Emirates Journal of Food and Agriculture. 2018. 30(9): 791-802
doi: 10.9755/ejfa.2018.v30.i9.1802
http://www.ejfa.me/

REGULAR ARTICLE

Frying influence on virgin olive oils by monitoring change of aliphatic monoaldehydes

Zhongyang Wang[1,2], Yaming Sun[1,2], Hui Li[1], Shuqing Dong[1,*], Liang Zhao[1,*]

[1]Key laboratory of Chemistry of Northwestern Plant Resources and Key laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences, Lanzhou 730000, PR China; [2]University of Chinese Academy of Sciences, Beijing 100039, PR China

ABSTRACT

When virgin olive oil (VOO) have been fried long time at high temperature, some aliphatic monoaldehydes including hexanal, heptanal, octaldehyde, nonanal and decanal were found in fried VOO and food samples. The HPLC method has been developed for determination of aliphatic monoaldehydes in VOO and food samples after different fried conditions. These five aliphatic monoaldehydes could be separated and analyzed in 60 min on reversed phase ODS column with methanol/0.1% acetic acid water solution mobile phase at 1.0 mL min^{-1} flow rate, after being derivatized with 2,4-dinitrophenylhydrazine (DNPH). The method was validated with recovery results ranging from 86% to 114%. Calibration plots of aliphatic aldehydes were linear ($r \geq 0.9991$) in the concentration range from 1.0×10^{-6} to 1.5×10^{-4} mol L^{-1}. The LODs were between 6.5×10^{-8} and 3.98×10^{-7} mol L^{-1}. The proposed method provides a reliable and sensitive quantitative evaluation for aliphatic monoaldehydes in fried oils and fried potatoes samples. The experiments results verify that the VOOs are not suitable to be fried long time at high temperature. The frying time should be less than 3.0 min, and the frying temperature should be below 150 °C for the healthy diet.

Keywords: Virgin olive oil; Eating safety; Aliphatic monoaldehydes; Derivatization; HPLC

INTRODUCTION

Virgin olive oil is a recognized health and original ecological edible oil which is extracted exclusively from the fresh fruit of *Olea europea* L. only by means of mechanical methods or other physical procedures to retain the natural nutrients (Alonso-Salces et al., 2011; Lioumbas et al. 2013). The outstanding features of VOO is rich in monounsaturated fatty acids which can effectively reduce blood lipids, but also contain nutritive antioxidants which has cosmetic effects and health benefits, i.e. polyphenols and tocopherols (Katragadda et al. 2010; Pereira-Caro et al. 2012). Therefore, with the development of the living standards, VOO was used for frying widely due to it has very nutritional value and strong antioxidation (Andrikopoulos et al. 2002).

As well known that frying is dietary traditions of many people and widely practiced in many culinary processes, this may be due to fried food have unique sensorial properties (Casal et al. 2010; Rui et al. 2014). However, during frying, the fried oils undergo oxidation, polymerization, isomerization, cyclization, and hydrolysis processes, its characteristic and properties will be changed, and produce a lot of toxic compounds, such as aldehydes, alcohols, and acid esters (Guillen et al. 2011; Guillen et al. 2012; Giuffre et al. 2017). These compounds will affect the health of people, so the oils frying conditions should be monitored to avoid the toxic compounds appearance. Two variables i.e. heating temperature and time of heating have an important influence on its physicochemical properties (Giuffre et al. 2017). So far, many analytical works of VOO mainly in the authentication and quality assessment of this high added value VOO, the detection of its adulteration with cheaper vegetable oils and refined (Frankel 2010). There was few report about the influence of frying process for VOO and VOO-containing foods. Therefore, based on the economic and people's health considerations, it is necessary for analysts to look for the specific index and set up a corresponding simple and feasible method to monitor change of quality for fried VOO and foods. The chemical changes of fried

***Corresponding author:**
Shuqing Dong, Key laboratory of Chemistry of Northwestern Plant Resources and Key laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, The Chinese Academy of Sciences, Lanzhou 730000, PR China. **Tel.:** +86 0931 4968273; Fax: +86 0931 8277088. **E-mail:** sqdong@licp.cas.cn (Shuqing Dong)

