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Adsorption of Fatty Acid Methyl Ester Derived from Squid Liver Lipid onto Silica Gel Adsorbent

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Abstract: The purpose of this study is the effective utilization of fisheries waste, squid liver, as a raw material of biodiesel. To obtain biodiesel from squid liver, extracted fatty acids are esterified with methyl alcohol. As the product of the esterification contains many by-products, the target product, fatty acid methyl ester of squid liver (SFAME), must be recovered from the products. SFAME is divided into three groups, which are saturated (SF), monounsaturated (MF), and polyunsaturated (PF) fatty acid methyl esters (FAMEs), based on the number of double bonds. In this study, the recovery of SFAME from the product of esterification through adsorption (i.e., dry washing) was investigated. Especially, the effect of solvents, toluene, and methyl alcohol on the recovery efficiency of SFAME using silica gel as an adsorbent was the focus. The competitive adsorption model successfully explained the present adsorption system, and the equilibrium adsorption constants and the saturated adsorption density could be determined by the model analysis. The equilibrium adsorption constant of PF was the largest among the SFAME (PF > MF > SF), and this order could correspond to the values of their dielectric constants. Methanol greatly affected the adsorption behavior of SFAME due to the fact of its hydrophilicity.

Keywords: fatty acid methyl ester; silica gel; biodiesel; squid liver; adsorption

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1. Introduction

The recent Sustainable Development Goals (SDGs) movement is interesting for several reasons, including the requirement of reproductive energy, the proceeding carbon neutrality, and the effective utilization of unused biomass. To address these issues, over the past decade, the utilization and production of biodiesel have been the focus of great interest. At first, edible oil was employed for biodiesel production; however, it gradually shifted to nonedible oil, waste cooking oil, and oils derived from terrestrial or aquatic plants and animals [1]. For the effective utilization of bioresources, we have been proceeding with the use of fisheries waste for biodiesel [2]. Biodiesel is chemically a fatty acid methyl ester (FAME), which can be obtained from the transesterification reaction of triglyceride (TG). Many types of lipid resources have been used and produced to make biodiesel. Among them, one of the most important resources is recognized as being vegetable oils, such as canola oil, rapeseed oil, palm oil, and olive oil. For biodiesel, they are mainly from waste oil used in frying cooking oil.

On the other hand, fisheries wastes, that is, the viscera of fishes or shells, have been known to contain proteins and lipids (TG). TGs derived from squid liver contain, particularly, docosahexaenoic acid (C22:6, DHA) and eicosapentaenoic acid (C20:5, EPA) [3], which are well known to be health-promoting substances, leading to their use in pharmaceuticals and health supplements. Other fatty acids, which are less available, can be used as a source of biodiesel if the EPA and DHA can be separated from them using several methods. However, fatty acids from fish waste have rarely been used as a source of biodiesel fuel. Little

research on this has been published over the last two decades. Although there have not been many attempts to use marine wastes as biodiesel feedstock, we developed a model [4] for the distribution of FAME in the solid phase (urea–FAME complex) using the urea inclusion method, which has long been used for the separation of FAME, and, in particular, we also developed a model for the distribution of FAME in the solid phase (urea–FAME complex) and divided FAMEs into three major categories (saturated, monounsaturated, and polyunsaturated) for modeling.

Recently, the wet washing method has been widely used as a purification method for biodiesel [5]. This method can process large quantities of crude biodiesel, which is one of its advantages, but it has several disadvantages, such as an increased cost and the generation of high effluents, including a high chemical oxygen demand (COD). On the other hand, dry washing with adsorption has also been attempted for the removal of contaminants and recovery of biodiesel (i.e., fatty acid alkyl esters).

Adsorptive separation is well known and has been used in various processes to remove/recover hazardous/available substances, etc. There are reports on the use of adsorbents such as the chemical products of adsorbents [6,7], biomaterial adsorbents [7], and activated carbon [8]. However, from the viewpoint of biodiesel fuel recovery, research on the adsorption behavior and properties of fatty acid alkyl ester on adsorbents has rarely been reported. The clarification of the adsorption behavior and properties is important for the design and operation of the separation apparatus from practical and chemical engineering aspects.

In addition, the extracted lipids are not always soluble in all types of organic solvents, so the difference in polarity between the lipids (TG) and these organic solvents causes the lipid oil droplets to be dispersed in the organic solvent. To solve this problem, a relatively highly polar solvent, such as toluene or benzene, must be added to the system. Lipids can be dissolved in the organic solvent to enhance the esterification reaction rate. When using the adsorption method (i.e., dry washing method) to recover FAME, it is necessary to understand the effect of these coexisting substances on the adsorption properties of the target material, i.e., FAME (i.e., biodiesel).

In a previous study [9], we investigated the influences of coexisting substances (methanol and toluene) on the adsorption behavior of methyl palmitate on silica gel and proposed a competitive adsorption model based on Langmuir-type adsorption equilibrium. The proposed competitive adsorption model, which is also treated in this study, is very different from the conventional competitive adsorption model for more than two adsorption target substances that are solutes. Most of the previous competitive adsorption models are based on and extend the Langmuir type to systems with more than two components, and many studies have dealt with metal ions and dyes as adsorption target substances [10–12]. The proposed competitive adsorption model differs from other competitive adsorption models in that it treats the adsorbate as a solute and the solvent as its medium. Such a treatment has rarely been used in the treatment of both aqueous adsorption and nonaqueous adsorption systems. It is well known that lipids derived from fishery products should contain many polyunsaturated fatty acids (PUFAs). The adsorbent used in this study was silica gel, which is commonly used for general analytical pretreatments and is relatively inexpensive and readily available. In previous studies, a molecular sieve 13X [6,8], an activated carbon from macaúba fruit kernel [8], and rice hull ash [7] have been used as adsorbents for FAME adsorption; in addition, silver-exchanged zeolite Y has been used for the separation of cis and trans geometric isomers of mono- and polyunsaturated fatty acid methyl esters [13].

