

Antioxidative Effects of Glycosyl-ascorbic Acids Synthesized by Maltogenic Amylase to Reduce Lipid Oxidation and Volatiles Production in Cooked Chicken Meat

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Glycosylated ascorbic acids were synthesized by using the transglycosylation activity of *Bacillus stearothermophilus* maltogenic amylase with maltotriose to show effective antioxidative activity with enhanced oxidative stability. The modified ascorbic acids comprised mono- and di-glycosyl transfer products with an α -(1,6)-glycosidic linkage. The antioxidative effects of the glycosyl derivatives of ascorbic acid on the lipid oxidation of cooked chicken breast meat patties were compared, and the synergistic effect when combined with α -tocopherol was determined in terms of thiobarbituric acid-reactive substances (TBARS) and volatiles production during storage. The results indicate that the glycosylated ascorbic acids had very effective antioxidative activity in preventing lipid oxidation, and were better in their synergistic effect in comparison to authentic ascorbic acid, with maltosyl-ascorbic acid being the most effective. Volatiles production was highly correlated with the TBARS values in the lipid oxidation of cooked meat. The antioxidative effect preventing the production of volatiles was particularly strong on pentanal, fairly strong on propanal and butanal, and not at all on ethanal. Propanal, pentanal, and the total volatiles thus provided a good representation of the lipid oxidation status of cooked chicken meat.

Key words: antioxidant; *Bacillus stearothermophilus* maltogenic amylase; glucosyl-ascorbic acid; maltosyl-ascorbic acid; lipid oxidation

Ascorbic acid, a well-known natural antioxidant, is commonly used in many food systems for maintaining organoleptic quality and protecting against oxidation.^{1,2} It functions both as a reducing agent and as a free radical scavenger by donating either one or two electrons to more-oxidized neighboring species.^{3–5} It readily scavenges such reactive oxygen and nitrogen species as superoxide and hydroperoxyl radicals, aqueous peroxy radicals, singlet oxygen, peroxynitrite, and nitroxide radicals. It can also act as a reducer by regenerating α -tocopherol from the α -tocopheroxyl radical that is produced via the scavenging of lipid-soluble radicals. Ascorbic acid has two major properties to make it an effective antioxidant.⁶ First is the low one-electron reduction potential of both ascorbate and its one-electron oxidation product, the ascorbyl radical. This low reduction potential enables ascorbate and its ascorbyl radical to reduce basically all physiologically relevant radicals and oxidants. Second is the stability and low reactivity of the ascorbyl radical that is formed. The ascorbyl radical readily dismutates to form ascorbate and dehydroascorbic acid. Dehydroascorbic acid is rapidly and irreversibly hydrolyzed to 2,3-diketogulonic acid, which then decomposes to oxalate, threonate, and many other products. Ascorbic acid has also recently been considered as a cosmetic ingredient for skin-care due to its beneficial role against skin aging by promoting collagen biosynthesis⁷ and inhibiting melanogenesis.⁸ Ascorbic acid can be used as a common antioxidant in many food systems.^{9,10} However, it easily undergoes

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Abbreviations: BSMA, *Bacillus stearothermophilus* maltogenic amylase; AA, ascorbic acid; G-AA, glucosyl-ascorbic acid; M-AA, maltosyl-ascorbic acid; Tc, α -tocopherol; TBARS, thiobarbituric acid-reactive substances

oxidation to 2,3-diketo-L-gulonic acid, a biologically inactive compound, and to 2-hydroxyfurfural under oxidative conditions such as heat, transition metals, and oxidases,^{11,12} and consequently loses its antioxidative activity. This instability against such oxidative environments is disadvantageous in food and other applications. To overcome this problem, many studies have been performed to synthesize more-stable ascorbic acid derivatives by many physico-chemical and enzymatic methods.¹²⁻¹⁴ Among them, 2-O- α -glucosyl ascorbic acid has been prepared by α -glucosidase.¹⁵ 2-O-glycosyl ascorbic acid derivatives showed greatly enhanced stability against oxidative degradation, but due to no reducibility, they were of no use as antioxidants in food. We have recently found that *Bacillus stearothermophilus* maltogenic amylase (BSMA), which is known to have a high degree of transglycosylation activity for various sugars and sugar-containing molecules, could transfer mono- or disaccharides to an acceptor, ascorbic acid, by forming an α -(1,6)-glycosidic linkage.¹⁶⁻¹⁸ The glycosyl-transfer products of ascorbic acid were structurally analyzed and also determined to show high oxidative stability against metal ions and oxidase while retaining active reducibility.

