Evaluation of the Mechanisms of Mayonnaise Phospholipid Oxidation

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INTRODUCTION

Mayonnaise consists primarily of vegetable oil, egg yolk and vinegar, and is widely used in foods. As mayonnaise is rich in lipids, it is susceptible to oxidation during the manufacturing process, which can result in loss of quality. Herein, we detected and analyzed phosphatidylcholine hydroperoxide (PCOOH) isomers present in fresh mayonnaise using LC-MS/MS. The PCOOH isomer composition suggests that mayonnaise phospholipid peroxidation is predominantly initiated by radical-oxidation (i.e., upon autoxidation), rather than singlet oxygen-oxidation (e.g., upon light exposure), during manufacturing, packaging, and/or storage. This LC-MS/MS method will be useful for elucidating the cause of lipid peroxidation in mayonnaise and related foods. Such information will be valuable in ensuring maintenance of product quality.

Key words: mayonnaise, oxidation, phospholipid, hydroperoxide isomer, LC-MS/MS

NOTE

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Abstract: Mayonnaise, which is widely used in foods, is rich in lipids and therefore susceptible to oxidation during the manufacturing process, which can result in loss of quality. Herein, we detected and analyzed phosphatidylcholine hydroperoxide (PCOOH) isomers present in fresh mayonnaise using LC-MS/MS. The PCOOH isomer composition suggests that mayonnaise phospholipid peroxidation is predominantly initiated by radical-oxidation (i.e., upon autoxidation), rather than singlet oxygen-oxidation (e.g., upon light exposure), during manufacturing, packaging and/or storage. This LC-MS/MS method will be useful for elucidating the cause of lipid peroxidation in mayonnaise and related foods. Such information will be valuable to ensure maintenance of product quality.

Key words: mayonnaise, oxidation, phospholipid, hydroperoxide isomer, LC-MS/MS

1 INTRODUCTION

Mayonnaise consists primarily of vegetable oil, egg yolk and vinegar, and is widely used in foods. As mayonnaise is rich in lipids, it is susceptible to oxidation during the manufacturing process, such as autoxidation (i.e., radical-oxidation) and/or light exposure (i.e., singlet oxygen-oxidation). The primary products of mayonnaise lipid peroxidation are lipid hydroperoxides, which then trigger a chain reaction that leads to odor problems caused by aldehydes. Moreover, free radicals derived from the chain reaction attack other molecules such as proteins, and deteriorate the color and nutritional value of mayonnaise. Hence, an improved understanding of the cause of mayonnaise lipid peroxidation could lead to a means of ensuring maintenance of the quality of the product.

Several classes of lipid hydroperoxides may be present in mayonnaise, such as triacylglycerol hydroperoxide, cholesterol hydroperoxide, and phospholipid hydroperoxide. Of these lipid hydroperoxides, we have recently developed a sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for detecting phosphatidylcholine hydroperoxide (PCOOH; Fig. 1), a primary oxidation product of phosphatidylcholine (PC). Determination of the hydroperoxide group position in PCOOH isomers may be helpful for elucidating the cause of mayonnaise lipid peroxidation, as demonstrated in our previous study in which we analyzed PCOOH isomers in human plasma and found that radical (and/or enzymatic) oxidation, rather than singlet oxygen-oxidation, is likely the cause of plasma PC peroxidation.

Based on these findings, we analyzed PCOOH isomers in commercial mayonnaise using LC-MS/MS in the present study. To the best of our knowledge, this is the first study to explore PCOOH isomers in commercial mayonnaise. Results of this study will be valuable in not only understanding the cause of mayonnaise lipid peroxidation during the production and sale of the product, but also in efforts aimed at developing preventive methods against oxidative deterioration of mayonnaise and related foods.
2 Materials and methods

2.1 Materials

Three different commercially available mayonnaise products (A, B and C) were purchased at a local market in Sendai, Japan. 1-Palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0/18:2 PC) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). PCOOH references (1-palmitoyl-2-(9-hydroperoxy-octadecadienoyl)-sn-glycero-3-phosphocholine (16:0/9-HpODE PC) and 1-palmitoyl-2-(9-hydroperoxy-octadecadienoyl)-sn-glycero-3-phosphocholine (16:0/9-HpODE PC); Fig. 1) were synthetically prepared according to our previous reports. All other reagents were of the highest grade available.

