Computational and experimental research on mechanism of cis/trans isomerization of oleic acid

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Abstract

The harm of trans-fatty acids to health has aroused public concern. It is believed that the main source of trans-fatty acids in diets is the isomerization of unsaturated fatty acids in edible oils during cooking. However, the information on the isomerization mechanism is very limited. In this paper, we used oleic acid, an unsaturated fatty acid, as a simplified model for edible oil and investigated the mechanism of cis/trans isomerization by computation and experiments. The computational results show that R\textsubscript{c}-O-O-H is a very important intermediate, and the cleavage of O-O bond in R\textsubscript{c}-O-O-H is the rate-controlling step during the cis/trans isomerization. Using the ATR-FTIR measurements, the contents of elaidic acid were measured quantitatively in sites. The experimental results indicate that the cis/trans isomerization of oleic acid can occur obviously only under oxidizing condition when the temperature is higher than 120 °C.

Keywords: Organic chemistry, Analytical chemistry, Theoretical chemistry, Food science
1. Introduction

Edible oil is an integral part of a healthy diet that provides essential fatty acids for the human body. However, partial hydrogenation [1, 2] and deep frying [3, 4] of edible oils can form trans-fatty acids (TFA). Recently, more and more evidence and cases show that high intake of TFA has the potential to induce cardiovascular disease [5, 6, 7]. The mechanism of cis/trans isomerization of fatty acids during the frying process has been widely concerned, however, information on how to produce TFA is rare.

Theoretically, the cis/trans isomerization of fatty acids can occur by direct breaking a C=C double bond. However, according to Dobbs’ calculations [8], the rotation along the carbon-carbon bond requires about 263–280 kJ/mol of high energy to destroy the π bond. Wolff et al. [9] studied the cis/trans isomerization of linoleic acid and suggested that the important intermediate for the cis/trans isomerization of the diene system is an unstable conjugated cis-cis radical. However, they did not know how this free radical is formed, and this assumption has not been supported by further experiments. Destaillats et al. [10] discussed the cis/trans isomerization of linoleic acid under oxidizing and non-oxidizing conditions. They proposed two different isomerization mechanisms. They argued that isomerization occurs under oxidizing conditions by free radical chain reactions, where free radicals can be produced by the oxidation of linoleic acid. On the other hand, linoleic acid can form conjugated isomers by sigma reaction under high temperature and non-oxidizing conditions. Tsuzuki [11] determined the isomerization rate of trioleate at different temperatures by gas chromatography and calculated the activation energy of cis/trans isomerization using the Arrhenius equation. The activation energy (106 kJ/mol) of the cis/trans isomerization of trioleate was relatively small compared to the cis-isomerization of 2-butene (262 kJ/mol) without catalyst. The value of 106 kJ/mol is similar to that of isomerization of 2-butene radicals catalyzed by exogenous free radicals (115 kJ/mol). Thus, they speculated that the cis/trans isomerization of fatty acids may be a free radical process.

Computational chemistry is a popular tool to solve some chemical problems (e.g., mechanism, energy requirement), which is widely used to predict the properties of chemical systems. Recently, the mechanism of formation of TFA has been studied by the density functional theory (DFT) [12, 13]. Li et al. [12] studied molecular mechanisms of the cis/trans isomerization of non-conjugated linoleic acids thermally induced by a combined experimental and computational study. They proposed two alternative isomerization schemes via the proton transfer paths under non-oxidizing conditions. According to this mechanism, a high-energy barrier (286 kJ/mol) must be overcome for the transition from cis to trans isomer. The hydrogen migration pathway complements the mechanism [10] of fatty acid cis/trans isomerization. However, the isomerization of the C=C double bond through a hydrogen migration did not produce a diradical product, contrary to the results by Barrows and Eberlein.
[14]. Li et al. [13] investigated the mechanism of formation of trans-fatty acids under heating conditions in triolein. Their studies suggest that the formation of fatty acid isomers follows a proton transfer pathway, and the cis/trans isomerization may occur at 150 °C. The DFT calculations at B3LYP/6-311++G** level show that the energy barrier of the transition from the cis to trans is 295 kJ/mol.

In general, the molecular mechanism of fatty acid cis/trans isomerization under oxidizing conditions is still limited. In this paper, the energy demand and reaction pathway of cis/trans isomerization under oxidizing conditions were determined by the combination of computational and experimental methods to supplement the cis/trans isomerization mechanism of fatty acids.

