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Influence of Temperature on Triacylglycerol Degradation in Camellia Seed Oil during Accelerated Thermal Oxidation

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Abstract A thermal oxidation test of Camellia seed oil (CO) was carried out at 120°C and 180°C by Rancimat instrument. The effects of temperature on the stability of CO were evaluated by measuring various chemical properties as well as the composition of nonpolar and polar triglycerides. The results showed that the rate of TAG degradation from CO during the first hour at 180°C was 4.16 times higher than at 120°C, and the formation of PTAG and TPC were 18.6 times and 8.15 times higher than at 120°C, respectively. This suggests that higher reaction temperature results in higher degree of degradation. The polymerization products (TGO and TGD), oxidation products (ox-TGM) and hydrolysates (DG and FFA) from CO were 27.67%, 59.05%, 13.32%, 66.15%, 29.28% and 5.21% after 10 hours oxidation at 120°C and 180°C, respectively, indicating that the reaction process of CO at the two temperatures was very different. The polymerization reaction was dominant at 180°C, while the oxidation reaction was the dominant reaction at 120°C. The degree of hydrolysis at 120°C was higher than at 180°C. In addition, polar compounds TGO and TGD are considered biologically toxic and cytotoxic, and, as temperature increases, the nutritional and safety characteristics of CO worsen. Therefore, the cooking temperature of CO should not be too high.

Keywords: *camellia seed oil, triacylglycerol, thermal oxidation*

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1. Introduction

Camellia oleifera Abel., an evergreen tree, grows only in middle and southern regions of China, such as Hunan, Jiangxi, Fujian, Anhui, Guangdong provinces, etc. [1]. The world's total plant area of camellia is around 3.3 million ha., of which approximately 3.0 million ha. in China standing about 90% of the global total. In addition to Southeast Asia, Japan and other State-owned very little distribution [2,3]. Camellia oleifera is considered to be nutritious and healthy, owing to its fatty acids composition and the presence of bioactive compounds, such as tea polyphenol, tea saponin and squalene, etc. [4].

Camellia oil (CO) is obtained from Camellia oleifera seeds, is characterized by a high proportion of oleic acid (74-89%) [5] and antioxidant compounds, such as sterols, triterpenes [6], tocopherol, squalene [7]and phenolics [8,9]. At present, quality research of CO is mainly focused on the oxidation stability under processing and storage, and research on the thermal oxidative stability under high temperature is relatively scarce. A significant number of papers on comparative thermal oxidative stability of different oils and fats are published every year, however, the results obtained are highly variable due to several reasons. First, one oil sample is not representative of every oil of the same type, because differences in the initial quality and in the contents of minor compounds are significant enough to alter oil quality. Second, important variables in the high-temperature process, i.e. additives or the surface-to-oil volume ratio, are often omitted. Furthermore, repeatability of the high-temperature process is very low [10]. Therefore, a comprehensive study on thermal oxidation stability of CO is needed.

Triacylglycerol (TAG) is the main component of vegetable oil (95-98%), which is composed of free fatty acids via the Kennedy pathway in glycerol skeletal molecules [11]. TAG analysis is widely used in oil and fat analysis. As the fingerprint of a fat, TAG molecular information can be combined with statistics to judge the variety [12,13], origin [14], treatment [15] and adulteration [16,17] of oil and fat. In addition, during the high-temperature treatment process, grease can produce volatile components, hydrolysates, triacylglycerol monomer, cyclized compounds, *trans*-structures and polymers due to oxidation, hydrolysis, isomerization and polymerization [18]. These compounds can be used to elucidate the thermal oxidation mechanism of greases during high-temperature treatment

In this study, commercial CO, acquired by a physical squeezing method, was used as a test sample and treated via accelerated oxidation by Rancimat oil oxidative

rancidity instrument at 120°C (temperature parameter at induced oxidation) and 180°C (recommended temperature for deep frying). The effects of temperature on the thermal oxidation stability of CO were analyzed via measuring various chemical properties as well as the composition of nonpolar and polar triglycerides.

2. Materials and Methods

2.1. Materials

Cold pressed CO were purchased from local supermarket (Changsha, China). The FFA, PV and *p*-AV of fresh CO were 0.22%, 3.13 mmol/kg and 4.91 respectively. The fresh CO contained (per 100 g): 8.42 g palmitic acid, 1.73 g stearic acid, 79.91 g oleic acid, 8 g linoleic acid, and 0.44 g linolenic acid. All chemicals and solvents used were either analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2. Sample Preparation

Thermal oxidation was carried out under strictly controlled conditions using Rancimat (Metrohm, Herisau, Switzerland) apparatus. The samples were treated under identical conditions. Briefly, 10.00 ± 0.01 g of oils were weighed in Rancimat reaction vessels and inserted into the heating block at 120°C or 180°C. The air flow rate was 10 L/h. Samples were analyzed in triplicate and heated for 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h. After heating, the samples were removed, drained under a stream of nitrogen, and stored in at -20°C for subsequent analysis.

2.3. Lipid Analysis

2.3.1. Determination of Fat Constants

The free fatty acid (FFA) of frying oils was determined according to PRC national standard methods GB/T 5538, 2005: Animal and vegetable fats and oils-determination of acid value and acidity [19]. Peroxide value (PV) was determined according to PRC national standard methods GB/T 5538-2005: Animal and vegetable fats and oils-determination of peroxide value [20]. *p*-Anisidine value (AV) was determined according to PRC national standard methods GB/T 24304-2009: Animal and vegetable fats and oils-determination of anisidine value [21]. Ultraviolet absorbance (K232 and K268) was determined according to PRC national standard methods GB/T 22500-2008: Animal and vegetable fats and oils-determination of ultraviolet absorbance expressed as specific UV extinction [22]. Carbonyl value (CV) was determined according PRC national standard methods GB/T 5009.37-2003: Method for analysis of hygienic standard of edible oils [23].

