

Effects of Ethanol Extract of *Bacillus polyfermenticus* SCD on the Physicochemical Properties of Cooked Ground Pork during Storage

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Bacillus polyfermenticus SCD 에탄올 추출물이 가열분쇄돈육의 저장 중 이화학적 특성에 미치는 영향

김학연 · 정종연¹ · 최지훈 · 최윤상 · 한두정 · 이미애 · 이장현 · 백현동 · 김천제*

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Abstract

The objective of this study was to determine the effects of 0.6% vitamin C (VC), 5% ethanol extract of *B. polyfermenticus* SCD (EB), and a mixture of 0.3% vitamin C and 2.5% *B. polyfermenticus* SCD ethanol extract (CB) on the physicochemical properties of cooked ground pork during storage. The changes in pH of VC, EB, and CB were smaller than was observed with the control (CON, no added antioxidant). VC, EB, and CB exhibited significantly lower TBARS values than CON during storage ($p < 0.05$). Longer storage periods resulted in higher TBARS values ($p < 0.05$). VBN values for VC, EB, and CB were significantly lower than CON ($p < 0.05$). The *L* values of CON and VC were higher than EB and CB ($p < 0.05$). The *a* value of VC was significantly lower than CON, EB, and CB during storage ($p < 0.05$). The *b* values of all samples significantly increased during storage ($p < 0.05$). The addition of vitamin C and *B. polyfermenticus* SCD to cooked ground pork did not significantly affect sensory evaluations during the storage period ($p > 0.05$). Further studies are needed to develop other meat products containing *B. polyfermenticus* SCD with acceptable physicochemical properties.

Key words : *Bacillus polyfermenticus* SCD, physicochemical properties, cooked ground pork, antioxidant

Introduction

Minced meat tends to become brown and rancid more rapidly than whole muscle cuts because of the high unsaturated fat content (Ho *et al.*, 1996). Meat product manufacturing processes prior to refrigerated storage disrupt muscle cell membranes, facilitating the interaction of unsaturated fatty acids with pro-oxidant substances such as non-heme iron, and thereby accelerate lipid oxidation leading to rapid qual-

ity deterioration and the development of rancidity (Tichivan-gana and Morrissey, 1985). The oxidation of fats and lipids decreases the quality and safety of foods, yielding peroxides, alkanes, alcohols, aldehydes and acids. Therefore, it is essential to control lipid oxidation to delay the development of lipid and fat oxidants since this is one of the major reasons for the spoilage of meat-based products.

Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate have been widely used in raw and ground meat products during storage to retard lipid oxidation (Shaidi *et al.*, 1987; McCarthy *et al.*, 2001). However, these synthetic antioxidants are under strict regulation due to the potential health hazards caused by such compounds (Hettiarachchy *et al.*, 1996). Natural antioxi-

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dants such as ascorbic acid, tocopherol and chitosan have been widely studied in raw and precooked ground poultry, beef and pork products during storage to retard lipid oxidation. However, there has been relatively little investigation of the use of natural antioxidants from microbes in raw meat and meat products.

Studies of lactic acid bacteria (LAB; *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus*) have provided evidence that they reduce the risk of some types of cancer and inhibit the growth of certain tumors and tumor cells (Romond *et al.*, 1997; Kim *et al.*, 2003). The genus *Bacillus* includes a variety of industrially important species which are commonly used in bioindustry. In particular, *B. polyfermenticus* SCD, which contains what are known as the "Bisroot" strains, has been used for the treatment of long-term intestinal disorders since live strains in the form of active endospores can successfully reach the target intestine (Jun *et al.*, 2000; Lee *et al.*, 2000). Several researchers have reported that *B. polyfermenticus* SCD has antioxidant and antitumor effects (Paik *et al.*, 2005; Kim *et al.*, 2006; Choi *et al.*, 2008). The addition of ethanol extract of *B. polyfermenticus* SCD retarded lipid oxidation of emulsion type sausage (Kim *et al.*, 2008). Kim *et al.* (2006a) also observed that ethanol extracts of *B. polyfermenticus* SCD had antioxidant effects in raw *Tteokalbi* during storage.

