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Antioxidant Activity of a Methanolic Extract from *Prunus mume* Byproduct in Cooked Chicken Breast Meat

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Abstract

The antioxidant properties of methanolic extracts (PM) from the fruit of *Prunus mume* after liquor manufacturing were determined in a chicken breast meat system. When PM was added to chicken breast meat, 2-thiobarbituric acid-reactive substances (TBARS) value at day 3 was decreased by about 25% compared to control meat without PM. PM did not significantly affect the color of chicken meat compared to the control. The amounts of volatile aldehydes and hydrocarbons were decreased by the addition of PM. Hexanal was the predominant volatile compound in the control, accounting for the majority of total volatiles; PM reduced the amount of hexanal to 81% of that in the control meat at 3 days.

Key words: *Prunus mume*, byproduct, chicken meat, antioxidant properties

INTRODUCTION

Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and *tertiary*-butylhydroquinone, have been widely used in foods for preventing oxidation. However, the use of these synthetic antioxidants in foods is discouraged because of their potential toxicity (1) and carcinogenicity (2). Such natural antioxidants (1,3-5) as flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolics, and terpenoids have attracted special interest because they can remove free radicals which may cause various diseases, carcinogenesis, and aging (6).

The *Prunus mume*, a deciduous tree of the genus *Rosaceae*, originated in central China, has more than 400 varieties worldwide. The fruit has been used in folk medicine to alleviate fever, cough, and intestinal disorders. However, the raw fruit is poisonous due to two types of cyanogenic glucosides, i.e., prunasin and amygdalin (7,8), making it necessary to remove or destroy the toxins by processing methods such as pickling in vinegar, preparing it as liquor or syrup, and making a fruit-juice concentrate. *P. mume* has been traditionally used for preparation of liquor in Korea, and thousands tons of byproducts of *P. mume* after manufacturing liquor are annually produced in Korea.

Cooked poultry meat products are highly susceptible to lipid oxidation and produce off-odor volatiles, and the use of antioxidants is commonly required to retard oxidative deterioration during storage. The need for natural antioxidants is increasing in the food and meat industries as consumers demand safer and more natural additives. Although a few plant extracts are widely used as safe antioxidants, their activities are not as strong as synthetic antioxidants such as BHA and BHT, and the manufacturing cost is relatively high (9).

The objective of this research was to determine the effects of *P. mume* byproduct from liquor extract manufacturing on lipid oxidation, volatile compounds, and color changes in cooked aerobically packaged chicken breast meat during refrigerated storage.

MATERIALS AND METHODS

Materials

Prunus mume byproduct after extraction with 98% of ethanol for liquor was kindly supplied from Muhak Co. (Masan, Gyeongnam, Korea). 2-Thiobarbituric acid (TBA) was purchased from Sigma Chemical Co. (St. Louis, MO., USA), and methanol and ethanol were purchased from Duksan Pure Chemical Co. (Sungkok-Dong, Ansan, Gyeonggi, Korea).

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Preparation of methanolic extract from *Prunus mume*

The *P. mume* byproduct was dried at 70°C, and crushed in an electric mixer (model FM-909T; Hanil Electric, Seoul, Korea). The crushed *P. mume* was passed through a 65 mesh sieve. Each 10 g of *P. mume* powder was extracted with 100 mL of methanol in a shaking incubator overnight at room temperature and filtered through Whatman No. 1 filter paper. The residue was re-extracted under the same conditions. The 1st and 2nd extracts were pooled and filtered through a Whatman nylon membrane filter (0.2-μm, Millipore filtration kit, Millipore Co., Bedford, UK). The methanol in the filtrate was evaporated using a rotary evaporator (Model Eyela N-1000; Tokyo Rikakikai Co., Tokyo, Japan) and the *P. mume* byproduct extract was designated as PM.