2.3.2. Analysis of TAG

Analysis of TAG profiles in CO was conducted by NARP-HPLC-EISD [24]. Briefly, oil samples were dried with anhydrous sodium sulphate and subsequently filtered through filter paper. A sample of oil (0.05 ± 0.01 g) was dissolved in 10 mL of isopropanol, and the flask was vortexed for approximately 1 min. A 1 mL aliquot was filtered through a 0.45 μm disposable LC filter disk and transferred to a sample vial via 2.5 mL disposable syringe. HPLC analysis was conducted on Shimadzu LC-20AD (Shimadzu, Japan) equipped with CMB-20A controller, LC-20AD binary pump, SIL-20A autosampler, and CTO-10AS column oven. Separation was achieved with an Agilent ZORBAX SB-C18 (4.6 mm × 250 mm; 5 μm, Agilent Technology, USA). The flow rate was set to 1.2 mL/min, and the injection volume was 20 μL. Mobile phases A and B were acetonitrile and isopropanol, respectively. The following gradient was employed: 0-1 min, 40% B; 115 min, 40-30% B; 15-60 min, 30% B; 60-75 min, 30-40% B; 75-85 min, 40% B. The eluent was monitored with 2000ES ELSD detector (Alltech, USA), with the following settings: 80°C drift tube temperature, gain of 1, and airflow rate of 2.8 L/min.

Quantification was based on the internal normalization method, assuming the sum of the peaks corresponding to various triglycerides is equal to 100%. The relative percentage of each triglyceride (T_i) was calculated to one decimal place, using equation (1):

$$T_i = \frac{\text{area of peak}}{\text{sum of all peak area}} \times 100\%. \quad (1)$$

2.3.3. Determination of Polymerized Triglycerides (PTAG)

Polymerized triglycerides (PTAG) were determined by high-performance size-exclusion chromatography (HPSEC) as described previously [25,26,27]. Briefly, 50.0 ± 0.1 mg samples were prepared in a conical flask. In order to dissolve the oil, 2 mL tetrahydrofuran were added followed by 50 mg anhydrous sodium sulfate. Approximately 2-5 minutes later, the solution was filtered through a 0.22 μm organic membrane. HPSEC analysis was conducted on GPC high performance liquid chromatograph (Agilent Technologies, USA) equipped with G1310A unit pump, G1328B manual injector, G1362A column oven and G1362A differential refractive index detector. A 500 Å Plgel single aperture chromatographic column (30 cm×0.75 cm I.D.) packed with high performance spherical gel made of styrene-divinylbenzene co-polymer (film thickness of 5 μm) (Agilent Technologies, USA) was used for separation. A sample volume of 20 μL was injected onto the HPLC, and tetrahydrofuran was selected as the mobile phase at a flow rate of 1 mL/min. The column temperature was maintained at 35°C, and the PTG was detected using a differential refractive index detector at 35°C (Agilent Technologies, USA). The W_{PTAG} was calculated as follows:

$$W_{PTAG} = \frac{\sum A_{PTG}}{\sum A_{tot}} \times 100\%. \quad (2)$$

where A_{PTAG} is the sum of the peak areas of all PTGs, A_{tot} is the sum of the peak areas of all acylglycerols (PTGs, TAGs, DAGs, and MAGs), W_{PTAG} is the percent contribution of polymerized triacylglycerols to the oil.

2.3.4. Quantification and Distribution of Total Polar Compounds

The total polar compound composition of frying oils was determined using classical column chromatography

[28,29,30] with modifications. Oil samples (1.00 g) were dissolved in 10 mL solvent (light petroleum/diethyl ether, 87%/13%, v/v). The solution was introduced to a glass column (20×400 mm) filled with a slurry of silica gel (70-230 mesh). In order to elute the nonpolar and polar compounds, 150 mL of solvent 1 (n-hexane/diethyl ether, 87/13, v/v) and 150 mL of solvent 2 (diethyl ether, b.p. 40-60°C) were used, respectively. A dropping funnel was used to maintain the flow rate at about 2.5 mL/min. The solvents were evaporated, and the contents of the nonpolar and polar fractions were determined gravimetrically. Efficiency of the separation was checked by thin layer chromatography [28]. The mass fractions of nonpolar compounds (W_{NPC}) and polar compounds (W_{PC}) are given by the following formulas:

$$W_{NPC} = \frac{M_{NPC}}{M} \times 100\% \quad (3)$$

$$W_{PC} = \frac{M_{PC}}{M} \times 100\% \quad (4)$$

where M_{NPC} and M_{PC} are the mass (g) of the nonpolar and polar fractions, respectively, and M is the mass (g) of the test portion added to the column.

The polar fractions, recovered in 10 mL tetrahydrofuran, were analyzed by means of HPSEC according to a previously published method [31,32]. The equipment configuration of HPSEC was identical to that of the PTG analysis. The separation of polar compounds, characterized by different polarity and molecular size, was performed on 100 Å and 500 Å PL gel columns (30×0.75 cm I.D.) packed with porous, highly cross-linked polystyrene-divinylbenzene copolymers (film thickness of 5 μm) (Agilent Technologies, USA) connected in series. HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. The sample concentration was 7-12 mg/mL in tetrahydrofuran. The temperature of the column and detector were kept constant at 35°C, and the analysis time was about 20 min. Under the above conditions, five groups of compounds were neatly separated and quantified, including triacylglycerols oligomers (TGO), triacylglycerols dimmers (TGD), oxidized triacylglycerols monomer (ox-TGM), diacylglycerols (DG), and free fatty acids (FFA). The calculation was performed with the assumption that all compounds in the sample were eluted. The content of the different groups of compounds, expressed as percentages of the oil, can be calculated as follows:

