Advance Journal of Food Science and Technology 13(3): 114-118, 2017
DOI:10.19026/ajfst.13.4147
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: March 21, 2016 Accepted: June 18, 2016 Published: March 25, 2017

Research Article

Research of Polar Compounds Formation in Three Different Oils during Frying and Thermoxidation

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Abstract: Three vegetable oils with different unsaturation degree, namely, unflower seed oil, peanut oil and rapeseed oil, were selected to evaluate the formation of polar compounds and their distribution during frying and thermoxidation. It was found that the levels of polar compounds mainly depended on oil unsaturation degree with same frying conditions. Relative molecular weights of triglyceride oligomers, triglyceride dimmers and oxidised triglyceride monomers were also detected by high performance sizeexclusion chromatography.

Keywords: Frying oil, high performance size exclusion chromatography, oxidized triglyceridepolymers, polar compounds

INTRODUCTION

There are several thermoxidative and hydrolytic reactions taking place during the deep-frying process, which results in quality deterioration of the frying oil (Zhang *et al*., 2012). Quantification of Polar Compounds (PCs) is considered to be the most reliable method for the evaluation of oil quality (Farhoosh and Tavassoli-Kafrani, 2011).

The PCs have been proven hazardous to human health for feeding trials showing that they are pretty well absorbed and presented toxic or other biological effects (Cao *et al*., 2013). In addition, the PCs also greatly affect the microstructure and texture of fried food. During the frying process, the total content of PCs shows statistically significant increases (Márquez-Ruiz *et al*., 1996). Formation of PCs also strongly depends on the fatty acid composition with same frying conditions (Crosa *et al*., 2014). And the PCs formed are practically identical in the frying oil and in the lipids extracted from the counterpart fried food (Marmesat *et al*., 2012). Analysis of PCs by HighPerformance SizeExclusion Chromatography (HPSEC) allows the separation and quantification of Triglyceride Oligomers (TGO), Triglyceride Dimmers (TGD), oxidised triglyceride monomers (ox-TG), diglycerides (DG) and Free Fatty Acid (FFA) (Kuligowski *et al*., 2010).The origin of deterioration can be evaluated from these different groups of alteration products. Especially TGO and TGD are regard as an objective endogenous parameter for characterizing the thermoxidation (Sebastian *et al*., 2014). The ox-TG is indicator of oxidative alteration and DG and FFA are related with hydrolytic alteration (Bansal *et al*., 2010).

A column chromatography coupled with HPSEC method was established in this study. Three vegetable oils with different unsaturation degree, namely, unflower seed oil, peanut oil and rapeseed oil, were selected to evaluate the influence of unsaturation degree on the formation of new compounds. Frying experiment at 180°C in three oils with bread sticks as fried food were performed to evaluate the formation of PCs at high temperature. In addition, relative molecular weights of TGD, TGO and ox-TG were detected by HPSEC.

MATERIALS AND METHODS

Reagents and instrument: Wheat flour (Fried bread stick flour, Jinyuan Flour Co., Ltd, China), peanut oil (First level squeeze peanut oil, Longda Foodstuff Group Co., Ltd, China), rapeseed oil (First level non-transgenic squeeze rapeseed oil, Henan Yifeng Oil Co., Ltd, China), unflower seed oil (First level non-transgenic squeeze unflower seed oil, China Oil and Foodstuffs Corporation, China). Petroleum ether, ethanol, diethyl ether (analytical pure, Tianjin Kemiou Chemical Reagent Co., Ltd, China), tetrahydrofuran (chromatographical pure, Tianjin Kemiou Chemical Reagent Co., Ltd, China).

Ultrapure water from Millipore Milli-Q (18.2MΩ·cm, equipped with a Millipack: 0.22μmfilter)

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was used for the preparation of solutions and mobile phase.

Standards of different molecular mass were used to perform the calibration curves. A Shodex polystyrene standard (range of molecular weights in kDa: 1.32, 1.86, 2.78, 4.83, 6.94) was obtained from ANPEL scientific instrument Co., Ltd (ANPEL, China).

EF-81 Electric Fryer (4L capacity,Wailaan Kitchen Equipment Co., Ltd, China), SHA-C thermostatic water bath oscillators (JingpinhuagongGongye Co., Ltd, China), DHG-9076A Eletric constant temperature drying oven (Shanghai Jinghong Laboratory Instrument Co., Ltd, China).

A CHEETAH MP100 preparative column chromatography system (Agela Technologies, China) was used for separation and collection of PCs in oils.

