EFFECT OF VARIOUS *KIMCHI* ETHANOLIC EXTRACT ON OXIDATIVE STABILITY IN COOKED GROUND PORK STORED REFRIGERATION

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Abstract— **antioxidant effects of various *kimchi* extracts in cooked ground pork during refrigerated was studied. Cooked gounr pork samples were treated with ascorbic acid, BHT, BK(*baechu kimchi*), LK(*leek kimchi*), MK(*mustard leaf kimchi*), and WK(*white kimchi*) and compared to cooked ground pork without antioxidants (NC). The radical scavenging activity of various *kimchi* extract using DPPH assay resulted MK>LK>BK>WK. The antioxidant activity of various *kimchi* extract on thiobarbituric acid reacting substances (TBARS), peroxide value, and hexanal conent of cooked ground pork was investigated. The most potent antioxidants on stabilizing oxidation was MK and the lowest antioxidant activity was found with the addition of WK to the meat. These results suggest that the application of *kimchi* extract could enhance oxidative stabiltiy of meat or other lipid containing food system.**

***Index Terms*—antioxidants, *kimchi*, lipid oxidation, ground pork**

I. INTRODUCTION

Lipid oxidation is the major cause of color, flavor, texture and nutritive value degradation of fats and lipids containing products (Frankel, 1996). Also, it can decrease nutritional quality and safety by the formation of secondary products after cooking and processing. To overcome this problem, synthetic antioxidants such as butylated hydroxyl tikyebe (BHT) and butylated hydroxyl anisole (BHA) have been widely used in food industry (Pokorny, 1981). However evidence of their toxicity effects has provided the impetus for the search of alternative antioxidants (Branen, 1975). As a group, natural polyphenols have been shown to be effective antioxidant because of their multiple polar functional and reactions with other compounds (Kim, Kim, Kim, Oh, & Jung, 1994). *Kimchi* is a traditional Korean fermented vegatable dish that is low in calories, carbohydrates, and fat. It also contains an abundance of organic acid and lactic acid producing bacteria that are produced during fermentation and provide several antioxidant effects. There are about 187 different types of *kimchi* according to raw material, processing method, harvest season and locality including Chinese cabbage *kimchi*, white *kimchi*, radish leaf *kimchi*, mustard leaf *kimchi*, and white radish *kimchi*(Sim & Han, 2008). Vegetables are good sources of natural antioxidants such as carotenoids, vitamins, flavonoids, and other phenolics compounds (Ismail, Marjan, & Foong, 2004). Recently, Kusznierewicz, Smiechowska, Bartoszek, and Namiesnik (2008) observed that fermentation processes increased the initial values of antioxidant activity of cabbage. The indicated that the antioxidant capacity of sauerkraut probably combines effects of wounding and chemical processes incurred by lactic acid bacteria.

The objective of this study was to establish the antioxidant properties of various *kimchi* ethanolic extracts and the effects of adding extracts obtained from *kimchi* extracts on cooked ground pork lipid oxidation, as indicated by thiobarbituric acid reactive substances (TBARS), peroxide value, conjugated dienes, hexanal content.

II. MATERIALS AND METHODS

1. Materials

Commercially produced *Baechu kimchi* (Chinese cabbage; *Brassica rapa*), *Leek kimchi* (Leek; *Allium tuberosum Rottler*, *Mustard leaf kimchi (*Mustard leaf; *Brassica juncea*), *White kimchi* (Chinese cabbage; *Brassica rapa*) were purchased from a local market and randomly packaged in PE/Nylon film bags. The packaged *kimchi* was fermented at 4±1℃ for 14 days. Fermented *kimchi* was blended with a cutter (C4 VV, Sirman, Marsango, Italy) and then packed in PE/Nylon film. Freeze drying of *kimchi* was frozen in PE/Nylon film bags at -70±1℃ and dried at -40℃ under 80×10^{-3} torr pressure using a freeze-dryer (PVTFD 20R, Ilshinlab, Yangju, Korea) for 24h.