$$W_{TGO} = \frac{A_{TGO}}{\sum A} \times W_{PC} \quad (5)$$

$$W_{TGD} = \frac{A_{TGD}}{\sum A} \times W_{PC} \quad (6)$$

$$W_{ox-TGM} = \frac{A_{ox-TGM}}{\sum A} \times W_{PC} \quad (7)$$

$$W_{DG} = \frac{A_{DG}}{\sum A} \times W_{PC} \quad (8)$$

$$W_{FFA} = \frac{A_{FFA}}{\sum A} \times W_{PC} \quad (9)$$

where W_{TGO}, W_{TGD}, W_{ox-TGM}, W_{DG} and W_{FFA} are the percent contributions of TGO, TGD, ox-TGM, DG, and FFA, respectively. A_{TGO}, A_{TGD}, A_{ox-TGM}, A_{DG} and A_{FFA} are the corresponding peak areas, ∑A is the sum of all peak areas, and W_{PC} is the percentage of polar compounds in the oil.

2.4. Statistical Analysis

We performed all experiments in triplicate, and present mean ±SD of the results. We used Origin Pro 8.0 and SPSS17.0 to calculate one-way analysis of variance (ANOVA) and regression. Significant differences among different oil aliquots and treatments were determined with LSD multiple range tests. Significant differences ($P \leqslant 0.05$) were identified using LSD procedures.

3. Results and Discussions

3.1. Chemical Parameters

Figure 1 shows the change in chemical parameters of CO during the process of accelerated oxidation for 10 h at 120°C and 180°C. The formation of FFA in CO at 180°C was strongly correlated with time (R^2 = 0.99) and FFA formation increased over time at a rate of 0.32%/h. At 120°C, the FFA content in CO did not change significantly after 3 h but slowly increased at a rate of 0.28%/h after 4 h. After 10 h, the amount of FFA in CO at 180°C was 2.03 times higher than at 120°C, indicating that the higher the temperature, the greater FFA content in CO. Abdulkarim [33] believes that the rise in FFA at elevated temperatures is related to the further oxidation of small molecules to organic acids by oxidative cleavage of unsaturated fatty acids in oils and fats. The rate of PV, AV and TOTOX formation at 180°C was 7.84, 6.64 and 6.71 times higher than at 120°C, respectively. In the process of accelerated oxidation, the PV, AV and TV content in the oil continuously increased at 120°C but gradually stabilized at 180°C, which was related to the further degradation of unstable hydroperoxides [34,35]. After 10 h, the PV and TOTOX content of CO at 120°C were 41.97 and 3.76 times higher than that at 180°C, respectively, which indicates that the accumulation level of primary oxidation products of CO at 120°C was higher than at 180°C. In addition, the increasing rates of CV, K232 and K268 at 180°C were 12.37, 17.70 and 9.39 times higher than at 120°C in the first 1 h, and the final levels of CV, K232 and K268 at 180°C were 1.39, 1.19 and 1.74 times higher than at 120°C after 10 h. Thus, it can be concluded that the accumulation of secondary oxidation products at 180°C is higher than that at 120°C. The chemical analysis of CO shows that the reaction process of CO at 120°C and 180°C was significantly different. At 120°C, the primary oxidation products in CO continued to accumulate, while the unstable primary oxidation products of CO at 180°C were involved in the subsequent reaction, resulting in large number of secondary oxidation products. The oxidation process of CO at 180°C may not be a typical free radical chain reaction that consists of initiation, proliferation and termination.

