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Effects of Ethanol Extracts of *Bacillus polyfermenticus* SCD on the Storage Stability of Emulsion Type Sausage

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Abstract The objective of this study was to determine storage stability and antioxidant effect of T1, ascorbic acid (0.6%); T2, ethanol extracts of *Bacillus polyfermenticus* (5%); T3, mixture with ascorbic acid (0.3%), and ethanol extracts of *B. polyfermenticus* (2.5%) during the storage period. The pH of sausage decreased constantly during storage ($p<0.05$). Thiobarbituric acid reaction substance (TBA) values were lower in all treated samples compared to the control ($p<0.05$). Longer storage periods resulted in higher TBA values ($p<0.05$). Volatile basic nitrogen (VBN) value in T1, T2, and T3 were significantly lower than control ($p<0.05$). The L^* value of T1, T2, and T3 had higher than control. The a^* value of T2 was significantly lower than control, T1, and T3 during storage period ($p<0.05$). The b^* value of T2 had significantly higher compared to the other sausage samples during storage period ($p<0.05$). Total microbial counts in the sausages samples significantly increased as storage period increased ($p<0.05$). Further studies are needed to develop various meat products to extend the storage stability using *B. polyfermenticus* SCD.

Keywords: antioxidant, *Bacillus polyfermenticus* SCD, storage stability, sausage

Introduction

Consumption of precooked and refrigerated ready-to-eat production such as sausage has been increased. However, pork meat oxidizes more rapidly than either beef or lamb because of its relatively high content of unsaturated fatty acid (1). Thus, control of lipid oxidation in sausage has become important (2). Lipid oxidation is one of the major cause deterioration meat product's quality during storage, because it changes color and rheological properties of meat, and increase microbiological spoilage with formation of toxic compounds such as 4-hydroxy-nonenal (3). Oxidation of fats and lipids has been known to be related to cancer, atherosclerosis, heart disease, and allergiy because it produces peroxides, alkanes, alcohols, aldehydes, and acids. For solving the problem the most common antioxidants commonly used in food industry are synthetic; butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ). Despite being high stability and low cost, use of these synthetic antioxidants are limited in food industry due to their carcinogenic potential (4,5). Therefore the demand for alternating antioxidants has recently increased (6).

It is well known that natural antioxidants have high antioxidant activity. Natural antioxidants such as rosemary, tocopherol, and chitosan have been studied in beef burgers to retard lipid oxidation during storage oxidation (7). However, antioxidant substances derived from *Bacillus polyfermenticus* SCD have been little studied in raw meat and meat products.

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Benefits of medical purposes have led to an increase in the incorporation of bacteria such as lactic acid bacteria (LAB; *Lactobacillus, Bifidobacterium, Streptococus,* and *Enterococcus*) in functional food (8). In particular, *B. polyfermenticus* SCD strains, commonly known as 'Bispan' strains, have been used for long-term intestinal disorders, since the live strains in the form of active endospore can reach the intestine (9).

The use of natural antioxidants to maintain high quality and safety is important to improve for human health and to prevent economical loss. In the literatures, effects of vitamin C, α-tocopherol, and natural antioxidant on meat products have been reported (7,10). However, there was little information available on antioxidants substances from *B. polyfermenticus* SCD. Therefore, the aims of this study were determined storage stability and effects of the antioxidants substances (ethanol extraction of *B. polyfermenticus* SCD) and vitamin C in emulsion type sausage during the storage period.

Materials and Methods

Production of *B. polyfermenticus* SCD *B. polyfermentixus* SCD production was performed as previously described (11). *B. polyfermenticus* SCD was inoculated into 1,500 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; Bioengeering AG, Wald, Switzerland) following incubation at 37°C for 7 hr with agitation at 500 rpm and 1 vvm (volume of air per medium per min) of aeration rate. During incubation, pH of the culture was maintained at 7.0±0.2 by adding 3 N H_2SO_4 and 3 N NaOH. Silicone oil which was used antifoam agent, added automatically whenever necessary. The culture was then centrifuged at 21,000×g at 4°C for 20 min.