2.2 Extraction of phospholipid from mayonnaise

Immediately after unsealing a fresh mayonnaise product, total lipid was extracted from 5 g of mayonnaise using 45 mL of a solution of chloroform: methanol: water (8:4:3, v/v/v) with 0.002% butylated hydroxytoluene. The extract was partitioned by centrifugation at 1,000 x g for 20 min at 4°C into 2 layers. The lower chloroform layer (lipid fraction) was collected. The remaining aqueous layer containing a semisolid interface was re-extracted with Folch’s theoretical lower phase and subjected to centrifugation at 1,000 x g for 20 min at 4°C. The combined lipid fraction was evaporated and dried under nitrogen gas. The weight of the obtained total lipids was measured.

Mayonnaise phospholipid was purified from the total lipid extract by solid phase extraction. Briefly, total lipid (0.1 g) was diluted with 0.7 mL chloroform: isopropanol (2:1, v/v), and 0.4 mL of this mixture was loaded onto a Sep-Pak Aminopropyl 6 cc Vac cartridge (1 g, Waters, Tokyo, Japan) equilibrated in chloroform: isopropanol (2:1, v/v). The cartridge was rinsed with 12 mL chloroform: iso-
propanol (2.1, v/v) and phospholipid was eluted with 12 mL methanol. The eluent was evaporated, and the residue was dissolved in 0.2 mL methanol. A 10 μL final aliquot was injected into the LC-MS/MS system for analysis of PCOOH isomers. The remaining aliquot was diluted 10,000 times with methanol, and a 10 μL aliquot was subjected to LC-MS/MS for analysis of 16:0/18:2 PC.

To evaluate the PCOOH extraction efficiency, we spiked 30 pmol references (16:0/13-HpODE PC and 16:0/9-HpODE PC) to the 0.07 mg of mayonnaise. PCOOH was then extracted as described above. Extract was quantified by LC-MS/MS and extraction recoveries were calculated using equation corresponding to the external standard curves.

2.3 LC-MS/MS determination of mayonnaise PCOOH and PC

Mayonnaise PCOOH isomers (16:0/13-HpODE PC, 16:0/9-HpODE PC and 16:0/10-HpODE PC) were analyzed by LC-MS/MS according to our previous report. Briefly, an ODS column (Atlantis T3 column, 3.0 μm, 2.1 x 100 mm; Waters) was used at 40°C. The mobile phase consisted of two components: A, water containing 0.1 mM sodium acetate and B, methanol containing 0.1 mM sodium acetate. The gradient profile was as follows: 0–4 min, 70–90% B linear; 4–10 min, 90% B; 10–17 min, 90–100% B linear; 17–30 min, 100% B; 30–30.1 min, 100–70% B linear; 30.1–35.0 min 70% B. The flow rate was 0.2 mL/min. PCOOH isomers were analyzed using a 4000QTRAP LC-MS/MS System (SCIEX, Tokyo, Japan). MS/MS parameters were optimized with synthesized PCOOH isomers under electrospray ionization (ESI) mode.

For analysis of 16:0/18:2 PC, the same column and mobile phases mentioned above were used. The gradient profile was as follows: 0–5 min, 98–100% B linear; 5–10 min, 100% B; 10–10.1 min, 100–98% B linear; 10.1–15 min, 98% B. The flow rate was 0.2 mL/min, and the column temperature was 40°C. MS/MS parameters were optimized with the standard 16:0/18:2 PC (m/z 780 > 147).

In this study, we individually quantified PCOOH isomers and 16:0/18:2 PC using external standard curves (0.01 to 10 pmol/injection).

2.4 Measurement of mayonnaise peroxide value (PV)

The peroxide value (PV) of mayonnaise lipid was measured according to the official method of the Japan Oil Chemists’ Society (JOCs) with little modification. The total lipid (2 g) was dissolved in 12.5 mL chloroform: acetate acid (2:3). Saturated potassium iodide solution (0.5 mL) was then added and gently mixed for 1 min, and the mixture was kept in the dark. After 5 min, distilled water (37.5 mL) and 1% starch solution (0.1 mL) were added, and the mixture was centrifuged (1,000 × g, 10 min, 4°C). The upper layer was then moved to another test tube and titrated with 10 mM sodium thiosulfate to measure the PV.