Preparation of chicken breast patties

Chicken breast meat from 8 different chickens was divided into two groups, and the muscles in each group were ground separately through a 3-mm plate and used as a replication. Two different chicken patty treatments were prepared: (1) control, no additive; (2) PM, 0.1%. PM was dissolved in ethanol (150 mg/mL) before addition. The same amounts of ethanol were added to the control patties to minimize solvent effect. Each additive was added to the ground chicken meat and mixed for 2 min in a bowl mixer (model KSM 90; KitchenAid Inc., St. Joseph, MI, USA). The mixed meats were ground again through a 3-mm plate to ensure even distribution of the additives. Chicken breast meat patties (~40 g/each) were prepared and individually vacuum-packaged in oxygen-impermeable vacuum bags (9.3 mL O_2/m^2/24 hr at 0°C; Koch, Kansas City, MO, USA). The meat samples were precooked in a water bath (90°C) to an internal temperature of 80°C. After cooking, chicken patties were chilled in running cold water for 10 min, and the vacuum bags were removed and repackaged individually in oxygen-permeable bags (polyethylene, 4″ ×6″, 2 MIL, Associated Bag Co., Milwaukee, WI, USA). The aerobically packaged samples were stored at 4°C. Lipid oxidation, volatiles compounds, and color of the samples were determined at 0, 1, and 3 days of storage.

2-Thiobarbituric acid-reactive substances (TBARS) values

Lipid oxidation was determined by measuring TBARS content (10). Minced sample (5 g) was placed in a 50-mL test tube and homogenized with 15 mL of deionized distilled water (DDW) using a Brinkman Polytron (type PT 10/35, Brikman Instrument Inc., Westbury, NY, USA) for 15 seconds at high speed. The meat homogenate (1 mL) was transferred to a disposable test tube (13× 100 mm), and butylated hydroxytoluene (7.2%, 50 μL) and thiobarbituric acid/trichloroacetic acid [20 mM TBA and 15% (w/v) TCA] solution (2 mL) was added. The sample was mixed using a vortex mixer and then incubated in a 90°C water bath for 15 min to develop color. After cooling for 10 min in cold water, the samples were mixed and centrifuged at 3000×g for 15 min at 5°C. The absorbance of the resulting upper layer was read at 531 nm against a blank prepared with 1 mL of DDW and 2 mL of TBA/TCA solution. The amounts of TBARS were expressed as milligram of malonedialdehyde (MDA) per kilogram of meat.

Analysis of volatiles compounds

A dynamic headspace analysis was performed using a Solatek 72 multimatrix vial autosampler and a purge and trap concentrator 3100 (Tekmar-Dohrmann, Cincinnati, OH, USA) connected to a gas chromatograph - mass spectrometer (GC-MS, Hewlett-Packard Co., Wilmington, DE, USA) according to the method of Ahn et al. (11). Minced sample (1 g) was placed in a 40-mL sample vial, and the vial was flushed with helium gas (40 psi) for 3 s, and capped airtight with a Teflon fluorocarbon resin/silicone septum (I-Chem Co., New Castle, DE, USA). The maximum holding time of the sample in a refrigerated (4°C) loading tray was 2 h or less to minimize oxidative changes during the waiting period before the start of the analysis. The meat sample was purged with helium gas (40 mL/min) for 14 min at 40°C. Volatiles were trapped using a Tenax/charcoal/silica column (Tekmar-Dohrmann) and desorbed for 2 min at 225°C, focused in a cryofocusing module (-80°C), and then thermally desorbed into a column for 60 s at 225°C.