Edible oil consists of a variety of fatty acid esters, its fried ingredients become more complex. Studying of TFA in this complex chemical composition is a very difficult task. Among the various vegetable oils, glycerol trioleate is the largest in proportion, which consists of 3 molecules of oleic acid and 1 molecule of glycerol. Among them, oleic acid is one of the simplest unsaturated fatty acids. Therefore, in order to promote isomerization mechanism of fatty acids, we use oleic acid to simulate edible oil.

### 2. Materials and methods

#### 2.1. Materials

Oleic acid of analytical pure grade (99%) was purchased from Aladdin (Shanghai, China).

#### 2.2. Heating procedures

10 ml of oleic acid was poured into a 25 ml round-bottomed glass flask which was heated to 180 °C for 12 hours. The effect of oxidation on oleic acid isomerization was investigated by controlling the access time of nitrogen. Non-oxidizing conditions were obtained by continuously adding N₂ to the flask.

In order to study the effect of thermal induction on the isomerization, 10 ml of oleic acid was added to a round-bottomed flask and separately heated in an oil bath at 60, 90, 120, 150, 180 °C for 8 hours. In addition, in order to observe in more detail the rule of the temperature effect in the temperature range of 120–150 °C, two additional observations were made at temperatures of 130 and 140 °C.

#### 2.3. Determination of elaidic acid content

TFA contains a double bond trans-configuration, and the C-H stretching attached to the trans-double bond belongs to the out-of-plane vibration, which has the largest
infrared absorption at 966 cm$^{-1}$. According to this feature, TFA can be qualitatively and quantitatively analyzed by infrared spectroscopy. This method was standardized by AOAC (2000) [15]. FTIR measurements were obtained on React IRTM 15 (Mettler Toledo, Switzerland) on site once per minute. The FTIR spectra were collected from 3000 to 600 cm$^{-1}$ by adding 256 interferograms at a resolution of 4 cm$^{-1}$. In the measurement, air is used as a reference background material.

2.4. Quantum chemical calculations

All calculations were performed using the Gaussian 09 software (Gaussian, Inc.) [16]. Geometry optimizations of the ground states, intermediates and transition states were conducted by using the DFT method with Becke’s 3 parameters (B3) and the Lee-Yang-Parr’s nonlocal correlation functional (LYP) at the 6-311++G** basis. IRCs were obtained to examine all the transition states and intermediates.

3. Results and discussion

3.1. The mechanism of isomerization via $\alpha$-radical

Previous experimental studies [9, 10, 11] had shown that the cis/trans isomerization of unsaturated fatty acid may involve $\alpha$-radical. Herein, $\alpha$-radical mechanism for oleic acid isomerization is promoted by DFT calculations. Since the 8-$\alpha$ radical of oleic acid and the 11-$\alpha$ radical of oleic acid is almost no difference in energy from our calculations, for simplicity, we refer to the 8-$\alpha$ radical of oleic acid as $\alpha$-radical in the following description. The energy and isomerization of $\alpha$-radical of oleic acid were calculated at the B3LYP/6-311++G** level. Optimized structure parameters of reactant, product and transition state are shown in Table 1. The vibration analysis showed that the optimized reactant and product structures were stable without imaginary frequency. The transition state has only one imaginary frequency (the imaginary frequency value is $-216.81$ cm$^{-1}$), confirming that the structure is an unstable transition state. The bond length (C9-C10) in cis/trans radical is greater than that of C=C (1.33Å), which means that the $\pi$ bond in the C=C has been broken.

| Species | Bond length (Å) (C9-C10) | Dihedral angle (°) (C8-C9-C10-C11) |
|---------|--------------------------|-----------------------------------|
| R$_{c}$ | 1.39                     | $-0.89$                           |
| TS      | 1.47                     | 97.88                             |
| R$_{t}$ | 1.39                     | 179.93                            |

The structures of R$_{c}$, TS, and R$_{t}$ are shown in Fig. 2.
when \( \alpha \)-radical is formed. According to the variation of dihedral angle (C8-C9-C10-C11) in oleic acid, we can clearly outline the reaction path information of isomerization of \( \alpha \)-radical of oleic acid.

