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Formation of Glycidol Fatty Acid Esters in Meat Samples Cooked by Various Methods

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Abstract

Glycidyl fatty acid esters (GEs) are found in some refined edible oils. It is thought that GEs may be broken down by lipase and release glycidol which has been classified as a genotoxic and carcinogenic compound. GEs are formed during deodorization step in the oil refining process. The deodorizing temperature occurs at temperatures of about 200 to 250°C. The cooking temperature is also around 200°C or higher. The aim of this study was to evaluate the formation of GEs in edible meat patties cooked using two methods in order to clarify the intake source of GEs. Three ground meat (beef, pork and chicken) patties were heated by gas fired and char-grilling cooking methods. GEs were formed in meat samples cooked with both heating treatments. In particular, a high concentration of GEs was contained in meat samples heated at high temperature using a charcoal grill. The concentration of each GE compound formed by heating treatment contributed to the amount of each corresponding fatty acid in non-treated raw meat samples. From these results, it is suggested that we may normally ingest GE compounds through cooked meat on a daily basis.

Keywords: Glycidol fatty acid ester; Glycidol; Edible meat; Cooking; Risk assessment

Introduction

Glycidyl fatty acid esters (GEs), such as glycidyl palmitate (C16:0-GE), glycisyl stearate (C18:0-GE), glycidyl oleate (C18:1-GE), glycidyl linoleate (C18:2-GE), and glycidyl linolenate (C18:3-GE), were contained in Diacylglycerol (DAG) oil at high concentration [1]. GEs were also found to be present in small amounts in other common oils rich in triacylglycerol [2]. It was thought that GEs may be carcinogenic [3,4]. This is because it is understood that GEs are broken down by the action of lipase to produce equimolal glycidol (G), which has a reactive epoxy site in the structure [5,6]. G was confirmed as a rodent carcinogen in National Toxicology Program (NTP) study [7]. The International Agency for Research on Cancer (IARC) also classifies G in Group 2A (Probably carcinogenic to humans) [8]. If we ingest 10.0 g per day of DAG oil containing GEs (269 μg/g) [1], the margin of exposure (MOE) of GEs is calculated as 342 value based on comparison of human exposure (0.012 mg/kg bw per day). A benchmark dose lower confidence limit (BMDL10) of G is estimated as 4.06 mg/kg bw per day from the data of mesotheliomas induction of the tunica vaginalis in rats [3]. Therefore, the sale of DAG oil was halted. It is important to estimate the human exposure and toxicities of GEs and G.

Hemoglobin (Hb) adducts have been applied for estimating human exposure to various reactive chemicals as biomarkers [9]. N-(2,3-dihydroxy-propyl) valine (diHOPrVal), which is a Hb adduct in the red blood cells of humans with exposure to G, is an useful biomarker for G and GEs exposure [10,11]. Honda et al. [11] demonstrated that there was no significant difference in diHOPrVal levels in the blood of DAG oil consumers and non-consumers. There was a report that the values of diHOPrVal in the blood of German subjects without G exposure were higher than those of the Japanese DAG oil user [10,12]. These results suggest that we might be exposed to GEs through different food sources other than DAG oil in daily life. Some studies demonstrated that GEs are formed during deodorization step in the oil refining process. The deodorizing temperature occurs at temperatures of about 200 to 250°C. The cooking temperature is also around 200°C or higher. It is reported that some mutagens and carcinogens, such as heterocyclic amines and acrylamide, are formed from meat and fish or potatoes cooked at high temperature [13-16]. Because edible meat and fish contain fatty materials, GEs may be generated in meat and fish heated at high cooking temperature. In the present study, we estimated the formation of GEs in some kinds of meat patties heated by two cooking methods.

Materials and Methods

Reagents

Methanol, 2-propanol, tert-butyl methyl ether, boron trifluoridemethanol complex methanol solution were purchased from Wako PureChemical Industries (Osaka, Japan). n-Hexane, ethyl acetate, diethyl ether, and dichloromethane were bought from Kanto Chemical (Tokyo, Japan). Lauric acid was purchased from Tokyo Chemical Industries (Tokyo, Japan). All reagents were used based on analytical grade reagent.

