DEMETHYLATION OF SESAMIN IN SUPERCRITICAL ETHANOL

Ming-Tsai Liang[1*] Yue-Jheng Jhuo[1], Chun-Lan Keng[2], Wang Hui-Ju[2], and Jer-Yi Liao[2]

[1] *Department of Chemical Engineering, I-Shou University Kaohsiung County, Taiwan 840*
[2] *JOJIA Bio-Tech Co., 308 Jwu Liau Rd. Ta-Hsu Village, Kaohsiung County, Taiwan 840*

** Corresponding author, e-mail : mtliang@isu.edu.tw*

Sesamin extracted by supercritical carbon dioxide from sesame seeds was purified up to 95% and demethylated in supercritical ethanol. The purpose of the demethylation was to transform the lyophilic sesamin into the hydrophilic compounds for wider applications. The demethylation was conducted in a continuous flow reactor. Sesamin was firstly dissolved in ethanol, and pressurized by HPLC pump. The pressurized liquid, then, flowed through a coiled reactor made of 1/8" tube with 18 m long. After demethylation, the liquid was cooled, depressurized by a back pressure regulator, and collected for HPLC analysis and antioxidation test. The studied temperature and pressure were 250 ~ 350℃ and 30 MPa. The reaction time of the decomposition was adjusted by changing the flow rate and calculated by the Peng-Robinson EOS for the ethanol density. It is found that the decomposition of sesamin was a series reaction, and the intermediated compounds were the active compounds for the antioxidation. This work revealed the sesamin decomposition mechanism and investigated the effect of temperature and reaction time on the distribution of the intermediated compounds. Further studies by isolating individual intermediated compound from the decomposed liquid effluent are also recommended to identify bioactive compounds and will be conducted in the future.

1. INTRODUCTION

Sesame oil is widely used in Asia. There are at least 20 species of sesame. The free fatty acids composition is reported and shown in Table I [1]. Other than the triglycerides, the sesame oil contains about 1 % of lignan, including sesamin, sesamolin, sesamol, and sesaminol and its glucosides. The lignan was reported as effective compounds for anticancer [2], protective liver from damaged caused by chemicals [3, 4], anti-inflammation [5], antihypertensive effect [6], and etc.. However, the application of sesamin is limited because it is lyophilic. In order to enlarge its applications, this study tried to change the lyophilic lignan into hydrophilic compounds. It has been reported that the methylenedioxyphenyl moiety in the structure of sesamin can be changed into the catechol moieties by demethylenation[7-9]. The demethylenation of sesamin is illustrate in Fig. 1. It is also found that the demethylenated lignans have extreme antioxidative activity[10, 11]. It is also known that catechol, $C_6H_6O_2$, is water soluble, and the solubility of its cyclized product, 1,3-benzodioxole, $C_7H_6O_2$, is reduced to about 0.2 wt %. It is, therefore, presumed that the products of sesamin demethylenation will dissolve into water in some extetent. In our prior study, the lyophilic sesamin can become water dissolvable after treated by supercritical water in a semi-continuous reactor. In order to conduct the demethylation in a continuous way, ethanol was used to dissolve the sesamin and the ethanol solution was continuously pumped into the high pressure system. This study reports the feasibility of the sesamin demethylenation in supercritical ethanol.

Table 1 The free fatty acid composition of sesame oil [1]

Free fatty acid	Dark Seeds	White Seeds
C16:0	12.02~12.43	10.78~12.03
C18:0	4.75~5.72	4.93~5.90
C18:1	38.84~45.46	41.14~47.03
C18:2	33.79~44.52	35.01~41.23
C20:0	1.006	0.94~1.001