For dry washing with silica gel, it is important to know the adsorption behavior and properties of these polyunsaturated fatty acid methyl esters (PUFAMEs) for dry washing with silica gel and the influence of the coexisting organic solvents on the adsorption behavior and properties of the PUFAMEs for dry washing with silica gel. For this purpose, it is necessary to verify whether the proposed competitive adsorption model can be extended to multicomponent systems. The FAME obtained from TG derived from natural products is

expected to be complicated due to the fact of its wide variety of components. For this reason, we decided to treat FAME derived from squid liver in the same way as in the modeling of FAME derived from fishery wastes using the urea inclusion method, i.e., by dividing FAME into three major categories: saturated, monounsaturated, and polyunsaturated. In this study, based on the obtained experimental results, we discuss the influence of coexisting substances on the adsorption behavior of FAME-derived squid liver by applying them to the proposed competitive adsorption model.

2. Materials and Methods

2.1. Materials

2.1.1. Japanese Squids (*Todarodes pacificus*)

Japanese squids (*Todarodes pacificus*) were captured at Tsugaru Strait near Hakodate Bay (Hokkaido Prefecture, Hakodate, Japan). The squid liver was separated from the body as soon as possible and stocked in a refrigerator (−20 °C).

2.1.2. Adsorbent

As an adsorbent, silica particles (silica gel 60, Kanto Chemical Co. Inc., Tokyo, Japan) were used in this study without further modification.

2.1.3. Reagents

Methyl alcohol (99.7%), toluene (99.5%), and chloroform (99.0%) were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). Hydrogen chloride methanol (10 wt.%) solution and hexane (96.0%) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). Methyl tricosanoate (99.9%) was purchased from AccuStandard (New Haven, CT, USA). They were used without further purification.

2.2. Preparation of Fatty Acid Methyl Ester

The liver was taken out and chopped using a homogenizer. The lipid was extracted using the Bligh and Layer method [14]. Forty grams of squid liver were weighed and homogenized in a homogenizer for 5 s. Eighty milliliters of methanol (99.7%) and forty milliliters of chloroform (99.0%) were added and homogenized for another 1 min. Then, 40 mL of chloroform was added and homogenized with a mixer for 30 s. Forty milliliters of distilled water were added and homogenized for another 15 s. The mixture was filtered under reduced pressure (quantitative filter paper 5C) using a Buchner funnel and a suction bottle. After allowing the filtrate to stand overnight in a 500 mL separating funnel, the lower chloroform layer was separated into a 500 mL beaker; then, nitrogen gas was blown in to evaporate the chloroform, and the mass of the lipid was weighed on an electronic balance. A portion of the lipid was placed in a test tube with a lid, and the air in the tube was replaced with nitrogen before storage in a refrigerator (−20 °C).

A certain amount of lipid was set in a 100 mL bottle with a lid. The desired amounts of toluene and lipid were added to the bottle, and it was shaken to dissolve the lipid in toluene. Then, a certain amount of hydrogen chloride methanol (10 wt.%) solution was added as a liquid catalyst of esterification with methanol. The bottle was placed in a water bath and heated at 90 °C for 120 min. After natural air-cooling, fatty acid methyl esters were collected by liquid extraction using hexane and stocked in a refrigerator (−20 °C).

2.3. Procedure of the Adsorption Experiment

All adsorption experiments in this study were conducted with a batch operation, and the manner was almost the same as the procedure us in the previous study [9]. Silica gel powder was washed with distilled water and dried at 180 °C for two hours in an incubator. After cooling, it was stocked in a desiccator. A certain amount of silica gel powder was set in a 100 mL Erlenmeyer flask. By pouring a small amount of hexane into the flask, the adsorbent was soaked in hexane and deaerated by decompression using an aspirator. A mixture of SFAME, methanol, and toluene was prepared in another glass vessel. The

adsorption experiment was started by adding the desired amount of the mixed solution to the flask. In most of the experiments, the final volume of the adsorption system was 49.5 mL. It was stirred with a magnetic stirrer for the time necessary to attain the adsorption equilibrium. Then, the silica gel powder was separated from the liquid phase by filtration with a membrane filter with an average pore size of 3 μm. The determination of the toluene, methanol, and methyl palmitate was conducted with ultraviolet spectrometry [15] (Jasco V-630, Tokyo, Japan), colorimetric spectrometry [16,17], and gas chromatography (Shimadzu GC-2014, Kyoto, Japan), respectively. The determination of the FAMEs was conducted using gas chromatography (Shimadzu GC-2014, Kyoto, Japan) with an internal standard method using methyl tricosanoate (C23:0). The measurement conditions of the gas chromatography were as follows: column—fused silica capillary column, Omegawax320 (Supelco, St. Louis, MO, USA, 30 m × 0.32 mm i.d., film thickness—0.25 μm); carrier gas—helium, 25 cm/s; detector—FID, 260 °C; injection—spilt 100:1, 1.0 mL, and 250 °C; and column temperature (oven)—140 °C (5 min) → 4 °C/min → 200 °C (50 min). The adsorbed amount of each substance onto the silica surface was determined from the difference between the concentrations in the initial and equilibrium states. All data for the evaluations were obtained in duplicate (n = 2), and the average values are shown in the figures.