Standard materials

The standard materials of glycidyl palmitate (C16:0-GE, purity 98.0%), glycidyl stearate (C18:0-GE, purity 98.0%), glycidyl oleate (C18:1-GE, purity 98.0%), glycidyl linoleate (C18:2-GE, purity 90.0%), and glycidyl linolenate (C18:3-GE, purity 85.0%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Individual GE solutions were prepared at 5.0 mg/ml using methanol/2-propanol(1:1 v/v). Each solution, mixed in equal proportion, was diluted with methanol/2-propanol (1:1 v/v). Standard mix solutions (0.005-1.0 ppm) were used for LC-MSanalysis. The standard materials of methyl palmitate (C16:0-MF, purity 98.0%), methyl stearate (C18:0-MF, purity 99.5%), and methyl oleate (C18:1-MF, purity 99.0%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methyl linoleate (C18:2-MF,

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purity 99.0%) and methyl linolenate (C18:3-MF, purity 98.0%), were bought from Tokyo Chemical Industries (Tokyo, Japan). Individual MF solutions were prepared at 5.0 mg/ml in equal quantity and diluted with dichloromethane. Finally, the standard mix solution was prepared by addition of methyl laurate (Tokyo Chemical Industries, Tokyo, Japan, C12:0-MF purity 99.5%) as an internal standard. Standard mix solutions (1.0-1000 ppm) were used for GC-FID analysis.

Heat treatment of edible meat samples by cooking methods

The pork, beef and chicken ground meats were purchased from a local supermarket in Shizuoka city and stored at -20°C before the experiment. At time of the experiment, ground meat samples were allowed to reach room temperature and thoroughly mixed by hand. One hundreds grams of mixed meat samples were used to form circular patties. The size of each patty was (1.0 cm thick and 10 cm indiameter). Gas fired frying pan cooking and charcoal barbecue cooking were used to cook meat patty samples. The pan cooking method was carried out with a commercial Teflon-coated flying pan (metal), which was preheated until the surface temperature was attained at 150 (low temperature condition) or 250°C (high temperature condition). Then, the meat patty samples were cooked for 2 min per side, for a total cooking time of 20 min (low temperature condition) or 10 min (high temperature condition), without adding oil. For the charcoal barbecue cooking, approximately 1.0 kg of charcoal was placed in the bottom of a barbecue oven. A firelighter was poured onto charcoal to start the fire. When all the flames had subsided, the charcoal was leveled by raking. The meat patty samples were then barbecued over the charcoal for 2 min per side, a total cooking time of 5 min. The distance between the samples and the charcoal was about 2 cm. Cooking temperature on surfaces of each heated meat patty sample was monitored with a thermometer (Hioki 3412-50 Temperature HiTester, HIOKI E.E. Corp, Nagano, Japan) for 1 min. The average temperature of charcoal open fire was about 400°C. The gas fired frying pan cooking experiments and charcoal barbecue cooking were respectively performed in quadruplicate and decaplicate.

Purification of meat samples for the instrumental analysis

Heated meat samples were crushed in a blender and freeze-dried with lyophilizer. Subsequently, 10 g of dry samples were oil-extracted by soxhlet extraction with diethyl ether. Extracted oil samples were evaporated with vacuum concentrator. Evaporated samples of 1.0 g were weighted accurately and dissolved in 5.0 ml tert-butyl methyl ether/ethyl acetate (4:1, v/v). Each solution was cleaned according to double solid-phase extraction (SPE) as described in the previous study [17]. The solution of 500 µl was loaded on the first reverse-phase (RP) SPE (Sep-Pak Vac RC C18 cartridge 500 mg, Waters) column preconditioned with 4 ml methanol just prior to use. Three elutions of 2.0 ml methanol each were then applied. The combined eluates (total volume: 6.0 ml) were dried using a nitrogen stream. The dried residues were dissolved in 2.0 ml n-hexane/ethyl acetate (95:5 v/v), and the solutions were loaded on the second normal-phase (NP) SPE (Sep-Pak Vac RC Silica cartridge 500 mg, Waters) preconditioned with 4.0 ml n-hexane/ethyl acetate (95:5 v/v) just prior to use. Three eluates of 2.0 ml n-hexane/ethyl acetate (95:5 v/v) each were then applied. The combined eluates (total volume: 6.0 ml) were dried using a nitrogen stream. The dried residues were then carefully dissolved in 1.0 ml methanol/2-propanol (1:1 v/v) and mixed by ultrasonic wave. The solutions were centrifuged (4000 rpm × 5 min) and the supernatants were used for the analysis of GEs using LC-MS.