Oxidative deterioration of unsaturated lipids generally produces an off-flavor and also decreases the nutritional quality of many lipid-containing foods. This oxidation can generate a carcinogenic initiator and mutagen which is a breakdown product of peroxidized polyunsaturated fatty acids.¹⁹ Hence, in cooked meat products, effective substances are required to be safe and stable to prevent lipid oxidation during storage. One of the important factors in preventing lipid oxidation in cooked meat during storage is blocking the availability of oxygen and removing the free radicals. These free radicals can be generated by oxygen reacting with the labile fatty acyl group of phospholipids in cooked meat. Ascorbic acid, even though it is susceptible to oxidative degradation, is known as a safe scavenger of oxygen and oxygen radicals; therefore, glycosylated ascorbic acids can be expected to be very effective for the stable storage of meat due to their dual properties of enhanced oxidative stability and antioxidative activity.

In the present study, the antioxidative effects of glycosyl derivatives of ascorbic acid are described on the storage stability of cooked chicken breast meat, measured as TBARS and volatiles produced, and their synergistic effect in combination with α -tocopherol is also reported.

Materials and Methods

Materials. *Bacillus stearothermophilus* maltogenic amylase (BSMA) was prepared from recombinant *Escherichia coli* DH5 α containing plasmid pSG12.¹⁶ This plasmid was constructed by inserting the BSMA gene into the HindIII site of pUC18. The cultivation of

recombinant *E. coli* and purification of BSMA has been previously reported.¹⁶ Ascorbic acid and maltotriose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of reagent grade.

Enzyme Assay. The activity of BSMA was assayed with 1% β -cyclodextrin in a 50 mM sodium citrate buffer (pH 6.0) at 55°C by using 3,5-dinitrosalicylic acid (DNS) according to the previously reported method.²⁰ The absorbance of the mixture was measured at 575 nm with an Ultrospec III spectrophotometer (Pharmacia LKB, Uppsala, Sweden). One unit of enzyme activity is defined as the amount of enzyme producing 1 μ mol of maltose per minute.

Transglycosylation of Ascorbic Acid by BSMA. The transglycosylation reaction was performed with 10% (w/v) maltotriose as a donor and 60% (w/v) ascorbic acid as an acceptor in a 25 mM sodium citrate buffer at pH 6.0. BSMA (1U/mg of maltotriose) was added to the reaction mixture which was incubated for 48 h at 55°C in the dark. The reaction mixture was then boiled for 5 min to stop the reaction. After centrifuging at 6,000 \times g for 10 min, the resulting supernatant was subjected to ultrafiltration by Ultrafree apparatus (Millipore, Bedford, MA, USA). The filtrate was used for further purification.

Analysis of the Transglycosylation Products by TLC and HPLC. The reaction products were analyzed by TLC on Whatman K6F silica gel plates (Fischer Scientific, Chicago, IL, USA) with n-butyl alcohol/acetic acid/water (3:1:1, v/v/v). After irrigating twice, the TLC plate was dried and visualized either by dipping in a solution containing 0.3% (w/v) N-(1-naphthyl)-ethylenediamine and 5% (v/v) H₂SO₄ in methanol and heating at 110°C for 10 min, or by UV detection at 254 nm (camag Reprostar 3, Muttenz, Switzerland). An HPLC analysis of the transfer products was carried out with a Nova-Pak C18 reverse-phase analytical column (3.9 mm ID \times 150 mm), using an isocratic solvent system at 0.7 ml/min of 0.1 M phosphoric acid (pH 2.0) as a mobile phase with detection at 265 nm using an SLC 200 instrument (Samsung, Seoul, Korea).

Purification of the Transfer Products. The reaction mixture was applied to a Q-sepharose anion exchange column (6 \times 30 cm; Pharmacia) that had been equilibrated with a 10 mM NaOH solution. Elution was performed with a linear NaCl gradient of 0–1 M in the same solution at a flow rate of 2 ml/min. The peak fractions of the transfer products were pooled and concentrated by Speed Vac SC-110 apparatus (Savant Instruments, Holbrook, NY, USA). The resulting concentrate was loaded into a Bio-Gel P-2 column (1.6 \times 100 cm; Bio-Rad) which was eluted with distilled water at a flow rate of 0.2 ml/min at room temperature.

The fractions containing the transfer products were confirmed by TLC, collected and lyophilized.

Liquid Chromatography/Mass Spectrometry (LC/MS). An LC/MS analysis was performed with a jeol LC instrument used in the atmospheric pressure chemical ionization (APCI) mode. A 5- μ l amount of the sample at 100 μ g/ml was directly injected into the instrument.

