Food Science of Animal Resources

Food Sci. Anim. Resour. 2019 December 39(6):943~952
DOI https://doi.org/10.5851/kosfa.2019.e84

pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

ARTICLE

Antioxidative and Sensory Properties of *Allium hookeri* Fermented by *Leuconostoc mesenteroides* in Pork Patties

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OPEN ACCESS

Received July 5, 2019
Revised October 30, 2019
Accepted November 5, 2019

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Abstract This study was performed to investigate the antioxidative and sensory properties of *Allium hookeri* fermented by *Leuconostoc mesenteroides* in pork patties. The patties were divided into three groups: CON, with no *Allium hookeri* powder; AH, containing 1% *Allium hookeri* powder; and FAH, containing 1% fermented *Allium hookeri* powder. *Allium hookeri* fermented by *L. mesenteroides* significantly increased the radical scavenging activities of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) compared to that in normal *Allium hookeri* ($p<0.05$). The cooking loss and expressible drip for FAH were significantly lower than the corresponding values of other groups ($p<0.05$). The pH and 2-thiobarbituric acid reactive substances value of the FAH were significantly lower than that of CON on the day 3 and 7 ($p<0.05$). In the texture analysis (TPA), FAH showed significantly decreased hardness and chewiness grades compared to the CON and AH ($p<0.05$). FAH had significantly higher color, flavor, tenderness, juiciness, and overall acceptability scores than the CON and AH ($p<0.05$). This study suggests that the adding fermented *Allium hookeri* effectively improved the quality characteristic of the food.

Keywords fermented *Allium hookeri*, *Allium hookeri*, antioxidant activity, fermentation, lipid oxidation

Introduction

Lipid oxidation usually results in deteriorated quality of meat products during their refrigerated storage, and it diminishes the tastes, increases the off-flavors, changes the color of the products, and alters their rheological properties (Juntachote et al., 2006). Previous studies on meat have usually attempted to enhance the quality of meat employing many different methods (Ju et al., 2016). Reducing lipid oxidation is a key route to improve meat quality. Therefore, many efforts have been made to decrease lipid oxidation in meat products through the addition of antioxidants. Recently, meat products have been manufactured with sulfur-containing additives that have shown significant antioxidant effects (Song et al., 2013).

Garlic, chives and onion such as *Allium* species are used to enrich flavor and taste of food (Cavallito et al., 1994). *Allium* spp. are also well known for demonstrating antioxidant activity owing to their rich phenolic and flavonoid contents (Kim et al., 2012). *Allium hookeri* is cultivated in Korea and used in many foods in different ways (Bae et al., 2012). It shows excellent anti-inflammatory and anti-oxidative effects and improves the quality of food (Bae et al., 2012; Kim et al., 2016). It is known for sweet, bitter, and spicy tastes and so is called "three-tastes namul" in Korea. It contains high amounts of sulfur compounds with unique flavor (Yang et al., 2016), due to which, it is not appreciated in sensory evaluation when it is used as additives in different foods (Jun et al., 2014).

Fermentation is the most traditional and common method to reduce unflavored tastes and flavor. Researchers have also reported that fermentation can be enhancement in antioxidant activity and total phenolic and flavonoid content in the food (Juan et al., 2010). *L. mesenteroides* is a common heterofermentative lactic acid bacteria used in spontaneous kimchi fermentation and in the production of lactate, carbon dioxide, ethanol, and acetate from carbohydrates (Jung et al., 2012a). *Leuconostoc* has been detected in garlic and onion and it has been found to be the predominant Lactic acid bacteria in garlic (Jung et al., 2012b). Anti-inflammatory effects and antioxidant and of *L. mesenteroides* have been reported previously (Jung et al., 2012a). Antioxidant and antibacterial activities have been induced in Chinese chives by fermentation using *L. mesenteroides* (Hong et al., 2016).

Numerous studies have proved the anti-inflammatory and antioxidant effect of *Allium hookeri* (Juntachote et al., 2006; Song et al., 2013), but hardly any have investigated the potential of fermented *Allium hookeri* in offering antioxidant activities and increasing the quality and lipid stability of meat products. Consequently, this study was conducted to regulate the lipid oxidation inhibitory effect and influence on food quality brought about by fermented *Allium hookeri* on pork patties during refrigerated storage.