LC-MS analysis

LC-MS was used with AOCS and JOCS conjunction testing methodology CD 28-10 to perform the measurement of GEs [18]. The mobile phases A (methanol/ distillated water 92:8) and B (2-propanol) were consecutively time-programmed as follows: isocratic elution of A 0% (B 100%) between 0 and 4 min, an isocratic elution of A 100% (B 0%) between 4 and 30 min, finally, an isocratic elution of A 0% (B 100%) between 30 and 60 min. For the selected ion monitoring measurement, each of the protonated molecular ions [M + H]+ were used: m/z 313.3 for C16:0-GE, m/z 341.4 for C18:0-GE, m/z 339.4 for C18:1-GE, m/z 337.4 for C18:2-GE and m/z 335.4 for C18:3-GE. Other parameters were as follows: instrument; API2000 LC-MS system (column; L-column ODS (4.6 mm diameter, 150 mm length, 5 µm packing materials, Chemical Evaluation and Research Institute, Tokyo, Japan), flow rate of mobile phases; 1.0 ml/min, injection volume; 2.0 µl, column temperature; 40°C, atmospheric pressure chemicalionization; positive ion mode, vaporizer temperature; 500°C, heater temperature of nitrogen gas; 350°C, flow of heated dry nitrogen gas; 5.0 L/min, nebulizer gas pressure; 0.241 MPa, corona current; 8.0 mA, and fragmenter voltage; 150 V.

Derivatization of samples and GC-FID analysis

Extracted oil samples by soxhlet extraction were derivatized by methylation for analysis fatty acids using gas chromatography. About 20 mg of extracted oil samples were dissolved in 0.5 M sodium hydrate methanol solution containing lauric acid as an internal standard. These samples were heated up at 100°C for 9.0 min using dried block bus. Continuously, samples of 2.0 ml of boron trifluoridemethanol complex methanol solution were added and heated up for 7.0 min. After heating, samples were refrigerated in ice and 2.0 ml of dichloromethane with vortex were added. After mixing for 3.0 min by ultrasonic agitation, 5.0 ml of saturated saline were added to the samples and they were centrifugalized for 10 min (4000 rpm). Lower layers were diluted tenfold with dichloromethane and used as GC-FID analysis test sample. The GC-FID system was a GC-18A-FID (Shimazu Tokyo, Japan) equipped with an auto sampler (AOC-20 Series (Shimazu Tokyo, Japan)) and a hydrogen generator (OPGU-2200S (Shimazu Tokyo, Japan)). Separations were conducted on a SLB-IL100 Capillary Column(60 m × 0.25 mm × 0.2 µm film thickness) from Supelco (Bellefonte, PA, USA). Helium (99.999%) was used as a carrier gas in constant flow mode of 0.4 ml/min. Injections (1.0 µl) were done at 240°C in the split mode (1.5:4.1). The oven temperature was held at 140°C for 5 min. Then it was increased at a rate of 2°C/min to 240°C (held for 10 min). Hydrogen gas was generated with a hydrogen generator for FID at a flow rate of 2.0 kgf/cm^2. The flow rate of air for FID was 4.0 kgf/cm^2. Primary gas (nitrogen) flow rate was 3.0 kgf/cm^2. This experiments was performed in triplicate.

Statistical analysis

Significant differences between the experimentalmeans were calculated by t-test and Tukey-Kramer method. A paired t-test and Tukey–Kramer method were used for the comparison of cooked meat samples in terms of GE and fatty acid contents. The evaluation of linearity was achieved by applying Microsoft Excel.

Results and Discussion

Figure 1 shows the typical LC-MS chromatogram of each GEs standard along with their characteristic ion. Each GE was clearly separated on LC-MS chromatogram. The recovery rates varied from 72 to 91%, which may be attributed to differing GEs. Figures 2-4 shows the