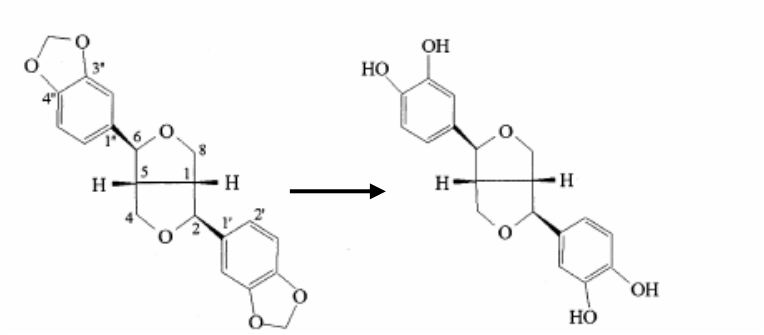

Fig. 1. The demethylenation of sesamin

2. EXPERIMENTS AND MATERIALS

Sesamin extracted by supercritical carbon dioxide from sesame seeds was purified up to 95% by JOJIA Biotech CO. The purified sesamin was firstly dissolved into ethanol, 500 ppm, as the feeding solution, and the solubility of sesamin in ethanol was also investigated as shown in figure 2. The reactor set up in a furnace was made of coiled 1/8" tube with 18 meters long. Pure water was pumped into the reactor and its pressure was regulated by a back pressure regulator equipped in the downstream. A cooler was used to cool the liquid effluent from the furnace to protect the regulator. The reaction temperature was controlled by the furnace. On reaching the designed temperature and pressure, the feeding solution was pumped into the system and the water pump was shut off. The space time for the reaction was controlled by the flow rate of HPLC pump, and it was calculated as the reactor volume divided by density of pure ethanol at the reaction condition which was found by Peng-Robinson equation of state. The liquid effluent flowed from the back pressure regulator was collected and dried and weighted. The dried solid was further dissolved into the mobile phase for the HPLC analysis.

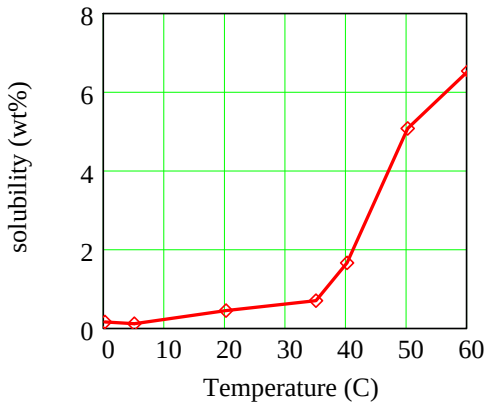

Fig. 2. The solubility of sesamin in ethanol

The packed column for HPLC was Develosil ODS-UG-5 with 150 mm x 4.6 mm. UV at 280 nm was used to detect the sesamin, and the concentration of sesamin was calculated by a calibration curve of ppm = 20091 x Area. The column was eluted with a linear gradient from solvent A (0.05% trifluoroacetic acid and 10% acetonitrile in water) to solvent B (0.05% trifluoroacetic acid and 90% acetonitrile in water) in 20 min at 1 ml/min.

A typical HPLC spectrum was illustrated in figure 3. Sesamin was eluted at 17.6-18.4 minute, and it is the strongest retention compound in the decomposed solution. After the alcothermal decomposition, the decomposed products become less retention. In this study, the sesamin and the decomposed compounds were named as group A to F according to the retention time listed in Table 2. The concentration of the six fractionated solution were calculated according to the same calibration curve as that for sesamin. The obtained concentrations were divided by the concentration of the sesamin in the feeding solution to study the reaction kinetics.

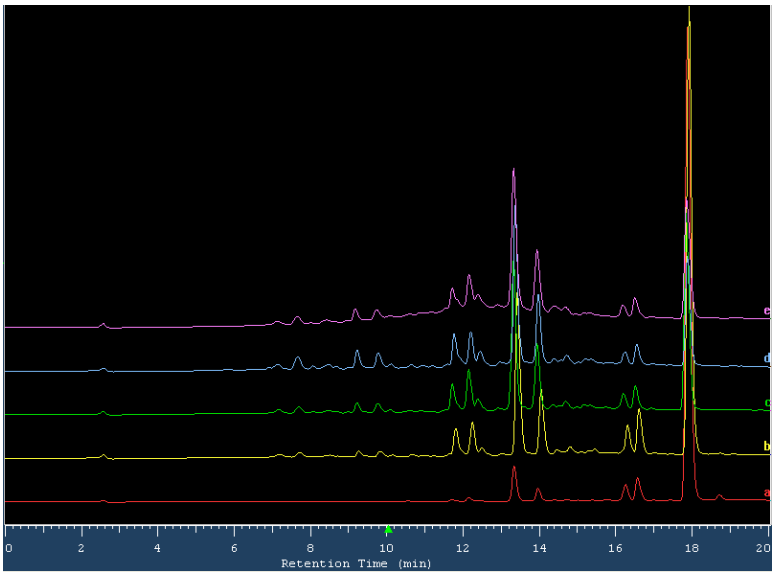

Fig. 3. The HPLC spectrum for the decomposed sesamin in supercritical ethanol.