It is well known that natural antioxidants have high antioxidant activity (Elliot, 1983; Mitosunoto *et al.*, 1991). The use of natural antioxidants to maintain high quality and safety is important for human health and for the prevention of economic losses. However, few studies have been performed on antioxidant substances from microbial sources. Also, the relationship between *B. polyfermenticus* SCD and cooked ground pork during storage has not been studied. The objective was to determine the effects of *B. polyfermenticus* SCD ethanol extracts and vitamin C on the physicochemical properties of cooked ground pork as measured by thiobarbituric acid-reactive substance (TBARS), volatile basic nitrogen (VBN), pH, instrumental color, and sensory evaluations during storage at 4°C.

Materials and Methods

Production of *B. polyfermenticus* SCD

B. polyfermenticus SCD was inoculated into 1500 mL of sterile tryptic soy broth and the seed culture (5%, v/v) was then transferred to a 50 L jar fermenter (30 L working volume; Bioengineering AG, Wald, Switzerland) followed by incubation at 37°C for 7 hr with agitation at 500 rpm and 1

vvm (volume of air per volume of medium per min) aeration rate. During incubation, the culture pH was maintained at 7.0 ± 0.2 by adding 3 N H_2SO_4 and 3 N NaOH. Silicone oil, which was used as an antifoam agent, was added automatically whenever necessary. The culture was then centrifuged at $21,000 \times g$ at 4°C for 20 min.

Adsorption to Diaion HP-20 column using polar solvent

Culture supernatants were injected into 5 cm \times 1 m (diameter \times length) Diaion HP-20 columns (100 g, Mitsubishi, Tokyo, Japan) at a ratio of 1:2 (volume of Diaion HP-20 resin:supernatant). The antioxidant fraction was eluted with 100% of ethanol. The fraction was concentrated (500 mg/mL) using a rotary vacuum evaporator and added to ground pork meat product.

Preparation of samples, packaging and storage

Fresh pork hams were purchased from a local processor at 48 h postmortem. Pork back fat was also collected. All subcutaneous and intermuscular fat and visible connective tissue were removed from fresh ham muscle. Lean materials were initially ground through a \emptyset 13-mm plate and the fat percentage was determined before blending. The pork fat was ground through an \emptyset 8-mm plate and added to ham (70%), fat (20%), NPS (1.5%), ice (10%), sugar (2.1%), pepper (0.3%), phosphate (0.3%), onion powder (2%), garlic powder (2%) and ISP (isolated soybean protein, 2%). The mixtures were mixed by hand for 3 min and subjected to 2 final grindings (\emptyset 3-mm plate). The mixtures studied were: CON, no added antioxidants; VC, vitamin C added to 0.6%; EB, ethanol extract of *B. polyfermenticus* added to 5%; CB, 0.3% vitamin C + 2.5% *B. polyfermenticus* ethanol extract. Each mixture was then vacuum packaged with Nylon/PE film and stored for 1, 3, 6, or 10 days at $4 \pm 1^\circ C$.

Experimental design

After heating in a water bath at 75°C for 35 min, each mixture package was stored at $4 \pm 1^\circ C$ for 1, 3, 6, or 10 days, then opened for subsequent analysis of pH, TBARS, VBN, color, and sensory evaluations.

pH determination

pH was determined by pH meter (Model 340. Mettler-Toledo GmbH Analytical, Schwerzenbach, Switzerland) following grinding and homogenization of 5 g of sample with 20 mL of distilled water for 60 sec (Ultra-Turrax® T25, Janke & Kunkel, Staufen, Germany).

Thiobarbituric acid reactive substances (TBARS)

Oxidative rancidity was evaluated in the cooked ground pork samples by measuring TBARS values after 1, 3, 6, or 10 days of storage. Malondialdehyde (MDA), is a secondary product of lipid oxidation, formed during lipid oxidation in the samples were measured and reported as the TBARS value in units of MDA equivalent/kg of sample. The amount of the pink colored TBARS complex was measured with a spectrophotometer at 538 nm using the methods of Tarladgis *et al.* (1960). Triplicate samples were analyzed. The TBARS value number was calculated as mg MDA/kg sample.