Materials and Methods

Reagents

The *Allium hookeri* was bought at Samchaenara Co. located Gyeongsangnamdo, Korea. The *Allium hookeri* was freeze dried to use as the sample at Dongil Cold Storage & Foods Co. (Icheon, Korea). Chemicals together with 1,1,3,3-tetraethoxypropane (TEP), ABTS, aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, TBA, quercetin, sodium carbonate and trichloroacetic acid (TCA) were bought at Sigma (Sigma-Aldrich, St. Louis, MO, USA). Supplementary chemicals including gallic acid and potassium persulfate were bought at Samchun (Samchun pure Chemical Co., Ltd., Pyeongtaek, Korea) and Kanto (Kanto Chemical Co., Inc., Tokyo, Japan).

Microorganisms and sample preparation

The *Leuconostoc mesenteroides* SK 1962 obtained from the Laboratory of Feed Biotechnology and Applied Molecular Microbiology of Konkuk University, Seoul. *L. mesenteroides* SK 1962 was cultivated in MRS broth (Oxoid Ltd., Basingstoke, UK) at 37℃ for a day, and then used as an inoculum that was incubated to get a population of 10^7 CFU/mL on regular. *Allium hookeri* powder was mixed with distilled water at a 1:10 (w/v) ratio and autoclaved for 15 min at 121℃. After chilling, it was inoculated (1%, v/v) with an activated strain of 10^7 CFU/mL on average. The mixed culture was cultivated in MRS broth at 37℃ and then incubated. The samples were collected after 16 h.

Determination of total phenolic and total flavonoid contents

Total phenol contents were measured by Folin-Ciocalteu's methods (Singleton et al., 1999) with a few modifications. The 0.5 mL of samples and 2.5 mL Folin-Ciocalteu's phenol reagent were assorted and then reacted for 5 min at the room temperature. Then, adding 2 mL of 75 g/L sodium carbonate to the sample and reacted at the room temperature for 2 h. The absorbance value was determined at 760 nm and for the blank the ethanol was used. To construct the standard curve garlic acid equivalents were used. Total flavonoid contents were measured by the Dowd method (Arvouet-Grand et al., 1994) with minor modifications. Temporarily, 1.5 mL of 2% aluminum chloride and 0.5 mL of diluted 10 times sample were mixed and reacted at the room temperature for 10 min. The samples were measured absorbance at 415 nm. For the standard curve the quercetin was used.

Analysis of free radical scavenging activity

The free radical scavenging activity of each group was determined by method of Blois (Blois et al., 1958), with a few modifications. The 5 mL of 0.1 mM DPPH in 95% ethanol was mixed to a 1 mL 10-fold-diluted aliquot of each sample. The mixture of the samples was vortexing and then reacted at the room temperature for 30 min in the darkroom. Later then, the sample was purified with the 0.45 μm nylon syringe filter. The sample was determined at 517 nm of absorbance at spectrophotometer. The ABTS free radical scavenging activity was measured by Re et al method. (Re et al., 1999). The 2 mM ABTS reagent was diluted in distilled water including 2.45 mM potassium persulfate and reserved at the room temperature for 12 h in the darkroom. The ABTS solution was accustomed with a sodium phosphate buffer (0.1 M, pH 7.4) to an original absorbance at 734 nm. Then, a 0.1 mL 10-fold-diluted aliquot of each sample was mixed with 3 mL of the ABTS solution. After incubating at the room temperature for 10 min, the samples were measured at 734 nm of absorbance.

The ABTS and DPPH scavenging activity values were calculated as follows:

$$\text{ABTS or DPPH scavenging activity (\%)} = \{1 - (\text{Absorbance sample} / \text{Absorbance control})\} \times 100$$

Preparation of pork patties

Pork loins and pork backfat were purchased from a local market (Seoul, Korea). Excluded the pork loin such as fat and connective tissue were removed. Lean pork (80%) and fat (20%) were ground through a 5-mm plate. The grounded mixture was mixed with 1.5 g/kg sodium nitrite. The patties were divided into three groups: CON, with no *Allium hookeri* powder; AH, containing 1% *Allium hookeri* powder; and FAH, containing 1% fermented *Allium hookeri* powder. The mixtures with different compositions were then formed into 80-g patties using a patty-maker (small round burger press, Spikomat Ltd., Nottingham, UK).