Received: 16 March 2018; Accepted: 22 July 2018

oils and food have been traditionally studied by free acidity, peroxide value, *p*-anisidine test, conjugated diene value, and iodine value, among others (Uriarte et al. 2010). Analysis of Free acidity, PV, K232, K268, ΔK, FAMEs, sterols, waxes, are necessary because required by the International Regulations and data are useful by a biological point of view for the human health and that aliphatic aldehydes analysis can increase the information about a fried olive oil. Polarity often changes in the moblie phase, which participates in the distribution balance between the components and the column. Compared to the GC-MS method, the HPLC method shows higher separation efficiency and speed. Therefore, the HPLC method is applied to separated and detect trace substances dissolved in solution.

Aldehydes can be formed quietly during the process of frying, baking, storing and sterilizing the foodstuffs in person's daily life (Ramirez-Jimenez et al. 2000; Rada-Mendoza et al. 2004; Zhang et al. 2011). During the olives crushing process, the polyunsaturated fatty acids are catalyzed into aldehydes by a series of lipoxygenases which are induced by tissue damage (Conde et al. 2008). Since various aldehydes have come into being in oils by frying treatment practice, the aliphatic monoaldehydes could be as specific index for the oil safety determination. Is known, aldehydes are highly reactive, volatile, polar compounds, and usually present at trace amounts and lacking significant chromophores in various materials, so the separation and detection of them is often a difficult assignment. However, this problem can be solved by chemical derivatization of aldehydes and the utilization of highly selective and specific detectors. The derivatization used most frequently for aldehydes is based on forming the corresponding thiobarbituric acid (Inoue et al. 1998; Al-Rimawi 2014), thiazolidine (Kataoka et al. 1995) or quinoxaline (Bravo et al. 2008) under relatively harsh conditions. The other derivatization method was to synthesize new derivatization reagent, for example, 2-[2-(7H-dibenzo[a,g]carbazol-7-yl)-ethoxy] ethyl carbonylhydrazine (You et al. 2009) 4- 2- (trimethylammonio) ethoxy benzenaminium halide (Eggink et al. 2008) and applied them for the different detection method. These approaches have potential drawbacks that the derivatization agents were not commercially available and need be synthesized in the laboratory, which apparently limited the application of these methods in aldehyde analysis.

In order to accurately detect and identify frying process effect on VOO, in the paper, we used HPLC coupled with UV detector to determine aliphatic aldehydes in different fried conditions VOO and VOO-containing foods samples. The 2,4-dinitrophenylhydrazine (DNPH) as derivatization agent was chose for aldehydes (Miyashita et al. 1991; Yukawa et al. 1993; Endo et al. 2001; Seppanen et al. 2002). In the present work, five aldehydes were detected simultaneously including hexanal, heptanal, octaldehyde, nonanal, decanal in real sample matrices. These five analytes could get good baseline separation in our research and the limits of detection were as lower as 10-7 or 10-8 mol L-1. These results proved that the method was a simple and economical means for the analysis of aldehydes comparing with other methods. The experiments presented here had been used for the identification the influence of frying for VOO and fried food, and supply the reference for right eating of VOO.

MATERALS AND METHODS

Reagents and solutions

Hexanal (≥98.0%), heptanal (≥99.0%), octaldehyde (≥98.0%), nonanal (≥98.0%), decanal (≥98.0%) were purchased from Sigma (St. Louis, MO, USA); 100-200 mesh silica gel for chromatography was obtained from Qingdao Puke separation materials Co. Ltd. (Qingdao, China); HPLC-methanol, DNPH (≥99.0%) were purchased from China National Pharmaceutical Group Corp. (Shanghai, China); they were all of analytical grade and used as received. Distilled water was used throughout the experiment. This article does not contain any studies with human or animal subjects, the parameters for virgin olive oil are established by European Union regulations and by the International Olive Council.

Preparation of aliphatic aldehydes derivatization products

Preparation of aliphatic aldehydes standard solutions

The five standard analytes were accurately weighed and dissolved in absolute ethyl alcohol to furnish each of the solution into 10 mmol L^{-1}; they were stored in the dark at 4 °C.

