Research Article

A Study on the Production Process Control of *Zanthoxylum bungeanum* Maxim Seed Kernel Oil without *Trans*-Fatty Acids

Zhongyi Yin,^{1,2} Shiyong Yao,^{1,2} Lixia Guo,^{1,2} and Xuxu Zheng^{1,2}

¹Chongqing Engineering Research Center of Processing, Storage and Transportation of Characterized Agro-Products, Chongqing, China

²Chongqing Key Lab of Natural Medicine Research, Chongqing Technology and Business University, Chongqing, China

Correspondence should be addressed to Xuxu Zheng; xuxuzheng@ctbu.edu.cn

Received 7 April 2016; Revised 10 August 2016; Accepted 6 September 2016

Academic Editor: Iciar Astiasaran

Copyright © 2016 Zhongyi Yin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The process control of the production of *Zanthoxylum bungeanum* Maxim seed kernel oil (ZSKO) with no *trans*-fatty acids (TFAs) was investigated. Results revealed that drying temperature and time had a small effect on TFA formation in ZSKO. And high concentrations of sodium hydroxide solution had some effect on TFA formation in ZSKO, but there were no TFAs when the concentration of the sodium hydroxide solution was lower than 20% and even at boiling temperature for one hour. The roasting temperature and duration for *Zanthoxylum bungeanum* Maxim seed (ZS) should be properly controlled at 100°C for six hours or 150°C for two hours. ZS, which has a moisture content of 18%, was pressed four times (two hours) at less than 60°C, and ZSKO was obtained by collection through centrifuge separation. This contained 90.84% unsaturated fatty acids, which mainly include 32.49% α -linolenic acid, 29.88% linoleic acid, and 27.52% oleic acids; and there was no TFA. Its acidic value and peroxide value conformed to China standards for edible oil. Therefore, ZSKO could be used as a healthy food for further development.

1. Introduction

The genus *Zanthoxylum* (*Rutaceae*) comprises a plenty of trees and shrubs. To date, approximately 250 species have been found in the temperate and tropical regions around the world. Furthermore, many of these are often used as condiments (due to the pungent taste of fruits, seeds, leaves, and bark) and therapeutic remedies, especially in East Asian countries [1]. *Zanthoxylum bungeanum* Maxim is one of these species that has been widely distributed in most parts of China and in some Southeast Asian countries. The fruits of this species are the most popular red huajiao commercial product, called “Da-Hong-Pao” (big red robe). Red huajiao, the pericarps of the fruits of *Zanthoxylum bungeanum* Maxim, have been utilized as pungent food stuff, as well as a kind of traditional Chinese medicine for the treatment of vomiting, toothache, stomach ache, abdominal pain, eczema, and diarrhea [2]. The seeds of the fruits of *Zanthoxylum bungeanum* Maxim have an estimated annual production potential of one million metric tons in China, and most of these seeds are used as muck and solid fuel [3].

The *Zanthoxylum bungeanum* Maxim seed (ZS) is semi-circular or oval in shape and can be divided into three parts: surface, hard shell, and kernel. The surface of ZS contains rich oil. The hard shell of ZS is mainly composed of cellulose, while the kernel of ZS has a milky and soft texture, which also contains rich oil, protein, essential mineral elements, and other valuable components [4]. The total oil content of ZS is 27–31%, in which surface oil accounts for approximately 10% and kernel oil accounts for approximately 20% [5]. Generally, *Zanthoxylum bungeanum* Maxim seed surface oil (ZSSO) cannot be used as edible oil, because it contains ester of saturated and unsaturated fatty acid including palmitic acid, palmitoleic acid, oleic acid, and linoleic acid. Furthermore, it also contains free fatty acid (FFA), colloid, waxes, and other impurity substances [5]. *Zanthoxylum bungeanum* Maxim seed kernel oil (ZSKO) can be prepared by pressing ZS when ZSSO is removed [4]. ZSKO mainly contains approximately 90% unsaturated fatty acid, in which the content of linoleic acids and α -linolenic acids is close to 70% [4, 6]. ZSKO functions in improving blood flow, adjusting blood lipids, and inhibiting the overoxidation of lipid substrates due to its

abundant unsaturated fatty acids [7, 8]. It is an abundantly nutritious, naturally green healthy food.

Recently, people have been more concerned about food safety and health. *Trans*-fatty acids (TFAs) have received global attention due to their hazard on human beings. TFAs may affect the digestion and assimilation to essential fatty acids, especially for the fetus. Furthermore, the deficiency of essential fatty acids would affect the growth of the fetus [9]. In addition, the overintake of TFAs can result in cardiovascular diseases. These could also increase blood viscosity and agglomeration, which causes the formation of arteriosclerosis and thrombus [10]. TFA intake also increases the risk of diabetes [11]. TFAs are greatly associated with prostate cancer [12], breast cancer [13], and female colon cancer [14]. Hence, TFAs are globally considered as stealth “killer” for human health, and measures to eliminate TFAs in food have spread all over the world. In November 2011, the Ministry of Health of the People’s Republic of China issued *prepackaged food nutrition labeling rules* in order to monitor the content of TFAs in the nutrient table of hydrogenated oil food. Therefore, from the point of view of oil processing enterprises, in order to reduce the formation of TFAs in the process of oil processing, these enterprises should conform to the trend of the times and improve the credibility of their products.

At present, in addition to our group [4], we have not yet seen a report on the production of ZSKO with no TFAs. This study aims to investigate the evolution of TFAs and its quantities and structures in the production of ZSKO. This research work may provide theoretical guidance for the industrial production of ZSKO.