Proximate composition of pork patties

Crude protein and crude fat contents of the patties were measured following to the AOAC methods (2012). The moisture content was measured by the drying method at 105℃ and the crude ash content was determined by the direct ash method at 550℃.

Determination of cooking loss and expressible drip

The cooking loss (%) was measured by weight changes in pork patties before and after cooking at 75℃ for 45 min in a water bath, as revealed following equation (Choi et al., 2009).

$$\text{Cooking loss (\%)} = (\text{Initial sample weight} - \text{Sample weight after cooking}) / \text{Initial sample weight} \times 100$$

The expressible drip was determined by modification of NGCS method (1987). The samples were set sandwiched between the Whatman No. 1 filter papers and forced with a force of 9.9 kg/cm^2 (IF 32B-S50; Ilshin Tech. Co. Ltd., Hwaseong, Korea) for 1 min and calculated with the formula as shown below.

$$\text{Expressible drip (\%)} = (\text{Initial sample weight} - \text{Sample weight after pressed}) / \text{Initial sample weight} \times 100$$

Measurement of pH

For the pH measurement, the sample (2 g) were homogenized with 18 mL of distilled water for 1 min using a pH meter (pH 900, Precisa Co., Dietikon, Switzerland).

TBARS values

The TBARS values of the samples were measured by the method reported by Witte et al. (1970). The 10 g of sample was homogenized with 50 mL of 10% (w/v) TCA for 1 min. After homogenized the samples were filled up to 50 mL with distilled water. The samples were purified with Whatman No.1 paper and then 5 mL of the filtrated sample was mixed with 5 mL of TBA (2.88 g/L H_2O), which was boiled in a boiling water bath at 80℃ for 10 min to develop a pink color. After cooling the samples at the room temperature, the samples were determined at 532 nm absorbance. The standard curve was measured with the malondialdehyde (MDA) prepared by acidification of TEP. The TBARS value was measured by the standard curve and presented by mg of MDA per kg of sample.

TPA evaluations

TPA of the pork patties was evaluated at the room temperature with a texture analyzer (CT31000, Brookfield Engineering Laboratories, Inc., Middleborough, MA, USA) and evaluate the hardness, springiness, cohesiveness, gumminess, and chewiness of the samples. TPA was measured with a few modifications of the Claus method (1995). The sample was cut into a 1 cm cube shape from the pork patties. The samples were crushed to 70% of its original height using a cylindrical probe with a diameter of 10 cm under a compression load of 15 kg and cross-head speed of 20 cm/min. The TPA values for hardness, springiness, cohesiveness, gumminess, and chewiness were measured as defined by Bourne (1978).

Sensory test

The 10 random trained panelists were joined the sensory evaluation. Each sample was graded 1 (not acceptable) to 10 (very acceptable) by the panelists. The sensory test was approved (approval number: 7001355-201910-E-102) by Konkuk Institutional Review Board (IRB). The sample patties were cut into a block with a thickness, length, and width of 1 cm each and were placed on a white plate at room temperature (Kim et al., 2015). After testing and graded one sample, the panelists were requested to wash their mouths with the pure water and wait a min before the grading the next sample for the evaluation. Grades for meat color, flavor, tenderness, juiciness, and overall acceptability were provided by the panelists. The sensory description used by the panelists was as described by Li et al. (2014).

Statistical analysis

All experiments data were evaluated by one-way analysis of variance using by SPSS software called PASW Statistics 18 (PASW 18, SPSS Inc., USA). Means were equated using the Tukey's multiple rage test at the significance level of $p<0.05$.

Results and Discussion

DPPH and ABTS scavenging activity of fermented *Allium hookeri*

Efficacies of the fermented *Allium hookeri* measured by the method used for DPPH and ABTS are presented in Table 1. According to the DPPH method, the radical scavenging activity of fermented *Allium hookeri* increased significantly compared to that of normal *Allium hookeri* ($p<0.05$). The ABTS radical scavenging activity of fermented *Allium hookeri* was as well significantly higher than that of normal *Allium hookeri* ($p<0.05$). Therefore, the addition of fermented *Allium hookeri* improved the DPPH and ABTS scavenging activities.

Total phenolic and total flavonoid contents of fermented *Allium hookeri*

Total phenolic and total flavonoid contents of fermented *Allium hookeri* are shown in Table 2. These contents were conducted to measure the relationship between different antioxidant activities of fermented *Allium hookeri*. Enrich phenols and flavonoid foods such as vegetables are famous for high antioxidant activities cause to many bioactive substances (Park et al., 2007).

