Conversion of Linoleic Acid to Its Conjugated Isomers in Subcritical Water

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Linoleic acid was heated in the presence or absence of water in the temperature range of 200 to 260°C using a pressure-resistant batch reactor to examine the possibility of its conversion to conjugated isomers. The conversion occurred at very low yields, and the major products were the c9,t11 and t10,c12 conjugated linoleic acids. The t10,c12 isomer seemed to be produced more than the c9,t11, and the ratio of the isomers did not depend on both the temperature and the ratio of linoleic acid to water. When a dilute potassium hydroxide was used as the aqueous solution, the yield of the conjugated isomers was lower than that of the isomers in the mixture of linoleic acid with distilled water.

Key words: conjugated linoleic acid, linoleic acid, subcritical water, isomerization

1. Introduction

Water that maintains its liquid state under a sufficient pressure at temperatures of 100 to 374°C is called subcritical water, and has unique properties. The relative dielectric constant and ion product are lower and higher, respectively, at the higher temperature [1-3]. The high ion product of water indicates the high concentrations of hydrogen and hydroxyl ions, and there is a possibility that the subcritical water acts as acid or base catalyst [4-6]. There are some reports of the hydrolysis and degradation of organic compounds in subcritical water, such as cellulose [7-9], vegetable oil [10], polycyclic aromatic hydrocarbon [11], and saccharides [12, 13] as well as condensation [14, 15]. The isomerizations of monosaccharide [16] and linolenic acid [10] in subcritical water have also been reported. The isomerization of linoleic acid into its conjugated isomers, to which much attention has been paid due to their physiological functionalities [17], has been reported in the absence of water [18].

For better understanding of the catalytic ability of subcritical water, it was determined, in this study, whether the isomerization of linoleic acid to its conjugated isomers occurs in subcritical water or not, and what type of isomer is apt to be formed if the isomerization occurs.

2. Materials and Methods

2.1 Materials

Palmitic acid (> 95%), which is used as the internal standard in gas chromatographic analysis, and linoleic acid (purity > 90%) were purchased from Tokyo Kasei Kogyo, Tokyo, Japan. The trans10, cis12 and cis9, trans11 conjugated linoleic acids, which are abbreviated t10,c12 and c9,t11 CLAs, respectively, and the purities of which were both > 96%, were purchased from the Cayman Chemical Co., Ann Arbor, MI, USA. The (trimethylsilyl)diazomethane solution of 2.0 mol/L in hexane, abbreviated TMSCHN₂, was purchased from Sigma Aldrich Japan, Tokyo.

2.2 Isomerization of linoleic acid in subcritical water

Specified volumes of linoleic acid and distilled water, which had been sonically degassed, were placed in a pressure-resistant vessel made of stainless steel SUS 316 (manufactured by Taiatsu Garasu, Osaka, Japan based on our design; inner volume, 5 mL). The volumes of linoleic acid and water were 0.1 mL and 2.0 mL, 1.0 mL and 1.0 mL, or 2.0 mL and 0.1 mL. The headspace of the vessel was filled with air. Linoleic acid with no water was also tested. The CLAs are produced by the isomerization of linoleic acid in the presence of a dense solution of potassium hydroxide at ca. 180°C [19]. In this study, linoleic acid (1.0 mL) was mixed with a dilute KOH solution (0.01 mol/L, 1.0 mL) and then treated at 200°C. The vessel was set in a GC-17A or GC-7A oven (Shimadzu, Kyoto) regulated at a
temperature from 200 to 320°C. After a specific time elapsed, the vessel was immersed in ice-water to stop the reaction.