Adsorption of Diaion HP-20 column using polar solvent Supernatants of the culture were injected into 5 cm×1 m (diameter×length) Diaion HP-20 columns (100 g, Mitzubishi, Tokyo, Japan) at a ratio of 1:2 (volume of Diaion HP-20 resin:supernatant). Antioxidant fraction was eluted with 100% of ethanol. The fraction was concentrated (concentration: 500 mg/mL) using rotary vacuum evaporator and added to ground pork meat product.

Preparation of samples, packaging, and storage Nine pork hams (initial pH 5.5-5.8) were obtained at 48 hr postslaughter from a local supplier, trimmed of external fat, and ground using an industrial grinding machine. Minced meat was separated into 4 batches, mixed with nitrite pickling salt (to a final concentration of 1.5%), ice (to a final concentration of 10%), ham (62%), fat (18%), phosphate (0.25%), Criste™ (0.5%), mono sodium glutamate (0.5%), and spice (0.2%) to ensure an even distribution of salt and antioxidants. The treatments studied were: control, no antioxidants; T1, ascorbic acid (0.6%); T2, ethanol extracts of *B. polyfermenticus* (5%); T3, ascorbic acid (0.3%)+ ethanol extracts of *B. polyfermenticus* (2.5%). The sausages were stuffed into fibrous casings, φ 50 mm and were boiling in water bath at 75°C for 35 min. Sausages were then vacuum packaged with nylon/poly ethylene film (Hankook Fujee, Suwon, Korea) after chilling. The sausages were stored for 5 weeks at 4±1°C. Each treatment pack was opened for subsequent analysis at intervals of 1 week (0, 1, 2, 3, 4, and 5 week).

pH The pH was determined, 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) and was measured with a pH meter (model 340; Mettler-Toledo GmbH Analytical, Schwerzenbach, Switzerland).

Thiobarbituric acid reaction substance (TBA) Oxidative rancidity was evaluated in the sausage samples by measuring TBARS at 1 day, 1, 2, 3, 4, and 5 week. Malondialdehyde (MDA) which was secondary product of lipid oxidation, formed during lipid oxidation in the samples were measured, and reported as TBA values in units of MDA equivalent/kg sample. The amount of the pink colored TBA complex was measured with a spectrophotometer at 538 nm using the methods of Tarladgis *et al.* (12). Triplicate samples were analyzed. TBA number was calculated as mg MDA/kg sample.

Volatile basic nitrogen (VBN) Volatile basic nitrogen was determined by the conway micro-diffusion method and was expressed as mg% of sample (13).

Instrumental color Sausage (about 90 mm, diameter) was sliced thickness of 15 mm. CIE L^* (lightness), a^* (redness), and b^* (yellowness) values were determined for each sample using a colorimeter (Chroma meter CR-210; illuminate C, calibrated with plate, L^*=+97.83, a^*=–0.43, b^*=+1.98, Minolta, Osaka, Japan) standardized using the white tile.

Microbiological evaluation To measure the microbial quality of the samples, duplicate packs from each treatment were taken, 10 g samples of the meat were aseptically transferred into a sterile stomacher bag, and 90 mL of sterile 0.1% peptone water (Difco Laboratories, Detroit, MI, USA) was added to each sample and macerated for 2 min in a stomacher. A decimal serial dilution in 0.1% peptone water was prepared. Mesophillic microorganisms were determined using Plate Count agar (PCA, Difco) at 35°C for 48 hr. Microbial colonies were counted and expressed as colony forming units (CFU) per gram.

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

Result and discussion

pH Changes in the pH values of sausage during storage period are given in Fig. 1. The pH value of T1 (added ascorbic acid; 0.6%), T2 (added ethanol extracts of *B. polyfermenticus* SCD; 5%), and T3 (added vitamin C; 0.3% and ethanol extracts of *B. polyfermenticus* SCD; 2.5%) were significantly lower than control (not added antioxidant) ($p<0.05$). During storage pH value of sausage sample constantly decreased ($p<0.05$). This decrease might be caused by the acid treatments (14). Shikama and Sugawara (15) showed that the rate of autoxidation and the total microbial counts increased markedly with decreasing pH value. Manju *et al.* (16) reported slight decrease in pH values may be caused by the dissolution of CO_2 in the fish muscle. Several authors observed a decrease of pH with increase in the concentration of CO_2 in the atmosphere (17).