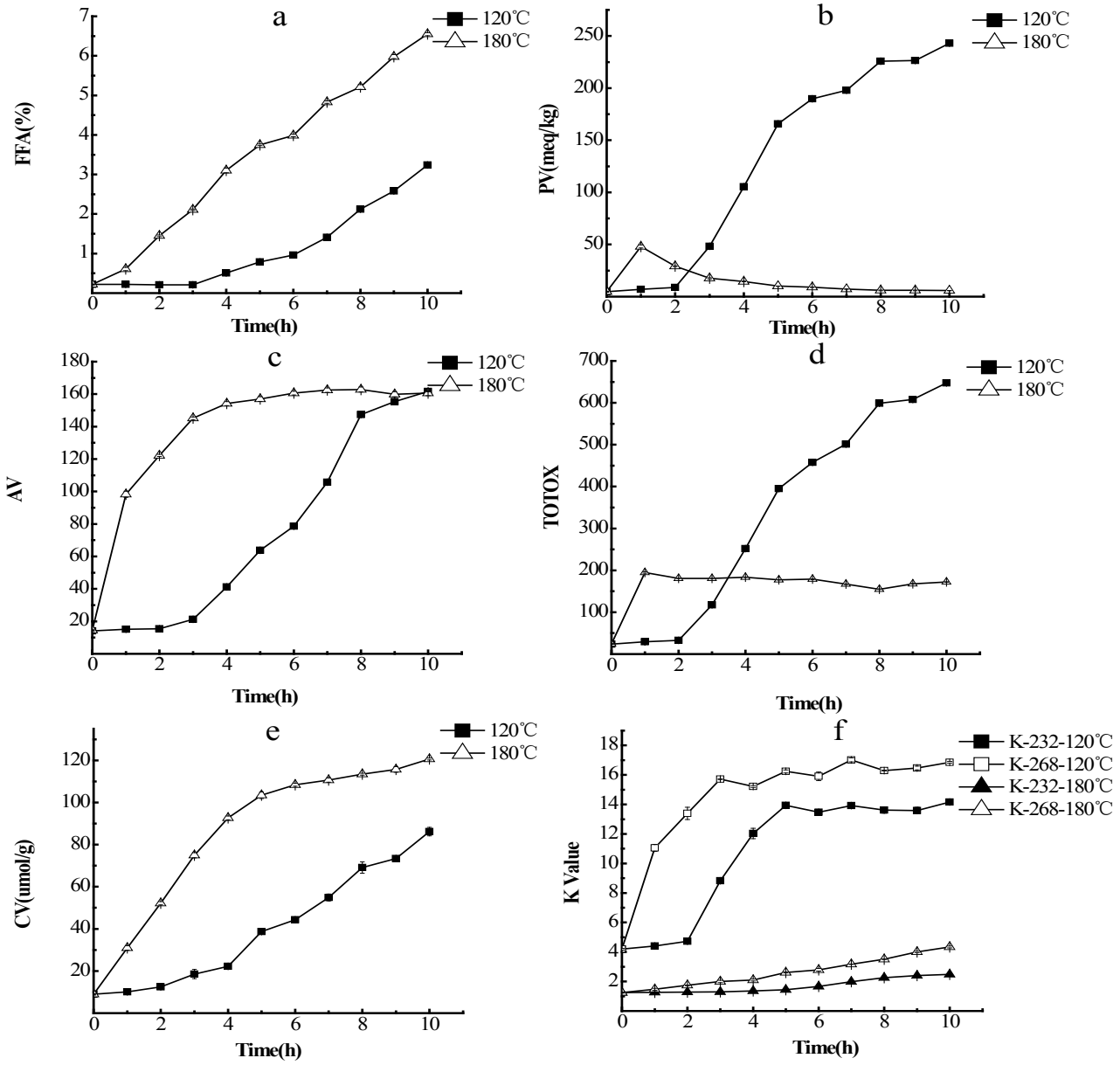

Figure 1. The change in chemical parameters of CO under accelerated oxidation at 120°C and 180°C. a, free fatty acid (FFA); b, peroxide value (PV); c, anisidine value (AV); d, total oxidation value (TOTOX); e, carbonyl value (CV); f, K value

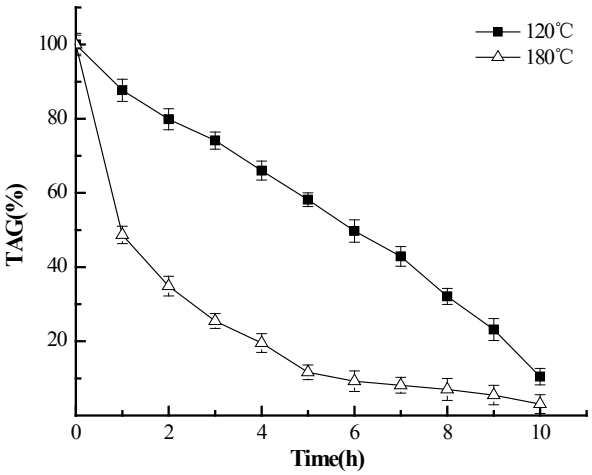

Figure 2. The degradation of CO TAG under 10 h of accelerated oxidation at 120°C and 180°C

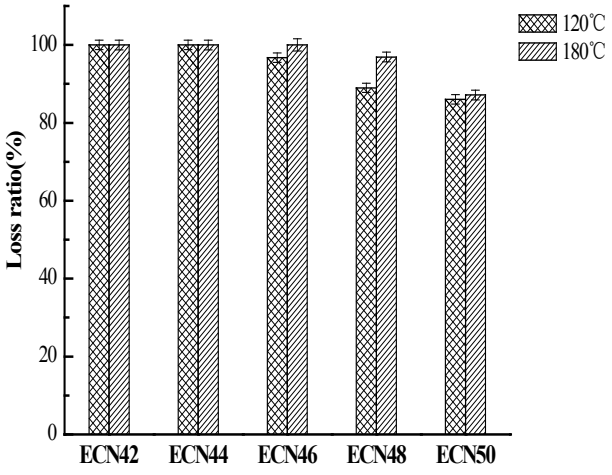

Figure 3. The loss ratio of camellia seed oil TAG with different equal carbon number under accelerated thermal oxidation at 120°C and 180°C

3.2. Glycerides

Figure 2 shows the overall degradation of CO TAG at 120°C and 180°C during the accelerated oxidation process. As shown in Figure 2, the total degradation of TAG in CO at 120°C was negatively correlated with time (R^2=0.99) and decreased at a rate of 8.80%/h. The TAG degradation rate of CO during the first 1 h at 180°C was 4.16 times higher than at 120°C, and the rate subsequently decreased at 3.59%/h. After 10 h, the total loss of CO in TGA at 120°C and 180°C was 89.50% and 96.92%, respectively, indicating that TAG degradation of CO was faster and more thorough at 180°C.

The degradation patterns of TAG in different regions of CO were also at the two different temperatures. At 120°C, TAG at different regions decomposed with time. The order of degradation is as follows: ECN48, ECN46, ECN50, ECN44 and ECN42, and the degradation rates were 7.90, 0.62, 0.18, 0.08 and 0.01%/h, respectively. The loss ratio of ECN42 and ECN44 were 100% after accelerated oxidation at 120°C for 9 h, and loss ratios of ECN46, ECN48 and ECN50 were 96.72%, 88.98% and 86.03%, respectively after 10 h. The degradation rates of ECN42, ECN44, ECN46, ECN48 and ECN50 at 180°C were 1.03, 1.80, 2.62, 4.45 and 4.47 times higher than at 120°C. The TAG in ECN42 and ECN44 regions completely degraded after accelerated oxidation for 2 h and 4 h, respectively, while TAG in ECN44, ECN46, ECN48 and ECN50 slowly degraded at rates of 0.07, 0.12, 3.31 and 0.13%/h, respectively. The loss ratios were 100%, 96.89% and 87.28% after 10 h, as shown in Figure 3. The TAG degradation rate in CO decreased with increasing ECN at 120°C and 180°C.