The reaction mixture was then transferred to a glass vial. The vessel was rinsed with 10 mL of a chloroform–methanol mixture (2:1 by vol.) and the washing was also placed in the vial. One milliliter of 3 mol/L HCl was added to the vial, and distilled water was further added to make the volume of aqueous phase 3 mL. No water was added when the reaction was carried out using 2 mL of water. After the mixture was vigorously shaken for 30 s, it was centrifuged at 3000 rpm for 5 min. The chloroform phase of the lower layer was added to another small vial, and evaporated under reduced pressure. Fatty acids in the vial were methylesterified with 30 μL of TMSCHN₂, 2.0 mL of benzene and 0.5 mL of methanol. After the solvent was evaporated under reduced pressure, the remainder was dissolved with 0.5 mL of hexane. The solution (1 μL) was applied to a GC-14B gas chromatograph (Shimadzu) with a hydrogen ionization detector to determine the fatty acids in the reaction mixture. The separation column was a DB1 capillary column with a liquid-phase film thickness of 1.0 μm (0.32 mm φ x 30 m; J & W Scientific, Folsom, CA, USA). The injection, column and detector temperatures were 250, 220 and 270°C, respectively. The flow rate of carrier helium gas was 3 mL/min with a sprit ratio of 20:1, and the flow rate of makeup nitrogen gas was 23.5 mL/min. The pressures of the hydrogen gas and air were 55 and 60 kPa, respectively.

3. Results and Discussion

Figures 1 (a) and (b) show the gas chromatograms for the mixture of linoleic acid (0.1 mL) and water (2.0 mL) and for the linoleic acid alone, respectively, treated at 200°C for 12 h. In both cases, small amounts of c9,t11 and t10,c12 CLAs, which were identified by comparing the retention times with those of the reagent CLAs, were formed. The base-line separation of the isomers could not be achieved under our analytical conditions. Because the amounts of the isomers were also small, we could not characterize the products by other instrumental analyses. Although reliability of the identification method may be low, further discussion will be made under the assumption of the identification. The molecular masses of the products were confirmed to coincide with those of the respective CLAs. The amount of c9,t11 CLA was greater than that of t10,c12 CLA.

![Fig. 1] Gas chromatograms of (a) the mixture of linoleic acid and water at the volume ratio of 0.1 to 2.0 and (b) linoleic acid alone, which were treated at 200°C for 12 h.

The effect of temperature on the formation of the CLAs at 12 h was examined for linoleic acid alone and for 0.1 mL of linoleic acid mixed with 2.0 mL of water (Fig. 2). The ordinate indicates the fraction of the sum of c9,t11 and t10, c12 CLAs to the total linoleic acids (C18:2; non-conjugated plus conjugated). More CLAs were formed at the higher
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temperatures. The presence of water did not influence the formation of the CLAs. The inset of Fig. 2 shows the temperature dependence of the ratio of the t10,c12 isomer to the c9,t11 one for the linoleic acids. The temperature scarcely affected the ratio as well as the presence of water, and the ratio was roughly 0.6 at any temperature.

The changes with time in the fraction of the sum of the c9,t11 and t10,c12 CLAs to the total linoleic acids were examined for the linoleic acids mixed with water at various ratios or with 0.01 mol/L KOH (Fig. 3). The isomerization seemed to be a very slow reaction and required a long time to reach equilibrium although the dispersion of the data was significant. The volume ratio of linoleic acid to water scarcely affected the isomerization of the linoleic acid to the CLAs. The yield of the CLAs from linoleic acid mixed with 0.01 mol/L KOH was lower than that of the CLAs from the linoleic acid mixed with or without water although the reason remains unclear.

It has been reported that CLAs of many types are formed through the KOH-catalyzed isomerization of linoleic acid and that the c9,t11 and t10,c12 isomers are the two major components among the CLAs [19]. Under our analytical conditions, only the c9,t11 and t10,c12 isomers could be determined under the assumption that identification by comparing the retention times between the produced and reagent CLAs was reliable. In order to estimate the selectivity during the isomerization, the fraction of the t10,c12 isomer is plotted versus the fraction of the c9,t11 one in Fig. 4 for the results shown in Fig. 3. All the plots lie on a line with a slope of 0.6. This indicates that the c9,t11 and t10,c12 isomers are formed at the molar ratio of 1.0 : 0.6 under the tested conditions. The ratio was identical to the value shown in the inset of Fig. 2. However, the ratio in the KOH-catalyzed isomerization of linoleic acid was estimated to be about 1 : 0 : 1.15 at 180°C from the HPLC chromatogram in the literature [19]. Although the reason for the discrepancy still remains unclear, a possible reason is that the equilibrium was not attained because of the extremely slow progress of the isomerization in our study.

References