^{13}C -NMR Analysis. The ^{13}C -NMR spectra of the transfer products were recorded with a JNM LA-400 FT-NMR spectrometer (Jeol, Tokyo, Japan) run in the heteronuclear multiple bond connectivity (HMBC) mode. The sample was dissolved in DMSO- d_6 at 24.9°C with tetramethylsilane (TMS) as the internal reference. Two transfer products synthesized were analyzed to be α -(1,6)-linked glucosyl-ascorbic acid (G-AA) and maltosyl-ascorbic acid (M-AA), as shown in Fig. 1.

Sample Preparation. Hand-deboned and skinless breast meat (2 kg) pieces were prepared from four chickens. The deboned breast meat was pooled, ground twice through a 3-mm plate, and used to make meat patties (30 g/patty) of about 35 mm in diameter and 5 mm in thickness. The experiment was designed with seven treatments to determine the antioxidative effects of ascorbic acid, glycosylated ascorbic acids, and their combinations with tocopherol on the lipid oxidation of cooked meat against a control with no added antioxidant. Eight sets of patty samples with four replications were prepared by adding a single antioxidant or antioxidant combination to the ground meat. The antioxidants of the glycosyl derivatives of ascorbic acid

were prepared in deionized distilled water (DDW), and an antioxidant stock of 1% (w/v) tocopherol was prepared as an emulsion by mixing 200 ml of DDW with 2 g of tocopherol in a Waring Blender for 1 min at high speed. The antioxidant solutions were added the ground meat to make patties (15% per patty, v/w) alone or in combination. A patty made with only DDW was used as the control. Each patty was put into an oxygen-impermeable nylon/polyethylene bag and cooked in a water bath at 80°C for 15 min. Immediately after cooking, each patty was individually vacuum-packed in the same type of bag and then stored at 4°C. Thiobarbituric acid-reactive substances (TBARS) and volatiles in the cooked meat patties, after their exposure to air, were determined every 6 h for 18 h at 15°C.

Lipid Oxidation. Lipid peroxidation was determined by the method of Buege and Aust²¹⁾ with a slight modification. A 1-g meat sample was placed in a 50-ml test tube and homogenized with 5 ml of DDW in a homogenizer for 15 s at speed 7–8. The meat homogenate (1 ml) was transferred to a disposable test tube (13 \times 100 mm) containing a butylated hydroxyanisole (50 μ l, 7.2%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (2 ml). The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. After the color had developed, the sample was cooled in cold water for 10 min and then centrifuged for 15 min at 2,000 \times g. The absorbance of the supernatant in each sample was determined at 531 nm against a blank containing a mixture of 1 ml of DDW and 2 ml of TBA/TCA. The TBARS number is expressed as milligrams of malondialdehyde (MDA) per kilogram of meat. The TBARS values were determined at the same time and under the same temperature conditions as those used in the volatile analysis.

Analysis of Volatiles. Purge-and-trap apparatus connected to a gas chromatograph (GC) was used to analyze the volatiles potentially responsible for the off-odor in meat.²²⁾ A Precept II and model 3000 purge-and-trap concentrator (Tekmar-Dorham, Cincinnati, OH, USA) were used to purge and trap the volatiles emitted from the samples. A model 6890 GC (Hewlett Packard Co., Wilmington, DE, USA) equipped with an HP 5973 mass selective detector (Hewlett-Packard) was used to characterize and quantify the volatile compounds influenced by headspace oxygen during the sample-holding period. A 1-g sample of cooked meat was placed in a sample vial (40 ml), and the vial was sealed tightly with a Teflon-lined cap. The sample in the vial was placed in a refrigerated (15°C) sample tray and purged with helium gas at 40 ml/min for 15 min by using the Precept II auto-sampling unit equipped with a robotic arm. The volatiles were trapped at 30°C in a Tenax/silica gel/charcoal column (Tekmar-Dorham) and desorbed for 1 min at 220°C. The temperature of the connecting transfer lines, the 3000 concentrator and the GC inlet was maintained