2. Materials and Methods

2.1. Materials. “Da-Hong-Pao” ZS was obtained from a local company located in Hancheng, Shanxi Province, China. A total of 37 types of standard methyl ester fatty acid samples and eight groups of the standard mixture of *trans*-fatty acids were supplied by American NU-CHEK Limited Co. The *cis*- and *trans*-standard mixture of methyl esters of linoleic acids of octadecanoic dienoic acids and methyl esters of α -linolenic acids were supplied by SIGMA Limited Co. Chromatographic grade isooctane, as well as petroleum ether, anhydrous methanol, potassium hydroxide, sodium chloride, and 50–52% (mass fraction) methanolic boron trifluoride were all of analytic grade.

2.2. Production Process of ZSKO with No TFAs. The production process of ZSKO with no TFAs includes generally four steps: drying of fresh ZS to the dry state, alkaline saponification of ZS to remove ZSSO, roasting of ZS, and pressing of ZS at a low temperature.

Fresh ZS $\Rightarrow$

Drying to Dry State $\Rightarrow$

Saponification in Sodium Hydroxide Solution $\Rightarrow$

Roasting ZS $\Rightarrow$

Pressing at Low Temperature $\Rightarrow$

ZSKO

(1)

2.3. Simulated Experiment for the Drying Process of ZS. An oven was adopted to simulate the drying process. With fresh ZS as the control group, eight kinds of 200 g of fresh ZS samples were weighed and placed onto an open stainless steel tray, respectively. These were dried for two, four, six, or eight hours under blowing at 50°C or 100°C, respectively. Then, each sample was extracted with petroleum ether to obtain ZSSO and ZSKO by the two-step extraction technology using the same kind of solvent [5]. The fatty acid composition of each sample was determined and three repeated measurements were conducted. The effect of drying temperature and time on the fatty acid compositions could be summarized.

2.4. Removal of ZSSO with Alkaline Saponification. A total of 16 kinds of 50 g of dried ZS samples were placed into a conical flask, and 10%, 14%, 18%, or 20% (w/w) sodium hydroxide solution was poured, respectively. These were saponified in water bath for one hour at 20°C, 50°C, 80°C, or boiling temperature, respectively. Next, each sample was washed, dried, pulverized, and extracted with petroleum ether. The fatty acid composition in each resultant ZSKO was determined in three parallel measurements. The effect of the sodium hydroxide solution concentration, saponification temperature, and saponification time on the fatty acid compositions could be summarized.

2.5. Experimental Simulation to Roasting of ZS. The roasting process was simulated using an oven. The ZS without ZSSO was used as the raw material. Twelve kinds of 50 g ZS samples were weighed and placed on an open stainless steel tray and were roasted for two, four, six, or eight hours under blowing at 50°C, 100°C, or 150°C, respectively. Then, each sample was pulverized and extracted with petroleum ether. The fatty acid composition in each resultant ZSKO was determined in three parallel measurements. The effect of roasting temperature and time on the fatty acid compositions could be summarized.

2.6. Pressing Test of ZS at Low Temperatures. A 2.5 kg ZS was pulverized into 20-mesh particles after drying at 100°C for six hours, boiled in 18% (w/w) sodium hydroxide solution, and roasted for two hours at 150°C to control the water content at 18% in turn. Then, it was pressed four times (two hours) in a machine with a 40 mm screw distance at less than 60°C [4]. Next, ZSKO was obtained by collecting through centrifuge separation. The fatty acid compositions, acidic value, and peroxide value of ZSKO were determined according to the Chinese National Standard GB/T 22479-2008.

2.7. Analysis of Fatty Acid Composition in Fats. The methylation treatment of ZSKO was carried out as follows: A 200 mg (accurate to 0.1 mg) well-mixed fat sample was placed into a 25 mL screw tube with a cap. A 25 mL methanolic potassium hydroxide was added into the tube. The tube was heated in a 75°C water bath for 20 minutes and cooled to ambient temperature. A 2 mL methanolic boron trifluoride was poured into the tube and was heated in a 75°C water bath for 30 minutes. Then, it was also cooled to ambient temperature. An additional 2 mL isooctane and 2 mL saturated aqueous

FIGURE 1: Gas chromatography of mixed fatty acid methyl ester standards. 1, octanoate methyl ester (C8:0); 2, decanoate methyl ester (C10:0); 3, undecanoate methyl ester (C11:0); 4, dodecanoate methyl ester (C12:0); 5, tridecanoate methyl ester (C13:0); 6, myristic acid methyl ester (C14:0); 7, myristoleate methyl ester (C14:1); 8, pentadecanoate methyl ester (C15:0); 9, *cis*-9-pentadecenoate methyl ester (C15:1); 10, palmitic acid methyl ester (C16:0); 11, *cis*-9-palmitoleate methyl ester (C16:1); 12, heptadecanoate methyl ester (C17:0); 13, *cis*-10-heptadecenoate methyl ester (C17:1); 14, stearate methyl ester (C18:0); 15, *trans*-9-octadecenoic acid methyl ester (C18:1, 9*t*); 16, *cis*-9-oleate methyl ester (C18:1, 9*c*); 17, linolelaidate methyl ester (C18:2, 9*t*12*t*); 18, linoleate methyl ester (C18:2, 9*c*12*c*); 19, arachidate methyl ester (C20:0); 20, γ -linolenate methyl ester (C18:3, 6*c*9*c*12*c*); 21, *cis*-11-eicosenoic acid methyl ester (C20:1); 22, linolenate methyl ester (C18:3, 9*c*12*c*15*c*); 23, heneicosanoate methyl ester (C21:0); 24, *cis*-11,14-eicosadienoic acid methyl ester (C20:2); 25, behenate methyl ester (C22:0); 26, *cis*-8,11,14-eicosatrienoic acid methyl ester (C20:3); 27, erucate methyl ester (C22:1); 28, *cis*-11,14,17-eicosatrienoic acid methyl ester (C20:3); 29, *cis*-5,8,11,14-arachidonate (C20:4); 30, *cis*-13,16-docosadienoic acid methyl ester (C22:2); 31, tetracosanoate methyl ester (C24:0); 32, *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (C20:5); 33, *cis*-15-tetracosenoate methyl ester (C24:1); 34, *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester (C22:6).

sodium chloride were added and mixed uniformly. The solution was rested in order to allow phase separation to take place, and the supernatant was collected for further characterization.