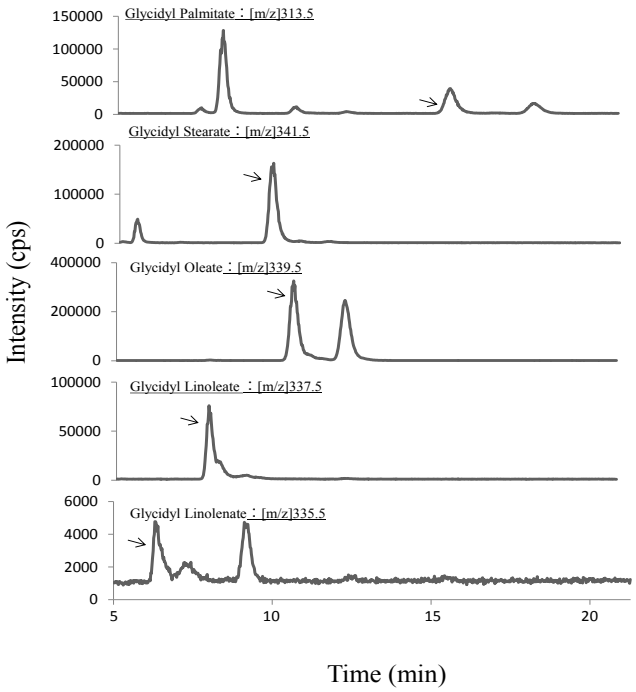

Figure 1: LC-MS chromatogram of each GEs standard (1 ppm) along with their characteristic ion.
For the selected ion monitoring measurement, each of the protonated molecular ions [M + H]+ were used: m/z 313.3 for C16:0-GE, m/z 341.4 for C18:0-GE, m/z 339.4 for C18:1-GE, m/z 337.4 for C18:2-GE and m/z 335.4 for C18:3-GE.

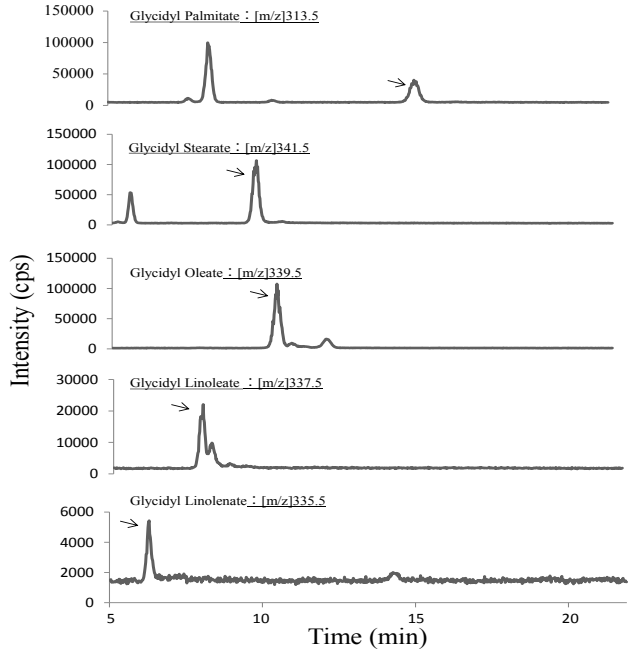

Figure 2: LC-MS chromatogram of each GEs compound in 0.05 g of extracted oil from pork meat patties cooked by charcoal grill. For the selected ion monitoring measurement, each of the protonated molecular ions $[M + H]^+$ were used: m/z 313.3 for C16:0-GE, m/z 341.4 for C18:0-GE, m/z 339.4 for C18:1-GE, m/z 337.4 for C18:2-GE and m/z 335.4 for C18:3-GE.