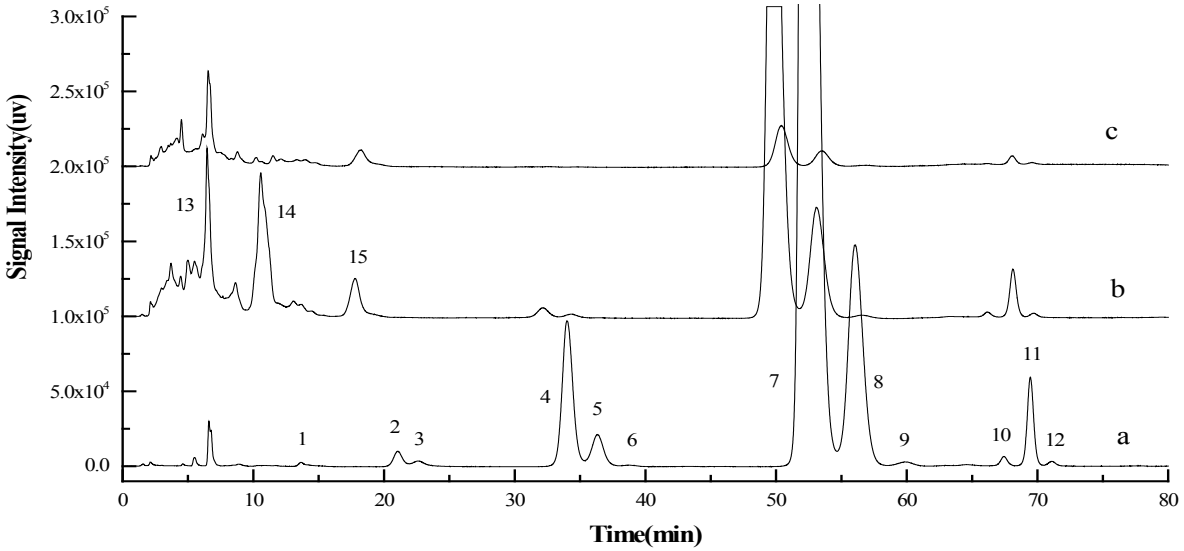

Figure 4. Chromatograms comparing CO TAGs composition in a high temperature system. a, fresh oil; b, accelerated oxidation for 10 h at 120°C; c, accelerated oxidation for 10 h at 180°C (1, LLL; 2, LLO; 3, PLL; 4, OOL; 5, POL; 6, PPL; 7, OOO; 8, OOP; 9, POP; 10, SOO; 11, SLS; 12, POS).

Table 1. Degradation of CO TAG under accelerated thermal oxidation at 120°C and 180°C (%)

ECN	TAG	Temperature (°C)	Time (h)					
			0	2	4	6	8	10
42	LLL	120	0.13 ± 0.01 ^{Aa}	0.08 ± 0.01 ^{B}	0.07 ± 0.00 ^{C}	0.05 ± 0.01 ^{D}	0.04 ± 0.00 ^{DE}	0.03 ± 0.02 ^{E}
		180	0.13 ± 0.02 ^{Aa}	ND	ND	ND	ND	ND
44	LLO	120	0.48± 0.03 ^{Aa}	0.28 ± 0.01 ^{Ca}	0.13 ± 0.01 ^{E}	0.07 ± 0.01 ^{G}	0.04 ± 0.01 ^{H}	ND
		180	0.48 ± 0.02 ^{Aa}	0.11 ±0.00 ^{Cb}	ND	ND	ND	ND
	PLL	120	0.26 ± 0.02 ^{Aa}	0.17 ± 0.01 ^{Ca}	0.06 ± 0.05 ^{E}	0.03 ± 0.00 ^{F}	ND	ND
		180	0.26 ± 0.03 ^{Aa}	0.04 ± 0.01 ^{Cb}	ND	ND	ND	ND
46	OOL+SLL	120	5.50 ± 0.14 ^{Aa}	3.39 ±0.16 ^{Ca}	2.10 ± 0.11 ^{Ea}	1.08 ± 0.01 ^{Fa}	0.52 ± 0.04 ^{Ha}	0.34 ± 0.06 ^{I}
		180	5.50 ± 0.24 ^{Aa}	0.83 ± 0.36 ^{Cb}	0.35 ± 0.18 ^{Eb}	0.10 ± 0.01 ^{FGb}	0.05 ± 0.02 ^{Gb}	ND
	POL	120	1.30 ± 0.07 ^{Aa}	0.81 ± 0.01 ^{Ca}	0.53 ± 0.03 ^{Ea}	0.33 ± 0.01 ^{Ga}	0.19 ± 0.01 ^{Ha}	0.11 ± 0.05 ^{I}
		180	1.30 ± 0.08 ^{Aa}	0.34 ± 0.04 ^{Cb}	0.15 ± 0.01 ^{Db}	0.10 ± 0.03 ^{Ea}	0.02 ± 0.00 ^{Gb}	ND
	PPL	120	0.07 ± 0.01 ^{Aa}	0.04 ± 0.00 ^{Ba}	0.03± 0.01 ^{BC}	0.02 ± 0.012 ^{C}	ND	ND
		180	0.07 ± 0.01 ^{Aa}	0.03 ± 0.01 ^{Ba}	ND	ND	ND	ND
48	OOO+SLO	120	78.58 ± 0.88 ^{Aa}	64.57 ± 0.26 ^{Ca}	54.11 ± 1.30 ^{Ea}	41.57 ± 0.94 ^{Ga}	26.31 ± 1.41 ^{Ia}	17.11 ± 1.25 ^{Ka}
		180	78.58 ± 2.96 ^{Aa}	27.01 ± 1.26 ^{Cb}	14.9 1± 1.03 ^{Eb}	6.34 ± 0.91 ^{Gb}	4.85 ± 0.52 ^{Hb}	1.90 ± 0.12 ^{Ib}
	OOP	120	11.19 ± 0.12 ^{Aa}	8.43 ± 0.04 ^{BCa}	7.25 ± 0.30 ^{Ca}	5.10 ± 0.10 ^{Da}	3.95 ± 0.19 ^{DEa}	2.68 ± 0.09 ^{Ea}
		180	11.19 ± 0.21 ^{Aa}	5.04 ± 0.07 ^{BCb}	3.08 ± 0.32 ^{CDb}	2.08 ± 0.11 ^{Db}	1.62 ± 0.09 ^{Db}	0.82 ± 0.01 ^{Db}
	POP	120	0.24 ± 0.01 ^{Aa}	0.16 ± 0.00 ^{Ca}	0.12 ± 0.00 ^{Ea}	0.09 ± 0.00 ^{Fa}	0.06 ± 0.00 ^{Ga}	0.04 ± 0.01 ^{Ha}
		180	0.24 ± 0.02 ^{Aa}	0.16 ± 0.00 ^{Ca}	0.15 ± 0.00 ^{CDa}	0.14 ± 0.02 ^{Da}	0.11 ± 0.01 ^{EFa}	0.08 ± 0.01 ^{Ga}
50	SOO	120	0.21 ± 0.01 ^{Aa}	0.18 ± 0.00 ^{Ba}	0.17 ± 0.00 ^{BCa}	0.15 ± 0.01 ^{Da}	0.11 ± 0.00 ^{Ea}	0.08 ± 0.02 ^{Ga}
		180	0.22 ± 0.05 ^{Aa}	0.18 ± 0.05 ^{Ba}	0.10 ± 0.01 ^{Da}	0.06 ± 0.00 ^{Fa}	0.05 ± 0.00 ^{Fa}	0.02 ± 0.00 ^{Ga}
	SLS	120	1.94 ± 0.06 ^{Aa}	1.70 ± 0.01 ^{Ba}	1.37 ± 0.01 ^{CDa}	1.16 ± 0.01 ^{Da}	0.83 ± 0.03 ^{Fa}	0.49 ± 0.03 ^{Ha}
		180	1.94 ± 0.06 ^{Aa}	1.02 ± 0.01 ^{Cb}	0.69 ± 0.05 ^{Eb}	0.35 ± 0.05 ^{Gb}	0.28 ± 0.09 ^{GHb}	0.22 ± 0.03 ^{Hb}
	POS	120	0.11 ± 0.01 ^{Aa}	0.10 ± 0.01 ^{ABa}	0.09 ± 0.01^{Ba}	0.08 ± 0.02 ^{BCa}	0.07 ± 0.01 ^{Ca}	0.06 ± 0.03 ^{Ca}
		180	0.12 ± 0.01 ^{Aa}	0.10 ± 0.00 ^{ABa}	0.10 ± 0.00 ^{Ba}	0.08 ± 0.00 ^{Ca}	0.06 ± 0.00 ^{Da}	0.05 ± 0.00 ^{Da}

