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

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Abstract

Glycidol 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

Glycidol fatty acid esters (GEs) are formed from meat and fish containing fatty materials, GEs may be generated in meat and fish during refining processing. 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 Pure Chemical 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), and glycidyl linolenate (C18:3-GE), were contained in Diacylglycerol (DAG) oil at high concentration [1]. GE standards 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 equimolar 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].

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 produced 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.

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purity 99.0%) and methyl linoleate (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 frying 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 alcohol/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 30 min by ultrasonic agitation, 5.0 ml of saturated saline were added to the samples and they were centrifuged 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/cm². The flow rate of air for FID was 4.0 kgf/cm². Primary gas (nitrogen) flow rate was 3.0 kgf/cm². This experiments was performed in triplicate.

**LC-MS analysis**

LC-MS was used with AOCS and JOCS conjuction 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 373.4 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 box. 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 30 min by ultrasonic agitation, 5.0 ml of saturated saline were added to the samples and they were centrifuged 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/cm². The flow rate of air for FID was 4.0 kgf/cm². Primary gas (nitrogen) flow rate was 3.0 kgf/cm². This experiments was performed in triplicate.

**Statistical analysis**

Significant differences between the experimental means 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 determined in pork meat samples cooked for 5 min (34.4 ± 1.5 ng) and 10 min (166.1 ± 6.8 ng/g). In the same condition, GEs were not detected in chicken cooked at high temperature (250°C). The concentrations of total GEs in cooked pork 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.

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: glycylid oleate was present in cooked pork meat samples at the highest content (481.4 ± 272.9 ng/g) and content percentage (44.7%), followed by palmitate acid (339.8 ± 213.8 ng/g (31.4%), stearate acid (163.5 ± 82.7 ng/g (15.0%), linoleate acid (80.0 ± 39.4 ng/g (7.4%) and linolenate acid (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. Glycylid 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 acid (291.6 ± 177.1 ng/g (32.8%), stearate acid (109.2 ± 84.9 ng/g (16.3%), linoleate acid (91.3 ± 11.1 ng/g (28.8%) and linolenate acid (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. Glycylid 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%)).

In the next experiments, the formation of GEs in meat samples cooked by charcoal grill was examined. 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: glycylid oleate was present in cooked pork meat samples at the highest content (481.4 ± 272.9 ng/g) and content percentage (44.7%), followed by palmitate acid (339.8 ± 213.8 ng/g (31.4%), stearate acid (163.5 ± 82.7 ng/g (15.0%), linoleate acid (80.0 ± 39.4 ng/g (7.4%) and linolenate acid (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. Glycylid 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 acid (291.6 ± 177.1 ng/g (32.8%), stearate acid (109.2 ± 84.9 ng/g (16.3%), linoleate acid (91.3 ± 11.1 ng/g (28.8%) and linolenate acid (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. Glycylid

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cooking treatment</th>
<th>Concentration of Glycidol Fatty Acid Esters (GEs) (ng/g meat samples)</th>
<th>Each GE content percentage in total GEs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (min)</td>
<td>Total GEs</td>
<td>Palmitate</td>
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<tr>
<td><strong>Pork</strong></td>
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<tr>
<td>Low (150)</td>
<td>5</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td></td>
<td>10</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td></td>
<td>15</td>
<td>N.D.</td>
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<td></td>
<td>20</td>
<td>N.D.</td>
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<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td>High (250)</td>
<td>5</td>
<td>65.7 ± 6.2</td>
<td>28.7 ± 3.9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cooking treatment</th>
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<th>Each GE content percentage in total GEs (%)</th>
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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) esters, which is a GE-related compound and has been classified as possibly 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. Nгоan 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 esters is metabolized to G by the pathway of dehalogenation in vivo [3]. We have also to examine the formation of 3-MCPD ester 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.

References