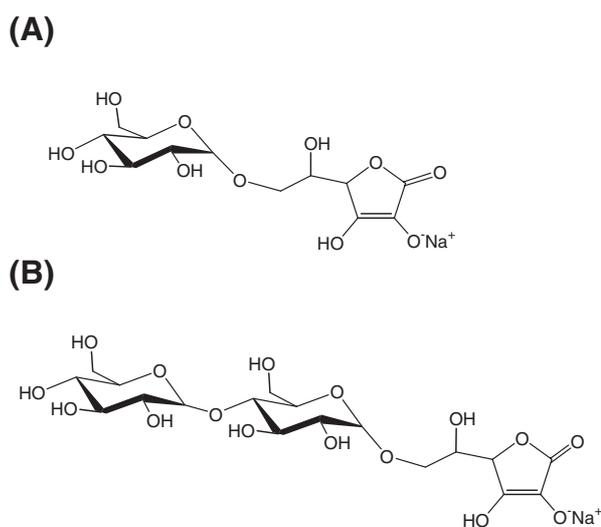


Fig. 1. Chemical Structures of the Transglycosylated Products of Ascorbic Acid.

(A) α -(1,6)-glucosyl-ascorbic acid; (B) α -(1,6)-maltosyl-ascorbic acid.

Table 1. Effects of Ascorbic Acid, Glycosyl Derivatives of Ascorbic Acid, α -Tocopherol, and Their Combinations with α -Tocopherol on Lipid Oxidation (TBARS) of Cooked Chicken Breast Meat Patties during Storage

Storage time	Control	AA ¹	G-AA	M-AA	Tc	AA+Tc	G-AA+Tc	M-AA+Tc	SEM ²
	TBARS (mg of MDA/ kg of meat) ³								
0h	0.973 ^a	0.521 ^c	0.694 ^b	0.712 ^b	0.61 ^b	0.445 ^c	0.658 ^b	0.558 ^c	0.0127
6h	2.834 ^a	0.831 ^b	1.083 ^b	0.986 ^b	0.904 ^b	0.566 ^c	0.701 ^c	0.64 ^c	0.0127
12h	4.001 ^a	1.122 ^{bc}	1.444 ^b	1.339 ^b	1.171 ^{bc}	0.659 ^c	0.688 ^c	0.603 ^c	0.0127
18h	5.542 ^a	2.021 ^b	2.667 ^b	2.376 ^b	1.826 ^b	0.774 ^c	0.865 ^c	0.735 ^c	0.0130

¹ Abbreviations for the treatments: AA, ascorbic acid; G-AA, glucosyl-ascorbic acid; M-AA, maltosyl-ascorbic acid; Tc, α -tocopherol; AA + Tc, ascorbic acid + α -tocopherol; G-AA + Tc, glucosyl-ascorbic acid + α -tocopherol; M-AA + Tc, maltosyl-ascorbic acid + α -tocopherol.

² SEM indicates the standard error of the mean.

³ TBARS indicate mean values expressed as milligrams of malondialdehyde (MDA) per kilogram of meat.

^{a-c} Different letters within a row indicate significant difference ($P < 0.05$).

at 135°C. A split inlet (49:1 split ratio) was used to inject the volatiles into a GC column (HP-5MS capillary, 0.25 mm i.d., 30 m, and 0.25 μ m film thickness, Hewlett-Packard) and subjected to an oven temperature program (30°C for 2 min, increased to 40°C at 2°C/min, increased to 50°C at 5°C/min, increased to 100°C at 10°C/min, increased to 140°C at 20°C/min, increased to 200°C at 30°C/min, and finally held for 4.5 min). The inlet temperature was 180°C, helium was used as the carrier gas, and the column flow was 1.1 ml/min. The ionization potential for MS was 70 eV with a scan range of m/z 45–450. The volatiles were identified by comparing their mass spectral data with those in the Wiley library (Hewlett-Packard). The area of each peak was integrated by using ChemStation software (Hewlett-Packard), and the total ion count ($\times 10^3$) is reported as an indicator of the volatiles generated from each meat sample. The TBARS values and selected volatile components of the cooked meat were statistically analyzed with SigmaStat software (SPSS, Illinois, USA), and the Student–Newman–Keuls test was used to compare the differences in the mean values of TBARS and volatiles affected by the antioxidants.