3. Results and Discussion

3.1. Composition of Fatty Acid Methyl Ester Derived from Squid Liver

After esterification, the composition of the SFAME was measured by gas chromatography. The result is shown in Table 1. Palmitic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid were found to be the main components, which is the most similar composition reported in the literature [3]. The average molecular weight (*Mwa*) of the SFAME used in this study was determined based on the results (Table 1) of the following comprehensive discussion.

$$\text{Mwa} = \sum \left[\left(\frac{\text{each composition } [\%]}{100} \right) \times \text{ each molecular weight} \right] \quad (1)$$

The value of *Mwa* was calculated as 293.52. In the following sections, this value is used, and the concentration of the SFAME is expressed as a molar concentration.

Damasceno et al. (2018) prepared FAMEs from commercially available macauba kernel oil for adsorption experiments and reported their composition in detail as follows: laurate (C12:0), methyl myristate (C14:0), methyl palmitate (C16:0), methyl staerate (C18:0), methyl oleate (C18:1), and methyl linoleate (C18:2) [8]. The composition is based on (1). According to this composition, the average molecular weight (*Mwa*) was calculated to be 245.1 using Equation (1). The FAMEs they used have relatively low carbon numbers, and there are few monounsaturated and polyunsaturated FAMEs, resulting in a slightly smaller average molecular weight than our value.

3.2. Influence of Coexisting Substances on the Adsorption of SFAME

The adsorption experiment was conducted at the same initial concentration (mol/L) ratio of methanol (ME), toluene (TL), and SFAME. In a previous study [9], glycerin was not affected by the amount of adsorbed ME, TL, and methyl palmitate (MP); therefore, glycerin was not added to the present adsorption system. Figure 1 shows the adsorption isotherm of ME, TL, and SFAME. The solid symbols correspond to the amount of adsorbed ME, TL, and MP, as reported in the previous study [9]. The amount of adsorbed ME and TL in this study was mostly the same as that reported in the previous study. Note that the relationships between the equilibrium concentration and equilibrium adsorbed amount are quite different for SFAME, TL, and ME. For example, the equilibrium amounts of the adsorbed SFAME and TL were approximately 1.3 mmol/g and 0.1 mmol/g at an equilibrium concentration of approximately 0.1 mol/L, respectively. However, ME had an adsorbed equilibrium amount of approximately 4.5 mmol/g at an equilibrium

concentration of 0.05 mol/L. This fact indicates that the binding strength of ME to silica gel is much higher than that of SFAME and TL. However, the amount of adsorbed SFAME was larger than that of MP. In the equilibrium concentration range of 0.1–0.3, the amount of adsorbed SFAME was mostly approximately 1.4-fold larger than that of MP. MP is saturated fatty acid methyl ester; however, SFAME consists of not only the methyl ester of saturated fatty acids but also the methyl ester of monounsaturated and polyunsaturated fatty acids. This is discussed later; however, the fact that SFAME contains not only SF but also MF and PF is related to the larger adsorbed amount compared to MP. The polarity of the molecule itself should have a significant effect on this phenomenon.

Table 1. The contents of fatty acids derived from squid liver in this study.

Fatty Acid Methyl Ester	M.W. [g/mol]	Content [%]
Myristic Acid (C14:0)	228.38	5.07
Myristoleic Acid (C14:1n5)	242.40	0.17
Pentadecanoic Acid (C15:0)	240.40	0.22
cis-10-Pentadecanoic Acid (C15:1n5)	256.43	0.48
Palmitic Acid (C16:0)	254.41	18.53
Palmitoleic Acid (C16:1n7)	270.46	5.74
Heptadecanoic Acid (C17:0)	268.43	0.67
cis-10-Heptadecanoic Acid (C17:1n-7)	284.48	0.92
Stearic Acid (C18:0)	282.46	3.55
Oleic Acid (C18:1n9c)	298.51	10.87
cis-11-Octadecenoic Acid (C18:1n7c)	296.50	4.77
Linoleic Acid (C18:2n6c)	296.50	1.02
Linolenic Acid (C18:3n3)	294.48	0.59
cis-6,9,12,15-Octadecatetraenoic Acid (C18:4n3)	292.46	0.95
cis-9-Eicosenoic Acid (C20:1n11)	276.41	5.66
cis-11-Eicosenoic Acid (C20:1n9)	324.54	3.45
cis-11,14-Eicosenoic Acid (C20:2n6)	324.54	0.37
Arachidonic Acid (C20:4n6)	322.53	2.10
cis-11,14,17-Eicosadienoic Acid (C20:3n3)	318.50	0.52
cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3)	322.53	11.60
cis-11-Docasenoic Acid (C22:1n11,13)	316.48	4.58
Behenic Acid (C22:0)	352.60	0.86
cis-13,16-Docosadienoic Acid (C22:2n6)	354.62	0.00
cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3)	342.51	12.65