2. Preparation of extracts

Dried various *kimchi* powder (5 g) was extracted with 100 ml of 75% ethanol overnight in a shaker at room temperature. The extracts filtered through 0.45μm of filter membrane and evaporated with a rotary evaporator (EYELA N-1000, RIKAKIKAI. Co. Ltd., Japan) below 50℃. After evaporation of ethanol solution, the extracts were used for further analysis and application. The dried residues of ethanolic extracts were dissolved in their extraction solvent

before use.

3. Analysis of antioxidant properteis

The effect of various *kimchi* ethanolic extracts on DPPH radicals was studied using the modified method of Brand-Williams, Cuvelier, and Berset (1995). The Folin–Ciocalteu reagent assay was used to determine the total phenolic content (Ozsoy, Can, Yanardag, & Akev, 2008). The total flavonoid content of the samples was determined using a modified colourimetric method described previously by Sakanaka, Tachibana, and Okada (2005), and using quercetin as a standard.

4. Preparation of pork patties and storage

Lean meat (5600 g) and fat (2400 g) were ground through an 8 mm plate. After mincing, the samples were assigned to one of the following 7 treatments. Each portion of ground pork (1500 g) was mixed with salt (1.5%), and then ascorbic acid (AC), BHT (butylated hydroxyl toluene) and 75% ethanolic extracts from *Baechu kimchi* (BK), *Leek kimchi* (LK), *Mustard leaf kimchi* (MK), *White kimchi*(WK) were added (w/w) according to the following formulation: negative control (NC: antioxidant added); positive control AC (PC-AC: 0.02% ascorbic acid); positive control BHT (PC-BHT: 0.02% BHT); Treatment BK (T-BK: 0.1% *baechu kimchi* ethanolic extract); Treatment LK (T-LK: 0.1% *leek kimchi* ethanolic extract); Treatment MK (T-MK: 0.1% *mustard leaf kimchi* ethanolic extract); Treatment WK (T-WK: 0.1% *white kimchi* ethanolic extract). Ground pork was packaed into polyethylene bags and evenly spread to a thickness of 1 cm. All samples were vacuum-packaged and then heated on an open electric water bath until a final internal temperature of 80℃ was reached. After cooling down to room temperature with running cold water for 10 min, the cooked pork samples were divided into smaller portion. Cooked ground pork was divided into 35 samples (7 ingredient treatments × 5 storage times) in smaller portions (about 200 g each) and anaerobically packed in PE/nylon film bags, stored at 4±1 ℃ for 14 days. The samples in each experiment were evaluated after 0, 4, 7, 10, and 14 days of storage.

5. Determination oxidant properties of cooked ground pork

5.1. Thiobarbituric acid reacting substances (TBARS)

Lipid oxidation was measured by the 2-thiobarbituric acid extraction method of Witte, Krause, and Bailey (1970) as modified. TBA values were calculated by multiplying the absorbance by 94%, the recovery of the standard from meat, resulting in a K value of 5.2. The TBA numbers were calculated as mg of malondialdehyde (MDA) per kg of sample.

5.2. Peroxide value and conjugated dienes

The method by Undeland, Harrod, & Lingner (1998) was used to extract lipids from ground. The peroxide value used to determine from Shantha & Decker (1994). Extracted pork lipid (0.05 g) was mixed with 9.8 ml chloroform-methanol (7:3 v/v) and vortexed for 2 to 4 s. 50 µl of 1 % ferrous iron solution were added and vortexed for 2 to 5 s. The samples were incubated for 5 min at room temperature and absorbance of 500 nm was measured spectrophotometer.