Table 2 The classification of the decomposed compounds

group	A	B	C	D	E	F
Retention time (min)	17.6~18.4	16.0~16.8	13.0~14.2	11.6~12.6	9.0~10.0	7.0~8.0

3. RESULTS AND DISCUSSION

3.1 The decomposition kinetics

The five spectrums in figure 3 represented five different residence time of the reaction. From the bottom to the top, the five residence times were 5, 15, 30, 45, and 60 minutes. From figure 3, it was presumed that the decomposition was a series reaction. The sequence of the series reaction could be in the order of A to F. It was also observed that the peaks become broader as the residence time increased. It is presumed that much of the small fragments would be produced after long time of heating. Those fragments could therefore broader the peaks. Further increasing the temperature to 350 ℃, the peaks became broader and additional peaks appeared. If the residence time of the reaction was hold for 60 minutes, the concentration of the sesamin and all decomposed were nearly less than 0.1 %. This implied that the products of B~F groups were all the intermediate products of the decomposition.

In this study, the three different reaction temperatures were set as 250, 300, and 350 ℃, and the pressure was hold at 30 MPa. Figure 4~6 illustrated the variation of the concentration of the six groups with the residence time at different temperature. It was observed that the rate of the decomposition of sesamin increased with temperature. However, the decomposition of sesamin was not the irreversible first order reaction, because no linearity of the logarithm of sesamin concentration vs. time was observed in figure 4~6. While the temperature was increased from 250 to 300 ℃, it was observed that the concentration of A~F groups increased. However, the concentration of A~F groups decreased after further heated up from 300 to 350 ℃. It was also noted that the concentration of all groups decreased with residence time at 350 ℃. This implied a fast decomposition reaction occurred at 350 ℃, and the reaction kinetics at 350 ℃ could not be the same as that at 250 and 300 ℃. It was concluded that 350 ℃ was not suited for obtaining the demethylated sesamin.

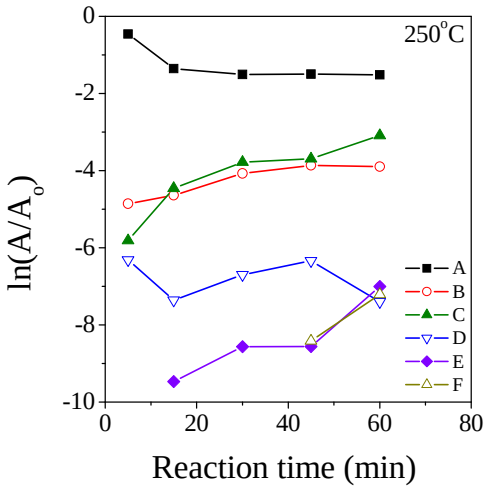

Fig. 4. The change of the products distribution along the residence time at 250 ℃.

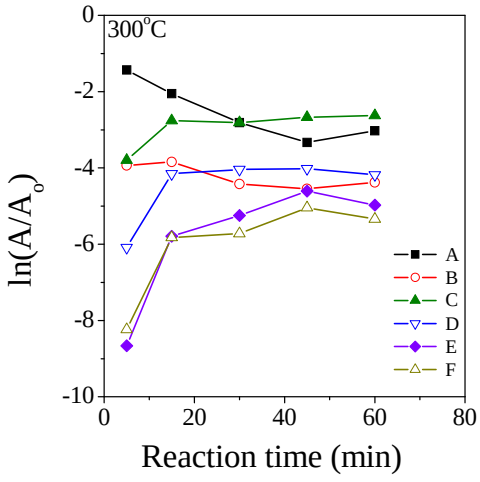

Fig. 5. The change of the products distribution along the residence time at 300 ℃.