An Agilent 6890 GC System equipped with a split/splitless injector, an Innowax capillary column (30 cm length, 0.25 mm i.d., 0.20μm film thickness) and a flame ionisation detector (FID) was used.

The HPSEC system was equipped with the following instruments: Kontron autosampler 360, Agilent quaternary pump system 1200 Series with online degasser, Agilent differential refractometer 1100 Series (RI detector), Jones chomatography thermostatic oven, Agilent HPLC control unit 1100 Series (console table) and Kontron Data System 450-MT2.

Frying process: 250g wheat starch, 8g leavening agent, 3.4g salt and 2.5g sugar was weighted and mixed together and then 170g water was added. Then, stirring of the mixture was continued for 7min in the mixer. At last, the dough was made into bread sticks with 10cm in length, 3cm width and 1cm thick. Bread sticks were fried in the oil. The oil (4L) was placed in the EF-81 Electric Fryer and heated to 180°C. One bread stick was fried each time at constant frying temperature. Bread sticks were fried at 7min intervals for 8 h per day for 4 consecutive days. At the end of each 2h, about 20g of the frying oil was filtered into a screw-cap vial and promptly stored in the dark at 4°C until use. The volume of oil was not replenished during the frying process. Frying experiments were conducted in duplicate.

Analytical determinations:

Fatty acid composition: The fatty acid composition was determined by GC after derivatization to Fatty Acid Methyl Esters (FAME) with 2N KOH in methanol, according to IUPAC Standard Methods 2.301 and 2.302. Hydrogen was used as carrier gas. The detector and injector temperatures were 250°C. The initial oven temperature was 180°C and a temperature gradient from 180 to 220°C at 3°C min^{-1} was applied. Injections were performed using a split ratio of 1:50.

Separation and collection of PCs in cooking oils: A 2.5g mass of oil was weighted into a 10mL volumetric flask, which was filled up to the mark with light petroleum afterwards. A CHEETAH MP100 column chromatography system was used for separation and collection of PCs in oils. The 10mL oil solution was placed on the preparative column and then the non-polar fraction was eluted with light petroleum-diethyl ether (87:13) for 60min at a rate of 3mL/min. Afterwards, the polar fraction was eluted with diethyl ether for 60min at a rate of 3mL/min. Polar fraction was evaporated under reduced pressure at a temperature of 60°C and dried in a vacuum drying oven at 40°C for 30min. Total PCs were determined gravimetrically according to IUPAC Standard Method 2.507.

Quantitation and distribution of total PCs: 0.1g PCs was redissolved in 10mL tetrahydrofuran for further analyses by HPSEC. Series of polystyrene standards (1.32, 1.86, 2.78, 4.83, 6.94 kDa) were used to calibrate the system. Each of these standards was separately filtered through 0.45μm cellulose acetate filters and injected at 5mg/mL (in tetrahydrofuran) in triplicate (n = 3). A standard calibration curve for the logaritham of the molecular weight versus the HPSEC retention time was obtained for each series of standards.

The separation was performed on a Styragel HR 1 THF column (7.8×300mm i.d.) and a Styragel HR 0.5 THF column (7.8×300 mm i.d.) with a Styragel (4.6×30mm) guard column. Highperformance liquid chromatography grade tetrahydrofuran served as the mobile phase at 35°C with a flow of 0.7mL/min. Standards and PCs were filtered through 0.45μm cellulose acetate filters and injected 10μL into the HPLC. Under the conditions used chromatographic runs took less than 25min.Amounts of each group of PCs, i.e., TGO, TGP, ox-TG and FFA, were calculated from the individual peak areas and percentage of total PCs, assuming equal response factors.

RESULTS AND DISCUSSION

Total content changes of PCs in three different frying oils: There are quite a number of parameters that contribute to PCs producing, such as frying time, frying temperature, frying oil type, frying food and so on. Among those factors, frying temperature and frying time have a significant impact. Table 1 shows the total content of PCs in three different frying oils heated at 180°C for 20 h, showing that total content of PCs all increased with the frying time up to 20h. The initial values were similar for three oils, ranging from 3.0 to 3.6%, which was a normal content for fresh refined vegetable oils.