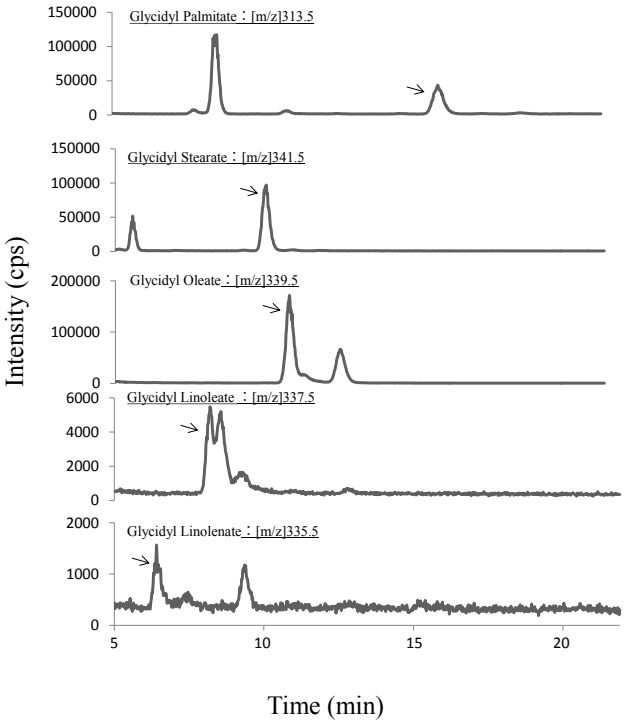

Figure 3: LC-MS chromatogram of each GEs compound in 0.05 g of extracted oil from beef meat patties cooked by charcoal grill. For the selected ion monitoring measurement, each of the protonated molecular ions $[M + H]^+$ were used: m/z 313.3 for C16:0-GE, m/z 341.4 for C18:0-GE, m/z 339.4 for C18:1-GE, m/z 337.4 for C18:2-GE and m/z 335.4 for C18:3-GE.

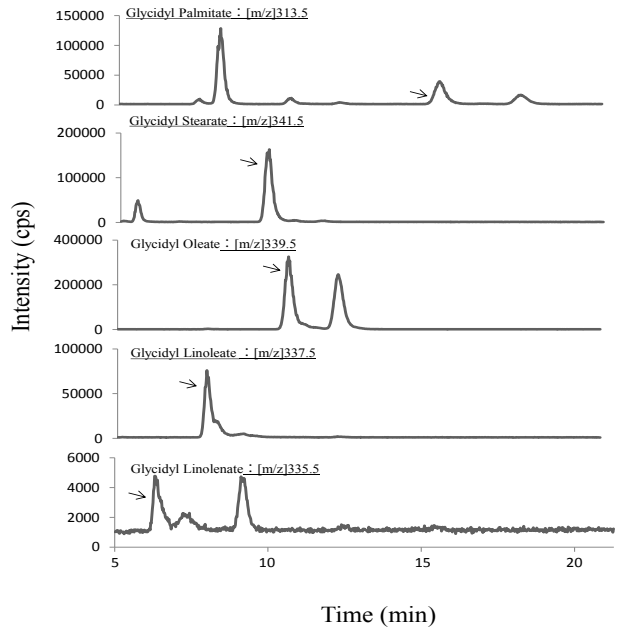

Figure 4: LC-MS chromatogram of each GEs compound in 0.05 g of extracted oil from chicken meat patties cooked by charcoal grill. For the selected ion monitoring measurement, each of the protonated molecular ions [M + H]+ were used: m/z 313.3 for C16:0-GE, m/z 341.4 for C18:0-GE, m/z 339.4 for C18:1-GE, m/z 337.4 for C18:2-GE and m/z 335.4 for C18:3-GE.

representative LC-MS chromatogram of each GEs compound in pork, beef and chicken meat patties cooked by charcoal grill by along with their characteristic ion. Peaks of each GE in heated meat samples was confirmed and clearly separated on LC-MS chromatogram.

Table 1 shows the concentration of total GEs in pork and beef meat samples cooked by gas fired frying pan. No GEs were detected in the pork and beef meat samples at low cooking temperature (150°C). At high temperature (250°C), GEs were determinedin pork meat samples cooked for 5 min (34.4 ± 1.5 ng) and 10 min (166.1 ± 6.8 ng/g) and beef meat samples cooked for 10 min (65.7 ± 6.2 ng/g), respectively. These results showed that the amount of GEs formed in meat samples might rise with increasing cooking temperature and time. However, GEs were not detected in chicken cooked at high temperature (250°C). The contents of fat and water in raw pork, beef and chicken meat samples were 0.15, 0.14 and 0.08 g fat/g, and 0.64, 0.65 and 0.70 g water/g respectively. The fat content is lower and water content is higher in raw chicken meat samples than those in pork and beef meat samples. Chicken meat samples could possibly not be sufficiently heated inside under gas fired frying pan cooking condition. Therefore, GEs could not be formed in chicken meat samples.