At the beginning of the isomerization, the cis-oleic acid radical as the reactant has a dihedral angle of \( -0.89^\circ \), at which the C8-C9 bond is almost parallel to the plane formed by C9-C10-C11. As the reaction progresses, the reactant becomes transition state when the isomerization is activated and the dihedral angle is 97.88°. The C8-C9 bond is then almost perpendicular to the C9-C10-C11 plane, indicating that the functional groups on both sides of the C9-C10 bond have been rotated. As the transition state is unstable, the molecules continue to rotate, and when the trans-\( \alpha \)-radical is finally formed, the C8-C9-C10-C11 is inverted completely and the dihedral angle is 179.93°.

The variation of the potential energy of the isomerization path is shown in Fig. 1. The 3D-structure of the transition state of the isomerization is attached in the supporting information 1 (S1). It can be seen that cis-\( \alpha \)-radical of oleic acid needs to overcome the energy barrier of 66.15 kJ/mol to reach the transition state and finally to trans-\( \alpha \)-radical. For cis/trans isomerization of \( \alpha \)-radical, the activation energy of 66.15 kJ/mol is a modest, and the frying process can meet this energy requirement. That is, in the process of frying, if \( \alpha \)-radical is present, it will undergo cis-trans isomerization, suggesting that Wolff’s \( \alpha \)-radical hypothesis for cis/trans isomerization of linoleic acid may be correct. However, where do \( \alpha \)-radicals come from?

On the basis of DFT calculations and FTIR experimental observations, we present a thermally induced cis/trans isomerization mechanism of oleic acid, as shown in Fig. 2. The optimized Cartesian coordinates of all species involved in the cis/trans isomerization as shown in Fig. 2 are provided in supporting information 2 (S2).

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**Fig. 1.** The variation of the potential energy of cis/trans isomerization of \( \alpha \)-oleic acid radical.
The isomerization of unsaturated fatty acids is a complex process involving initiation of \( \alpha \)-radicals, propagation and termination. The initiation step (R1) involves the cleavage of a carbon-hydrogen bond to form a \( \alpha \)-radical, which requires very high energy (362.62 kJ/mol) to activate, so that the heating in frying cannot initiate such a reaction. The cleavage is likely to initiate by light, a little metal ions and enzyme [17, 18, 19, 20]. Therefore, this reaction (R1) occurs rarely, resulting in a low concentration of \( \text{Rc}^*_{\text{C15}} \). Propagation usually begins with the formation of \( \text{Rc}^*_{\text{C15}}\text{OO} \) through the reaction of oxygen with \( \text{Rc}^*_{\text{C15}} \) (R2). Then, the \( \text{Rc}^*_{\text{C15}}\text{OO} \) obtains hydrogen atom from \( \text{Rc}^*_{\text{C15}}\text{H} \) to produce \( \text{Rc}^*_{\text{C15}}\text{O-O-H} \) and another radical \( \text{Rc}^*_{\text{C15}} \) (R3). In general, the O-O bond in \( \text{Rc}^*_{\text{C15}}\text{O-O-H} \) is more unstable than the C-C and C-H bonds.

In frying, the cleavage of the O-O bond can produce two new radicals (\( \text{Rc}^*_{\text{C15}}\text{O} \) and \( \text{Rc}^*_{\text{C15}}\text{OH} \) (R4), and the two radicals will capture a hydrogen atom from \( \text{Rc}^*_{\text{C15}}\text{H} \) to produce a new \( \text{Rc}^*_{\text{C15}} \) radical, respectively (R5, R6). Next, \( \text{Rc}^*_{\text{C15}} \) radical will be isomerised to \( \text{Rc}^*_{\text{C15}} \) by rotation (R7), and \( \text{Rc}^*_{\text{C15}} \) will react with \( \text{Rc}^*_{\text{C15}}\text{H} \) to produce the final product—\( \text{Rc}^*_{\text{C15}}\text{H} \) (elaidic acid) and another \( \text{Rc}^*_{\text{C15}} \) (R8). As the reaction continues, there are 4 sources of \( \text{Rc}^*_{\text{C15}} \) from R3, R5, R6, R8 shown in Fig. 2, and the number of \( \text{Rc}^*_{\text{C15}} \) grows rapidly, resulting in an increasing the rate of reaction R7.

Fig. 2 also gives the activation energies of eight possible reactions during oleic acid isomerization. It is easy to see that the dissociation energy (R4, 160.97 kJ/mol) of the O-O bond in \( \text{Rc}^*_{\text{C15}}\text{O-O-H} \) is significantly higher than those of the other reaction steps in addition to the reaction R1, suggesting that when the thermally induced energy is greater than 160.97 kJ/mol, the cis/trans isomerization of oleic acid can proceed.
smoothly, and the cleavage of O-O bond is the rate-controlling step of the isomerization reaction.