Results and Discussion

Lipid Oxidation

Hydroxyl radicals and other reactive oxygen species can interact with lipids in meat and form lipid hydroperoxides. Lipid hydroperoxides are broken down to form lipid alkoxy radicals that can initiate and propagate the chain reaction of lipid peroxidation.^{23,24} Phospholipids are generally considered to be responsible for about 90% of lipid oxidation in meat.²⁴ Subsequent breakdown of such hydroperoxides generates volatiles, which may partially contribute to the off-odor of oxidized meat. The extent of lipid peroxidation can be represented by the TBARS number which indicates the amount of malondialdehyde (mg/kg of lipid) produced from lipid peroxidation. In the present work, the antioxidative effects of glycosylated ascorbic acids and the synergistic effects with α -tocopherol were evaluated

as TBARS values on lipid oxidation in cooked chicken breast meat (Table 1). The TBARS values for G-AA and M-AA with or without Tc were significantly different in comparison with those of the control with increasing storage time. Cooked chicken breast meat with no treatment in the sample vial (control) gave TBARS values of meat samples held in the autosampler at 15°C that increased during the first 6 h of sample-holding time by 2.9-fold over those at 0 h, and that continued to increase and give significantly higher TBARS values throughout the whole storage period of 18 h. The development of lipid oxidation in cooked meat patties has been reported to be very rapid after exposure to air. Cooking not only disrupts the membrane structure and destroys the endogenous antioxidative system, but also facilitates the release of iron from carrier proteins or storage proteins in food.²⁴ The increments of TBARS values per hour were evaluated as follows: 0.248 (correlation coefficient, $r^2=0.993$) for the control, 0.080 ($r^2=0.915$) for the AA treatment, 0.104 ($r^2=0.902$) for the G-AA treatment, 0.0891 ($r^2=0.897$) for the M-AA treatment, 0.0652 ($r^2=0.949$) for the α -tocopherol (Tc) treatment, 0.018 ($r^2=0.998$) for the AA+Tc treatment, 0.011 ($r^2=0.712$) for the G-AA+Tc treatment, and 0.0082 ($r^2=0.7224$) for the M-AA+Tc treatment. These results show that the 6- α -glycosyl ascorbic acids acted as antioxidants and suppressed the lipid oxidation in cooked chicken breast meat by up to around 60% of the oxidation in the control during storage, similarly to ascorbic acid. This result is in agreement with the report that ascorbic acid was effective in preventing lipid peroxidation in plasma and low-density lipoprotein.⁵ The antioxidative effects of the glycosylated ascorbic acids on lipid oxidation in the cooked meat were very strong in comparison to ascorbic acid, even though small variations were observed. When the meat samples were treated by the combinations with α -tocopherol, the antioxidative effects were highly synergistic, the most effective being the combination of M-AA and α -tocopherol. The TBARS values with that combined treatment did not significantly change, maintaining their level up to 18 h. This is believed to have been due to the regeneration of

Table 2. Effects of Ascorbic Acid, Glycosyl Derivatives of Ascorbic Acid, α -Tocopherol, and Their Combinations with α -Tocopherol on the Production of Total Volatiles in Cooked Chicken Breast Meat Patties during Storage

Storage time	Control	AA ¹	G-AA	M-AA	Tc	AA+Tc	G-AA+Tc	M-AA+Tc	SEM ²
	Total volatiles (total ion count $\times 10^{-3}$) ³								
0 h	4290	3378	2681	4082	5618	2554	2912	2441	622
6 h	17090 ^a	10568 ^b	9948 ^b	18298 ^a	8353 ^b	6784 ^b	7041 ^b	4020 ^b	672
12 h	30445 ^a	18484 ^b	15947 ^b	17982 ^b	10162 ^c	11482 ^c	8429 ^c	7013 ^c	707
18 h	49462 ^a	26692 ^b	23819 ^b	23781 ^b	13033 ^c	13742 ^c	12755 ^c	8892 ^c	740

¹ Abbreviations of the treatments: see the legend to Table 1.

² SEM indicates the standard error of the mean.

³ Total volatiles are presented as the mean value expressed as the total ion count of volatiles generated.

^{a-c} Different letters within a row indicate significant difference ($P < 0.05$).

α -tocopherol from the α -tocopheroxyl radical by the glycosyl-ascorbic acids. TBARS values of the cooked meat samples at 0 h fluctuated to some extent. This is presumed to have been due to the initial oxidation status of the cooked meat samples; this can be influenced by the degree of lipid oxidation, that is, significant amounts of primary and secondary lipid oxidation by-products, in the homogenized raw meat before cooking.