TBA Values of TBA, which are indicators of lipid oxidation, are shown Fig. 2. As expected, the TBA values of all treatments significantly increased with as pasting storage time at 4°C refrigerator ($p<0.05$). TBA values were lower in all treated samples compared to those of the

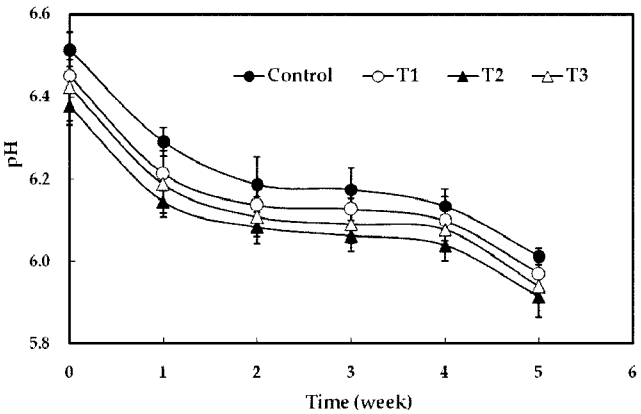

Fig. 1. Change of pH in emulsion type sausage containing vitamin C and ethanol extracts of *B. polyfermenticus* SCD during storage period. Control, no antioxidant treatment; T1, vitamin C treatment; T2, ethanol extracts of *B. polyfermenticus* SCD treatment; T3, vitamin C and ethanol extracts of *B. polyfermenticus* SCD treatment.

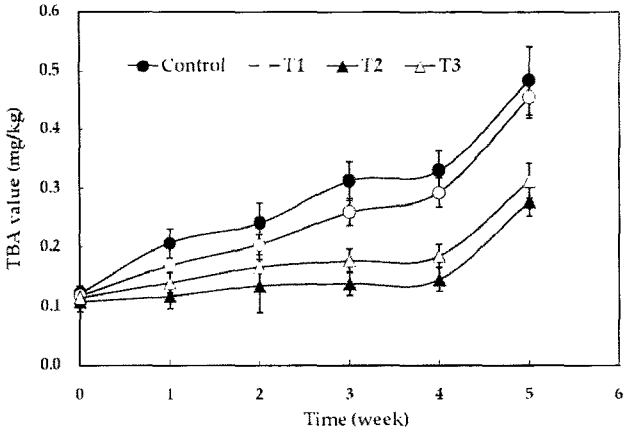

Fig. 2. Change of TBA values in emulsion type sausage containing vitamin C and ethanol extracts of *B. polyfermenticus* SCD during storage period. Control, no antioxidant treatment; T1, vitamin C treatment; T2, ethanol extracts of *B. polyfermenticus* SCD treatment; T3, vitamin C and ethanol extracts of *B. polyfermenticus* SCD treatment.

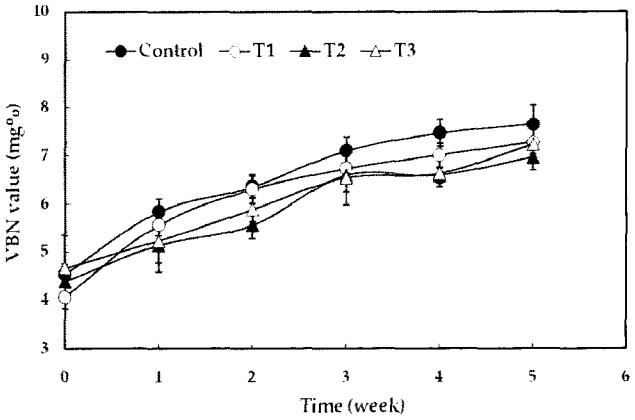

Fig. 3. Change of VBN value in emulsion type sausage containing vitamin C and ethanol extracts of *B. polyfermenticus* SCD during storage period. Control, no antioxidant treatment; T1, vitamin C treatment; T2, ethanol extracts of *B. polyfermenticus* SCD treatment; T3, vitamin C and ethanol extracts of *B. polyfermenticus* SCD treatment.