5.3. Hexanal analysis

Three g of cooked meat samples was placed in 25ml headspace glass vials and sealed with Teflon-faced silicone septums and aluminum capsSample vials were removed from the water bath and allowed to come to room temperature prior to extraction of the headspace volatiles by solid-phase microextraction (SPME) fiber (85 µm carboxen/pdms stable fles – Supelco, Bellefonte, Penn., U.S.A.) was used to extract the headspace volatiles. An Agillent 7890 (Hewlett-Packard Co., Palo, Calif, USA) with a capillary column (DB-5MS, 15 m × 0.248 mm internal diameter, 0.25 ㎛ film, J&W Scientific Inc., CA, USA) was used for separation. Column flow was 1 ml/min and split flow was 100 ml/min at 25 spi head pressure. Injector and detector temperature were 250 ℃ and 280 ℃, respectively. The column temperature was maintained at 35 ℃ for 5 min, increased up to 75 ℃ at 8 ℃/min and then up to 200 ℃ at 40 ℃/min, and finally held at 200 ℃ for 5 min.

5.4. Statistical analysis

A 5 × 5 factorial design with three replicates was employed for storage with treatments and storage time as main effects using two-way analysis of variance (ANOVA). Analysis of variance was performed on all the variables using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Inc., 2001). Duncan's multiple range test ($P < 0.05$) was used to determine differences between treatment means.

III. RESULTS AND DISCUSSION

3.1.Antioxidant properties of ethanolic extracts from various kimchi

Table 1 shows the contents of total phenolics and total flavonoids expressed as µg of gallic acid and quercetin, respectively, per mg of extract. The results given in Table 1 showed that the total phenol compound content of BK, LK,

MK, and WK of ethanolic extracts from various *kimchi* were 34.99±1.35, 46.73±1.30, 41.53±0.78, and 32.52±1.09 μg gallic acid/mg extract in fermented samples, repectively, while the flavonoids content of BK, LK, MK, and WK of ethanolic extracts were 9.07±1.05, 16.25±0.83, 25.83±2.54, and 5.87±0.95 μg quercetin/mg extract in fermented samples, respectively. The results indicated that ethanolic extracts from LK and MK exhibited slightly greater antioxidant activity compared to ethanolic extracts from BK and WK. Radical scavengers were evaluated by their reactivity toward a stable free radical, DPPH. DPPH radical scavenging activity (IC_{50}) of ethanolic extracts from MK was the highest among other ethanolic extracts from BK, LK, and WK.

Table 1. Total phenolic content, total flavonoid, DPPH radical scavenging ability of ethanolic extracts for BK, LK, MK, and WK

Activity sample	BK	LK	MK	WK
DPPH radical scavenging ability	1.96±0.05^{B}	0.71±0.06^{C}	0.40±0.04^{D}	2.41±0.09^{A}
Total phenolic (mg of GAE.g)	34.99±1.30^{C}	46.73±1.30^{A}	41.53±0.78^{B}	32.52±1.09^{D}
Total flavonoid (μg quercetin/mg)	9.07±1.05^{C}	16.25±0.83^{B}	25.58±2.54^{A}	5.87±0.95^{D}

Values are means ± standard deviation of three replicate analyses ($P < 0.05$).
$^{A-E}$ Means sharing different letters in the same row are significantly different ($P < 0.05$).
EC_{50} value: the effective concentration at which DPPH radicals were scavenged by 50%. EC_{50} value was obtained by interpolation from linear regression analysis.
BK: baechu *kimchi* ethanolic extract; LK: leek *kimchi* ethanolic extract; MK: mustard leaf *kimchi* ethanolic extract; WK: white *kimchi* ethanolic extract

3.2.Changes in thiobarbituric acid reacting substances of cooked ground pork during storage

TBARS analysis measures the formation of secondary products of lipid oxidation, mainly malondialdehyde, which may contribute off-flavor to oxidized fat. Table 2 showed the effect of various *kimchi* ethanolic extracts on TBARS values of cooked ground ground pork during refrigerated storage (4 ℃) for 14 days. In general, the storage period had a significant influence on the development of lipid oxidation in the cooked ground pork resulting in extensive increase of TBARS values during the 14 days of refrigerated storage. TBARS values of all treatment samples were considerably lower ($P < 0.05$) than that of the negative control (NC) and positive control (PC) for each day studied during the storage period, thus indicating high protection of various *kimchi* ethanolic extracts against lipid oxidation in cooked ground pork. The lipid oxidation inhibition effect was the highest ($P < 0.05$) in MK compared to BK, LK, and WK at all storage time.