Volatile basic nitrogen (VBN)

Volatile basic nitrogen was determined by the Conway micro-diffusion method and expressed as mg% of sample (Pharmaceutical Society of Japan, 1980).

Instrumental color

CIE *L* (lightness), *a* (redness), *b* (yellowness) values were determined for each sample using a reflectance spectrophotometer (Minolta Chroma meter CR-210; illuminate C, calibrated with plate, $L = +97.83$, $a = -0.43$, $b = +1.98$, Japan) standardized using the white tile.

Sensory evaluation

Sensory evaluations were performed in duplicate on each sample by a 10-member trained panel. Sliced samples were served at a temperature of approximately 60°C to each panelist. Panelists were given warm water (30°C) to consume between samples. Samples were evaluated for flavor, tenderness, juiciness, and oiliness on a 10-point horizontal scale (1 = extremely undesirable and 10 = extremely desirable for flavor; 1 = extremely tough and 10 = extremely tender for tenderness; 1 = extremely dry and 10 = extremely juicy for juiciness; 1 = no oily feeling and 10 = extremely oily for oiliness).

Statistical analysis

Data were analyzed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC, 1999) of a 5% level of significance. Difference between mean values were determined using Duncan's multiple range test.

Results and Discussion

pH

Changes in pH values of cooked ground pork after vari-

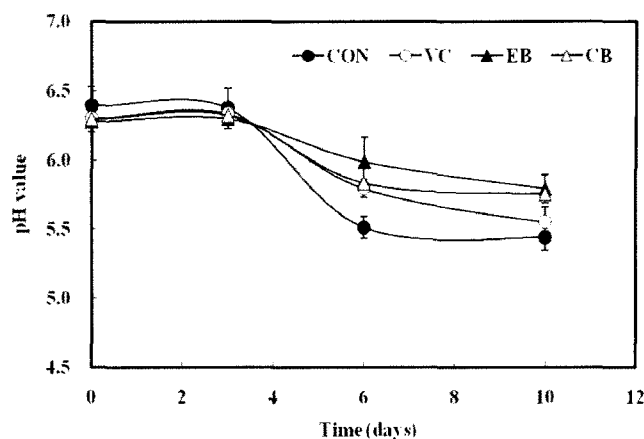


Fig. 1. Changes in pH of cooked ground pork containing vitamin C and ethanol extract of *B. polyfermenticus* SCD during storage. CON, no antioxidant added; VC, vitamin C added; EB, ethanol extract of *B. polyfermenticus* SCD added; CB, vitamin C and ethanol extract of *B. polyfermenticus* SCD added.

ous periods of storage at 4°C are given in Fig. 1. The pH values of VC (supplemented with 0.6% vitamin C), EB (supplemented with 5% *Bacillus polyfermenticus* SCD), and CB (supplemented with 0.3% vitamin C and 2.5% *Bacillus polyfermenticus* SCD ethanol extract) cooked ground pork were significantly lower than CON (the control with no added antioxidant) during storage up to 3 days ($p < 0.05$). However, VC, EB and CB had significantly higher pH values than CON from 6 days to 10 days of storage ($p < 0.05$). The pH of CON samples sharply decreased at 6 days of storage ($p < 0.05$). The pH values of VC, EB, and CB significantly decreased from 6 days to 10 days of storage ($p < 0.05$). This sharp decrease might be attributed to production of organic acids by bacteria (Goddard *et al.*, 1996). Lan-nelongue *et al.* (1982) showed that a decrease in pH correlates with increases in the concentration of CO₂ in the atmosphere. Kim *et al.* (2006a) reported that the pH value of *Teokgalbi* dramatically decreased after 1 week of storage. Several other authors have also observed that pH values fall during the storage (Jose *et al.*, 1984; Kim *et al.*, 2007). Park and Kim (2007) showed that the pH values of ground pork meat containing fresh paprika decreased dramatically from approximately 6.5 to 5.0 during storage. Shikama and Sugawara (1978) showed that pH values decreased markedly with increasing rates of autoxidation. The change in pH of VC, EB, and CB were smaller than CON. These results support the antioxidant characteristics of vitamin C and *B. polyfermenticus* SCD.