Proximate composition, cooking loss, and expressible drip of pork patties

Effects of *Allium hookeri* and fermented *Allium hookeri* on the proximate composition, cooking loss, and expressible drip of pork patties are presented in Table 3.

Table 1. DPPH and ABTS radical scavenging activity (%) of *Allium hookeri* and fermented *Allium hookeri*

	AH	FAH	SEM	p-value
DPPH radical scavenging activity	10.39^{b}	12.15^{a}	0.65	0.012
ABTS radical scavenging activity	22.07^{b}	25.60^{a}	0.34	0.002

Each value is the mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
AH, pork patties with *Allium hookeri* root powder; FAH, pork patties with fermented *Allium hookeri* root powder; DPPH, 2,2-diphenyl-1-picrylhydrazyl, ABTS, activities of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

Table 2. Changes in total phenol and flavonoid contents in *Allium hookeri* and fermented *Allium hookeri*

	AH	FAH	SEM	p-value
Total phenol content (mg GAE/g)	16.63	17.47	0.58	0.130
Total flavonoid content (mg QE/g)	0.13	0.15	0.01	0.114

All values are mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
AH, pork patties with *Allium hookeri* root powder; FAH, fermented *Allium hookeri* root powder.

Table 3. Effects of *Allium hookeri* and fermented *Allium hookeri* on proximate composition, cooking loss, and expressible drip of pork patties

Proximate composition (%)	CON	AH	FAH	SEM	p-value
Moisture	59.07	60.00	60.21	0.54	0.138
Crude fat	22.53	21.41	21.06	0.65	0.318
Crude protein	15.61	16.38	15.47	0.29	0.358
Ash	1.98	2.38	2.03	0.13	0.176
Cooking loss	32.76^{ab}	33.24^{a}	31.56^{b}	0.30	0.019
Expressible drip	37.50^{a}	34.91^{b}	34.50^{b}	0.53	0.014

Each value is the mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
CON, normal pork patties; AH, pork patties with *Allium hookeri* root powder; FAH, pork patties with fermented *Allium hookeri* root powder.

In the proximate composition result, no significant difference was shown between the all groups. However, the cooking loss and expressible drip of FAH was significantly lower than those of other groups ($p<0.05$). Similar trends in cooking loss and expressible drip have been previously noted after the addition of *Allium* spp. in meat products (Kim et al., 2015).

pH values of pork patties

Effects of *Allium hookeri* and fermented *Allium hookeri* on pH values of pork patties are summarized in Table 4. The pH values of all treated groups were significantly lower than in CON on days 1, 3, and 7 ($p<0.05$). On day 14, the pH value of FAH was significantly lower than that of other groups ($p<0.05$). The pH influences an effect on different parameters of the food product such as the shelf life, texture, and other quality characteristics. Low pH values of meat products prolong the shelf life by inhibiting the growth of microorganisms. Previous studies have indicated that adding high-antioxidant-activity additives result in a low pH value for products, as was the case in this study (Cho et al., 2015).

2-Thiobarbituric acid (TBA) values of pork patties

Effects of *Allium hookeri* and fermented *Allium hookeri* on TBARS values of pork patties are shown in Table 5. The TBARS values of FAH were significantly lower than those of CON on days 3 and 7 ($p<0.05$). The TBA values denote lipid oxidation of the meat products. High TBA values in meat products suggest sensory quality degradation such as rancidity and odor. A previous study reported that supplementing *Allium* spp. improves the TBA value by enhancing its phenolic content (Cho et al., 2015). In this study, FAH maintained the lowest TBA values among all groups. These results indicate that

Table 4. Effects of *Allium hookeri* and fermented *Allium hookeri* on pH values of pork patties

pH value	CON	AH	FAH	SEM	p-value
Day 1	6.43^{a}	6.32^{b}	6.15^{b}	0.03	0.002
Day 3	6.29^{a}	6.12^{b}	6.02^{c}	0.20	0.000
Day 7	6.44^{a}	5.68^{b}	6.02^{c}	0.05	0.000
Day 14	6.70^{a}	6.56^{a}	6.53^{b}	0.02	0.003

Each value is the mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
CON, normal pork patties; AH, pork patties with *Allium hookeri* root powder; FAH, pork patties with fermented *Allium hookeri* root powder.