An HP-624 column (7.5 m, 0.25 mm i.d., 1.4 μm nominal), an HP-1 column (60 m, 0.25 mm i.d., 0.25 μm nominal; Hewlett-Packard Co., Wilmington, DE), and an HP-Wax column (7.5 m, 0.25 mm i.d., 0.25 μm nominal) were connected using zero dead-volume connectors (J&W Scientific, Folsom, CA, USA). Ramped oven temperature was used to improve volatile separation. The initial oven temperature of 0°C was held for 1.5 min. After that, the oven temperature was increased to 15°C at 2.5°C/min, increased to 45°C at 5°C/min, increased to 110°C at 20°C/min, and then increased to 170°C at 10°C/min and held for 2.25 min at that temperature. A constant column pressure of 21.5 psi was maintained. The ionization potential of the mass selective detector (model 5973; Hewlett-Packard Co.) was 70 eV, and the scan range was m/z 19.1~350. Identification of volatiles was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.). The area of each peak was integrated using ChemStation

software (Hewlett-Packard Co.) and the total peak area (total ion counts $\times 10^4$) was reported as an indicator of volatiles generated from the meat samples.

Color measurement

CIE color values were measured on the surface of samples using a LabScan colorimeter (Hunter Associated Laboratories, Inc., Reston, VA, USA) that had been calibrated against black and white reference tiles covered with the same packaging materials as used for the samples. The CIE L^* (lightness), a^* (redness), and b^* (yellowness) values were obtained using an illuminant A (light source). Area view and port size were 0.63 and 1.02 cm, respectively. The values from 4 random locations of upper and bottom surfaces were obtained, averaged, and used for statistical analysis.

Statistical analysis

The experimental design was to determine the effects of *P. mume* byproduct extracts and storage time on lipid oxidation, volatile compounds, and color of the chicken breast meat patties. Analysis of variance was conducted according to the procedure of the General Linear Model using SAS software 1995 (12). Student-Newman-Keuls' multiple-range tests were used to compare the significant differences among the mean values of treatments ($p<0.05$). Mean values and standard error of the means (SEM) were reported.

RESULTS AND DISCUSSION

TBARS values in cooked chicken breast meat

TBARS have been used to quantify malondialdehyde (MDA) in meat compounds (13) which are produced as a result of lipid oxidation. The methanol extract of *Prunus mume* byproduct (PM) reduced TBARS values in cooked chicken breast meat (Table 1).

At day 0, PM did not significantly affect lipid oxidation. As storage time increased, the overall lipid oxidation was drastically accelerated due to the denatured structure of the meats by cooking and aerobic storage conditions. At 3 days of storage, PM had lower TBARS values than the control by about 25%.

Table 1. TBARS values of cooked chicken breast meat with the addition of *Prunus mume* byproduct (PM) during refrigerated storage (mg of MDA/kg of meat)

Storage (day)	Treatment		
	Control	PM	SEM
0	$0.095^{z1)}$	0.098^{z}	0.003
1	0.530^{ay}	0.477^{by}	0.010
3	0.934^{ax}	0.705^{bx}	0.057
SEM	0.065	0.014	

[1)]Different letters (a~b) within a row indicate that values are significantly different ($p<0.05$), n=4. Different letters (x~z) within a column with the same meat are significantly different ($p<0.05$).

The 80% methanolic extract and ethanolic extract from fruits of *P. mume* showed antioxidative and free radical scavenging activity (14,15). *P. mume* contains several flavonoids such as naringenin (16), and rutin which have been identified as antioxidant components of the fruit of *P. mume* (17). Like grape seed and green tea extracts (18), which contain a larger amount of polyphenolic and phenolic compounds, *P. mume* extract reduced lipid oxidation in chicken breast meats. This could be due to either inhibition of the formation of free radicals during the initiation step or interruption of the propagation of the free radical chain reaction by acting as an electron donor (19,20).

