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Glycidyl esters in refined palm (*Elaeis guineensis*) oil and related fractions. Part I: Formation mechanism

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ABSTRACT

Glycidyl esters (GE) are process contaminants generated during the deodorisation step of edible oil refining. In particular, GE are found in high abundance in refined palm oil. Palm oil is unique in that it contains a high amount of diacylglycerols (DAG, 4–12%). In the present study, a series of model reactions mimicking palm oil deodorisation has been conducted with pure tri-, di- and mono-acylglycerols (MAG). Results showed that GE are formed from DAG and MAG, but not from TAG, at temperatures (T) above 200 °C. Our observations suggest that GE are formed predominantly by intramolecular elimination of a fatty acid from DAG. In addition, isomers of GE, formed from DAG heated at $T > 140$ °C, were identified as oxopropyl esters. These new isomers were found to represent approximately 10% of GE levels in refined palm oil. Based on these considerations, the final GE content of palm oil could be limited by reducing DAG levels before oil processing and minimising deodorisation temperatures.

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1. Introduction

Thermal treatment of lipids can trigger the formation of undesired compounds such as *trans* fatty acids (Wolff, 1993), cyclic fatty acid esters (Destaillats & Angers, 2005), or acylglycerol polymers (Beljaars, van Dijk, & Houwen-Claassen, 1994). The two main thermal processes in which oils are submitted to relatively high temperatures ($T > 160$ °C) are deep-frying and deodorisation. Deep-frying results in the formation of thermo-oxidised products and polymers through the reaction of lipids with water, oxygen, and various components of food matrices (Batista & Sánchez-Muniz, 2001). Deodorisation, however, can be considered as a pure thermal treatment of lipids without the addition of other food components. During edible oil refining, deodorisation is typically conducted under vacuum (3–7 mbar) at temperatures ranging from 180 to 270 °C *via* the use of injected stripping steam (Dijkstra & Segers, 2007).

It has been found that chlorination of acylglycerols can occur during deodorisation of edible oils, resulting in the formation of fatty acid esters of 3-chloropropane-1,2-diol [MCPD, (Franke, Strijowski, Fleck, & Pudel, 2009; Zelinková, Svejkovská, Velíšek, & Doležal, 2006)]. More recently, fatty acid esters of glycidol [or glycidyl esters (GE)] have been detected in refined oils (Masukawa et al., 2010; Weissharr & Perz, 2010). It has been observed that both GE and MCPD esters are found in particularly high abundance in refined palm (*Elaeis guineensis*) oil and palm-based fractions (Hrncirik & van Duijn, 2011; Pudel et al., 2011; Weissharr & Perz, 2010). Safety aspects of both GE (as glycidol) and MCPD esters have recently been reviewed (Bakhiya, Abraham, Gürtler, Appel, & Lampen, 2011; Schilter, Scholz, & Seefelder, 2011). Although, data regarding the bioavailability of glycidol when ingested as glycidyl esters are missing, the authors confirmed (as previously reported) that glycidol is considered as a genotoxic carcinogen (Schilter et al., 2011).

Several reports in the literature aim at associating GE formation with the formation of MCPD esters through common intermediates (Hamlet et al., 2011; Rahn & Yaylayan, 2011a, 2011b; Weissharr & Perz, 2010). Currently, effective mitigation routes for GE and MCPD esters, during oil refining, are limited by the available information related to the formation mechanisms of GE and MCPD esters.

The present study aimed at ascertaining the formation mechanism(s) of GE during oil refining. Possible connection of GE with MCPD ester formation was also investigated. For this purpose, a series of model reactions with pure acylglycerols was carried out, mimicking the thermal conditions employed during the deodorisation of palm oil. Reaction products were analysed by liquid chromatography–mass spectrometry and chemical formation pathways have been proposed.

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2. Materials and methods

2.1. Samples and reagents

cis-Trivaccenin, triheptadecanoin, *cis*-divaccenin, diheptadecanoin, monoheptadecanoin, and heneicosanoic acid were obtained from Nu-Chek-Prep (Elysian, MN, USA). Glycidyl-oleate, palmitate, linoleate and linolenate were obtained from Wako Chemicals GmbH (Neuss, Germany). Glycidyl stearate was synthesised and purified according to a published procedure for the synthesis of glycidyl esters [see synthesis of glycidyl palmitate, (Haines et al., 2011)]. Briefly, 1 g of methyl stearate (99% purity; Sigma, St. Louis, MO, USA) was mixed with 0.29 g (R)-(−)-glycidol (97% purity, Sigma) and 0.1 g Lipase Acrylic resin (Novozyme® 435, Sigma) in a 7 ml amber vial. The reaction mixture was heated in an oil bath at 70 °C with a stream of nitrogen and stirred for 24 h. Purification was performed on a silica gel column (400 × 23 mm glass column, filled with glass wool and 45 g of silica gel 60 with a particle size range 0.063–0.2 mm; Merck, Darmstadt, Germany) with 80:20 (v/v) petroleum ether:diethyl ether as eluent. Methyl stearate eluted the column within 60–105 ml elution volumes, whereas glycidyl stearate eluted between 130 and 190 ml. These fractions were pooled and concentrated under vacuum, resulting in 0.25 g of product. Palmitoyl-(Sn1)-monoester and palmitoyl-(Sn1,2)-diester MCPD were obtained from Campro Scientific (Berlin, Germany) and $^{13}C_3$-Sn1-palmitoyl-Sn2-stearyl-MCPD was obtained from Atlanchim Pharma (Nantes, France). Hydroxyacetone, *cis*-vaccenic acid, oleic acid, anhydrous sodium sulphate, and concentrated sulphuric acid were obtained from Sigma. Ultra pure water, methanol, ammonium acetate, and formic acid were obtained from Chemie Brunschwig AG (Basel, Switzerland). For ULC–TOF experiments, solvents used were water, methanol, 2-propanol, acetone, and formic acid, obtained from Merck, and ammonium formate buffer, from Sigma. Commercially available fully-refined palm oil samples were procured from a Nestlé laboratory (NQAC Weiding, Polling, Germany).