Note: ECN-Equal carbon number; ND-not detected; All the values are mean ± SD (n = 3); Values with the different uppercase letters (A–F) within the same row are significantly different at $P < 0.05$; Lowercase letters (a–d) within the same column of each index represent values that are significantly different at $P \leq 0.05$.

Figure 4 shows the chromatogram of TAG composition after accelerating oxidation of CO for 10 h. Table 1 shows the trend of each type of TAG in accelerated oxidation process of CO. At 120°C, the relative content of TAG in CO decreased with time, and the degradation rate of each TAG was correlated with its content. TAG at a higher percent composition had a higher degradation rate. The percent composition of OOO was the highest and its degradation rate was 6.95 %/h, while the degradation rate of PPL, with the lowest percent composition, was only 0.63%/h. The degradation rates of OOO + SLO, OOP and OOL + SLL at 180°C were 4.90, 2.62, and 2.98 times higher than at 120°C, respectively. The loss ratio of each TAG in the process of accelerated oxidation is related to its composition. More unsaturated fatty acids in TAG compounds resulted in a higher loss ratio, and the loss ratio was different at the two different temperatures. LLL and LLO were completely degraded after accelerated oxidation for 9 h at 120°C and completely degraded at 180°C for 2 h and 4 h, respectively. At 180°C, the loss ratio of PPL and PLL reached 100% 3 h faster than at 120°C. OOL + SLL and POL reached 100% degradation after 10 h of accelerated oxidation at 180°C, and they reached 96.91% and 95.77% degradation after 10 h at 120°C, respectively. The loss ratios of OOO+SLO, OOP, POP, SOO, SLS, and POS at the end of accelerated oxidation at 180°C were 1.09, 1.01, 0.19, 1.2, 1.01 and 1.24 times higher than at 120°C, respectively.

As shown in Figure 4, the area of peak 13 increased after accelerated oxidation for 10 h. According to the results of TAG analysis of soybean oil via the IUPAC method, it was deduced that peak 13 is glyceride and diglyceride [36]. The increase in glyceride and diglyceride content during the process of accelerated oxidation of Camellia oil may be related to the oxidation and hydrolysis of TAG. The peaks 14 and 15 are newly generated compounds in the accelerated oxidation process of CO. According to Zeb [37], these are epoxy groups or individual oxidized TAGs. As shown in Figure 4, the peak area of peaks 13, 14 and 15 are significantly higher at 120°C than at 180°C, indicating that the mechanism of TAG degradation at different temperatures is significantly different.