Inhibition of off-odor volatiles

Preventing the production of warmed over flavor is the most critical problem and a major role of antioxidants in storing cooked meat. In general, hexanal was the most highly correlated compound with the TBARS values in cooked meats, and it can be a good indicator for lipid oxidation (21,22). Hexanal was the most predominant volatile compound in the control meat, accounting for more than 50% of the total volatiles, and it was produced in the same amounts as the control in the meat with added PM at the beginning of storage (Table 2). At day 1, the hexanal contents of the control chicken meat increased by about 2.7 times compared with day 0 (Table 3). The amounts of most volatile aldehydes in the control meats were statistically almost the same with those of the PM samples. At day 3, the hexanal contents of the

Table 2. Volatiles profiles of cooked chicken breast meat with addition of *Prunus mume* byproduct (PM) at 0 day

Compounds	Total ion count $\times 10^4$		
	Control	PML	SEM
Hydrocarbons			
Pentane	$816^{b1)}$	1498^{a}	11
Hexane	86	115	8
Heptane	258	416	53
Octane	153^{ab}	250^{a}	57
Carbonyls			
Propanal	1345	1465	117
Butanal	0^{b}	175^{a}	30
Pentanal	822	982	78
Hexanal	10423	10531	859
Heptanal	72^{b}	127^{a}	14

[1)]Different letters (a~b) within a row indicate significant differences ($p<0.05$), n=4.

Table 3. Volatiles profiles of cooked chicken breast meat with addition of *Prunus mume* byproduct (PM) at 1 day

Compounds	Total ion count × 10^4		
	Control	PM	SEM
Hydrocarbons			
Pentane	1835[b1)]	2377[a]	123
Hexane	77	110	14
Heptane	474	483	50
Octane	233[b]	413[a]	49
Carbonyls			
Propanal	5106	5349	307
Butanal	0[b]	232[a]	49
Pentanal	2424	2747	209
Hexanal	28371	30652	1325
Heptanal	288	335	35

[1)]Different letters (a~b) within a row indicate significant differences ($p<0.05$), n=4.

Table 4. Volatiles profiles of cooked chicken breast meat with addition of *Prunus mume* byproduct (PM) at 3 days

Compounds	Total ion count × 10^4		
	Control	PM	SEM
Hydrocarbons			
Pentane	3753[a1)]	2965[ab]	361
Hexane	303[a]	162[b]	25
Heptane	1103[a]	647[b]	131
Octane	782[a]	645[ab]	71
Carbonyls			
Propanal	11688[a]	9245[ab]	815
Butanal	480	470	114
Pentanal	8710[a]	6137[b]	790
Hexanal	67623[a]	55051[b]	3469
Heptanal	809	892	88

[1)]Different letters (a~b) within a row indicate significant differences ($p<0.05$), n=4.

control increased by about 6.5 times compared with the 0 day. However, the addition of PM reduced the hexanals to 81% of the control (Table 4). Moreover, other volatile aldehydes (propanal, pentanal, and heptanal) and hydrocarbons (pentane, hexane, heptane, and octane) were also significantly decreased by the addition of PM. Typical gas chromatographs of cooked chicken meat with and without PM after 3 days of storage at 4°C are shown at Fig. 1. These results indicate that PM effectively reduced the off-odor volatiles in cooked chicken breast meat.

When the same amount (0.1%) of methanolic extract of rice hull (RHE) was added to cooked turkey meat, hexanal contents and TBARS values of the meat were decreased to 92% and 75% of the non-added control after 7 days of storage at 4°C, respectively (23). Though it is difficult to compare directly the antioxidant activities of PM and RHE, PM shows a little bit higher antioxidant