A good correlation between the total content of PCs formed at 20h and the percentages of unsaturated

Table 1: Total PCs (% on oil wt) in samples heated at 180°C

	Heating time (h)										
Sample	0	2	4	6	8	10	12	14	16	18	20
Peanut oil	3.0	3.4	3.9	6.3	10.1	12.5	16.0	19.8	24.0	27.5	29.0
Rapeseed oil	3.4	4.1	5	7.2	8.8	11.2	14.3	18.1	22	25.7	27.2
Unflower seed oil	3.6	4.3	6.1	8.8	12.4	15.1	18.3	22.3	25.2	28.5	30.9

Table 2: Fatty acid composition of three different frying oils (%)

	Major fatty acid (%)								
Sample	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	SFA	MUFA	PUFA
Unflower seed oil	6.3	0.2	3.6	31.6	56.8	0.1	9.90	31.8	56.9
Rapeseed oil	4.5	0.2	1.6	63.5	18.8	8.1	6.10	63.7	26.9
Peanut oil	11.7	0.2	3.5	43.2	36.5	0.8	15.2	43.4	37.3

(SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids)

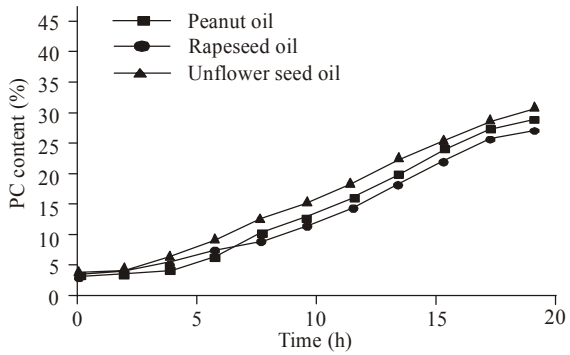

Fig. 1: Total content of PCs in three different frying oils heated at 180°C

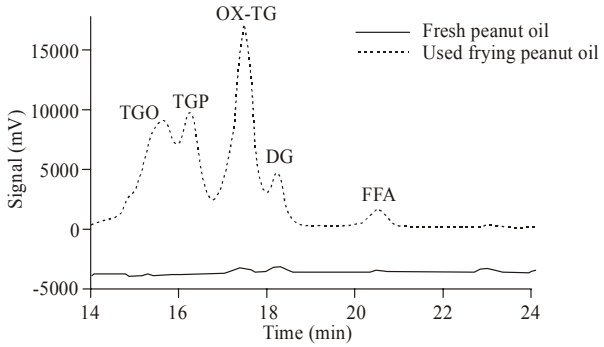

Fig. 2: HPSEC chromatograms of PCs in fresh peanut oil and used frying peanut oil

fatty acid in the oils was found. As Table 2 shown, unsaturated fatty acid content of unflower seed oil was highest, that of peanut oil was medium and that of rapeseed oil was relatively lower. The result shows that total PCs content was higher when the degree of unsaturation increased (Casal *et al*., 2010). PCs content of unflower seed oil with the highest degree of unsaturation reached the maximum value allowed for human consumption (25%) in most countries all over the world. This value was higher than that obtained from rapeseed oil, which was 22.0% under the same thermal oxidation conditions. Probably because unsaturated fatty acids were more susceptible to degradation than saturated fatty acids (Guillén and Uriarte, 2013).

PCs content of unflower seed oil was the highest during the whole frying process and PCs content of peanut oil was higher than that of rapeseed oil at frying time of over 8h, as the line graph in Fig. 1 shown. Therefore, we come to a conclusion that rapeseed oil has best performance, peanut oil takes a second place and unflower seed oil is relatively worse.

PCs content of peanut oil was a little lower than that of rapeseed oil at frying time of less than 6h and family frying time generally did not exceed 6h. In addition, food fried by peanut oil tasted much better than by rapeseed oil or unflower seed oil. Therefore, peanut oil was an appropriate frying oil for family use.

Quantitative determination of PCs: Further application of HPSEC to the isolated polar fractions offered substantial advantages for the quantitation of specific groups of PCs differing in molecular weight. A typical example of HPSEC chromatogram of PCs from a peanut oil was presented in Fig. 2. Five main peaks, elutining inverse order of molecular weight, were resolved: the TGO, TGD, ox-TG, DG and FFA. Table 3 shows the total amount of PCs in three frying oils heated at 180°C and their distribution in TGO, TGD, ox-TG, DG and FFA. The distribution of PCs showed the quantitative importance of TGP (TGO+TGD) and ox-TG, while hydrolysis compounds, such as DG and FFA, remained at the same levels as those found in the initial oils. As can be observed, TGP(TGO+TGD) were the major compounds and their proportion among the new compounds formed increased as the level of degradation increased (Juárez *et al*., 2011).