2.5 Statistics

For measurement of PCOOH isomers and 16:0/18:2 PC, extraction and LC-MS/MS analysis were performed 3 times for each sample, and data are expressed as means ± SDs. One-way ANOVA was used to compare the overall differences among the 3 mayonnaise products. If a statistically significant difference was detected, the Turkey-Kramer test was performed for comparison between individual groups. Differences were considered significant at p < 0.05.

3 Results and discussion

During the production and sale of mayonnaise, lipids in mayonnaise may be oxidized by radical-oxidation (i.e., upon autoxidation) and/or singlet oxygen-oxidation (e.g., upon light exposure). Analysis of lipid hydroperoxides, such as PCOOH, may lead to elucidation of the cause of lipid peroxidation, as information on the position of the hydroperoxide group provides insight into the processes that initiate lipid peroxidation. An improved understanding of the cause of mayonnaise lipid peroxidation could then lead to ensuring proper maintenance of the quality of the product.

PCOOH (e.g., 16:0/HpODE PC, m/z 790) is generally measured by LC-MS/MS MRM for the transition of the precursor ion to the phosphocholine (m/z 184) product ion. However, MRM (790/184) cannot distinguish hydroperoxide positional isomers (i.e., 16:0/13-HpODE PC or 16:0/9-HpODE PC). In our previous work, we found that sodiated 16:0/HpODE PC (m/z 832) generates unique product ions (i.e., m/z 541 for 16:0/13-HpODE PC and m/z 388 for 16:0/9-HpODE PC), and achieved specific measurement of 16:0/13-HpODE PC (MRM 812/541) and 16:0/9-HpODE PC (MRM 812/388). The m/z 147 (sodiated cyclophosphane) allows for detection of total 16:0/HpODE PC (M RM 812/147), regardless of the hydroperoxide position. Hence, subtraction of the concentrations of 16:0/13-HpODE PC and 16:0/9-HpODE PC (radical oxidation products) from the total 16:0/HpODE PC concentration yields the concentrations of other 16:0/HpODE PC isomers (16:0/12-HpODE PC and 16:0/10-HpODE PC; singlet oxygen-oxidation products) (Fig. 1). In the present study, we analyzed 16:0/13-HpODE PC, 16:0/9-HpODE PC and total 16:0/HpODE PC in commercial mayonnaise using LC-MS/MS, and obtained insight into the mechanism of mayonnaise lipid oxidation.

Firstly, we checked PCOOH recovery, because it has been reported that lipid hydroperoxide is easily decomposed by several factors, such as acids, metal ions, and high temperature. In this study, we spiked standard
PCOOH (16:0/13-HpODE PC and 16:0/9-HpODE PC) into mayonnaise and checked PCOOH recovery. PCOOH recovery of 3 commercial mayonnaise were 16:0/13-HpODE PC, 79.0% ± 11.3 and 16:0/9-HpODE PC, 77.6% ± 12.9 for mayonnaise A; 16:0/13-HpODE PC, 83.0% ± 12.2 and 16:0/9-HpODE PC, 77.0% ± 18.6 for mayonnaise B; 16:0/13-HpODE PC, 60.8% ± 11.7 and 16:0/9-HpODE PC, 61.9% ± 12.8 for mayonnaise C. The slightly low recoveries for mayonnaise C may be accounted for its ingredient (e.g. whole egg- or egg yolk-mayonnaise).

Once extraction recovery was satisfied, PCOOH analysis was carried out with all 3 commercial mayonnaise products (A, B and C) immediately after unsealing. A typical chromatogram of mayonnaise 16:0/HpODE PC is shown in Fig. 2A, which exhibits a clear peak at 17.2 min. For example, for mayonnaise A, the total 16:0/HpODE PC concentration was determined to be 256 ± 12 pmol/g (mean ± SD, n = 3), and the concentration of 16:0/HpODE PC across the different products decreased in the following order: C ≥ A ≥ B (Table 1). The concentration of 16:0/18:2 PC was determined to be 2371 ± 125 nmol/g (mayonnaise A), with the concentration of 16:0/18:2 PC decreasing in the following order: C > A > B (Table 1). From these results, it was estimated that about 0.01 ~ 0.03% of 16:0/18:2 PC had already been oxidized to hydroperoxide prior to opening. To the best of our knowledge, this is the first report demonstrating that oxidized phospholipid was detected in mayonnaise, despite of the freshness.