The overall amount adsorbed of the FAMEs increased up to an equilibrium concentration of approximately 0.1 mol/L and decreased at a higher equilibrium concentration range; in other words, the maximum amount of FAME adsorption was observed at approximately an equilibrium concentration of 0.1 mol/L. This trend was also observed in the single methyl palmitate system described in the previous paper, and it was found that a similar trend was observed. Adsorption equilibrium relationships with maxima in the adsorption isotherm may seem strange at first glance, but they are often reported in systems with more than two adsorbents and adsorbates. The following examples have been reported: activated carbon and a binary system of reactive yellow and reactive black dyes [18]; acid soil and a binary system of Cu^{2+}/Zn^{2+} [10]; mesoporous silica compound [19] (MCM-41 and AlMCM-41) and a binary system of methylene blue and rhodamine B dyes [20]; goethite and gibbsite and two binary systems of phosphate/arsenate and phosphate/selenite [11]; multiwalled carbon nanotubes and a $Pb^{2+}/Cu^{2+}/Cd^{2+}$ systems [21]; iminodiacetic acid (IDA) chelating resin and $Pb^{2+}/Cu^{2+}/Cd^{2+}$ systems [22]; and rice husk ash and an aqueous binary system of Cd^{2+}/Ni^{2+} [23]. Moreover, the following results have been reported in the literature regarding the adsorption kinetics process that was observed to show not only an adsorption equilibrium relationship but also the maximum adsorption amount in the adsorption kinetics process, leading to equilibrium, for example, a cation exchange resin (IRN-77) and a binary system of Co^{2+}/Cr^{3+} and a ternary system of $Co^{2+}/Ni^{2+}/Cr^{3+}$ [24]; iminodiacetic acid (IDA) chelating resin and a binary system of Pb^{2+}/Cu^{2+} (at an initial

concentration of 2 mmol/L) [22]. As described above, many adsorption isotherms with maxima have been reported for divalent metal ion adsorption systems. We considered that methanol has a stronger binding force with silica gel. The extremely high abundance of methanol in the system and its strong binding strength are thought to be responsible for the change in the adsorption isotherm of SFAME, which has a maximum value.