Table 1 shows the concentration and content percentage of each glycidyl fatty acid in cooked meat samples. Glycidyl oleate was present in pork meat samples cooked for 5 min at the highest content (13.4 ± 0.4 ng/g) and content percentage (39.1%), followed by palmitate (11.0 ± 0.7 ng/g (32.0%)), stearate (7.7 ± 0.2 ng/g (22.3%)), linoleate (1.8 ± 0.0 ng/g (5.2%)) and linolenate (0.5 ± 0.2 ng/g (1.5%)). In the case of cooking for 10 min, the concertation and content percentage of glycidyl oleate was also highest (57.3 ± 2.4 ng/g (34.5%)), followed by palmitate (53.0 ± 1.9 ng/g (32.1%)), stearate (41.8 ± 1.7 ng/g (25.1%)), linoleate (12.3 ± 0.7 ng/g (7.4%)) and linolenate (1.3 ± 0.5 ng/g (0.8%)). In beef meat samples cooked for 10 min, glycidyl oleate was contained at the highest content (28.7 ± 3.6 ng/g) and content percentage (43.6%), followed by palmitate (26.7 ± 3.9 ng/g (30.7%)), stearate (20.2 ± 2.1 ng/g (24.2%)), linolenate (0.6 ± 0.2 ng/g (0.9%)), linoleate (0.3 ± 0.1 ng/g (0.5%)). From these results, the concentration of each GE compound varied widely in cooked pork and beef meat samples.

Oleic acid, palmitic acid, stearic acid, linoleic acid and linolenic acid are contained in raw pork, beef and chicken at high concentrations. GEs might be generally formed from corresponding fatty acids in raw meat samples. Therefore, the concentrations of five main fatty acids in raw meat samples were detected using GC-FID. Table 2 shows the concentration and content percentage of each fatty acid in unheated raw meat samples. Oleate acid was present in raw pork and beef meat samples at the highest content (50.4 ± 1.0 mg/g (46.6%), 48.4 ± 1.5 mg/g (52.9%)), followed by palmitate acid (27.6 ± 0.5 mg/g (25.5%), 27.3 ± 1.0 mg/g (29.8%)), stearate acid (16.4 ± 0.3 mg/g (15.2%), 13.8 ± 0.4 mg/g (15.1%)), linoleate (12.3 ± 0.2 mg/g (11.4%), (1.6 ± 0.2 mg/g (1.7%)) and linolenate (1.4 ± 0.2 mg/g (1.3%), 0.4 ± 0.1 mg/g (0.5%)), respectively. In raw chicken meat samples, oleate acid was also detected at the highest concentration (25.4 ± 2.2 mg/g (52.1%)), followed by palmitate acid (12.2 ± 1.1 mg/g (25.0%)), linoleate (7.2 ± 0.6 mg/g (14.9%)), stearate acid (3.4 ± 0.3 mg/g (6.9%)) and linolenate (0.5 ± 0.1 mg/g (1.0%)). As compared with the content percentage of each GE in cooked meat samples and fatty acid in raw meat samples, GEs might be generally formed from corresponding fatty acids in raw meat samples.

In the next experiments, the formation of GEs in meat samples cooked by charcoal grill was examined. The temperature under the charcoal grill condition was higher than that under the pan frying condition. The range of cooking temperature using charcoal grill was from 350°C to 600°C. As it was difficult to control the temperature of charcoal grill fire, experiments were done under the same condition ten times. Table 3 shows the amount of total GEs and each GE in three ground meat samples cooked by charcoal grill. The average contents of total GEs in cooked pork meat samples was 1083.5 ± 602.9 ng/g meat sample. Regarding the average contents of each GE compound: glycidyl oleate was present in cooked pork meat samples at the highest content (481.4 ± 279.2 ng/g) and content percentage (44.7%), followed by palmitate (339.8 ± 213.8 ng/g (31.4%)), stearate (163.5 ± 82.7 ng/g (15.0%)), linoleate (80.0 ± 39.4 ng/g (7.4%)) and linolenate (18.8 ± 11.3 ng/g (1.8%)). The average contents of total GEs in beef meat samples cooked by charcoal grill were 669.5 ± 526.0 ng/ g meat sample. Glycidyl oleate was detected in cooked beef meat samples at the highest content (311.1 ± 262.7 ng/g) and content percentage (46.4%) of GEs compounds, followed by palmitate (219.6 ± 177.1 ng/g (32.8%)), stearate (109.2 ± 84.9 ng/g (16.3%)), linoleate (19.3 ± 11.1 ng/g (2.8%)) and linolenate (10.3 ± 9.0 ng/g (1.5%)). The average contents of total GEs in cooked chicken meat samples were 1106.6 ± 475.3 ng/ g meat sample. Glycidyl