2.2. Thermal-reaction experiments

Thermal reaction experiments were conducted in sealed glass ampoules under nitrogen for 1–3 h at temperatures, ranging from 100 to 280 °C in an Agilent 6890 gas-chromatograph oven (Agilent Technologies, Palo Alto, CA, USA). Glass ampoules were fabricated from glass Pasteur pipettes.

2.3. Synthesis of oxo-propyl cis-vaccenate and oleate

Hydroxyacetone acidified with 2% H_2SO_4 was prepared by adding concentrated sulphuric acid (0.5 ml), dropwise under constant stirring, to hydroxyacetone (20 ml) at very low temperature (cold bath with dry ice and acetone). The acidified hydroxyacetone (2 ml) was added to free *cis*-vaccenic or oleic acid (15 mg) and heated at 50 °C for 2 h. After cooling to room temperature, water (4 ml) and *n*-hexane (4 ml) were added sequentially. The mixture was shaken vigorously for 10 s. The organic phase was recovered and dried over anhydrous sodium sulphate.

2.4. Standard solutions

Pure glycidyl palmitate, palmitoyl-(Sn1)-monoester and palmitoyl-(Sn1,2)-diester MCPD at 5 μM concentration in methanol were used as analytical standards to optimise the ULC–MS/MS system. A solution of 200 μg/ml of $^{13}C_3$-Sn1-palmitoyl-Sn2-stearyl-MCPD in methanol: acetone, at 1:4 (v:v), was used as internal standard for measurements in positive-ion mode. A solution of 100 μg/ml of heneicosanoic acid in acetone was used as internal standard for measurements in negative-ion mode. Optimisation of the ULC–TOF MS system was performed by using 200 ng/ml solutions of glycidyl-stearate, oleate, palmitate, linoleate, and linolenate dissolved in acetone. Sample purification was optimised by using palm oil samples spiked with these compounds at 1 μg/g concentration. Quantification was based on constructing calibration curves with palm oil extract samples (see 'sample preparation for analysis of palm oil samples') spiked with the target analytes at 0, 0.5, 1 and 5 μg/g concentrations. The recovery of sample purification was assessed by comparing the amounts of analytes measured after extraction to the original spiked values and was found to be around 100 ± 15 % (data not shown).

2.5. Sample preparation for in vitro experiments

A 20 μl aliquot of sample was diluted in a glass vial along with 975 μl of acetone and 5 μl internal standard solution. Next, 100 μl of this solution were transferred into a new glass vial and 900 μl methanol were added; 25 μl were injected for analysis. For the preparation of heptadecanoic TAG, DAG, and MAG samples, a different dilution was applied, due to the poor solubility of samples. First, an aliquot of 20 μl of sample was diluted in a glass vial, along with 975 μl of acetone:*n*-hexane at 1:1 (v/v) and 5 μl of internal standard solution. Next, 100 μl of this solution were transferred into a new glass vial and 900 μl of acetone were added. Then, 100 μl of this solution were transferred into a new glass vial and 900 μl of methanol were added. For analyses of free fatty acids, a 10 μl aliquot of 100 μg/ml of heneicosanoic acid in acetone was also added. A 25 μl volume was injected for each analysis.

2.6. Sample preparation for analyses of palm oil samples

Oil samples were diluted in *n*-hexane (1 g oil in 10 ml of *n*-hexane) and 2 ml were then added to 1 g of C18 resin (Bakerbond Octadecyl 40 μm Prep LC Packing; J.T. Baker, Mallinckrodt Baker B.V., Deventer, Netherlands) in a beaker. The resin, mixed with sample solution, was dried under a stream of nitrogen and then transferred onto a C18 SPE cartridge (2 g, Bakerbond SPE Octadecyl, J.T. Baker). Glycidyl esters were eluted with 15 ml of methanol. The eluted fraction was dried under nitrogen and reconstituted in 400 μl of acetone. A 1 μl aliquot was injected into the LC–TOF system.

2.7. Ultrahigh performance liquid chromatography–tandem mass spectrometry (ULC–MS/MS)

Relative quantifications of glycidyl esters, free fatty acids, as well as MCPD monoesters and diesters formed in the "*in vitro* experiments", were carried out using a ThermoFisher Accela 1250 liquid chromatograph (ThermoFisher Scientific, Inc., San Jose, CA, USA) coupled to a TSQ Quantum Access Max mass spectrometer (ThermoFisher). A silica-based octadecyl phase (Waters Acquity HSS C18, 1.7 μm; 2.1 × 150 mm) was found adequate for the separation of analytes, using a buffered methanol–isopropanol gradient, as seen in Table 1. A slightly different stationary phase (Waters Atlantis dC18 2.1 × 150 mm) was used for the separation of free fatty acids, with acidified methanol (*i.e.*, with 0.001% formic acid) as solvent A and isopropanol as solvent B; gradient is summarised in Table 2. Electrospray ionisation (ESI) in positive-ion mode, followed by triple quadrupole-based tandem mass spectrometry, was used to detect glycidyl and MCPD esters, whereas negative-ion mode was used to detect free fatty acids. Applied transitions for the selected reaction monitoring (SRM) experiments are given in Table 3. For all transitions, a dwell time of 150 ms and a span

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