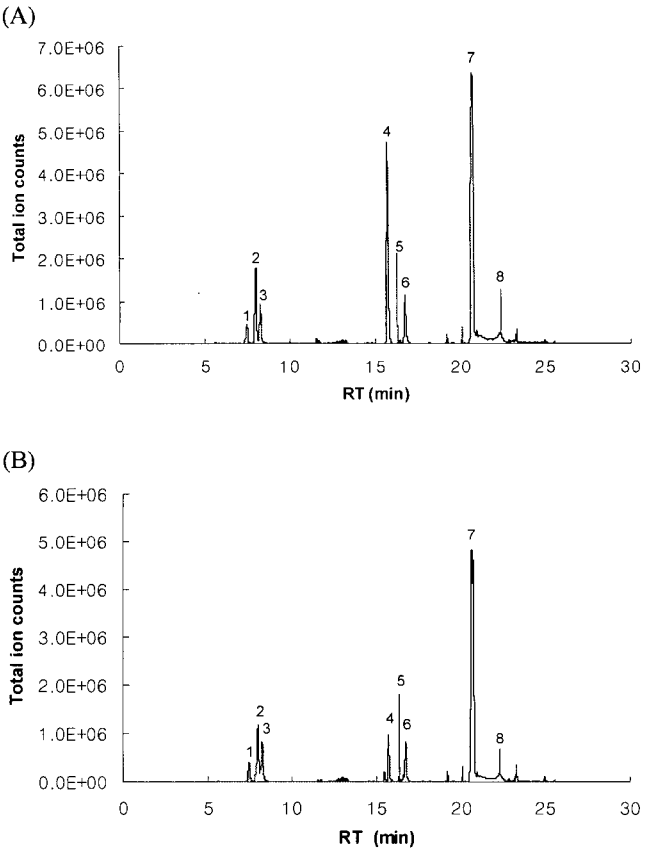

Fig. 1. Typical gas chromatograms of cooked chicken meat after 3 days of storage at 4°C, where the chicken meat patties (A) without additives, and (B) containing methanolic extract of *Prunus mume* byproduct after liquor manufacture. Peaks in (A) and (B) are 1: pentane; 2: propanal; 3: 2-propanone; 4: heptane; 5: oxirane; 6: pentanal; 7: hexanal; 8: heptanal. Peaks in (B) are 1: pentane; 2: propanal; 3: 2-propanone; 4: heptane; 5: oxirane; 6: pentanal; 7: hexanal; and 8: heptanal.

activities. Both PM and RHE could be reasonable candidates for natural antioxidants in poultry meats.

Color change by *Prunus mume* extract

All treatments resulted in very few significant changes in redness (a^*), yellowness (b^*), and lightness (L^*) values (Table 5). The a^* value with added-PM was lower than the control because of the light green color of PM. Little color change should be a desirable aspect of added PM in terms of the color of chicken breast meat, because consumers usually expect the color of cooked poultry breast meat to be unchanged for L^* and b^* values. In the case of RHE, the added RHE significantly changed the color of cooked turkey meat; the color became darker (lower L^* value), whereas both a^* and b^* values were higher than those of the non-added control (23). In this respect, PM is more attractive than RHE for the application in meats.

In conclusion, the methanolic extract of *P. mume* byproduct after liquor manufacturing showed antioxidant

Table 5. Color values of cooked chicken breast meat with the addition of *Prunus mume* byproduct (PM) during refrigerated strage

Storage (day)	Control	PM	SEM
	L^* value		
0	$84.14^{y1)}$	$83.76^{}$	0.50
1	84.50^{axy}	83.62^{ab}	0.58
3	85.28^{ax}	84.61^{ab}	0.47
SEM	0.41	0.59	
	a^* value		
0	6.12^{a}	5.65^{b}	0.14
1	6.24^{a}	5.55^{b}	0.16
3	6.24^{a}	5.65^{b}	0.13
SEM	0.10	0.18	
	b^* value		
0	20.19^{bx}	20.14^{bx}	0.23
1	18.98^{z}	18.74^{y}	0.30
3	19.80^{y}	20.03^{x}	0.31
SEM	0.19	0.35	

[1)]Different letters (a~b) within a row indicate significant differences ($p<0.05$), n=4. Values with different letters (x~z) within a column with the same color value are significantly different ($p<0.05$).

activities in cooked chicken breast meat. If more efficient ways are developed to increase the antioxidant activities by concentrating more antioxidant components and/or excluding the unnecessary portions, the extract from *P. mume* byproduct has the potential to be an excellent natural antioxidant source.

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