Thiobarbituric acid reactive substances (TBARS)

The results of TBARS testing for secondary oxidation

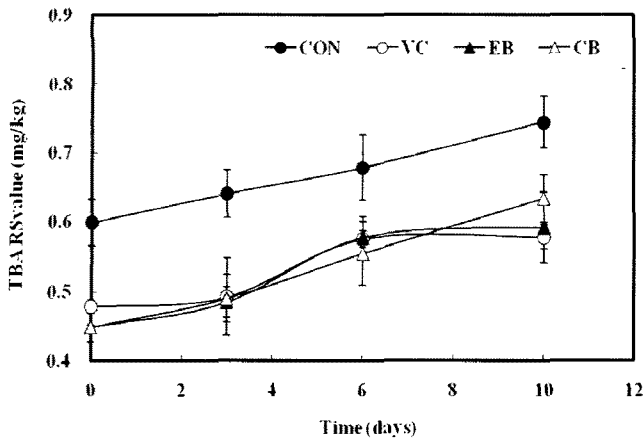


Fig. 2. Changes in TBARS values of cooked ground pork containing vitamin C and ethanol extract of *B. polyfermenticus* SCD during storage. CON, no antioxidant added; VC, vitamin C added; EB, ethanol extract of *B. polyfermenticus* SCD added; CB, vitamin C and ethanol extract of *B. polyfermenticus* SCD added.

products are shown in Fig. 2. TBARS values were lower in all supplemented samples relative to CON ($p < 0.05$). VC, EB, and CB exhibited significantly lower TBARS values than CON during storage ($p < 0.05$). Furthermore, TBARS values of all samples significantly increased during storage. These results suggest that antioxidant substances derived from *B. polyfermenticus* SCD and vitamin C retard lipid oxidation during storage ($p < 0.05$). The antioxidant effects of *B. polyfermenticus* SCD have been reported by many researchers (Paik *et al.*, 2005; Heddur *et al.*, 2005; Kim *et al.*, 2006b). In terms of TBARS values, CB was not significantly different than VC and EB ($p > 0.05$). Nevertheless, many researchers have reported that vitamin C used in combination with other antioxidants functions synergistically to promote greater antioxidant effects (Chang *et al.*, 1997). In fact, various natural antioxidants (α -tocopherol and herb extracts) have been used together with Vitamin C to prevent food oxidation (Shahidi and Wanasundara, 1992). However, the CB sample containing Vitamin C and *B. polyfermenticus* SCD in our study had higher TBARS values than VC or EB. These results agree with those of Kim *et al.* (2006a).

Volatile basic nitrogen (VBN)

The measurement of volatile basic nitrogen (VBN) compounds, which result from the decomposition of protein by microorganisms, can be an important indicator of deterioration in meat products during storage. Changes in VBN values of the various cooked ground pork samples during storage are shown in Fig. 3. The VBN values of VC, EB, and CB were lower than CON ($p < 0.05$). The VBN value of

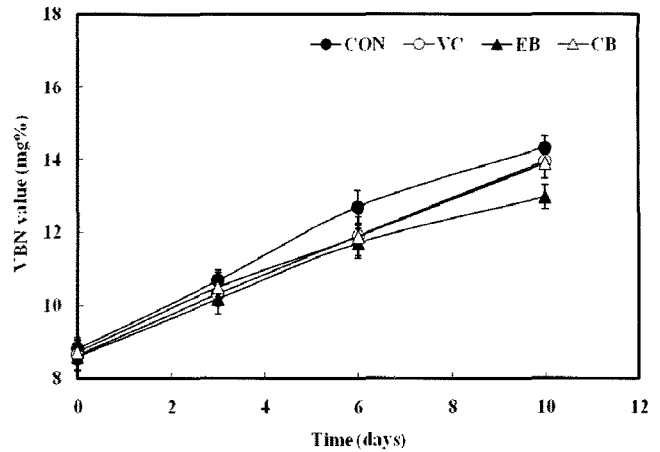


Fig. 3. Changes in VBN of cooked ground pork containing vitamin C and ethanol extract of *B. polyfermenticus* SCD during storage. CON, no antioxidant added; VC, vitamin C added; EB, ethanol extract of *B. polyfermenticus* SCD added; CB, vitamin C and ethanol extract of *B. polyfermenticus* SCD added.