In the above isomerization, oxygen is involved mainly in R2, leading to the accumulation of \( \text{Rc-O-O-H} \), the so-called induction period of free radical reaction [21]. On the one hand, without the participation of oxygen, the number of \( \text{Rc} \) radicals cannot be increased and the reaction will cease at the initiation step. On the other hand, the activation energy of the rate-controlling step is relatively high and requires heat induction to maintain the reaction. Thus, we speculate that cis/trans isomerization of oleic acid is associated with oxygen and induced temperature.

3.2. Effect of oxygen on isomerization of oleic acid

The effect of oxygen on oleic acid isomerization was studied experimentally. The relative concentration of elaidic acid was determined by on-line infrared spectroscopy according to AOAC (2000.10) standard, and the variations of elaidic acid in the reaction were observed and analyzed. Quantitative analysis of infrared spectroscopy is based on the Lambert-Beer law \( A = kbc \) (\( A = \text{Absorbance}; k = \text{Molar absorption coefficient}; c = \text{Concentration}; b = \text{Path Length} \)), using the intensity of absorption peak in the spectrum. It is noted that as the temperature increases, the width of absorption peak increases, and the position of the highest peak also changes, therefore we choose the peak area of the characteristic vibration mode to quantitative processing. All infrared spectra were processed with iC IRTM software. Supporting information 3 (S3) is the FTIR spectra of oleic acid and elaidic acid.

To simulate the frying temperature, we chose the experimental temperature to be 180 °C. The reaction time of the sample under non-oxidizing conditions was controlled by the access of nitrogen in the round bottom flask. In the experiments, 4 groups of oleic acid samples were heated at 180 °C for 12 hours, in which the oxidation time was 0, 3, 6, 12 h, respectively. The peak area of trans-double bond of elaidic acid (peak at 966 cm\(^{-1}\)) varies with time as shown in Fig. 3. It can be seen that the peak area is dependent on the oxidation time. During the oxidation heating, the three characteristic peak curves almost overlap and increase linearly for 3, 6, 12 h oxidative heating samples. However, when the oxidation conditions were terminated after 3 h and 6 h, that is, when the nitrogen gas was adding, it was found that the two curves almost instantly became a horizontal line. Correspondingly, the curve has been kept increasing linearly for the 12 h oxidative heating sample.

On the other hand, in the non-oxidizing condition, i.e., nitrogen was introduced into the reaction flask and heated for 12 hours, the peak area curve increases a little, indicating that there was small quantity of elaidic acid, which is almost negligible compared with those under oxygen conditions. Above observations are consistent
with the discovery of Destaillats [10], which suggests that oxygen is one of the necessary conditions for oleic acid isomerization.

The variations in characteristic IR spectra for 4 groups samples indicate that the oxidation of C=C double bond is important for cis/trans isomerization of oleic acid. The intermediates of the oxidation reaction may initiate and participate in the thermal induced cis/trans isomerization. Next, we will try to use the mechanism proposed above to make a reasonable explanation of these variations.

In the α-radical mechanism for oleic acid isomerization, oxygen is essential for its involvement in the propagation of free radicals. In the absence of oxygen, the number of radicals is reduced and the reaction is stopped at the initiation step. However, since a \( \text{R}_c^* \) radical can form a \( \text{R}_t^* \) radical (R7) and \( \text{R}_t^* \) radicals react further with \( \text{R}_c\text{H} \) to produce a new \( \text{R}_c^* \) radical (R8), which means reaction R7 and R8 can form a closed spontaneously oleic acid isomerization pathway. Dynamically, the activation energy of reaction R8 is much larger than that of reaction R7, the yield of elaidic acid depends on the rate at which \( \text{R}_t^* \) obtains hydrogen from \( \text{R}_c\text{H} \). So a trace amount of elaidic acid generated under heating without oxygen. It is expected that \( \text{R}_t^* \) radicals generated by \( \text{R}_c^* \) isomerization will grow rapidly due to 4 sources of \( \text{R}_t^* \) from reaction R3, R5, R6, R8 at oxidation conditions, and the production rate of eladic acid is rapidly increasing. There is an unexpected phenomenon that the content of elaidic acid showed a slowly decline during a few hours of non-oxidized state after the oxidative heating was terminated. We speculate that this phenomenon is caused by chemical equilibrium [22] of reaction R7. At the beginning of non-oxidative conditions \( \text{R}_c^* \) was richer and \( \text{R}_c\text{H} \) increased gradually over time. However, as the reaction progressed, the amount of \( \text{R}_c^* \) decreased rapidly, and the rate of the reverse reaction was higher than that of the forward, resulting in a decrease of the amount of elaidic acid. This process lasted 2—3 hours until it reached equilibrium, the content of elaidic acid remained stable.