Table 5. Effects of *Allium hookeri* and fermented *Allium hookeri* on TBARS values of pork patties

TBARS value (mg MDA/kg)	CON	AH	FAH	SEM	p-value
Day 1	0.37	0.38	0.37	0.01	0.347
Day 3	0.35^{a}	0.33^{ab}	0.32^{b}	0.01	0.029
Day 7	0.29^{a}	0.24^{b}	0.24^{b}	0.01	0.013
Day 14	0.41	0.40	0.40	0.00	0.251

Each value is the mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
CON, normal pork patties; AH, pork patties with *Allium hookeri* root powder; FAH, pork patties with fermented *Allium hookeri* root powder.

fermented *Allium hookeri* can be used as a natural antioxidant additive to enhance the sensory properties.

Texture profile analysis (TPA) measurement of pork patties

Effects of *Allium hookeri* and fermented *Allium hookeri* on textural properties of pork patties are shown in Table 6. FAH showed significantly decreased hardness and chewiness values ($p<0.05$). The moisture content affects the hardness and chewiness. However, in this study, even though the moisture content was not significantly different for the samples, the hardness and chewiness of FAH were significantly lower than those of other groups ($p<0.05$). A previous study indicated that addition of some additives improved the textural properties of a meat product by lowering its hardness (Yang et al., 2007). Furthermore, supplementing *Allium* spp. ingredients can soften the texture (Kotwaliwale et al., 2007). Therefore, this study suggests that supplanting fermented *Allium hookeri* can improve the texture properties by decreasing the hardness of meat products.

Sensory evaluation of pork patties

Changes in sensory characteristics of cooked pork patties with *Allium hookeri* and fermented *Allium hookeri* are shown in Table 7. FAH had significantly higher color, flavor, tenderness, juiciness, and overall acceptability scores compared to the other groups ($p<0.05$). Previous reports indicated that adding *Allium* spp. in the meat product improves the flavor by masking off-flavors due to its antioxidant properties (Tang et al., 2007).

Conclusion

These results suggest that the adding fermented *Allium hookeri* effectively improves the quality characteristics of meat

Table 6. Effects of *Allium hookeri* and fermented *Allium hookeri* on textural properties of pork patties

Parameter	CON	AH	FAH	SEM	p-value
Hardness (kg)	8.74^{a}	7.25^{ab}	6.42^{b}	0.41	0.019
Springiness (mm)	1.58	1.58	1.38	0.17	0.627
Cohesiveness	0.50	0.39	0.41	0.06	0.470
Gumminess (kg)	1.93	1.76	1.77	0.05	0.636
Chewiness (mJ)	2.33^{a}	1.17^{b}	1.23^{b}	0.18	0.009

Each value is the mean of three replicates.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
CON, normal pork patties; AH, pork patties with *Allium hookeri* root powder; FAH, pork patties with fermented *Allium hookeri* root powder.

Table 7. Changes in sensory characteristics of cooked pork patties with *Allium hookeri* and fermented *Allium hookeri*

Parameter	CON[1)]	AH	FAH	SEM	p-value
Color	2.80^{b}	4.40^{a}	4.20^{a}	0.28	0.001
Flavor	3.00^{b}	4.00^{a}	4.00^{a}	0.23	0.005
Tenderness	3.40^{b}	4.00^{ab}	4.60^{a}	0.30	0.032
Juiciness	3.60^{b}	4.00^{ab}	4.80^{a}	0.32	0.041
Overall acceptability	3.20^{b}	4.40^{a}	4.60^{a}	0.20	0.000

N=10.
^{a,b} values in each row with different letters are statistically different ($p<0.05$).
CON, normal pork patties; AH, pork patties with *Allium hookeri* root powder; FAH, fermented *Allium hookeri* root powder.

products and extends their shelf life during refrigerated storage owing to its high antioxidative activity. Therefore, fermented *Allium hookeri* can be used as a natural antioxidant additive that also provides beneficial sensory improvement.

Conflict of Interest

The authors declare no potential conflict of interest.

Author Contributions

Conceptualization: Lee NY. Data curation: Lee NY, Lee CH. Investigation: Lee NY. Writing - original draft: Lee NY. Writing - review & editing: Lee NY, Lee CH.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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