The fatty acid composition of ZSKO was determined according to the following conditions: chromatographic column, CD-2560 gaseous capillary column (100 m $\times$ 0.25 mm $\times$ 0.20 μ m); detector, hydrogen flame ionized detector; process of temperature, constant 140°C for five minutes (then temperature was increased at 4°C/min up to 240°C, constant at 240°C for 25 minutes); sample injection, 1 μ L; injection temperature, 250°C; detector temperature, 280°C; branching injection, branching ratio 32 : 1, 99.999% nitrogen carrier at a constant 130 kPa; hydrogen combustion gas, constant at 50 kPa; air aid combustion, constant at 50 kPa. Qualitative analysis was carried out through the relative retention time of the referred samples, while quantitative analysis was performed by unifying the peak area.

3. Results and Analysis

3.1. Gas Chromatographic Analysis of Reference Substance. Gas chromatographic diagrams for the 37 types of methyl fatty acid methyl esters, as well as eight kinds of TFAs methyl esters, linoleic acid methyl ester isomer, and linolenic acid methyl ester isomer, are shown in Figures 1, 2, 3, and 4, respectively. It was concluded that ZSKO mainly contained C16:0, C16:1 9*c*, C18:0, C18:1 9*c*, C18:2 9*c*12*c*, C18:3 9*c*12*c*15*c*, C20:0, C20:1, C22:0, and C22:1 [15]. As shown in Figure 1, it was proven that C16:0, C16:1, C17:0, C17:1, C18:0, C18:1 9*t*, C18:1

9*c*, C18:2 6*t*, C18:2 6*c*, C20:0, C18:3, C20:1, C18:3, C22:0, C21:0, and C22:1 could be completely separated.

ZSKO contains abundant unsaturated fatty acids such as oleic acids, linoleic acids, and α -linolenic acids. These are susceptible to oxidation into TFAs. Figures 2, 3, and 4 revealed an obvious separation among C18:1 6*t*/9*t*/11*t*, C18:2 9*t*12*t*, C18:2 9*c*12*t*, C18:2 9*t*12*c*, C18:2 9*c*12*c*, C18:3 9*t*12*t*15*t*, C18:3 9*t*12*t*15*c*/C18:3 9*t*12*c*15*t*, C18:3 9*c*12*t*15*t*/C18:3 9*c*12*c*15*t*, C18:3 9*c*12*t*15*c*/C18:3 9*t*12*c*15*c*, and C18:3 9*c*12*c*15*c*, which could be used to accurately analyze the structure and quantity of TFAs.

3.2. Effect of Drying Temperature and Time on the Fatty Acid Compositions of ZSKO and ZSSO. Eight kinds of fresh ZS samples were dried with different temperatures and time conditions, respectively. The fatty acid compositions of ZSSO and ZSKO are listed in Tables 1 and 2, respectively.

The second column in Table 1 was the control group. The major components of ZSSO were 39.04 $\pm$ 0.18 g/100 g of oleic acid (C18:1 9*c*), 28.31 $\pm$ 0.09 g/100 g of palmitic acid (C16:0), 16.45 $\pm$ 0.13 g/100 g of palmitoleic acid (C16:1 9*c*), and 13.34 $\pm$ 0.07 g/100 g of linoleic acid (C18:2 9*c*12*c*). This indicated that the main components of ZSSO were oleic acid, linoleic acid, palmitic acid, and palmitoleic acid [16]. Nonetheless, considerable amount of *trans*-oleic acids (*trans*-C18:1; 0.21 $\pm$ 0.02 g/100 g) was presented. This is probably due to the long time exposure of ZS to sunlight, which induced the thermal isomerization reaction of ZSSO, that is, *cis*-isomers into *trans*-isomers [17, 18].

The second column in Table 2 comprises the control group. The major components of ZSKO were 29.24 $\pm$

TABLE 1: Effect of drying temperature and duration on the fatty acid composition of ZSSO.