Volatiles

The volatile compounds produced from the cooked meat during storage were analyzed by gas chromatography (GC) with the purge-and-trap apparatus. The volatiles mainly consisted of hydrocarbons (butane and pentane) and aldehydes (ethanal, propanal, butanal, pentanal and hexanal) in the cooked chicken breast meat with no added antioxidant after oxygen exposure for 18 h. In the cooked chicken breast meat with no treatment (control) and with the other treatments, the production of total volatile compounds due to lipid oxidation gradually increased throughout the sample holding time of 18 h (Table 2). The total volatiles produced from lipid oxidation in the cooked meat patties were considerably less after treatment with the glycosyl derivatives of AA, G-AA and M-AA, being the range of 40-50% lower. The synergistic suppression of total volatiles by the combination with α -tocopherol (Tc) was also clearly apparent, the most effective being the combination of M-AA and Tc which is in good agreement with the result for TBARS. This is assumed to have been due to the better stability of M-AA against an oxidative environment in comparison with AA and G-AA. The linear correlation between TBARS and total volatiles in the cooked meat patties was confirmed (Fig. 2). The correlation coefficients (r^2) were as follows: 0.902 (control), 0.769 (AA), 0.714 (G-AA), 0.798 (M-AA), 0.701 (Tc), 0.764 (AA+Tc), 0.741 (G-AA+Tc) and 0.744 (M-AA+Tc). Total volatiles were highly correlated with lipid oxidation (TBARS values) of the cooked meat patties for the control and treated samples. The respective production of aldehydes and hydrocarbons in the volatiles of the cooked meat patties throughout the storage period also gradually increased (Tables 3 and 4). The volatile values for G-AA and M-

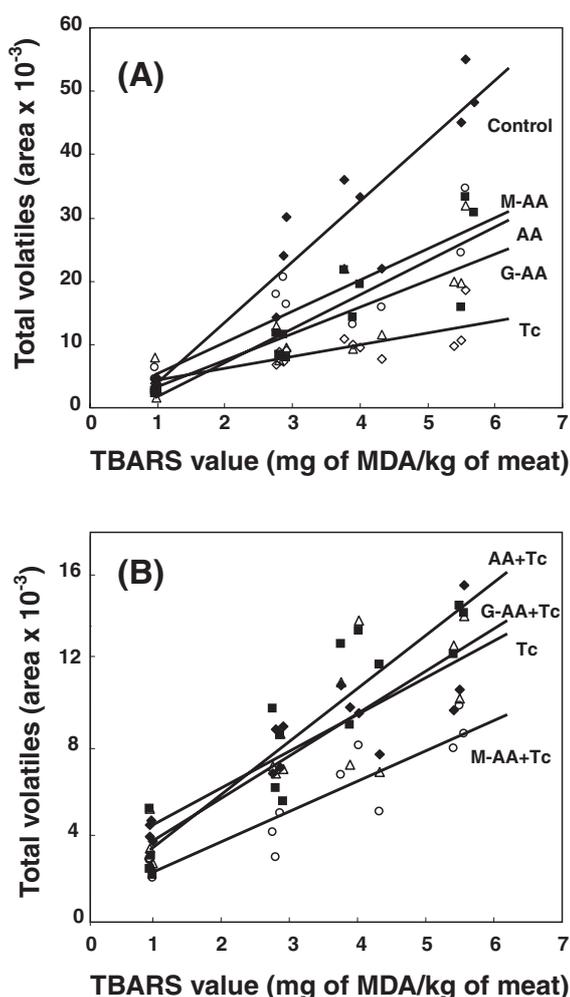


Fig. 2. Relationships between TBARS Values and Total Volatiles in the Control and Antioxidant-treated Cooked Meat Patties.

(A) Antioxidative effects for the control (\blacklozenge), AA (\blacksquare), G-AA (\triangle), M-AA (\circ) and Tc (\diamond); (B) synergistic effects of Tc (\blacklozenge), AA+Tc (\blacksquare), G-AA+Tc (\triangle) and M-AA+Tc (\circ). The area of volatiles on the ordinate indicates the total ion count ($\times 10^3$) of volatiles generated. Straight lines were drawn by a linear least-squares regression.

AA with or without Tc were significantly different from those of the control with increasing storage time up to 18 h. Aldehydes were the major components (around

Table 3. Effects of Ascorbic Acid, Glycosyl Derivatives of Ascorbic Acid, α -Tocopherol, and Their Combinations with α -Tocopherol on the Production of Hydrocarbons (butane, pentane, and hexane) in Cooked Chicken Breast Meat Patties during Storage

