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A Study on Triacylglycerol Composition and the Structure of High-Oleic Rapeseed Oil

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ABSTRACT

The composition of fatty acids in triacylglycerides (TAGs) and their position on the glycerol backbone determine the nutritional value of vegetable oil. In this study, gas chromatography and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) were used to analyze the composition and distribution of fatty acids in TAGs of different rapeseed oils. Our results show the content of oleic acid in high-oleic-acid rapeseed oil to be about 80%. In terms of the number of acyl carbon atoms (CN), TAGs with CN52–54 were most abundant, with a maximum concentration at CN54 (80%). The main type of TAG was oleic-oleic-oleic (OOO), accounting for 71.75%, while oleic-oleic-linoleic (OOL) accounted for 7.56%, oleic-oleic-linolenic (OOLn) accounted for 4.81%, and stearic-oleic-oleic (SOO) accounted for 4.74%. Oleic acid in high-oleic-acid rapeseed oil was distributed in the following order of preference: *sn*-2 > *sn*-1/3. In high-erucic-acid rapeseed oil, however, oleic acid was enriched at the *sn*-1/3. These data show that the content of oleic acid can be as high as about 80% in high-oleic-acid material. This finding suggests that high-oleic-acid rapeseed oil has high nutritional value.

1. Introduction

In our increasingly aging society, the prevention of cardiovascular diseases, Alzheimer's disease and other chronic diseases has become a significant issue for physicians worldwide. Voluminous research indicates that lipid nutrition has a significant impact on the development of cardiovascular diseases. Excessive fat intake is considered an important risk factor for cardiovascular diseases. In contrast, the dietary intake of edible oils with suitable structure and function could delay the onset of cardiovascular and other chronic diseases. Fatty acids from different sources have different carbon chain lengths, numbers of double bonds, and double-bond positions, and therefore exhibit different chemical and physical properties. Due to the high content of fatty acids (about 95%) in oils, the composition, saturation, chain length, and double-bond location of the fatty acids in triacylglycerides (TAGs) have been thought to be associated with the nutrition of edible oils for some time [1]. Moreover, because of variable stereospecific distributions, fatty acids sharing the same structure and composition may exhibit different effects on the absorption and metabolism of the TAG [2,3].

With the development of techniques to measure the acylated position of different fatty acids (i.e., the stereospecific number, SN), the position of fatty acids on the glycerol backbone can now be effectively determined [4,5]. Measuring the distribution of acylated fatty acids in a TAG is important for improving the TAG structure, optimizing its physicochemical properties, and enhancing the nutritional value of an edible oil in order to develop more healthy oils for human consumption. Modifying the fatty-acid composition of rapeseed oil is an important goal of rapeseed breeding [6]. With previous success in breeding low-erucic-acid and low-glucosinolate rapeseed cultivars, current research focuses

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on breeding high-oleic cultivars because high-oleic oil can reduce low-density lipoprotein (LDL) and lower cholesterol levels in the blood—effectively reducing the risk of developing cardiovascular diseases, and in some cases, cancer. In addition, vegetable oils with high-oleic-acid content are highly desirable with respect to thermal stability, easy oxidation, and longer shelf life. Our group has successfully developed a rapeseed strain by means of γ-60 ionizing radiation that can be used to produce high-oleic oil (more than 80% oleic acid content) [7–10]. After continuous self-breeding and the selection of agronomic traits, several inbred cultivars with stable characteristics were established. However, the molecular structures of the TAGs and the nutritional quality of these strains have not been studied until now.

2. Materials and methods

2.1. Materials

A total of six types of inbred seeds were included in the study. Samples 1 and 2 contained high oleic acid content (more than 80% oleic acid), samples 3 and 4 had medium oleic acid content (about 60% oleic acid) and low erucic acid content, and samples 5 and 6 had low oleic acid content (about 30% oleic acid) and high erucic acid content. All the seeds were provided by Hunan Agricultural University, China.

2.2. Methods

2.2.1. Extraction of oil from rapeseed

Oil was extracted according to Ref. [4]. In brief, a small quantity of dry rapeseed was homogenized using a microglass pestle to a particle size less than 0.5 mm. Next, the homogenized samples were extracted in a microcentrifuge tube by 2 mL petroleum ether at 45 °C in a water-bath applied with ultrasound for 1.5 h. After centrifugal separation, the supernatants were dried by evaporating the petroleum ether with a gentle stream of nitrogen.