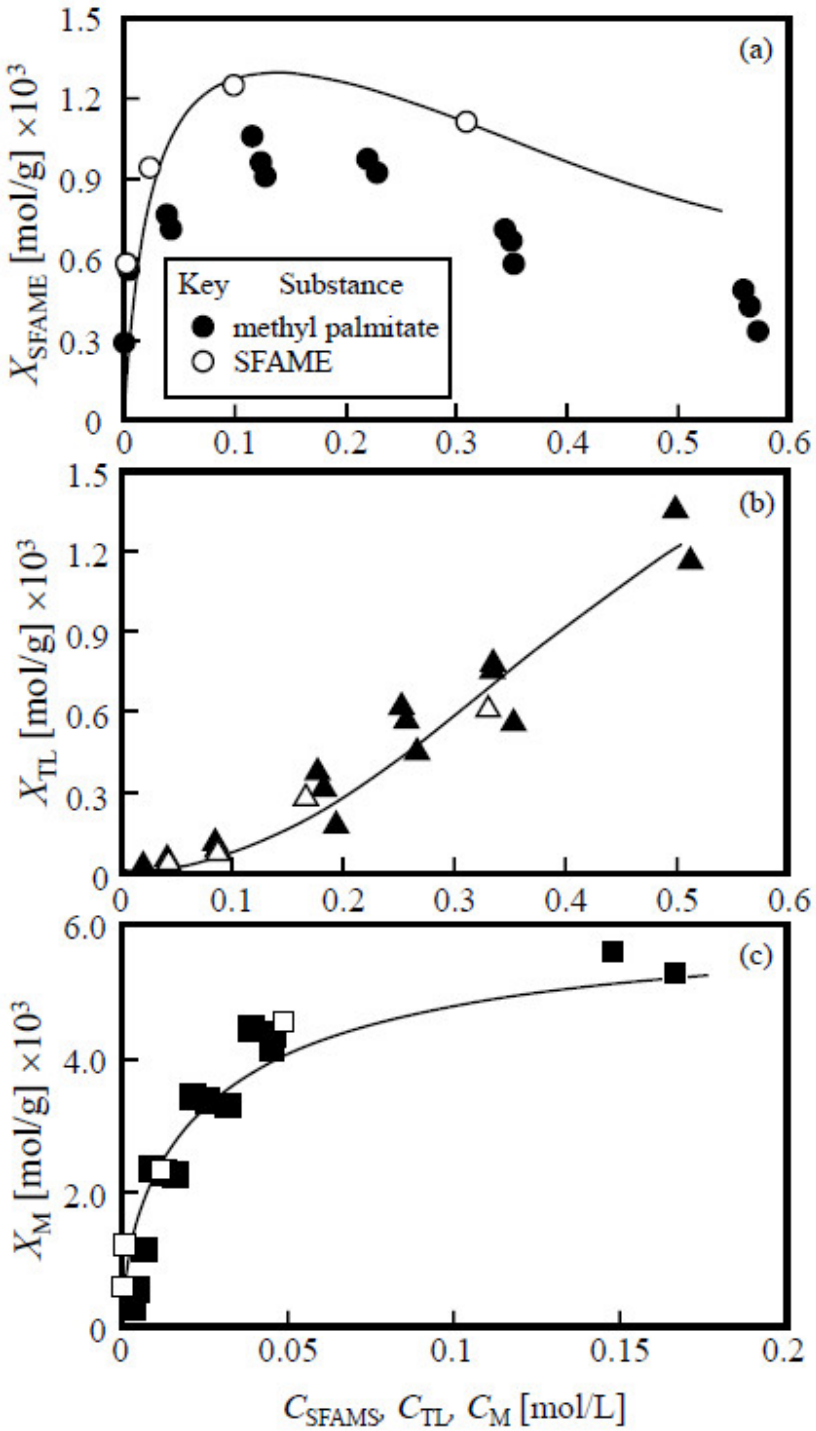

Figure 1. The adsorption isotherms of (**a**) SFAME, (**b**) TL, and (**c**) ME. The solid symbols correspond to the previous data of the methyl palmitate/methanol/toluene system [6]. The solid lines are values calculated using Equations (2)–(4) and the parameters in Table 2.

Üstün (1996) conducted adsorption experiments on FAME derived from tall oil using the molecular sieve X13 as an adsorbent [6]. Nine solvents (methanol, ethanol, isopropanol, petroleum ether, petroleum naphtha, hexane, isooctane, acetone, and benzene) were used to dissolve the FAME. The results report that the equilibrium amounts of the adsorbed FAME at the equilibrium concentrations in the neighborhood range for each solvent were mainly 345 g/g-X13 for methanol (76.9 g/L); 331 g/g-X13 for ethanol (84.4 g/L); 240 g/g-X13 for isopropanol (77.7 g/L); 170 g/g-X13 for acetone (81.1 g/L); 108 g/g-X13 for isooctane (79.5 g/L); and 101 g/g-X13 for hexane (85.7 g/L). Özgül-Yücel and and Türkay (2003) conducted FAME (crude methyl ester derived from rice bran) adsorption experiments using rice hull ash and silica gel as adsorbents to purify the crude FAME (removal of FFA (free fatty acid)) [7]. The adsorption experiments were performed at 25 °C in 50 mL of hexane in a batch system. The FFA equilibrium concentration and the equilibrium amount of the adsorbed FFA were in the range of approximately 0.2–1.6 g/L and 5–45 mg/g-silica, respectively. The amount of adsorbed FFA onto the silica gel was higher than that onto the rice hull ash. They reported that the addition of too many adsorbents (rice hull ash and silica gel) may increase the amount of adsorbed FAME itself and that the adsorption equilibrium relationship between adsorbent (rice hull ash and silica gel) FFAs was expressed by the Freundlich adsorption isotherm.

Our results show that the maximum adsorption volume adsorbed in the SFAME experiment was approximately 1.3×10^{-3} mol/g (0.382 g/g-silica) at an equilibrium concentration of approximately 0.1 mol/L (29.4 g/L), which is a very small adsorption volume compared to Ustun's results with the FFA adsorption reported by Özgül-Yücel and Türkay (2003), which is not a FAME, and the maximum adsorption value obtained in their experiments was 0.045 g/g-silica [7], which is smaller than our adsorbed amount. However, the equilibrium concentration shown in this study was quite larger than that reported by Özgül-Yücel and Türkay (2003), suggesting the great influence of the presence of methanol.

The competitive adsorption model was applied to the present results. The adsorption model is based on the following two reactions: (1) competitive adsorption between MP and FAME onto the hydrogen atom of the silanol functional group on the silica surface; (2) competitive adsorption between FAME and TL onto the methyl functional group of the methanol adsorbed on the silanol group on the silica surface. Finally, each adsorbed amount, X, is represented as follows.

$$X_{\mathrm{SFAME}} = \frac{X_{\mathrm{S}} K_{\mathrm{SFAME1}} C_{\mathrm{SFAME}}}{1 + K_{\mathrm{SFAME1}} C_{\mathrm{SFAME}} + K_{\mathrm{M1}} C_{\mathrm{M}}} + \frac{X_{\mathrm{M}} K_{\mathrm{SFAME2}} C_{\mathrm{SFAME}}}{1 + K_{\mathrm{SFAME2}} C_{\mathrm{SFAME}} + K_{\mathrm{TL}} C_{\mathrm{TL}}} \quad (2)$$

$$X_{\mathrm{M}} = \frac{X_{\mathrm{S}} K_{\mathrm{M1}} C_{\mathrm{M}}}{1 + K_{\mathrm{SFAME1}} C_{\mathrm{SFAME}} + K_{\mathrm{M1}} C_{\mathrm{M}}} + \frac{X_{\mathrm{S}} K_{\mathrm{M2}} C_{\mathrm{M}}}{1 + K_{\mathrm{M2}} C_{\mathrm{M}}} \quad (3)$$

$$X_{\mathrm{TL}} = \frac{X_{\mathrm{M}} K_{\mathrm{TL}} C_{\mathrm{TL}}}{1 + K_{\mathrm{SFAME2}} C_{\mathrm{SFAME}} + K_{\mathrm{TL}} C_{\mathrm{TL}}} \quad (4)$$

where X_{SFAME} is the overall amount of adsorbed SFAME; X_{M} and X_{TL} are the amounts of adsorbed methanol and toluene, respectively; X_{S} represents the total number of adsorption sites existing on the silica surface, which correspond to all silanol groups on the silica surface; K_{SFAME1} and K_{SFAME2} are the overall equilibrium adsorption constants of SFAME with sites 1 and 2; K_{M1} and K_{M2} are the equilibrium adsorption constants of ME with sites 1 and 2, respectively; K_{TL} is the equilibrium adsorption constant of TL; C represents the equilibrium concentration of each adsorbate. As for SFAME and TL, the maximum adsorbed amounts of SFAME and TL are regarded as the same as the amount of adsorbed ME. By fitting the data to Equations (2)–(4) using a least squares regression, the equilibrium adsorption constant and the saturated adsorption density were determined. The estimated adsorption parameters are listed in Table 2. The calculated lines using these estimated adsorption parameters are shown as the solid lines in Figure 1. As seen in Figure 1, good agreements between the experimental data and the calculated values are confirmed. In

comparison with the previously determined adsorption parameters of the MP-ME-TL system, K_1 for SFAME was 2.4-fold larger than K_1 for PM.