all samples gradually increased as the total microbial counts increased during storage. Furthermore, the VBN values of all supplemented samples were lower than CON due to lower total microbial counts during storage. The VBN values of cooked ground pork significantly increased as the storage time increased ($p < 0.05$). These results are similar to those of several groups (Wang, 2000; Kim *et al.*, 2006a; Kim *et al.*, 2008).

Instrumental color

Changes in the CIE *L*, *a* and *b* value of samples during storage are shown in Table 1. The *L* value of CON and VC were higher than EB and CB ($p < 0.05$). These results are shown similar to the study of Kim *et al.* (2006a), since ethanol extracts of *B. polyfermenticus* SCD have a brown color. The samples tested did not necessarily exhibit consistent changes in *L* values during storage. The *a* value of VC containing added vitamin C was significantly higher than the other samples during storage ($p < 0.05$). Vitamin C plays a role in color stability through metmyoglobin reduction, thus improving the red color in meat products (Djenane *et al.*, 2001; Naveena *et al.*, 2006). All mixture had higher *a* values after 6, 10, and 15 days than during the initial 3 days of storage. The *b* value of all samples were significantly increased during storage ($p < 0.05$). These results do not show a general trend regarding acceptable color values of samples during storage.

Sensory evaluations

The sensory evaluations of cooked ground pork meat products from 1 to 6 days of storage are shown in Table 2.

Table 1. Changes in color of cooked ground pork containing vitamin C and ethanol extract of *B. polyfermenticus* SCD during storage

Trait	Time (days)	Treatments ¹⁾			
		CON	VC	EB	CB
CIE <i>L</i>	1	60.49±1.71 ^{Aa2)}	59.65±0.56 ^{Ab}	57.25±1.4 ^{Bab}	59.13±0.47 ^{Aa}
	3	60.61±0.93 ^{Aa}	60.51±0.74 ^{Aa}	58.24±0.83 ^{Ca}	59.41±0.61 ^{Ba}
	6	60.74±1.13 ^{Aa}	59.18±0.23 ^{Bb}	55.99±0.90 ^{Dc}	57.94±0.26 ^{Ch}
	10	59.25±1.04 ^{Ab}	59.39±1.06 ^{Ab}	57.41±0.57 ^{Bab}	57.47±0.79 ^{Bb}
CIE <i>a</i>	1	10.26±1.87 ^{Ba}	11.93±0.44 ^{Ab}	11.08±1.17 ^{ABb}	10.72±0.81 ^{ABc}
	3	9.74±0.61 ^{Bab}	13.07±0.23 ^{Aa}	11.75±0.21 ^{Bb}	11.54±0.78 ^{Bb}
	6	8.45±0.54 ^{Cc}	13.69±0.28 ^{Aa}	12.32±0.39 ^{Ba}	12.03±0.15 ^{Bab}
	10	8.57±0.32 ^{Cbc}	13.06±0.91 ^{Aa}	11.77±0.21 ^{Bab}	11.60±0.26 ^{Bb}
CIE <i>b</i>	1	6.75±0.56 ^{Cb}	7.27±0.65 ^{BCc}	8.11±0.55 ^{Ab}	7.66±0.31 ^{ABb}
	3	6.87±0.37 ^{Bb}	7.44±0.49 ^{ABc}	7.58±0.44 ^{Ab}	7.54±0.60 ^{Ab}
	6	8.24±0.80 ^{Ca}	9.54±0.53 ^{Ab}	9.47±0.31 ^{ABa}	8.86±0.20 ^{BCa}
	10	8.31±1.39 ^a	9.17±1.02 ^b	8.97±0.81 ^a	8.49±0.63 ^a

¹⁾CON, no antioxidant added; VC, vitamin C added; EB, ethanol extract of *B. polyfermenticus* SCD added; CB, vitamin C and ethanol extract of *B. polyfermenticus* SCD added.