Fatty acids (g/100 g)	Control group	Drying at 50 °C				Drying at 100 °C			
		2 hr	4 hr	6 hr	8 hr	2 hr	4 hr	6 hr	8 hr
C14:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14:1, 9c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	28.31 ± 0.09	29.06 ± 0.05	28.08 ± 0.25	28.27 ± 0.12	29.20 ± 0.06	27.19 ± 0.09	27.51 ± 0.31	28.68 ± 0.13	29.05 ± 0.05
C16:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:1, 9c	16.45 ± 0.13	16.79 ± 0.11	17.04 ± 0.09	17.21 ± 0.09	17.22 ± 0.05	17.81 ± 0.14	17.01 ± 0.17	17.38 ± 0.09	16.24 ± 0.35
C18:0	1.22 ± 0.05	1.19 ± 0.02	1.13 ± 0.06	1.11 ± 0.02	1.11 ± 0.02	1.07 ± 0.11	1.21 ± 0.09	1.33 ± 0.11	1.36 ± 0.03
C18:1, 6t/9t/11t	0.21 ± 0.02	0.24 ± 0.01	0.23 ± 0.03	0.25 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.39 ± 0.03	0.49 ± 0.02	0.61 ± 0.04
C18:1, 9c	39.04 ± 0.18	38.42 ± 0.32	38.96 ± 0.08	39.04 ± 0.08	38.52 ± 0.33	39.14 ± 0.21	39.21 ± 0.07	38.63 ± 0.40	39.03 ± 0.21
C18:2, 9t12t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9t12c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12c	13.34 ± 0.07	12.63 ± 0.17	13.25 ± 0.17	13.42 ± 0.11	12.73 ± 0.09	13.39 ± 0.09	13.51 ± 0.12	12.52 ± 0.15	12.61 ± 0.09
C18:3, 9t12t15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9t12t15c or 9t12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15t or 9c12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15c or 9t12c15c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12c15c	1.15 ± 0.21	1.67 ± 0.08	1.32 ± 0.21	0.95 ± 0.09	0.93 ± 0.02	1.12 ± 0.08	1.16 ± 0.04	0.97 ± 0.08	1.10 ± 0.12
C22:1, t	ND	ND	ND	ND	ND	ND	ND	ND	ND
Subtotal trans	0.21 ± 0.02	0.24 ± 0.01	0.23 ± 0.03	0.25 ± 0.02	0.29 ± 0.01	0.28 ± 0.01	0.39 ± 0.03	0.49 ± 0.02	0.61 ± 0.04

Data are expressed as means ± SD from at least three independent experiments. ND means not detected.

TABLE 2: Effect of drying temperature and duration on the fatty acid composition of ZSKO.

Fatty acids (g/100 g)	Control group	Drying at 50 °C				Drying at 100 °C			
		2 hr	4 hr	6 hr	8 hr	2 hr	4 hr	6 hr	8 hr
C14:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14:1, 9c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	14.71 ± 0.09	15.38 ± 0.13	15.35 ± 0.17	15.5 ± 0.03	15.96 ± 0.15	15.36 ± 0.17	15.28 ± 0.13	16.26 ± 0.02	15.95 ± 0.08
C16:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:1, 9c	7.06 ± 0.31	7.84 ± 0.21	7.67 ± 0.13	7.92 ± 0.35	7.62 ± 0.08	7.69 ± 0.13	7.59 ± 0.05	7.26 ± 0.28	7.18 ± 0.16
C18:0	1.45 ± 0.13	1.26 ± 0.06	1.27 ± 0.07	1.35 ± 0.01	1.22 ± 0.12	1.28 ± 0.06	1.35 ± 0.01	1.45 ± 0.11	1.40 ± 0.06
C18:1, 6t/9t/11t	ND	ND	ND	ND	ND	ND	ND	ND	0.15 ± 0.01
C18:1, 9c	29.24 ± 0.24	28.52 ± 0.11	28.16 ± 0.11	28.58 ± 0.13	28.23 ± 0.13	27.86 ± 0.28	28.42 ± 0.06	28.26 ± 0.1	27.98 ± 0.31
C18:2, 9t12t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9t12c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12c	24.62 ± 0.07	24.17 ± 0.09	24.29 ± 0.08	23.84 ± 0.06	23.52 ± 0.24	24.08 ± 0.13	24.17 ± 0.17	22.95 ± 0.35	23.63 ± 0.13
C18:3, 9t12t15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9t12t15c or 9t12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15t or 9c12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15c or 9t12c15c	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12c15c	22.92 ± 0.02	22.84 ± 0.07	23.25 ± 0.05	22.80 ± 0.19	23.45 ± 0.15	23.72 ± 0.21	23.19 ± 0.11	23.82 ± 0.51	23.71 ± 0.09
C22:1, t	ND	ND	ND	ND	ND	ND	ND	ND	ND
Subtotal trans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.01

Data are expressed as means ± SD from at least three independent experiments. ND means not detected.

FIGURE 2: Gas chromatography of the eight components of TFA methyl ester mixture standards. 1, *trans*-9-myristelaidate methyl ester (C14:1, 9*t*); 2, *trans*-9-palmitelaidate methyl ester (C16:1 9*t*); 3/4/5, *trans*-11-transvaccenate methyl ester (C18:1, 11*t*)/*trans*-6-petroselaidate methyl ester (C18:1, 6*t*)/*trans*-9-elaidate methyl ester (C18:1, 6*t*); 6, C_{18:2} 9*t*12*t*; 7, *trans*-11-eicosenoate methyl ester (C20:1, 11*t*); 8, *trans*-brassidate methyl ester (C22:1*t*).

FIGURE 3: Gas chromatography of linoleic acid methyl ester isomer mixture standards. 1, C18:2, 9*t*12*t*; 2, *cis*-9,*trans*-12-octadecadienoic acid methyl ester (C18:2, 9*c*12*t*); 3, *trans*-9,*cis*-12-octadecadienoic acid methyl ester (C18:2, 9*t*12*c*); 4, C18:2, 9*c*12*c*.

0.24 g/100 g of oleic acid (C18:1 9*c*), 24.62 ± 0.07 g/100 g of linoleic acid (C18:2 9*c*12*c*), 22.92 ± 0.02 g/100 g of α -linolenic acid (C18:3 9*c*12*c*15*c*), and 14.71 ± 0.09 g/100 g of palmitic acids (C16:0). There was no TFA detected, indicating that ZSKO could be developed into healthy edible fats.