Storage time	Control	AA ¹	G-AA	M-AA	Tc	AA+Tc	G-AA+Tc	M-AA+Tc	SEM ²
	Hydrocarbon volatiles (total ion count $\times 10^{-3}$) ³								
Butane									
0h	60 ^a	22 ^b	16 ^b	29 ^b	22 ^b	20 ^b	16 ^b	16 ^b	3
6h	174 ^a	85 ^b	64 ^b	102 ^b	49 ^c	70 ^b	41 ^c	33 ^c	8
12h	231 ^a	144 ^b	113 ^b	149 ^b	73 ^c	86 ^c	57 ^c	45 ^c	11
18h	259 ^a	193 ^b	161 ^b	188 ^b	130 ^{bc}	131 ^{bc}	79 ^c	55 ^c	12
Pentane									
0h	1030 ^a	445 ^b	428 ^b	511 ^b	381 ^b	256 ^c	292 ^c	236 ^c	49
6h	2532 ^a	881 ^b	857 ^b	1519 ^b	998 ^b	403 ^c	448 ^c	455 ^c	133
12h	3655 ^a	1577 ^b	1996 ^b	1930 ^b	1428 ^b	477 ^c	468 ^c	537 ^c	185
18h	3807 ^a	2056 ^b	2008 ^b	2581 ^b	2181 ^b	529 ^c	426 ^c	527 ^c	212
Hexane									
0h	42	55	54	34	45	43	21	29	2
6h	94 ^a	59 ^a	46 ^a	84 ^a	57 ^a	51 ^a	26 ^b	31 ^b	5
12h	119 ^a	55 ^{bc}	92 ^b	94 ^b	41 ^{bc}	23 ^c	22 ^c	20 ^c	8
18h	159 ^a	112 ^{ab}	80 ^b	72 ^b	52 ^{bc}	31 ^c	37 ^c	39 ^c	9

¹ Abbreviations of the treatments: see the legend to Table 1.

² SEM indicates the standard error of the mean.

³ Hydrocarbon volatiles are presented as the mean value of the total ion count.

^{a-c} Different letters within a row indicate significant difference ($P < 0.05$).

Table 4. Effects of Ascorbic Acid, Glycosyl Derivatives of Ascorbic Acid, α -Tocopherol, and Their Combinations with α -Tocopherol on the Production of Aldehydes (ethanal, propanal, butanal, and pentanal) in Cooked Chicken Breast Meat Patties during Storage

Storage time	Control	AA ¹	G-AA	M-AA	Tc	AA+Tc	G-AA+Tc	M-AA+Tc	SEM ²
	Aldehyde volatiles (total ion count $\times 10^{-3}$) ³								
Ethanal									
0h	1474	1009	929	970	1695	1128	1202	727	58
6h	4557 ^a	3859 ^a	3343 ^a	2966 ^a	2720 ^a	3358 ^a	2983 ^a	1499 ^a	157
12h	5761 ^a	6063 ^a	5102 ^a	3600 ^a	3850 ^a	6188 ^a	4357 ^a	2302 ^a	250
18h	6209 ^a	6359 ^a	6926 ^a	4287 ^a	5548 ^a	8599 ^a	6097 ^a	2967 ^a	365
Propanal									
0h	910 ^a	153 ^b	238 ^b	756 ^{ab}	318 ^a	151 ^b	127 ^b	119 ^b	53
6h	5483 ^a	2066 ^b	2747 ^b	4346 ^{ab}	1275 ^b	410 ^c	401 ^c	292 ^c	232
12h	7508 ^a	3398 ^b	3304 ^b	5689 ^{ab}	2075 ^b	725 ^c	475 ^c	306 ^c	359
18h	9963 ^a	5248 ^b	4783 ^b	6351 ^b	2910 ^{bc}	563 ^c	756 ^c	635 ^c	589
Butanal									
0h	31	35	56	52	18	20	32	6	13
6h	284 ^a	167 ^{ab}	179 ^{ab}	438 ^a	67 ^c	50 ^c	52 ^c	29 ^c	25
12h	679 ^a	368 ^b	467 ^b	480 ^b	146 ^c	98 ^c	99 ^c	49 ^c	42
18h	1074 ^a	622 ^b	572 ^b	800 ^{ab}	172 ^c	68 ^c	73 ^c	51 ^c	68
Pentanal									
0h	ND ⁴	ND	ND	ND	ND	ND	ND	ND	—
6h	2291 ^a	77 ^b	163 ^b	184 ^b	ND	ND	ND	ND	61
12h	3047 ^a	271 ^b	519 ^b	503 ^b	ND	ND	ND	ND	113
18h	4518 ^a	915 ^b	786 ^b	947 ^b	616 ^b	ND	ND	ND	268

¹ Abbreviations of the treatments: see the legend to Table 1.

² SEM indicates the standard error of the mean.

³ Aldehyde volatiles are indicated by the mean value of the total ion count.

⁴ ND indicates not detected.

^{a-c} Different letters within a row show significant difference ($P < 0.05$).