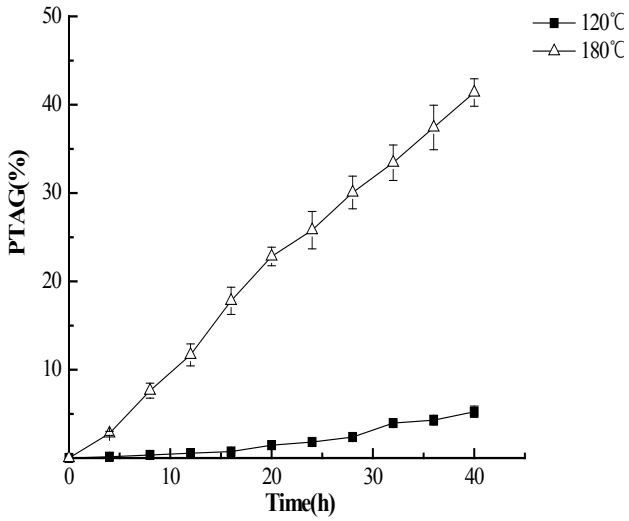

Figure 5. Formation of PTAG during accelerated thermal oxidation at 120°C and 180°C in CO

3.3. Polymerized Triglycerides

No PTAG was detected in the fresh CO. After 1 h of accelerated oxidation at 120°C and 180°C, PTAG started to be produced. At this time, the generated amount at 180°C was 18.6 times higher than at 120°C. As shown in Figure 5, the PTAG generation of CO showed an upward trend over time, and the rate of PTAG generation was elevated at 180°C and was 8.02 times higher than at 120°C. After 10 h, the content of PTAG was only 5.14% at 120°C, while the PTAG content at 180°C was 41.38%, which indicated that the degree of polymerization of CO was higher at 180°C than at 120°C. Bansal *et al.* [38] concluded that the formation of PTAG at high temperature is related to the free radical chain reaction and automatic oxidation.

3.3. Distribution of Polar Triglycerides in Polar Components

The total polar compound (TPC) content in the fresh CO was 1.01%. After 1 h of accelerated oxidation, the content of TPC at 120°C and 180°C was 1.76 times and 13.05 times the initial content, which indicated that the degradation rate of CO was faster at 180°C. The formation of TPC in CO at 120°C showed a continuous upward trend and was significantly correlated with time. However, at 180°C, the rate of TPC production increased rapidly and then decreased, and the rate of TPC formation in the first 5 h was 2.28 times that in the last 5 h. The rate of TPC production in CO at 180°C was 3.08 times higher than at 120°C. After 10 h of accelerated oxidation, the TPC content at 180°C was 3.09 times higher than at 120°C, indicating that the higher the temperature, the higher the degradation rate of CO. TGO, TGD, ox-TGM, DG and FFA were the main compounds of the modified polar fraction during the accelerated oxidation process at 120°C and 180°C. The distribution of these five compounds is shown in Table 2.

TGD and TGO are polymers formed by ox-TGM through C-C, C-O, O-O and other covalent bonds [39]. The molecular weight of TGD and TGO was significantly higher than the TAG monomer, about 2 to several times of TAG monomer [40]. It can be seen from Table 2 that the increase of TGD in CO at 120°C is only 1.5 times the initial value, while it is 40.63 times at 180°C. In the accelerated oxidation process, the TGDs of CO showed an increasing trend under both temperature conditions, but certain difference existed. The formation rate at the later stage was 3.4 times higher than at 120°C, while it is 14 times greater at 180°C. The overall formation rate of TGD at 180°C was 4.04 times higher than at 120°C. After 10 h, the cumulative amount of TGD at 180°C was 3.77 times higher than at 120°C. While TGO was not detected at 120°C, it was detected at a percent composition of 1.60% after accelerated oxidation at 180°C for 3 h. TGO increased rapidly at a rate of 2.10%/h over time, reaching a peak level at 16.74% after 10 h.

Ox-TGM is a TAG monomer formed by the oxidation of TAG in the processing and storage of oil, which contains HOO-, epoxy, ketone, HO- and other oxygenated groups [41]. Ox-TGM content can provide effective information regarding the oxidation process, and it can be

used as a good indicator to evaluate the early oxidation of oil [42]. The content of ox-TGM in CO increased at a rate of 0.99%/h at 120°C. The ox-TGM increased rapidly to 11.85% at the rate of 5.77%/h in the first 2 h, which was 40.86 times greater than the initial content. It increased slowly at the rate of 1.03%/h in the late stage. The ox-TGM production rate at 180°C was significantly higher than that of 120°C. After 10 h, the accumulation of ox-TGM at 180°C was 1.53 times greater than at 120°C.

Table 2. TPC and their percent distribution in CO during accelerated thermal oxidation at 120°C and 180°C