Table 1 shows that the drying of ZS could generate TFAs obtained from the surface oil. Moreover, all these TFAs were *trans*-C18:1, which is probably due to the high oleic acid content and low linoleic and linolenic acids. The latter two

acids produced a trace amount of TFAs that could be hardly detected. After being dried at 50°C for 2, 4, 6, and 8 hours, 0.24 ± 0.01 , 0.23 ± 0.03 , 0.25 ± 0.02 , and 0.29 ± 0.01 g/100 g of *trans*-C18:1 could be detected, respectively, in ZSSO. Compared to the control group, the total TFA only slightly increased. However, if the ZS was dried at 100°C for 2, 4, 6, and 8 hours, the total TFA would be obviously increased. For instance, 0.39 ± 0.03 g/100 g of *trans*-C18:1 was detected after four hours of drying at 100°C, which is above the National

FIGURE 4: Gas chromatography of linolenic acid methyl ester isomer mixture standards. 1, *trans*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (C18:3, 9*t*12*t*15*t*); 2, *trans*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (C18:3, 9*t*12*t*15*c*)/*trans*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (C18:3, 9*t*12*c*15*t*); 3, *cis*-9,*trans*-12,*trans*-15-octadecatrienoic acid methyl ester (C18:3, 9*c*12*t*15*t*)/*cis*-9,*cis*-12,*trans*-15-octadecatrienoic acid methyl ester (C18:3, 9*c*12*c*15*t*); 4, *cis*-9,*trans*-12,*cis*-15-octadecatrienoic acid methyl ester (C18:3, 9*c*12*t*15*c*)/*trans*-9,*cis*-12,*cis*-15-octadecatrienoic acid methyl ester (C18:3, 9*t*12*c*15*c*); 5, C18:3, 9*c*12*c*15*c*.

Standard Limit (0.30 g/100 g). The increase in *trans*-C18:1 indicated that the drying process accelerated the TFA formation of ZSSO. In addition, the higher the drying temperature was and the longer the duration was, the more the TFAs would be formed.

In Table 2, a trace amount of TFA was formed in ZSKO; that is, 0.15 ± 0.01 g/100 g of *trans*-C18:1 was detected after being dried at 100°C for eight hours. This was probably because the hard shell had protected ZSKO from being oxidized to some extent.

3.3. Effect of Alkaline Solution Concentration and Temperatures on the Fatty Acid Compositions of ZSKO. As mentioned in Section 1, ZSSO could not be used as edible oil [5]. Therefore, the surface oil must be removed prior to the production of ZSKO to ensure high quality. When ZS was pretreated using a sodium hydroxide solution with different concentrations and temperatures, ZSKO was extracted. Its fatty acid compositions are listed in Table 3.

In Table 3, TFAs were produced while boiling for one hour in 20% (w/w) sodium hydroxide solution. These mainly comprised of *trans*-linolenic acids, including 0.05 ± 0.01 g/100 g of C18:3 9*t*12*t*15*t* and 0.09 ± 0.01 g/100 g of C18:3 9*c*12*t*15*t* or C18:3 9*c*12*c*15*t*. Nonetheless, the total TFA content was less than the National Standard Limit (0.30 g/100 g). The possible reason was that the high concentration of the alkaline solution could easily damage the hard shell of ZS, resulting in exposing the kernel to a high temperature alkaline solution. The boiling temperature of higher concentrations of alkaline solution was higher, and the *cis*-linolenic acids were more easily isomerized to *trans*-linolenic acids.

Table 3 shows that there were no TFAs produced from the *cis*-isomers of ZSKO if the concentration of sodium

hydroxide solution was lower than 20% (w/w) and even at boiling temperature for one hour. Therefore, a low concentration sodium hydroxide solution could be used to pretreat ZS in order to ensure the production of ZSKO with no TFAs.

3.4. Effect of Roasting Temperature and Duration on the Fatty Acid Compositions of ZSKO. There was a roasting process of ZS before the traditional pressing method was used to produce ZSKO. After alkaline pretreatment, the ZS was roasted at 50°C, 100°C, and 150°C for 2, 4, 6, or 8 hours, respectively. The fatty acid compositions of ZSKO are listed in Table 4.

In Table 4, the roasting ZS at 100°C for eight hours generated a trace amount of *trans*-linoleic acids, namely, 0.08 ± 0.01 g/100 g of C18:2 9*c*12*t*. There was no *trans*-oleic acid or linolenic acid. When the ZS was roasted at 150°C for 2, 4, or 8 hours, 0.06 ± 0.02 g/100 g, 0.09 ± 0.02 g/100 g, or 0.11 ± 0.01 g/100 g of C18:2 9*c*12*t* was detected, respectively. These results indicate that the TFAs of ZSKO increased with increasing roasting duration and temperature. However, the total content of TFAs in ZSKO was at a low level, which was lower than the Chinese National Standard Limit (0.30 mg/100 g). Therefore, TFAs could be controlled when roasting below 100°C for less than six hours or roasting at 150°C for less than two hours.

3.5. Effect of Pressing at Low Temperatures on ZKSO's Fatty Acid Compositions, Acidic Value, and Peroxide Value. ZSKO was obtained by drying, saponification, roasting, pressing, and centrifugal separation processes in turn. ZSKO's fatty acid compositions, acidic value, and peroxidation value were measured, as described in Table 5.

In Table 5, a self-made ZKSO contained 90.84% unsaturated fatty acids, mainly including 32.49 ± 0.21 g/100 g of

TABLE 3: Effect of alkaline solution concentration and temperatures on the fatty acid compositions of ZSKO.