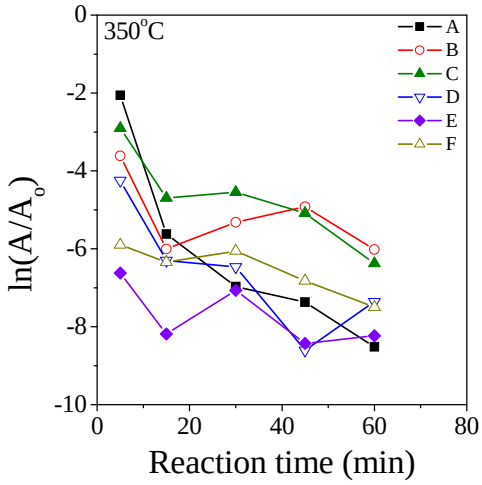

Fig. 6. The change of the products distribution along the residence time at 350 ℃.

3.2 The antioxidation test

The decomposed solution was firstly dried to obtain the solid products. The solid products were then weighted and dissolved into 95 % ethanol upto15 ppm for the antioxidation test. 1 ml of the sample and 0.25 ml of DPPH (0.01 g of DPPH in 50 ml of ethanol) were mixed for 30 minutes in the dark. The absorption at OD 517 for the samples and the references was measured to calculate the DPPH-scavenging ratio. The results of the antioxidation test were listed in Table 4, and the scavenging ratio for catechin at 10 ppm was found as 56 %. It was concluded that the decomposed sesamin by supercritical ethanol can produce high antioxidation activity compounds. From Table 3, it was found that the antioxidation activity of the decomposed products increase with reaction time at 250 and 300 ℃. However, the antioxidation activity showed a maximum along the reaction time as decomposed at 350 ℃. This implied that the active compounds could be the intermediate products of the decomposition. The trend of the antioxidation test of the decomposed products was similar to that decomposed in supercritical water in our primary study. However, the HPLC spectrum of the decomposed sesamin in supercritical ethanol showed different patterns from that in supercritical water. Further study is recommended to identify the active compounds either in supercritical water or in supercritical ethanol.

Table 3 The antioxidation test by the DPPH-scavenging ratio

Residence time	Temperature		
	250℃	300℃	350℃
5 min	9.0%	13.5%	41.0%
15 min	9.5%	38.3%	79.2%
30 min	14.6%	56.5%	81.9%
45 min	14.5%	72.5%	77.7%
60 min	29.0%	76.7%	29.0%

3. CONCLUSIONS

This work demonstrated that the decomposed product of sesamin by supercritical ethanol had the antioxidation activity, and they were hydrophobic. From the kinetics study, it was also implied that the active compounds were the intermediated products of the sesamin decomposition.

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REFERENCES

[1] H. Bahkali, M. A. Hussain, A. Y. Basahy, International Journal of food Sciences and Nutrition, 49 (1998) 409-414
[2] N. Hirose, F. Doi, T. Ueki, K. Akanwa, K. Chijiiwa, M. Sugano, K. AKimoto, Shinimizu. Anticancer Res, 12 (1992) 1259-1265
[3] K. Akimoto, Y. Kitagawa, T. Akamatsu, N. Hirose, M. Sugano, S. Shimizu, H. Yamada, Anni Nutr Metab 37 (1993) 218-224
[4] Young Y. Y., So Y. K., Hae Y.P., Sang H. S., Sun J. L., Sun Y. K., Young, C. K., J. Pharm. Pharmacol 52 (2000) 11638-11639
[5] S. R. Chavali, R. A. Forse, Prostaglanding Leukot Essent Fatty Acids, 61 (1999) 347-52
[6] D. Nakano, Vascular Disease Prevention, 1 (2004) 233-241
[7] K.P. Kreth, K.A. Kovar, M. Schwab, and U.M. Zanger, Biochemical Pharmacology, 59 (2000) 1563-1571
[8] Y. Kumagai, K.A. Wichkham, D.A. Schmitz, and A.K. Cho, ibid, 42 (1991) 1061-1067
[9] L.D. Paul, D. Springer, R.F. Staack, T. Kraemer, H.H. Maurer, European J. Pharmacology, 485 (2004) 69-79
[10] M. Nakai, M. Harada, K. Nakahara, K. Akimoto, H. Shibata, W. Miki, Y. Kiso, J. Agric. Food Chem., 51 (2003) 1666-1670
[11] Y. Miyake, S. Fukumoto, M. Okada, K. Sakaida, Y. Nakamura, T. Osawa, ibid, 53 (2005) 22-27
[12] M. Nakai, N. Kageyama, K. Nakahara, W. Miki, Biosci. Biotechnol. Biochem., 70 (2006) 1273-1276