Molecular weight of TGO, TGP, ox-TG: The linearity of the method was calibrated using polystyrene standards of different molecular weight. Table 4 shows retention time of the standards and the calibration curve

Table 3: Total PCs and their distribution (% on oil wt) in frying oils

			PCs distribution					
Sample	Heating time	PCs	TGO	TGD	TGO+TGD	ox-TG	DG	FFA
Peanut oil	0h	3.0	nd	0.40	0.40	1.60	0.8	0.3
	6h	6.3	0.20	0.90	4.40	3.30	1.4	0.5
	12h	16.0	1.30	2.70	4.00	7.90	2.0	1.1
	20h	29.0	5.70	6.80	12.5	12.8	2.2	1.5
	35h	38.3	11.7	8.10	19.8	14.1	2.4	2.0
Rapeseed oil	20h	27.2	5.70	11.5	17.2	7.00	1.9	1.1
Unflower seed oil	20h	30.9	8.60	13.0	21.6	7.20	1.4	0.7

Table 4: Retention time, relative response factors and calibration curve equations of the polystyrene standards by HPSEC method

Standards	MW (kDa)	Retention time (min)	Calibration curve
PS1200	1.32	17.7	lgM_p= -0.0412X^3+2.1088X^2
PS1900	1.86	11.1	- 36.242X +212.3
PS2500	2.78	16.4	r = 0.9999
PS4000	4.83	15.8	
PS6000	6.94	15.5	

Table 5: Molecular weights of TGO, TGD and ox-TG within PCs

Item	TGO	TGD	ox-TG
Retention time (min)	15.67	16.25	17.41
Peak point molecular weight (Da)	-	2708	1270
Weight average molecular weight (Da)	3862	2641	1283
Corrected molecular weight (Da)	2526	1727	839

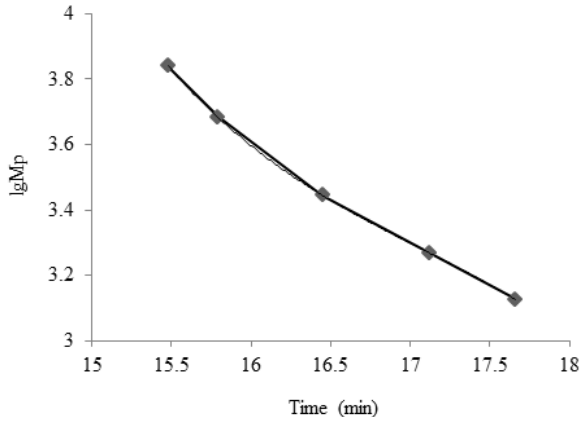

Fig. 3: Standard curve of polystyrene Mp molecular weight with retention time

equations obtained. The calibration curves of polystyrene were plotted as the molecular weights on a log scale versus the retention time (Fig. 3). Polystyrene calibration curves showed good linearity (coefficient of correlation, r = 0.9999) in the range of 1320 to 6940.

PCs was injected in triplicate and the peak point molecular weight was obtained from their retention time using the calibration curves of polystyrene (Table 5). The peak point molecular weight of TGO could not be obtained because of irregular peak shape. Weight average molecular weight of TGO, TGD and ox-TG were also calculated by the software. Triglyceride standard (Mw = 885Da) was also injected in triplicate and the calculated molecular weight by the calibration curves was 1352. So a correction index (0.654) should be multiplied to obtain accurate molecular weights. Corrected molecular weights of TGO, TGD and ox-TG were listed in Table 5. Molecular weight of TGO (2469) was about triple molecular weight of TG (850), so it was deduced that TGO was mainly trimer. Also molecular weight of TGD (1727) was about twice molecular weight of TG (850), so TGD was considered to be dimer (Marcato and Cecchin, 1996). Therefore, molecular weight results of TGO, TGD and ox-TG implied good agreement with literature result (Zhang *et al*., 2012).

CONCLUSION

In conclusion, total content of PCs in three different frying oils heated at 180℃all increased with the frying time. A good correlation between the total content of PCs and the percentages of unsaturated fatty acid in the oils was found. Total PCs content was higher when the degree of unsaturation increased. Distribution in TGO, TGD, ox-TG, DG and FFA shows that TGP (TGO+TGD) were the major compounds and their proportion among the PCs formed increased as the level of degradation increased. Relative molecular weights of TGD, TGO and ox-TG were also detected by HPSEC. Results showed that TGO was mainly trimer and TGD was mainly dimer.

ACKNOWLEDGMENT

Financial support granted by Doctor Scientific Research Foundation of Henan University of Technology (2013BS029) and Scientific and echnological project of Henan Province (162102310408) are gratefully acknowledged.

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