Fatty acids (g/100 g)	10% NaOH treatment for 1 hr				14% NaOH treatment for 1 hr				18% NaOH treatment for 1 hr				20% NaOH treatment for 1 hr				Boiling
	20 °C	50 °C	80 °C	Boiling	20 °C	50 °C	80 °C	Boiling	20 °C	50 °C	80 °C	Boiling	20 °C	50 °C	80 °C	Boiling	
Cl4:1, 9f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl4:1, 9c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl6:0	7.23 ± 0.04	7.14 ± 0.08	6.88 ± 0.11	7.28 ± 0.07	7.19 ± 0.05	7.14 ± 0.06	7.19 ± 0.04	7.02 ± 0.07	6.88 ± 0.11	7.32 ± 0.11	7.07 ± 0.05	6.82 ± 0.15	7.01 ± 0.09	7.32 ± 0.09	6.71 ± 0.26	7.51 ± 0.24	7.51 ± 0.24
Cl6:1, 9f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl6:1, 9c	0.74 ± 0.02	0.71 ± 0.03	0.69 ± 0.02	0.72 ± 0.04	0.72 ± 0.04	0.70 ± .01	0.72 ± 0.02	0.69 ± 0.04	0.69 ± 0.02	0.73 ± 0.04	0.72 ± 0.01	0.69 ± 0.03	0.69 ± 0.03	0.72 ± 0.03	0.69 ± 0.03	0.89 ± 0.13	0.89 ± 0.13
Cl8:0	1.28 ± 0.04	1.28 ± 0.05	1.25 ± 0.05	1.31 ± 0.02	1.17 ± 0.09	1.31 ± 0.05	1.31 ± 0.05	1.31 ± 0.05	1.25 ± 0.05	1.31 ± 0.01	1.30 ± 0.02	1.28 ± 0.02	1.27 ± 0.06	1.28 ± 0.00	1.32 ± 0.04	1.51 ± 0.09	1.51 ± 0.09
Cl8:1, 6f/9f/11f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:1, 9c	28.02 ± 0.11	28.28 ± 0.11	28.13 ± 0.09	27.72 ± 0.13	27.68 ± 0.12	27.69 ± 0.07	27.59 ± 0.12	27.56 ± 0.03	28.13 ± 0.09	27.86 ± 0.23	27.85 ± 0.07	27.98 ± 0.14	28.13 ± 0.08	28.16 ± 0.22	27.96 ± 0.12	27.76 ± 0.04	27.76 ± 0.04
Cl8:2, 9f12f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:2, 9c12f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:2, 9f12c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:2, 9c12c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:2, 9c12c	30.31 ± 0.12	30.47 ± 0.09	30.95 ± 0.16	30.88 ± 0.09	30.83 ± 0.18	30.41 ± 0.31	30.84 ± 0.03	30.77 ± 0.12	30.85 ± 0.16	30.67 ± 0.12	30.62 ± 0.06	30.67 ± 0.06	30.73 ± 0.14	30.62 ± 0.05	30.69 ± 0.07	29.04 ± 0.08	29.04 ± 0.08
Cl8:3, 9f12f15f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05 ± 0.01
Cl8:3, 9f12f15c or 9f12c15f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:3, 9c12f15f or 9c12c15f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.09 ± 0.01
Cl8:3, 9c12f15c or 9f12c15c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cl8:3, 9c12c15c	32.44 ± 0.13	32.13 ± 0.17	32.11 ± 0.21	32.08 ± 0.18	32.43 ± 0.11	32.75 ± 0.11	32.35 ± 0.27	32.65 ± 0.30	32.20 ± 0.21	32.11 ± 0.31	32.43 ± 0.21	32.56 ± 0.15	32.17 ± 0.23	31.90 ± 0.31	32.63 ± 0.21	33.15 ± 0.21	33.15 ± 0.21
C22:1, f	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Subtotal trans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.02	0.14 ± 0.02

Data are expressed as means ± SD from at least three independent experiments. ND means not detected.

TABLE 4: Effect of roasting temperature and duration on the fatty acid composition of ZSKO.