2.2.2. Analysis of composition of fatty acids and total acyl carbon number of triacylglycerides (TAGs)

The methyl esterification method used was the same as that given in Ref. [4], and the analysis of the composition of the fatty acids was done according to ISO standards [4,5], under the category of animal and vegetable fats and oils—that is, analysis by gas chromatography (GC) of the methyl esters of fatty acids. We analyzed the composition of the fatty acids using an Agilent 7890A gas chromatograph (American Agilent Technologies, Columbia, MD, USA), an Agilent HP-FFAP capillary column (30 m × 0.25 mm, 0.25 μm, for the separation and analysis of fatty-acid methyl esters), and a flame ionization detector (FID). The total number of acyl carbons in the fatty acids of the TAGs was analyzed using an Agilent 7890A gas chromatograph, a Varian VF-5ht column (30 m × 0.32 mm, 0.1 μm, Crawford Scientific™ Ltd., Scotland, UK), and an FID.

2.2.3. Analysis of TAGs in rapeseed oil

Qualitative analysis of the TAGs was performed according to Ref. [11]. In brief, the rapeseed oil was dissolved in hexane at about 65 mg·mL^{-1}, and this solution was then diluted with acetonitrile (ACN)/isopropanol containing 0.5% ammonia (7:3 v/v) to 0.65 mg·mL^{-1}. The analysis of the composition of the TAGs in rapeseed oil was performed using electrospray ionization (ESI) tandem mass spectrometry, neutral loss (NL) scan mode, and enhanced product ion (EPI) scan mode with auxiliary judgment. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used for the quantitative analysis of the TAGs. The column was ZORBAX Eclipse Plus C18 (150 mm × 4.6 mm (inner diameter), 5 μm particle size). For the separation of TAGs in the rapeseed oil, the following gradient mode was used: 0–20 min, 70%–30% A; 20–23 min, 30% A; 23–23.1 min, 30%–70% A; and 23.1–28 min, 70% A, where solvent A (100% ACN) and solvent B (0.5% aqueous ammonia in isopropanol) were used as the mobile phase. The flow rate was 1 mL·min^{-1}, the injection volume was 20 μL, and the column temperature was 35 °C. An atmospheric-pressure chemical ionization (APCI), multiple reaction-monitoring (MRM) mode, and C18 column were adopted. The quadrupole linear ion-trap mass spectrometer (API 4000 Q-Trap) was purchased from American AB Sciex (Framingham, MA, USA). An Agilent 1200 series HPLC system was purchased from American Agilent Technologies.

3. Results

3.1. Fatty-acid composition of rapeseed oil

Table 1 presents the composition of fatty acids and their relative percentages in the six tested samples. The results showed the oleic acid content to be 85.31% and 74.67% in samples 1 and 2, respectively, while in samples 3 and 4, the oleic acid content was 60.48% and 64.90%, respectively. The oleic acid content was 23.05% and 24.07% in samples 5 and 6, respectively. The content of erucic acids and eicosenoic acids was rich in samples 5 and 6.

3.2. The number of acyl carbon atoms in the fatty acids of TAGs in rapeseed oil

The total carbon number (CN value) of the acyl chains in the fatty acids of TAGs in the six samples of rapeseed was analyzed using a VF-5ht capillary column with GC-FID detection. The results showed that the CN values of the TAGs in samples 1–4 were 52 and 54, while samples 5 and 6 had a wide range of CN values (from 52 to 62), as shown in Table 2.

Table 1
Composition and percentage of fatty acids in six rapeseed oils after acylation treatment.

Composition	1	2	3	4	5	6
Palmitic acid (C16:0)	3.63%	4.03%	4.40%	5.28%	3.98%	3.68%
Stearic acid (C18:0)	2.32%	2.33%	1.72%	2.61%	1.85%	1.74%
Oleic acid (C18:1)	85.31%	74.67%	60.48%	64.90%	23.05%	24.07%
Linoleic acid (C18:2)	3.41%	11.74%	21.44%	18.91%	14.52%	14.30%
Linolenic acid (C18:3)	4.40%	6.20%	10.72%	8.30%	10.61%	10.24%
Eicosenoic acid (C20:1)	0.94%	1.03%	1.25%	—	15.32%	16.21%
Erucic acid (C22:1)	—	—	—	—	30.66%	29.76%