Table 2. Effect of various *kimchi* ethanolic extracts on TBARS values (mg malondialdehydr/kg meat) in cooked pork during refrigerated storage for 14 days

Storage periods (days)	NC [1)]	PC-AC	PC-BHT	T-BK	T-LK	T-MK	T-WK
0	0.79±0.06^{Ad}	0.40±0.05^{Ce}	0.54±0.05^{Be}	0.30±0.04^{De}	0.20±0.02^{Ee}	0.20±0.03^{Ee}	0.22±0.02^{Ee}
4	0.99±0.06^{Ac}	0.65±0.01^{Bd}	0.68±0.02^{Bd}	0.46±0.04^{Dd}	0.41±0.04^{Ed}	0.38±0.03^{Ed}	0.56±0.04^{Cd}
7	1.10±0.05^{Ab}	0.75±0.04^{Bc}	0.76±0.08^{Bc}	0.61±0.07^{Dc}	0.48±0.03^{Ec}	0.44±0.02^{Ec}	0.70±0.03^{Cc}
10	1.17±0.03^{Aa}	0.86±0.02^{Bb}	0.82±0.05^{Cb}	0.74±0.03^{Eb}	0.53±0.03^{Fb}	0.48±0.03^{Gb}	0.77±0.04^{Db}
14	1.20±0.03^{Aa}	0.93±0.03^{Ba}	0.91±0.04^{Ba}	0.79±0.02^{Da}	0.64±0.04^{Ea}	0.56±0.03^{Fa}	0.86±0.04^{Ca}

All values are mean ± SD of the three replicates.
$^{A-E}$ Means sharing different letters in the same row are significantly different ($P < 0.05$).
$^{a-d}$ Means sharing different letters in the same column are significantly different ($P < 0.05$).
[1)]NC(negative control): no antioxidant; PC-AC(positive control): cooked pork containing ascorbic acid 0.02%; PC-BHT(positive control): cooked pork containing BHT 0.02%; T-BK(treatment): cooked pork containing *bechu kimchi* extract 0.1%; T-LK(treatment): cooked pork containing *leek kimchi* extract 0.1%; T-MK(treatment): cooked pork containing *mustard leaf kimchi* extract 0.1%; T-WK(treatment): cooked pork containing *white kimchi* extract 0.1%.

3.3.Changes in peroxide value of cooked ground pork during storage

Table 3 shows the changes of POV as affected by the various *kimchi* ethanolic extracts. As shown in Table 3, POV gradually increased for all treatments during storage time. The initial POV of each sample was significantly different ($P < 0.05$). POV for all treatments were significantly lower than those of control ($P < 0.05$) during the refrigerated storage period. These results indicate that various *kimchi* ethanolic extracts showed antioxidative properties to retard lipid oxidation in cooked ground pork. Lipid oxidation in the controls was more intense compared to the other treatments; maximum values for POV were reached on day 7, after which decline was observed. The POV increase with time to a maximum level and the POV decrease thereafter because it decomposes rapidly to secondary products leading to a subsequent.

3.4.Changes in hexanal content of cooked ground pork during storage

The most important volatiles isolated from cooked ground pork treated with various source of various *kimchi* ethanolic extracts and stored under refrigerated condition for 14 days are showed in Table 4. In general, the concentration of hexanal increased with storage time. All samples treated with various *kimchi* had reduced volatile formation when compared to both the negative control (NC) for all 14 days of refrigerated storage. The MK and LK treatment resulted in significantly lower hexanal content as compared to both the negative control (NC) and positive control (PC). At the end of the storage (14 days). The stability of the cooked ground pork treated with various *kimchi*

ethanolic extracts was in the following order: MK>LK>BK>WK>PC-BHT>PC-AC>NC. The increase in hexanal content in all treatments during storage may indicate persistent formation of aldehydes in the meat.