Fatty acids (g/100 g)	Control group	50°C				100°C				150°C			
		2 hr	4 hr	6 hr	8 hr	2 hr	4 hr	6 hr	8 hr	2 hr	4 hr	6 hr	8 hr
C14:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14:1, 9c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	7.63 ± 0.03	7.67 ± 0.04	8.03 ± 0.08	6.84 ± 0.31	7.1 ± 0.08	7.36 ± 0.09	8.31 ± 0.03	6.98 ± 0.24	7.2 ± 0.13	7.02 ± 0.35	7.13 ± 0.09	7.23 ± 0.13	7.35 ± 0.02
C16:1, 9t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:1, 9c	1.24 ± 0.02	1.23 ± 0.02	1.89 ± 0.04	0.97 ± 0.27	1.08 ± 0.14	1.12 ± 0.13	1.59 ± 0.27	1.03 ± 0.14	0.93 ± 0.27	1.21 ± 0.02	0.99 ± 0.13	0.93 ± 0.14	0.92 ± 0.15
C18:0	1.73 ± 0.01	1.73 ± 0.05	1.52 ± 0.13	1.38 ± 0.13	1.44 ± 0.06	1.48 ± 0.04	1.43 ± 0.05	1.57 ± 0.06	1.19 ± 0.21	1.65 ± 0.06	1.41 ± 0.09	1.73 ± 0.23	1.28 ± 0.22
C18:1, 6t/9t/11t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:1, 9c	21.26 ± 0.11	21.73 ± 0.03	21.83 ± 0.10	22.86 ± 0.08	22.23 ± 0.35	20.58 ± 0.43	21.08 ± 0.15	21.94 ± 0.43	21.43 ± 0.32	22.01 ± 0.14	21.43 ± 0.26	21.13 ± 0.35	22.31 ± 0.04
C18:2, 9t12t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12t	ND	ND	ND	ND	ND	ND	ND	ND	0.08 ± 0.01	ND	0.06 ± 0.02	0.09 ± 0.02	0.11 ± 0.01
C18:2, 9t12c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:2, 9c12c	30.02 ± 0.22	29.71 ± 0.16	28.66 ± 0.24	30.02 ± 0.04	29.95 ± 0.07	30.26 ± 0.26	29.66 ± 0.13	29.92 ± 0.07	30.41 ± 0.08	30.52 ± 0.11	30.08 ± 0.17	29.61 ± 0.17	30.07 ± 0.05
C18:3, 9t12t15t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9t12t15c or 9t12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15t or 9c12c15t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12t15c or 9t12c15c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3, 9c12c15c	37.63 ± 0.09	37.45 ± 0.18	37.63 ± 0.09	37.42 ± 0.11	37.73 ± 0.23	38.68 ± 0.45	37.46 ± 0.55	38.08 ± 0.03	38.29 ± 0.04	37.1 ± 0.42	38.43 ± 0.03	38.82 ± 0.05	37.43 ± 0.18
C22:1, t	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:0	0.15 ± 0.01	0.15 ± 0.02	0.13 ± 0.03	0.16 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.13 ± 0.03	0.15 ± 0.00	0.12 ± 0.04	0.16 ± 0.00	0.17 ± 0.01	0.13 ± 0.02	0.14 ± 0.02
C20:1	0.34 ± 0.02	0.33 ± 0.03	0.31 ± 0.05	0.35 ± 0.02	0.33 ± 0.04	0.36 ± 0.02	0.32 ± 0.04	0.31 ± 0.04	0.36 ± 0.01	0.35 ± 0.02	0.30 ± 0.05	0.32 ± 0.04	0.38 ± 0.00
Subtotal trans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.01	0.00 ± 0.00	0.06 ± 0.02	0.09 ± 0.02	0.11 ± 0.01

Data are expressed as means ± SD from at least three independent experiments. ND means not detected.

TABLE 5: Fatty acids compositions and quality index of self-made ZSKO.

Fatty acids (g/100 g) and quality index	ZSKO
C14:1, 9t	ND
C14:1, 9c	ND
C16:0	7.64 ± 0.08
C16:1, 9t	ND
C16:1, 9c	0.95 ± 0.05
C18:0	1.52 ± 0.11
C18:1, 6t/9t/11t	ND
C18:1, 9c	27.52 ± 0.04
C18:2, 9t12t	ND
C18:2, 9c12t	ND
C18:2, 9t12c	ND
C18:2, 9c12c	29.88 ± 0.27
C18:3, 9t12t15t	ND
C18:3, 9t12t15c or 9t12c15t	ND
C18:3, 9c12t15t or 9c12c15t	ND
C18:3, 9c12t15c or 9t12c15c	ND
C18:3, 9c12c15c	32.49 ± 0.21
C22:1, t	ND
C20:1	ND
C20:3, n6	ND
Total content of TFAs	0.00 ± 0.00
Acidic value/mg KOH/g	0.92 ± 0.02
Peroxide value/mmol/Kg	1.15 ± 0.04

Data are expressed as means ± SD from at least three independent experiments. ND means not detected.

α -linolenic acid, 29.88 ± 0.27 g/100 g of linoleic acid, and 27.52 ± 0.04 g/100 g of oleic acid; and there was no TFA. ZSKO could be classified into nourishing healthy edible lipids with polyunsaturated fatty acids [19]. Its acidic value was 0.92 ± 0.02 mg KOH/g, and its peroxide value was 1.15 ± 0.04 mmol/kg. Furthermore, these complied with the Chinese Primary National Standards. The oil had a natural scent of *Zanthoxylum bungeanum* Maxim.

4. Conclusion

- (1) Drying temperature and time had a great effect on the TFA formation of ZSSO, and temperature had a larger effect than time. However, drying temperature and time had a small effect on TFA formation in ZSKO; that is, only a small quantity of TFAs were developed after drying at 100°C for eight hours.
- (2) ZSSO was removed with alkaline saponification. High concentrations of sodium hydroxide solution had some effect on TFA formation in ZKSO, but there was no TFA when the concentration of the sodium hydroxide solution was lower than 20% and even at boiling temperature for one hour.
- (3) The roasting temperature and duration for ZS should be properly controlled at 100°C for six hours or 150°C for two hours. High temperatures or prolonged time

accelerates the formation of TFAs, which is unfavorable to the quality of ZSKO.

- (4) ZS, which has a moisture content of 18%, was pressed four times (two hours) at less than 60°C, and ZSKO was obtained by collection through centrifuge separation. This contained 90.84% unsaturated fatty acids, which mainly include 32.49% α -linolenic acid, 29.88% linoleic acid, and 27.52% oleic acids; and there was no TFA. Its acidic value and peroxide value conformed to China standards for edible oil. Therefore, ZSKO could be used as a healthy food for further development.

Competing Interests

The authors declare that they have no conflict of interests.

Acknowledgments

This work was supported by grants from the Key Projects of Outstanding Achievement Transformation Fund in University of Chongqing (KJZH14105), the Chongqing 100 Leading Scientists Promotion Project, Chongqing Technological Innovation Leading Talent, and the Chongqing Science & Technology Commission (cstc2016shmszx80028).