Table 2. Estimated adsorption equilibrium constants.

K_{SFAME1} [L/mol]	K_{SFAME2} [L/mol]	K_{M1} [L/mol]	K_{M2} [L/mol]	K_{TL} [L/mol]	X_S [mmol/g]
21.9	0.01	212	38.7	0.61	3.24

3.3. Influence of the Degree of Unsaturation of SFAME on Adsorption

In a previous study, we pointed out that the difference in the amount of adsorbed ME, TL, and MP is strongly related to their dielectric constant [25]. The silanol group on the silica surface rather strongly binds the substances that have a larger dielectric constant. In Table 3, the dielectric constants of octadecanoic fatty acids [25] are listed. It is found that the value of the dielectric constant becomes larger with an increase in the number of the double bonds of the adsorbates. The reason why the amount of adsorbed SFAME became larger than that of MP was considered to be that the existence of unsaturated fatty acid methyl esters contribute to an increase in the amount of adsorbed SFAME. We divided SFAME into three groups: saturated (SF), monounsaturated (MF), and polyunsaturated (PF) FAME. The three categorized groups are summarized in Table 4. Similar to the case of MP or to the overall SFAME, each categorized SF, MF, and PF could be adsorbed competitively onto ME and TL. For each amount of adsorbed SF, MF, and PF, Equation (2) can be extended for the three categorized SFAMEs as follows.

$$X_{SF} = \frac{X_S K_{SF1} C_{SF}}{1 + K_{SF1}C_{SF} + K_{MF1}C_{MF} + K_{PF1}C_{PF} + K_{M1}C_M} + \frac{X_M K_{SF2} C_{SF}}{1 + K_{SF2}C_{SF} + K_{MF2}C_{MF} + K_{PF2}C_{PF} + K_{TL}C_{TL}} \quad (5)$$

$$X_{MF} = \frac{X_S K_{MF1} C_{MF}}{1 + K_{SF1}C_{SF} + K_{MF1}C_{MF} + K_{PF1}C_{PF} + K_{M1}C_M} + \frac{X_M K_{MF2} C_{MF}}{1 + K_{SF2}C_{SF} + K_{MF2}C_{MF} + K_{PF2}C_{PF} + K_{TL}C_{TL}} \quad (6)$$

$$X_{PF} = \frac{X_S K_{PF1} C_{PF}}{1 + K_{SF1}C_{SF} + K_{MF1}C_{MF} + K_{PF1}C_{PF} + K_{M1}C_M} + \frac{X_M K_{PF2} C_{PF}}{1 + K_{SF2}C_{SF} + K_{MF2}C_{MF} + K_{PF2}C_{PF} + K_{TL}C_{TL}} \quad (7)$$

$$X_M = \frac{X_S K_{M1} C_M}{1 + K_{SF1}C_{SF} + K_{MF1}C_{MF} + K_{PF1}C_{PF} + K_{M1}C_M} + \frac{X_S K_{M2} C_M}{1 + K_{M2}C_M} \quad (8)$$

$$X_{TL} = \frac{X_M K_{TL} C_{TL}}{1 + K_{SF2}C_{SF} + K_{MF2}C_{MF} + K_{PF2}C_{PF} + K_{TL}C_{TL}} \quad (9)$$

Table 3. Dielectric constant * of octadecanoic fatty acids.

C18:0	C18:1	C18:2	C18:3	
2.3	2.5	2.7	2.9	F/m

* Ref. [25].

The data shown in Figure 1 were applied to Equations (5)–(9), and using a least squares regression, the adsorption parameters could be determined. The values of the parameters are summarized in Table 5. Figure 2 shows the fitting of the data of Figure 1 to Equations (5)–(9). The solid, dashed, and dotted lines are the calculated values using the adsorption parameters in Table 5. As for ME and TL, they seems to be almost the same as in Figure 1b,c; in addition, they show good agreement between the experimental and calculated values. As for the SFAME, the amount of adsorbed PM was larger than that of SF and MF. In comparison to these values, K_{PF1} and K_{PF2} were 2.3- and 3.1-fold larger than K_{SF1} and K_{SF2}. The value of X_S was almost the same in the case of the overall SFAME adsorption. In this case of K_{M1} and K_{M2}, they were 1.4-fold larger than the values in the

case of the overall SFAME adsorption. The degree of unsaturation, that is, the number of double bonds between carbons, of the FAMEs was found to significantly affect their adsorption behavior on the silica surface.

Table 4. The categories of fatty acid methyl esters derived from squid liver in this study.