Table 3. Effect of various *kimchi* ethanolic extracts on peroxide value (meq of active O_2/kg meat) in cooked pork during refrigerated storage for 14 days

Storage periods (days)	NC [1)]	PC-AC	PC-BHT	T-BK	T-LK	T-MK	T-WK
0	6.23±0.24^{Ae}	3.84±0.34^{Bd}	3.51±0.13^{Cd}	3.39±0.13^{Cc}	3.87±0.19^{Be}	3.86±0.25^{Bd}	3.84±0.23^{Bd}
4	11.88±0.34^{Ac}	8.30±0.93^{Bc}	8.50±0.78^{Bc}	8.24±0.52^{Bb}	6.09±0.70^{Cd}	6.19±0.24^{Cc}	8.11±0.62^{Bc}
7	18.76±0.59^{Aa}	14.05±0.48^{Cb}	15.05±1.03^{Bb}	13.04±1.47^{Da}	8.93±0.89^{Ec}	8.43±0.28^{Eb}	13.40±0.63^{CDb}
10	13.32±0.37^{Db}	15.86±0.76^{Aa}	15.34±1.10^{ABb}	13.85±1.25^{CDa}	11.09±0.77^{Eb}	10.51±0.17^{Ea}	14.55±0.57^{BCa}
14	8.32±1.10^{Dd}	16.01±0.54^{Aa}	16.39±1.35^{Aa}	13.67±0.77^{Ba}	11.90±0.59^{Ca}	10.99±1.31^{Ca}	14.28±0.29^{Ba}

All values are mean ± SD of the three replicates.
[A-E] Means sharing different letters in the same row are significantly different ($P < 0.05$).
[a-e] Means sharing different letters in the same column are significantly different ($P < 0.05$).
[1)] Treatments are the same as in Table 2.

Table 4. Effect of various *kimchi* ethanolic extracts on hexanal content (ppm) in cooked pork during refrigerated storage for 14 days

Storage periods (days)	NC [1)]	PC-AC	PC-BHT	T-BK	T-LK	T-MK	T-WK
0	24.98±0.79^{Ae}	7.58±0.45^{Be}	6.77±0.32^{Ce}	5.17±0.26^{De}	3.39±0.22^{Ee}	3.51±0.31^{Fe}	7.20±0.08^{BCe}
4	39.87±1.18^{Ad}	18.62±0.49^{Bd}	12.73±0.52^{Dd}	15.52±0.89^{Cd}	11.71±1.45^{Dd}	12.32±1.34^{Dd}	17.55±1.69^{Bd}
7	53.63±1.01^{Ac}	35.18±1.67^{Bc}	27.82±1.11^{Cc}	27.95±0.91^{Cc}	21.93±1.72^{Ec}	18.96±0.79^{Fc}	25.84±1.69^{Dc}
10	73.75±1.69^{Ab}	42.17±1.16^{Bb}	38.37±1.78^{Cb}	35.68±1.95^{Db}	25.72±1.02^{Eb}	23.64±2.12^{Eb}	34.95±1.98^{Db}
14	97.50±1.58^{Aa}	62.41±1.35^{Ba}	46.14±1.31^{Ca}	44.76±1.82^{Ca}	36.87±1.19^{Da}	32.82±2.65^{Ea}	47.07±1.38^{Ca}

All values are mean ± SD of the three replicates.
[A-D] Means sharing different letters in the same row are significantly different ($P < 0.05$).
[a-e] Means sharing different letters in the same column are significantly different ($P < 0.05$).
[1)] Treatments are the same as in Table 2.

IV. CONCLUSION

The results demonstrate the effectiveness of various *kimchi* on antioxidant properties and lipid oxidation of cooked ground pork during storage at 4℃ for 14 days. Because of the purported health benefits of these highly effective natural antioxidant extracts, their application in the meat industry may be very valuable a desirable.

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