²⁾All values are mean±SD.

^{a-c} Means in the same column with different letters are significantly different ($p<0.05$).

^{A-C} Means in the same row with different letters are significantly different ($p<0.05$).

Table 2. Changes in sensory evaluations of cooked ground pork containing vitamin C and ethanol extract of *B. polyfermenticus* SCD during storage period

Trait	Time (days)	Treatments ¹⁾			
		CON	VC	EB	CB
Color	1	8.67±0.52 ²⁾	8.50±0.55	8.83±0.41	8.83±0.41
	3	8.67±0.52	8.50±0.55	8.67±0.52	8.67±0.52
	6	8.33±0.82	8.33±0.52	8.17±1.17	8.50±0.55
Flavor	1	8.67±0.52 ^a	8.67±0.52 ^a	8.00±0.63 ^a	8.33±0.82 ^a
	3	8.67±0.52 ^a	8.67±0.52 ^a	8.17±0.75 ^a	8.50±0.55 ^a
	6	5.50±0.55 ^b	5.67±0.52 ^b	6.17±0.75 ^b	5.83±0.75 ^b
Tenderness	1	8.33±0.52 ^{ab}	8.50±0.55 ^a	8.50±0.55	8.67±0.52 ^a
	3	8.50±0.55 ^a	8.50±0.55 ^a	8.67±0.52	8.50±0.55 ^a
	6	7.67±0.82 ^b	7.50±0.55 ^b	7.83±0.98	7.67±0.67 ^b
Juiciness	1	8.50±0.55 ^a	8.33±0.52 ^a	8.67±0.52 ^a	8.67±0.52 ^a
	3	8.33±0.52 ^a	8.33±0.52 ^a	8.67±0.52 ^a	8.33±0.52 ^a
	6	6.50±0.55 ^b	6.83±0.41 ^b	6.83±0.75 ^b	6.50±0.55 ^b
Overall acceptability	1	8.67±0.52 ^{Aa}	8.17±0.41 ^{ABa}	7.83±0.75 ^{Ba}	8.17±0.75 ^{ABa}
	3	8.17±0.75 ^a	7.83±0.75 ^a	8.00±0.63 ^a	8.17±0.75 ^a
	6	6.50±0.55 ^b	6.50±0.55 ^b	6.83±0.75 ^b	6.33±0.52 ^b

¹⁾CON, no antioxidant added; VC, vitamin C added; EB, ethanol extract of *B. polyfermenticus* SCD added; CB, vitamin C and ethanol extract of *B. polyfermenticus* SCD added.

²⁾All values are mean±SD.

^{a,b} Means in the same column with different letters are significantly different ($p<0.05$).

^{A,B} Means in the same row with different letters are significantly different ($p<0.05$).

Sensory evaluations after 10 and 15 days of storage could not be carried out because all samples emitted an unpleasant odor. CON samples had significantly higher *L* values than the others samples ($p<0.05$). However, the sensory color scores did not reflect the discoloration of any samples during

the storage period. EB had lower sensory flavor scores than the other samples, though the differences were not significant ($p>0.05$). The hardness of EB and CB was significantly higher than CON and VC during storage ($p<0.05$). Differences in tenderness among the test samples were not

observed ($p>0.05$). Even though EB and CB had significantly higher chewiness relative to the others samples ($p<0.05$), the differences of juiciness were not large enough to be significant ($p>0.05$). Overall acceptability scores did not reveal any significant differences ($p>0.05$). These results indicate that the addition of vitamin C and *B. polyfermenticus* SCD to cooked ground pork does not significantly affect sensory evaluations after various periods of storage at 4 °C ($p>0.05$).

In conclusion, the addition of *B. polyfermenticus* SCD ethanol extract retarded lipid oxidation in cooked ground pork, however there was no evidence of synergic effects in the combination with vitamin C.

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