70%) in total volatiles of the cooked oxidized meat with no treatment (control), with propanal being the most abundant volatile among the aldehydes. Hydrocarbons amounted to about 10% of the total volatiles, pentane being the major hydrocarbon. The linear correlation between TBARS and each volatile in the control was

confirmed as shown in Fig. 3. Ethanal and propanal of the aldehydes and butane and pentane of the hydrocarbons showed high correlation ($r^2 > 0.8$) with TBARS values. The reduction in the amounts of hydrocarbons, including butane, pentane and hexane, by treating with the glycosyl derivatives of AA was in the range of 40–

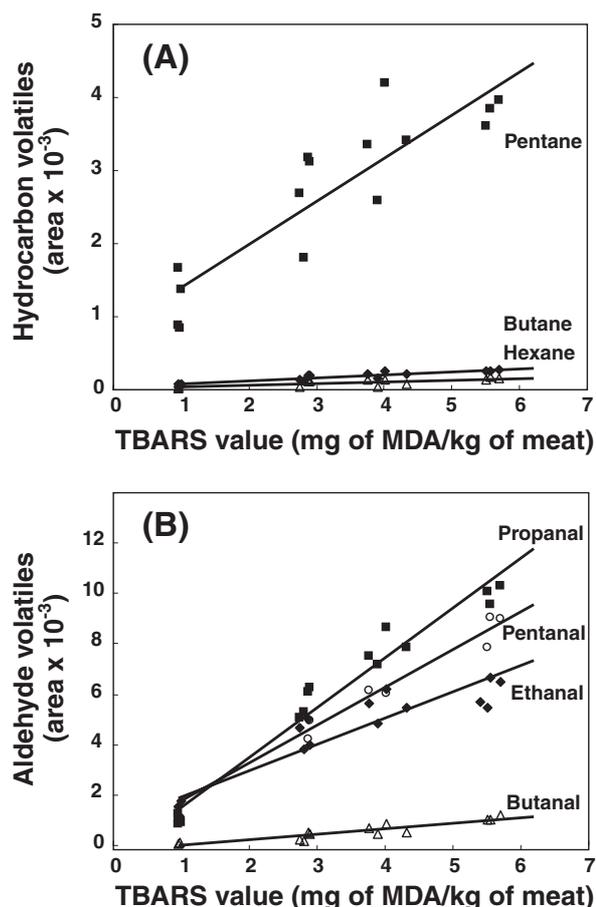


Fig. 3. Relationships between TBARS Values and Volatile Components for Hydrocarbons and Aldehydes.

(A) Hydrocarbons: butane (◆), pentane (■) and hexane (△); (B) aldehydes: ethanal (◆), propanal (■), butanal (△) and pentanal (○). The area of volatiles on the ordinate indicates the total ion count ($\times 10^3$) of volatiles generated. Straight lines were drawn by a linear least-squares regression.

50%, without being a function of the species of hydrocarbon. However, the antioxidative effects of the glycosylated AA derivatives and Tc on the production of aldehydes in cooked meat patties were different, depending on the aldehyde species. Hexanal was hardly detectable after the antioxidant treatments, while pentanal was substantially reduced by more than 80%. Propanal and butanal were reduced in the range of 30–50%, but the production of ethanal was not much affected by the treatments. Therefore, the differences in total volatiles after the treatments were mainly due to a decrease in the aldehydes (propanal, butanal, pentanal and hexanal), the other volatiles being relatively less affected. With all the treatments, pentane of the hydrocarbons ($r^2 > 0.8$) and propanal of the aldehydes ($r^2 > 0.9$) were correlated best with the TBARS values of cooked meat during storage. The antioxidative effects from the treatments on lipid oxidation were thus additionally confirmed by the reduction in the amounts of the respective volatiles, aldehydes and hydrocarbons. This is very likely to have been due to the lipids in food being

generally oxidized and easily degraded at the scissible double bond of unsaturated fatty acyl moieties to produce several short-chain volatile compounds.²⁵) In addition, pentane and propanal were better indicators than butane, hexane and butanal, showing the synergistic effect of the antioxidants by combined treatment with Tc during storage in the present study. In conclusion, glycosylated ascorbic acids showed a considerable antioxidative effect on the lipid oxidation of cooked chicken meat, and acted synergistically when the antioxidant was combined with α -tocopherol which has easy accessibility to the phospholipids in meat due to its lipophilicity. Propanal, pentanal, pentane and total volatiles were highly correlated with the TBARS values of cooked meat during lipid oxidation, implying that these compounds would provide a good representation of the lipid oxidation status of cooked meat.

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