We then analyzed the 16:0/HpODE PC isomers. 16:0/13-HpODE PC (Fig. 2B) and 16:0/9-HpODE PC (Fig. 2C) were detected at 17.3 min and 17.1 min, respectively, and the concentrations were determined to be 145 ± 60 pmol/g and 142 ± 70 pmol/g, respectively (mayonnaise A). As mentioned above, these results suggest that other isomers (e.g., 16:0/10- and 12-HpODE PC) scarcely exist in mayonnaise. Because of the lack of 16:0/10- and 12-HpODE PC, mayonnaise phospholipid peroxidation is considered to be initiated by radical-oxidation, but not singlet oxygen-oxidation (Fig. 1). Hence, antioxidants such as tocopherol (radical trapping reagent), rather than carotenoids (singlet oxygen trapping reagent), can be more useful to prevent mayonnaise phospholipid oxidation²⁰.

Furthermore, 16:0/13(9)-HpODE PC peaks consist of 16:0/13(9)-hydroperoxy-9Z(10E), 11E(12E)-octadecadienoic acid PC and 16:0/13(9)-hydroperoxy-9E(10E), 11E(12E)-octadecadienoic acid PC. Porter et al. reported that the ratio of 13(9)-hydroperoxy-9Z(10E), 11E(12Z)-octadecadienoic acid to 13(9)-hydroperoxy-9E(10E), 11E(12E)-octadecadienoic acid decreases when linoleic acid is oxidized under high temperature²¹. Hence, in future

| Table 1 Concentrations of mayonnaise 16:0/HpODE PC and 16:0/18:2 PC. |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 16:0/HpODE PC | 16:0/9-HpODE PC | 16:0/13-HpODE PC | 16:0/18:2 PC | 16:0/HpODE PC/PLPC % |
| A | 256 ± 12⁷ | 142 ± 7⁷ | 145 ± 6⁷ | 2371 ± 125⁷ | 0.0108 ± 0.0001⁷ |
| B | 255 ± 3⁷ | 151 ± 4⁷ | 153 ± 9⁷ | 1027 ± 106⁷ | 0.0251 ± 0.0030⁷ |
| C | 479 ± 151⁷ | 250 ± 82⁷ | 260 ± 73⁷ | 3267 ± 162⁷ | 0.0146 ± 0.0042⁷ |

Values are means ± SD (n = 3). Means without a common letter differ, p < 0.05.
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studies, the determination of cis-trans isomers will be helpful for the elucidation of the cause of mayonnaise lipid peroxidation.

The peroxide values (PVs) of the mayonnaises used in this study were 1.03 ± 0.28 (meq/kg), 2.38 ± 0.51, and 0.51 ± 0.20 for A, B and C, respectively. PV did not correlate with the PCOOH concentration. As mayonnaise is primarily composed of vegetable oil\(^1\),\(^2\), mayonnaise PV is predominantly indicative of triacylglycerol (TG) peroxidation, rather than PC peroxidation. The present results suggest two potential hypotheses: (1) PC-TG emulsion structure in mayonnaise affects oxidation susceptibility (2) initial ingredients, such as vegetable oil or egg yolk, had been already oxidized. To further elucidate these hypotheses, TG hydroperoxide (TGOOH) quantification will be required in future trials.

In this study, peroxidized phospholipid (16:0/HpODE PC) was detected in fresh mayonnaise and subsequently analyzed by using LC-MS/MS. Insofar as we know, this is the first report about the analysis of peroxidation product of dietary phospholipid, which provides the insight into dietary phospholipid peroxidation mechanisms. Dietary phospholipid is contained in not only mayonnaise, but also meats or dairy products. It has reported that meat phospholipid oxidation is involved in discoloration or flavor deterioration\(^2\),\(^3\). Taken together, our analytical methods are expected to be useful for the elucidation of the cause of these food phospholipid peroxidation, and the resulting information will be valuable for ensuring maintenance of the product quality.

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