	Fatty Acid Methyl Ester
Saturated FAME	Myristic Acid (C14:0) Pentadecanoic Acid (C15:0) Palmitic Acid (C16:0) Heptadecanoic Acid (C17:0) Stearic Acid (C18:0) Behenic Acid (C22:0)
Monounsaturated FAME	Myristoleic Acid (C14:1n5) cis-10-Pentadecanoic Acid (C15:1n5) Palmitoleic Acid (C16:1n7) cis-10-Heptadecanoic Acid (C17:1n-7) Oleic Acid (C18:1n9c) cis-11-Octadecenoic Acid (C18:1n7c) cis-9-Eicosenoic Acid (C20:1n11) cis-11-Eicosenoic Acid (C20:1n9) cis-11-Docasenoic Acid (C22:1n11,13)
Polyunsaturated FAME	Linoleic Acid (C18:2n6c) Linolenic Acid (C18:3n3) cis-6,9,12,15-Octadecatetraenoic Acid (C18:4n3) cis-11,14-Eicosenoic Acid (C20:2n6) Arachidonic Acid (C20:4n6) cis-11,14,17-Eicosadienoic Acid (C20:3n3) cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3) cis-13,16-Docosadienoic Acid (C22:2n6) cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3)

Table 5. Estimated adsorption equilibrium constants for the categorized FAMEs and the saturated adsorption density.

K_{SF1} [L/mol]	K_{SF2} [L/mol]	K_{MF1} [L/mol]	K_{MF2} [L/mol]	K_{PF1} [L/mol]	K_{PF2} [L/mol]	K_{M1} [L/mol]	K_{M2} [L/mol]	K_{TL} [L/mol]	X_S [mmol/g]
35.4	0.072	38.6	0.198	82.0	0.230	293.0	56.0	0.548	3.14