Samples	Cooking treatment		Concentration of Glycidol Fatty Acid Esther (GEs) (ng/g meat samples) (Each GE content persentage in total GEs (%))					
	Temp (°C)	Time (min)	Total GEs	Palmitate	Stearate	Oleate	Linoleate	Linolenate
Pork	Low (150)	5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		15	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	High (250)	5	34.4 ± 1.5^{b}	11.0 ± 0.7^{b} (32.0%)	7.7 ± 0.2^{b} (22.3%)	13.4 ± 0.4^{b} (39.1%)	1.8 ± 0.0^{b} (5.2%)	0.5 ± 0.2^{a} (1.5%)
		10	166.1 ± 6.8^{a}	53.0 ± 1.9^{a} (32.1%)	41.8 ± 1.7^{a} (25.1%)	57.3 ± 2.4^{a} (34.5%)	12.3 ± 0.7^{s} (7.4%)	1.3 ± 0.5^{a} (0.8%)
Beef	Low (150)	5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		15	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	High (250)	5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		10	65.7 ± 6.2^{ab}	26.7 ± 3.9^{ab} (30.7%)	20.2 ± 2.1^{ab} (24.2%)	28.7 ± 3.6^{ab} (43.6%)	0.3 ± 0.1^{ab} (0.5%)	0.6 ± 0.2^{a} (0.9%)

N.D. : Not Detected; Each value is expressed as mean ± standard deviation (n=4); Values are shown as mean ± standard deviation. Symbols bearing different letters in the same column are significantly different ($p<0.05$).

Table 1: Concentration and content percentage of glycidol fatty acids in meat samples cooked by gas fired frying pan.

Meat samples	Concentration of fatty acid (mg/g meat samples) (Each fatty acid content persentage in total fatty acids (%))					
	Total Fatty acids	Palmitate	Stearate	Oleate	Linoleate	Linolenate
Pork	108.1 ± 2.2[a]	27.6 ± 0.5[a] (25.5%)	16.4 ± 0.3[a] (15.2%)	50.4 ± 1.0[a] (46.6%)	12.3 ± 0.2[a] (11.4%)	1.4 ± 0.2[a] (1.3%)
Beef	91.5 ± 3.2[a]	27.3 ± 1.0[a] (29.8%)	13.8 ± 0.4[b] (15.1%)	48.4 ± 1.5$_a$ (52.9%)	1.6 ± 0.2[c] (1.7%)	0.4 ± 0.1[b] (0.5%)
Chicken	48.7 ± 0.5[b]	12.2 ± 1.1[b] (25.0%)	3.4 ± 0.3[c] (6.9%)	25.4 ± 2.2[b] (52.1%)	7.2 ± 0.6[b] (14.9%)	0.5 ± 0.1[b] (1.0%)

Each value is expressed as mean ± standard deviation (n=3).
Values are shown as mean ± standard deviation. Symbols bearing different letters in the same column are significantly different (p<0.05).

Table 2: Concentration and content percentage of fatty acids in raw meat samples.

Meat samples	Concentration of Glycidol Fatty Acid Esther (GEs) (ng/g meat samples) (Each GE content persentage in total GEs (%))					
	Total GEs	Palmitate	Stearate	Oleate	Linoleate	Linolenate
Pork	1083.5 ± 602.9[b*]	339.8 ± 213.8[a] (31.4%)	163.5 ± 82.7[a] (15.0%)	481.4 ± 279.2[a] (44.4%)	80.0 ± 39.4[a] (7.4%)	18.8 ± 11.3[a] (1.8%)
Beef	669.5 ± 526.0[c*]	219.6 ± 177.1[b] (32.8%)	109.2 ± 84.9[a] (16.3%)	311.1 ± 262.7[b] (46.4%)	19.3 ± 11.1[b] (2.8%)	10.3 ± 9.0[a] (1.5%)
Chicken	1106.6 ± 475.3[a]	332.8 ± 160.8[c] (30.1%)	98.1 ± 54.9[b] (8.9%)	518.4 ± 230.6[c] (46.8%)	139.4 ± 57.7[c] (12.6%)	17.8 ± 6.6[a] (1.6%)

Each value is expressed as mean ± standard deviation (n=10).
Values are shown as mean ± standard deviation. Symbols bearing different letters in the same column are significantly different (p<0.05). (*vs gas fired frying pan High 10 min p<0.05)