References

- [1] C.-F. Wang, C.-X. You, K. Yang et al., "Antifeedant activities of methanol extracts of four *Zanthoxylum* species and benzophenanthridines from stem bark of *Zanthoxylum schinifolium* against *Tribolium castaneum*," *Industrial Crops and Products*, vol. 74, pp. 407–411, 2015.
- [2] K. Li, R. Zhou, W. W. Jia et al., "*Zanthoxylum bungeanum* essential oil induces apoptosis of HaCaT human keratinocytes," *Journal of Ethnopharmacology*, vol. 186, pp. 351–361, 2016.
- [3] L. Zhang, H.-T. Wu, F.-X. Yang, and J.-H. Zhang, "Evaluation of Soxhlet extractor for one-step biodiesel production from *Zanthoxylum bungeanum* seeds," *Fuel Processing Technology*, vol. 131, pp. 452–457, 2015.
- [4] T. Liu, S.-Y. Yao, Z.-Y. Yin, X.-X. Zheng, and Y. Shen, "Study on preparation method of *Zanthoxylum bungeanum* seeds kernel oil with zero trans-fatty acids," *Environmental Science and Pollution Research*, vol. 23, no. 8, pp. 7132–7137, 2016.
- [5] X. X. Zheng, Z. Y. Yin, R. Y. Du, M. J. Fang, X. M. Wang, and Q. Li, "Extraction of crust oil and kernel oil from pepper seeds by two-step extraction technology," *Food Science*, vol. 31, no. 22, pp. 180–184, 2010.
- [6] S. Y. Yao, F. Wang, C. Shi, R. Cui, and X. X. Zheng, "Changes in trans fatty acids composition, acid value and peroxide value in *Zanthoxylum bungeanum* seed kernel oil during heating process," *Food Science*, vol. 16, no. 36, pp. 32–37, 2015.
- [7] V. Scislowski, D. Bauchart, D. Gruffat, P.-M. Laplaud, and D. Durand, "Effect of dietary n-6 and n-3 polyunsaturated fatty acids on peroxidizability of lipoproteins in steers," *Lipids*, vol. 40, no. 12, pp. 1245–1256, 2005.
- [8] Y. Y. Liu, D. N. Wang, Y. S. Liu et al., "Preventive and therapeutic effect of the kernel of *Zanthoxylum* seed oil on experimental hyperlipidemia in rats," *Journal of the Fourth Military Medical University*, vol. 28, no. 5, pp. 411–413, 2007.

- [9] P. C. Calder, "Functional roles of fatty acids and their effects on human health," *Journal of Parenteral & Enteral Nutrition*, vol. 39, no. 1, supplement, pp. 18S–32S, 2015.
- [10] A. Erkkilä, V. D. F. de Mello, U. Risérus, and D. E. Laaksonen, "Dietary fatty acids and cardiovascular disease: an epidemiological approach," *Progress in Lipid Research*, vol. 47, no. 3, pp. 172–187, 2008.
- [11] S. K. Gebauer, J.-M. Chardigny, M. U. Jakobsen et al., "Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies," *Advances in Nutrition*, vol. 2, no. 4, pp. 332–354, 2011.
- [12] I. B. King, A. R. Kristal, S. Schaffer, M. Thornquist, and G. E. Goodman, "Serum Trans-fatty acids are associated with risk of prostate cancer in β -carotene and retinol efficacy trial," *Cancer Epidemiology Biomarkers & Prevention*, vol. 14, no. 4, pp. 988–992, 2005.
- [13] L. E. Voorrips, H. A. M. Brants, A. F. M. Kardinaal, G. J. Hiddink, P. A. van den Brandt, and R. A. Goldbohm, "Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer," *The American Journal of Clinical Nutrition*, vol. 76, no. 4, pp. 873–882, 2002.
- [14] M. L. Slattey, J. Benson, K.-N. Ma, D. Schaffer, and J. D. Potter, "Trans-fatty acids and colon cancer," *Nutrition & Cancer*, vol. 39, no. 2, pp. 170–175, 2001.
- [15] F. X. Bian, X. X. Zheng, Z. Y. Yin, and Y. J. Zhang, "Optimization of alkaline removal of oil from Sichuan pepper (*Zanthoxylum bungeanum*) pericarp before oil pressing and oxidative stability of Sichuan pepper oil," *Food Science*, vol. 34, no. 16, pp. 46–51, 2013.
- [16] C. G. Ma, Y. L. Bi, M. G. Lin, and X. Y. Li, "Analysis of chemical components in husk and kernel oil of *zanthoxylum bungeanum* seed," *Journal of Zhengzhou Grain College*, vol. 23, pp. 39–42, 2002.
- [17] M. Y. Xie, J. H. Xie, M. Y. Yang, and Z. Y. Deng, "A review of the research progress on trans fatty acids," *Journal of Chinese Institute of Food Science and Technology*, vol. 10, no. 4, pp. 14–26, 2010.
- [18] C. Li, Y. Zhang, S. Li et al., "Mechanism of formation of trans fatty acids under heating conditions in triolein," *Journal of Agricultural and Food Chemistry*, vol. 61, no. 43, pp. 10392–10397, 2013.
- [19] S. Y. Yao, T. Liu, R. Liu, Z. Y. Yin, and X. X. Zheng, "Research progress in polyunsaturated fatty acids and their anti-cancer role," *Journal of Chongqing Technology and Business (Natural Sciences Edition)*, vol. 31, no. 12, pp. 81–91, 2014.