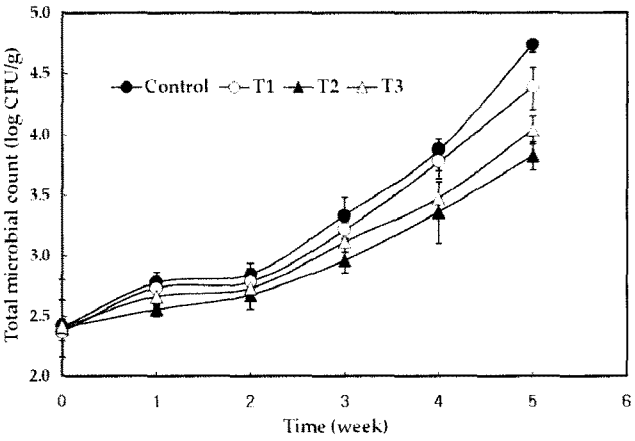

Fig. 4. Change of total microbial count in emulsion type sausage containing vitamin C and ethanol extracts of *B. polyfermenticus* SCD during storage period. Control, no antioxidant treatment; T1, vitamin C treatment; T2, ethanol extracts of *B. polyfermenticus* SCD treatment; T3, vitamin C and ethanol extracts of *B. polyfermenticus* SCD treatment.

control from 1 day to 4 week ($p<0.05$). These results suggested that antioxidant substances derived from *B. polyfermenticus* SCD and vitamin C retarded lipid oxidation during storage. Formanek *et al.* (18) reported that minced beef with natural antioxidants under modified atmosphere packaging (MAP) and illuminated display at 4°C for 8 days improved the oxidative stability. Samples treated with T1, T2, and T3 exhibited significantly lower TBA values than control ($p<0.05$), exception at the 5 weeks of storage. The effect of antioxidant, and antitumor of *B. polyfermenticus* SCD has been reported by many researchers (19-23). T3 had resulted in higher TBA values at 5 week than T1 and T2 treatments had significantly higher TBA value than T2 during storage period ($p<0.05$). In our study, combination use of vitamin C and antioxidant substances of *B. polyfermenticus* SCD did not caused a significantly reduced TBA value compared with vitamin C. However, many works have reported that vitamin C when used in combination with other antioxidants, functions synergistically to promote their antioxidative effect (24).

VBN Increase amounts of VBN, which is the result of decomposition of protein during storage by microorganisms, can be an index of meat product freshness. Value of VBN in sausage samples during storage are shown in Fig. 3. VBN values of T1, T2, and T3 were significantly lower than those of control ($p<0.05$). While VBN values in treatments significantly increased with increasing storage time ($p<0.05$), but T3 had lower VBN value than other treatment. This increase of VBN value in samples was probably due to increasing total microbial counts as storage time increased. These results agreed with result from a study of Wang (25).

Microbiological evaluation The total microbial counts recovered from sausage samples during storage are shown in Fig. 4. Total plate counts of T1, T2, and T3 had lower than control ($p>0.05$). Similar result was previously observed in a study of Naveena *et al.* (10), as buffalo meat treated with antioxidants had lower total plate counts than samples not treated with antioxidants. Shilvas *et al.* (26) and Djenane *et al.* (27) also reported that microbial populations were not affected by an ascorbic acid treatment. Initial total plate count of control and antioxidants treated samples ranged from 2.3 to 2.4 log CFU/g with counts increasing to a rage of 3.8-4.7 log CFU/g after 5 weeks of storage. Total microbial counts in the sausage samples significantly increased as storage period increased ($p<0.05$). Decreasing pH value of and increasing VBN value of all treatments was probably because total microbial count increased as storage time increased. Mahmoud *et al.* (28) reported that there is a relationship between the increase of the pH value and the deterioration of food material due to microbial activity.