Table 3: Concentration and content percentage of glycidol fatty acids in meat samples cooked by charcoal grill.

oleate was detected in cooked beef meat samples at the highest content (518.4 ± 230.6 ng/g meat samples) and content percentage (46.8%), followed by palmitate (332.8 ± 160.8 ng/g (30.1%)), linoleate (139.4 ± 57.7 ng/g (12.6%)), stearate (98.1 ± 54.9 ng/g (8.9%)) and linolenate (17.8 ± 6.6 ng/g (1.6%)). From these results of cooked pork, beef and chicken meat samples, the levels of GEs in meat samples cooked by charcoal grill were very higher than those in meat samples cooked by gas fired frying pan. This was because the cooking temperature in a charcoal grill was much higher than that of a gas fired frying pan. The temperature range was from 350°C to 600°C. The formation of GEs in cooked meat samples might be increased with the temperature rise, especially under very high temperature conditions such as charcoal grill cooking. The level of each GE in meat samples cooked by charcoal grill might also be proportional to the contents of each fatty acid in raw meat samples as with gas fired frying pan. Therefore, GEs might be formed from each corresponding fatty acid directly under high temperature conditions. Cooking meat by charcoal fire was improper for eating in terms of visual presentation, burn deposits, hardness and the formation of GEs. However, meat cooking by frying pan is conducive to a good diet when done on a routine basis and decreases the risk of exposure of mutagens. It was reported that rice oil contains approximately 2 μg/g of GEs, and 1 g of DAG oil has approximately 200 μg/g of GEs. The concentration of GEs in 100 g meat patties cooked by charcoal grill in the present study is equivalent to the amount of GEs in 1 g of DAG. Though the sale of DAG oil has been discontinued, the present results show that GEs are contained in cooked meat even without DAG oil or other refined oils.

From these results, it was demonstrated that GEs were formed in meat samples cooked at high temperature. There are many studies about the examinations of mutagens and carcinogens formed from heat cooking from the 1970s. Derived mutagens and carcinogens were identified and characterized as heterocyclic amines (HCAs) polycyclic aromatic hydrocarbon (PAH) from cooked meat and fish. It was reported that char-grilled beef cooked in similar heating condition to ours contained HCAs and PAH at high concentration [19,20]. Some scientists demonstrated that lengthening cooking time and raising temperatures increase the formation of HCAs in meats. In the present study, the formation of GEs was increased with the cooking time elongation and temperature rise. Therefore, excessive heating and feeding treatment might generate not only HCAs and PAH but also GEs as novel mutagens in cooked meat. 3-Monochloropropane-1,2-diol (3-MCPD) esthers, which is a GE-related compound and has been classified as possiblly carcinogenic to humans by IARC, is also formed from a model oil under deodorization conditions at high temperature [21]. Before now, ecological studies have shown that high exposure to cooked meat containing mutagens, such as HCAs and PAH, increases the risk of human cancers. Ngoan reported a high intake frequency of cooked food is well correlated with sundry cancer [22]. From our results, GEs in cooked meats might also be involved in an increased risk of human cancer.

G is liberated from GEs by action of lipase within an organism. Released G is metabolized by conjugation with glutathione and is excreted as conjugates in urine [5,23]. Formed G also combines with hemoglobin to form a hemoglobin adduct as a biomarker of G invivo. Eckert et al. [24] also reported that 2,3-dihydroxypropyl mercapturic acid (DHPMA) is a useful biomarker as a biological exposure index of G. However, it is reported that 3-MCPD esthers is metabolized to G by the pathway of dehalogenation *in vivo* [3]. We have also to examine the formation of 3-MCPD esther in cooked meats in order to research the precursor of G in cooked meat. and to research G *in vivo*. In the present study, it is thought that we might be exposed by GEs through cooked meats. Exposure to GEs from regular diets, such as cooked meat dishes using these biomarkers of G and GEs in humans, should be examined to estimate the risk of mutagens containing GEs in diets holistically.

Conclusions

Overall, the present study shows that meat cooked by heat treatment contains GEs. Especially, highly concentrated GEs were generated in meat by cooking at high temperature using an open fire charcoal grill. Therefore, we might be exposed to GEs through regular diet other than refined edible oils on a daily basis. As a result, assessment of the risk of GEs for humans must involve in research into the concentration of GEs in various foodstuffs and the level of comprehensive GE intake in regular diets.

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