Index	Temperature (°C)	Time (h)					
		0	2	4	6	8	10
Total	120	2.03 ± 0.12 ^{Fa}	2.59 ± 0.03 ^{Eb}	5.87 ± 0.21 ^{Db}	9.33 ± 0.31 ^{Cb}	11.65 ± 0.65 ^{Bb}	16.73 ± 0.76 ^{Ab}
	180	1.98 ± 0.04 ^{Fb}	21.10 ± 0.73^{Ea}	33.66 ± 0.98 ^{Da}	39.55 ± 1.21 ^{Ca}	46.09 ± 2.01 ^{Ba}	51.69 ± 2.51 ^{Aa}
TGO	120	ND	ND	ND	ND	ND	ND
	180	ND	ND	3.24 ± 0.11 ^{D}	8.59 ± 0.42 ^{C}	11.68 ± 0.19 ^{B}	16.74 ± 0.87 ^{A}
TGD	120	0.10± 0.01 ^{Fa}	0.16 ± 0.01 ^{Eb}	0.77 ± 0.04 ^{Db}	1.55 ± 0.09 ^{Cb}	2.41 ± 0.34 ^{Bb}	4.63 ± 0.31 ^{Ab}
	180	0.08±0.01 ^{Fb}	7.03 ± 0.54 ^{Ea}	12.83 ± 0.54 ^{Da}	14.47 ± 0.65 ^{Ca}	16.64 ± 0.43 ^{Ba}	17.45 ± 1.44 ^{Aa}
ox-TGM	120	0.64± 0.04 ^{Fa}	1.11 ± 0.21 ^{Eb}	3.16 ± 0.16 ^{Db}	5.75 ± 0.06 ^{Cb}	7.43 ± 0.52 ^{Bb}	9.88 ± 0.67 ^{Ab}
	180	0.29±0.03 ^{Fb}	11.84 ± 0.42 ^{Ea}	14.71 ± 0.76 ^{Da}	14.14 ± 0.62 ^{Ca}	14.67 ± 1.45 ^{Ba}	15.14 ± 1.36 ^{Aa}
DG	120	1.26± 0.04 ^{Fa}	1.33 ± 0.05 ^{Eb}	1.88 ± 0.04 ^{Db}	2.09 ± 0.06 ^{Ba}	1.99 ± 0.34 ^{Cb}	2.23 ± 0.45 ^{Aa}
	180	1.62 ±0.06 ^{Fb}	2.21 ± 0.26 ^{Ea}	2.85 ± 0.09 ^{Ba}	2.63 ± 0.43 ^{Da}	3.01 ± 0.03 ^{Aa}	2.7 ± 0.57 ^{Ca}
FFA	120	0.04± 0.01 ^{Ab}	0.02 ± 0.02 ^{A}	ND	ND	ND	ND
	180	0.07±0.01 ^{a}	ND	ND	ND	ND	ND

Note: TGO-triacylglycerols oligomers; TGD-triacylglycerols dimmers; ox-TGM-oxidized triacylglycerols monomers; DG-diacylglycerols; FFA-fatty acid value; ND-not detected; All the values are mean ± SD (n = 3); Uppercase letters (A–F) within the same row represent values that are significantly different at $P \leq 0.05$; Lowercase letters (a–d) within the same column of each index represent values that are significantly different at $P \leq 0.05$.

The contents of DG and FFA in fresh CO were 1.44% and 0.05%, respectively. FFA was not detected after 1 h of accelerated oxidation at 180°C, while FAA was detected at 120°C after 3 h. At 120°C, DG was slowly increased at a low rate of 0.07%/h. At 180°C, DG content increased to about 2.23% at a rate of 0.75%/h for 1 h and increased with the same rate at 120°C. The formation of DG at 180°C was only 1.21 times greater than at 120°C after 10 h. The low levels and low formation rate of DG and FFA indicate that the hydrolysis of CO at high temperature is weak.

As shown in Figure 6, the polymerization products (TGO and TGD), oxidized products (ox-TGM), and hydrolysates (DG and FFA) of CO at 120°C and 180°C had the following percent compositions: 27.67%, 59.05%, 13.32%, 66.15%, 29.28%, and 5.21%, respectively. The results showed that the reaction process was significantly different at 180°C. The polymerization reaction was dominant at 180°C, while the oxidation reaction was dominant at 120°C, and the degree of hydrolysis at 120°C was higher than at 180°C.

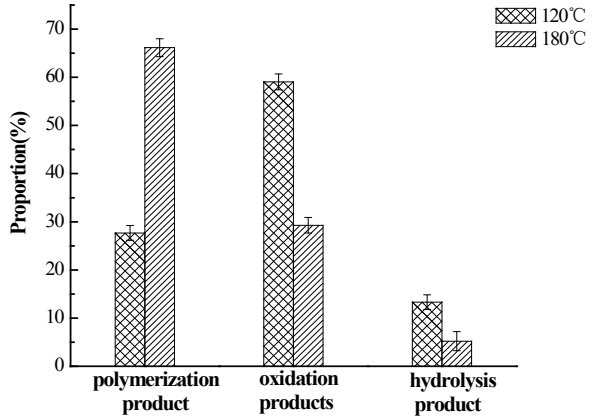

Figure 6. Distribution of polymerization production (TGO and TGD), oxidation product (ox-TGM) and hydrolysis product (DG and FFA) after 10 h of accelerated oxidation of CO

4. Conclusion

Chemical analysis showed that the reaction process of CO at 120°C and 180°C was significantly different. The oxidation process of CO at 180°C may not follow a typical chain reaction process, such as initiation, proliferation and termination. PTAG analysis showed that the degree of polymerization of CO at 180°C was significantly higher than at 120°C. The results of HPSEC analysis showed that there were significant differences in the distribution of polymeric products (TGO and TGD), the oxidized product (ox-TGM) and hydrolysates (DG and FFA) during the accelerated oxidation of CO at 120°C and 180°C, indicating the reaction process of CO was different at the two temperatures. The polymerization reaction was dominant at 180°C, while the oxidation reaction was dominant at 120°C, and the degree of hydrolysis at 120°C was higher than at 180°C. It is noteworthy that the triglyceride polymeric products TGO and TGD have biological toxicity and cytotoxicity. The higher the temperature, the more products accumulated in the oil, thus affecting the nutritional characteristics and safety of CO.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

CO	Camellia seed oil
HPSEC	High performance size exclusion chromatography
FFA	Free fatty acid value
PV	Peroxide value
AV	p-Anisidine value
CV	Carbonyl value
PTG	Polymerized triacylglycerides
TPC	Total polar compounds
TGO	Triacylglycerol oligomers
TGD	Triacylglycerol dimmers
ox-TGM	oxidized-Triacylglycerol monomers
DG	Diacylglycerols
ECN	Equal carbon number
TAG	Triacylglycerol
PTAG	Polymerized triacylglycerol
LLL	Trilinolein
LLO	1,2-linolein-3-olein;
PLL	1-palmitin-2,3-linolein;
OOL	1,2-olein-3-linolein;
POL	1-palmitin-2-olein-3-linolein;

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