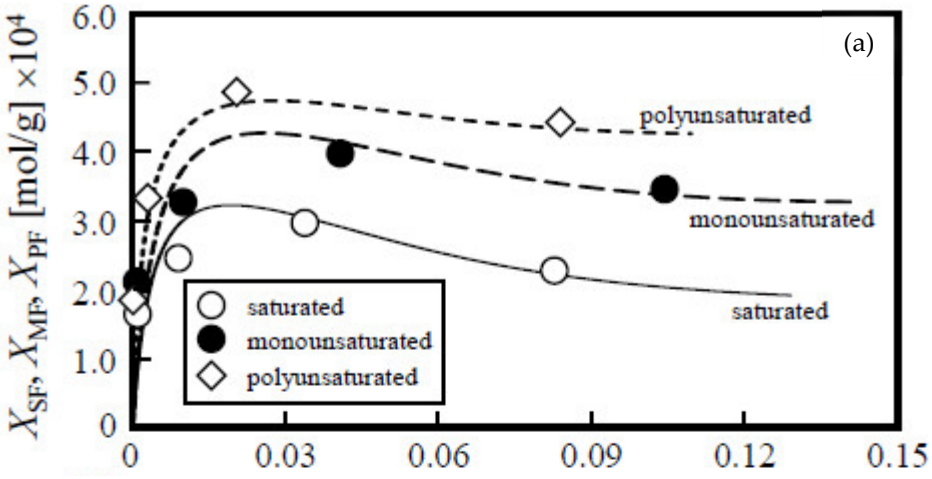

Figure 2. *Cont.*

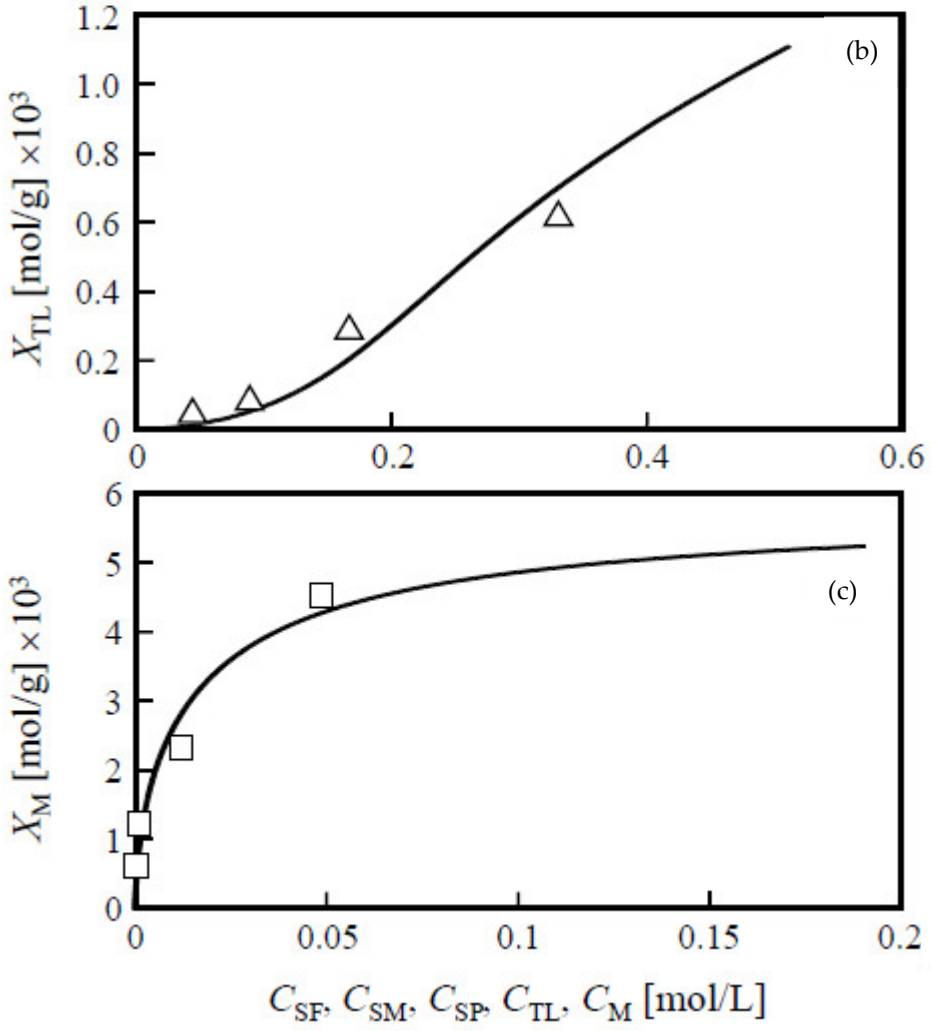

Figure 2. The adsorption isotherms: (**a**) SF, MF, and PF; (**b**) TL; (**c**) ME. The solid lines are values calculated using Equations (5)–(9) and the parameters in Table 5.

4. Conclusions

The influence of coexisting substances on the adsorption behavior of FAME derived from squid liver, as a model substance of biodiesel fuel, onto a silica surface in the presence of methanol and toluene was investigated, assuming the adsorption of diesel fuel from the solution after the reaction in the biodiesel production process.

The overall and categorized FAME adsorption behaviors could be explained well by the competitive adsorption model based on Langmuir-type adsorption. The equilibrium adsorption constant and the saturated adsorption density were determined by fitting the data to the model equations using a least squares regression. The equilibrium constants of K_1 and K_2 for PF were larger than those of SF and MF, which correspond to 2.3- and 3.2-fold larger than that of PF. The occupied surface area of a single silanol group estimated from X_S was mostly the same as the values reported in the literature. It was found that the presence of the double bond of carbon molecules in the molecular structure significantly affected the adsorption behavior onto the silica surface.

In a further study, for the confirmation of the applicability of this model, we will conduct adsorption experiments using samples with different FAME compositions and investigate the influence of the application of the proposed model on the estimation of the adsorption parameters, especially the adsorption equilibrium constants. In particular, when considering three categories, as in the present study, it is desirable to use lipids extracted from other organisms, such as scallop midgut or fish viscera, and when confirming whether

the adsorption behavior of the individual FAMEs is compatible with the present proposed model in detail, a model system in which reagents are artificially prepared is desirable.

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Abbreviations

C_M	Equilibrium concentration of methanol (mol/m^3)
C_{SFAME}	Equilibrium concentration of fatty acid methyl ester derived from squid liver (mol/m^3)
C_{TL}	Equilibrium concentration of methyl toluene (mol/m^3)
K_{M1}	Equilibrium adsorption constant of methanol binding with the hydrogen atom of the silanol group (m^3/mol)
K_{M2}	Equilibrium adsorption constant of methanol binding with the oxygen atom of the silanol group (m^3/mol)
K_{MF1}	Equilibrium adsorption constant of monounsaturated SFAME binding with the hydrogen atom of the silanol group (m^3/mol)
K_{MF2}	Equilibrium adsorption constant of monounsaturated SFAME binding with the methyl group of methanol adsorbed onto the silica group (m^3/mol)
K_{PF1}	Equilibrium adsorption constant of polyunsaturated SFAME binding with the hydrogen atom of the silanol group (m^3/mol)
K_{PF2}	Equilibrium adsorption constant of polyunsaturated SFAME binding with the methyl group of methanol adsorbed onto the silica group (m^3/mol)
K_{SF1}	Equilibrium adsorption constant of saturated SFAME binding with the hydrogen atom of the silanol group (m^3/mol)
K_{SF2}	Equilibrium adsorption constant of saturated SFAME binding with the methyl group of methanol adsorbed onto the silica group (m^3/mol)
K_{SFAME1}	Overall equilibrium adsorption constant of SFAME binding with the hydrogen atom of the silanol group (m^3/mol)
K_{SFAME2}	Overall equilibrium adsorption constant of SFAME binding with the methyl group of methanol adsorbed onto the silica group (m^3/mol)
K_{TL}	Equilibrium adsorption constant of toluene binding with the methyl group of methanol adsorbed onto the silica group (m^3/mol)
X_M	Total equilibrium adsorbed amount of methanol (mol/kg)
X_{MF}	Equilibrium adsorbed amount of monounsaturated SFAME (mol/kg)
X_{M1}	Equilibrium adsorbed amount of methanol binding with the hydrogen atom of the silanol group (mol/kg)
X_{M2}	Equilibrium adsorbed amount of methanol binding with the oxygen atom of the silanol group (mol/kg)
X_{PF}	Equilibrium adsorbed amount of polyunsaturated SFAME (mol/kg)
X_S	Total number of silanol groups onto the silica gel (mol/kg)
X_{SF}	Equilibrium adsorbed amount of saturated SFAME (mol/kg)
X_{SFAME}	Overall equilibrium adsorbed amount of SFAME (mol/kg)
X_{TL}	Equilibrium adsorbed amount of toluene binding with the methyl group of methanol adsorbed onto the silica group (mol/kg)

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