R group. This result might be due to the inhibitory effect of vacuum packaging on microorganism growth under anaerobic conditions. Similar results were observed in the studies by Wang et al. (2004) and Ramane et al. (2010), which reported that vacuum packaging prevented spoilage of the product because of the hurdle effect of anaerobic conditions and some chemical reactions. The coliform count in the S group was significantly (p<0.05) lower than that in the R group. Coliform counts in both groups were in the range of 1.26-2.39 until 5 d of storage, and then coliforms were not detected after 7 d of storage at 4°C. As a general guideline, total coliform counts were considered to be satisfactory when they were less than log 2 CFU/g (Solberg et al., 1990). High concentration of coliforms in food is regarded as inadequate hygiene, failure of heat treatment, or post-processing contamination (Nkere et al., 2011). But total coliforms, including about 20 species and also gastrointestinal tract bacteria in humans and animals, are a less specific indicator of fecal contamination compared to *E. coli* (Odonkor and Ampofo, 2013). *Salmonella* spp., which is largely associated with contamination of chicken meat, was not detected in samples of both groups during the studied refrigerated storage period.

This study investigated the changes in meat quality characteristics of sous vide cooked chicken breast that was stored before sous vide cooking as raw meat and after sous-vide cooking as cooked meat at 4°C during 14 d of storage. Implication from this research will not only afford a potential value of sous vide chicken meat but will also provide technical information on preliminary data that could be applied to the food industry including the ready-to-eat meal industry. Further studies are needed to find a means to reduce the pink color of sous vide cooked chicken breast that can be used in the food industry for improving the consumers satisfaction.

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Table 4. The total viable counts (TVC), coliform counts, and Salmonella spp. in raw meat storage samples (R) and Sous-Vide meat storage samples (S) stored at 4°C (Log10 CFU/g)

Storage period	TVC		Coliform counts		Salmonella spp.	
(d)	R group	S group	R group	S group	R group	S group
0	4.38±0.11°	4.38±0.11 <sup>d</sup>	$2.29\pm0.23^{NS}$	$2.61\pm0.10^{b}$	ND	ND
3	$4.18\pm0.07^{c^{**}}$	$3.83\pm0.08^{c}$	$2.39\pm0.08^{**}$	$1.26\pm0.24^{a}$	ND	ND
5	$4.22\pm0.14^{c**}$	$2.84\pm0.21^{a}$	$2.31\pm0.11^{**}$	$1.26\pm0.24^{a}$	ND	ND
7	$2.26\pm0.24^{b**}$	$3.52\pm0.20^{bc}$	ND	ND	ND	ND
10	$1.93\pm0.01^{ab^{**}}$	$3.16\pm0.15^{ab}$	ND	ND	ND	ND
14	1.63±0.11 <sup>a**</sup>	$3.01\pm0.21^{a}$	ND	ND	ND	ND
SEM	0.109	0.137	0.130	0.170	-	-
<i>p</i> -value	< 0.001	< 0.001	0.695	< 0.001	-	-

All values are presented as means±SD (n=15).

ND: Not detected.

<sup>&</sup>lt;sup>a-d</sup>Mean values with different superscripts within the same column differ significantly (p<0.05).

<sup>\*\*\*</sup>Mean values with an asterisk mark within a row during the same storage period differ significantly (\*p<0.05, \*\*p<0.01).

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