Instrumental color Color of sausage is an important factor for consumer acceptance of sausage product. Storage

Table 1. Changes of color values in emulsion type sausage containing vitamin C and ethanol extracts of *B. polyfermenticus* SCD during storage period

Trait	Storage (week)	Treatment[1)]			
		Control	T1	T2	T3
CIE *L**	0	70.17±0.23$^{a2)}$	70.53±0.42^{b}	70.36±0.39^{b}	70.31±0.11^{ab}
	1	70.16±0.49^{a}	70.42±0.31^{b}	70.41±0.41^{b}	70.13±0.46^{abc}
	2	69.76±0.28^{Cab}	69.44±0.18^{Dc}	70.89±0.15^{Aa}	70.48±0.11^{Ba}
	3	70.19±0.38^{a}	70.49±0.31^{b}	70.24±0.55^{bc}	70.02±0.23^{bc}
	4	69.79±0.31^{Bab}	70.25±0.38^{Ab}	69.83±0.40^{Bc}	69.34±0.16^{Cd}
	5	69.39±0.49^{Cb}	71.20±0.23^{Aa}	70.45±0.40^{Bab}	69.83±0.50^{Cc}
CIE *a**	0	11.91±0.57^{Abc}	12.07±0.12^{Ad}	11.55±0.19^{Bc}	11.90±0.16^{Ac}
	1	12.19±0.27^{Bab}	12.70±0.16^{Aa}	11.99±0.24^{Bb}	12.73±0.08^{Aa}
	2	12.36±0.15^{Aa}	12.41±0.14^{Abc}	11.99±0.07^{Bb}	11.65±0.15^{Cd}
	3	12.23±0.20^{Aab}	12.33±0.08^{Ac}	11.90±0.23^{Bb}	12.29±0.18^{Ab}
	4	12.27±0.17^{Bab}	12.54±0.05^{Ab}	11.89±0.12^{Cb}	12.40±0.13^{ABb}
	5	11.54±0.42^{Cc}	12.42±0.15^{ABbc}	12.20±0.16^{Ba}	12.64±0.08^{Aa}
CIE *b**	0	7.52±0.09^{Da}	7.62±0.06^{Cb}	8.80±0.09^{Ac}	8.32±0.10^{Bc}
	1	7.66±0.23^{Ca}	7.61±0.17^{Cb}	8.84±0.16^{Abc}	8.11±0.13^{Bd}
	2	7.68±0.12^{Ca}	7.73±0.05^{Ca}	8.47±0.07^{Bd}	9.06±0.07^{Aa}
	3	7.58±0.10^{Ca}	7.61±0.10^{Cb}	9.17±0.11^{Aa}	8.53±0.13^{Bb}
	4	7.64±0.10^{Ca}	7.70±0.07^{Cab}	8.94±0.14^{Ab}	8.42±0.13^{Bbc}
	5	7.08±0.27^{Cb}	7.25±0.04^{Cc}	8.60±0.10^{Ad}	8.10±0.17^{Bd}

[1)]Control, no antioxidant treatment; T1, vitamin C treatment; T2, ethanol extracts of *B. polyfermenticus* SCD treatment; T3, vitamin C and ethanol extracts of *B. polyfermenticus* SCD treatment.
[2)]All values are mean±SD; letters of a-e and A-D are significantly different in the same column and the same row, respectively ($p<0.05$).

properties and quality of cooked meat products are strongly influenced by initial meat quality, additives, packaging parameters, and storage conditions (29). Change in CIE *L**, *a**, and *b** value of samples during storage period are shown in Table 1. The *L** value of treatments added antioxidants had generally higher than control. The *L** value of T1 was significantly higher comparing to other samples ($p<0.05$). The *a** value of T2 had significantly lower than control ($p<0.05$), T1 and T3 during storage period. However, the *b** value of T2 had significantly higher compared to other sausage samples during storage period ($p<0.05$). These results maybe were because ethanol extracts of *B. polyfermenticus* SCD had brown color which could be was evident that addition of ethanol extracts of *B. polyfermenticus* SCD improve the stability of color.

In conclusion, the addition of the ethanol extracts of *B. polyfermenticus* SCD retarded lipid oxidation of sausage, however, there was no evidence found in synergic effect of the combination treatment with vitamin C.

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