![Fig. 3. The variations of peak area of the trans-double bond (966 cm⁻¹) with oxidation time.](https://doi.org/10.1016/j.heliyon.2018.e00768)
3.3. Effect of heating temperature on the isomerization of oleic acid

In order to study the effect of reaction temperature on cis/trans isomerization of oleic acid, five oleic acid systems (cis-18:1n-9; 10 ml) were prepared at different heating temperatures (60, 90, 120, 150, 180 °C, respectively) for 8 hours, and the variations in the peak area at 966 cm⁻¹ during heating were monitored using on-line IR spectroscopy, as shown in Fig. 4.

The experimental results show that oleic acid isomerization depends on the heating temperature and reaction time, and oleic acid isomerization is a thermal induced process. When the heating temperature was 60, 90 and 120 °C, the difference of the peak area is not obvious, indicating that the production rate of elaidic acid did not change obviously in the flask below 120 °C. Such a conclusion can also be easily obtained from the slope of the peak area curves labeled as k in Fig. 4. With the increasing of the heating temperature (above 120 °C), the area of the peak increases approximately linearly with reaction time, and the higher the heating temperature, the larger the slope of the rising curve for the peak area, indicating that raising the heating temperature contributes to the yield of elaidic acid.

In order to further obtain the threshold temperature for isomerization of oleic acid, the variations of peak area (966 cm⁻¹) with reaction temperature at different reaction time were shown in Fig. 5. It is clearly shown that the increasing of peak area is slow when the reaction temperature is below 120 °C. However, the peak area increases rapidly when the reaction temperature is higher than 120 °C. It is indicated that the reaction temperature is one of important factors of oleic acid isomerization.

The threshold temperature of thermal isomerization of cis/trans oleic acid may be about 120 °C, which is similar to the decomposition temperature of two peroxides.

![Graph showing the variations of peak area of the trans-double bond (966 cm⁻¹) with time at different reaction temperatures.](https://doi.org/10.1016/j.heliyon.2018.e00768)
This indicates that when the heating temperature is over 120 °C, the thermal energy is high enough to activate the reaction R4, i.e., the O-O bond in peroxide (Rc-O-O-H) may break, leading to initiation of the isomerization.

The experimental results support reaction R4 in our proposed mechanism, i.e., homogeneous break of O-O bond in Rc-O-O-H is the rate-controlling step for oleic acid isomerization. On the other hand, when the reaction temperature is lower than 120 °C, it is impossible to produce sufficient Rc radical because the O-O bond in Rc-O-O-H cannot be broken (R4), resulting in a very slow rate of oleic acid isomerization in the flask. Therefore, in order to reduce the production of TFA, we should control temperature of edible oil in the production, storage and consumption in daily life.

4. Conclusions

The frying process produces a small amount of TFA that is harmful to human health. In this paper, the mechanism of thermal induction of oleic acid isomerization under oxidizing conditions is proposed by combining computational and experimental studies. The mechanism explains the source of free radicals, which confuse researchers for years. Oxygen and reaction temperature are two important factors in the cis/trans isomerization of fatty acids. The presence of oxygen is a necessary condition to activate cis/trans isomerization. Heating above the threshold temperature can accumulate the energy for the peroxide intermediates, so that the O-O bond can be broken (the reaction-controlling step) and accelerate the isomerization. This work describes the relationship between oxidation of C=C double bonds and cis/trans isomerization, which is beneficial for inhibiting the formation of TFA. For human health, it is necessary to further study the isomerization of various fatty acids.
unsaturated fatty acids. There is still a long way to go to reduce the production of TFA in our daily life.

**Declarations**

**Author contribution statement**

Ningning Cheng: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jingjing Zhang: Contributed reagents, materials, analysis tools or data.

Jingmei Yin: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Shenmin Li: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

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