Preparation of DNPH derivatization solution

For the aldehydes derivatization reaction, the derivatization solution was prepared. 0.5 g DNPH was accurately weighed and added in the solution containing 1.0 mL 30% H_2SO_4, 15 mL ethanol and 9.0 mL H_2O. The derivatization solution was stored in the dark at 4 °C.

Derivatization reaction

The five aldehydes standard solutions 10.0 mL were added in 20.0 mL derivatization solution dropwise, respectively. The every resulting mixture was capped and shaken slowly, and the reaction was allowed to proceed for 40 min at room temperature. Each aldehydes hydrazone was purified by recrystallized with ethanol, and then dried at 40 °C for further quantitative use.

The dried derivatization products were accurately weighed and dissolved in dichloromethane and absolute ethyl alcohol (1:1, v/v) to furnish each of the solution into 10 mmol L^{-1}; they were stored in the dark at 4 °C. All

solutions were filtered through 0.45 μm polypropylene acrodisc syringe filter (Xinya Purification Instrument Factory, Shanghai, China) and degassed by agitation in an ultrasonic bath for 5 min to remove bubbles.

Sample preparation and derivatization

Ten oils samples were used in the study, which are all brands of virgin olive oil in the local retailers in Lanzhou (China). The oil samples were heated to 150 °C, 160 °C, 180 °C and boiling point and samples were taken for 3 min, 3.5 min, 4 min, 5 min. Every oil sample was repeated 5 times. The frying oil samples were enriched by passing column of silica gel column. 50 mL oil samples were solved in 80 mL normal hexane, 100 g 100-200 mesh silica gel column for separation. The gradient elution was 500 mL normal hexane and 500 mL ethyl acetate and absolute ethyl alcohol (10:1, v: v), respectively. The flow rate was 1.5 mL min^{-1}. Then the part of ethyl acetate and absolute ethyl alcohol eluents were concentrated to 10 mL by rotary evaporator. The preconcentration samples were filtered through 0.45 mm nylon filters prior to derivatization. The derivatization reaction procedures were same as the aldehydes standard derivatization. A 1.0 mL aliquot of sample was added in 200 μL DNPH derivatization solution. The resulting mixture was shaken slowly, and the reaction was allowed to proceed for 40 min at room temperature.

The fried potato was mashed, and accurately weighed 50.0 g. Then 500 mL ethyl acetate and absolute ethyl alcohol (10:1, v: v) was used as solvent to extract the aldehydes in the fried potato samples. The extract solution was concentrated to 10 mL by rotary evaporator. The preconcentration samples were filtered through 0.45 mm nylon filters prior to derivatization. The derivatization reaction procedures were same as the oil samples derivatization. A 1.0 mL aliquot of sample was added in 200 μL DNPH derivatization solution. The resulting mixture was shaken slowly, and the reaction was allowed to proceed for 40 min at room temperature.

Chromatography analysis procedure

Samples were analyzed with a Waters 2847 HPLC equipped with a diodearray UV detector. The five analytes were separated on a 150 mm × 4.6 mm, 5-μm particle, Symmetry C18 column (Waters ODS-2). The mobile phase was 0.1% acetic acid water: methanol (25:75/v: v), pH value was at about 3.4, injection volume was 20 μL, the mobile phase flow rate was 1.0 mL min^{-1}, column temperature was room temperature, and the detection wavelength was 356 nm.

RESULTS AND DISCUSSION

Optimization of derivatization reaction

Aliphatic aldehydes do not contain a chromophore absorbing in the wavelength range useful for liquid chromatography with UV detection. As a derivatization reagent, DNPH reacts with the aliphatic aldehydes, which significantly improved the UV absorbance and the sensitivity in quantification of aliphatic aldehydes.

The effect of the amount of coupling agent on the derivatization was investigated to obtain the optimum DNPH concentration. In general, higher reagent ratio will shift the reaction in favor of product formation. In our experiment, accurate amount of 10 mmol L^{-1} aldehyde 10 mL was transferred into a flask and then different volume (10.0, 20.0, 30.0, 40.0 and 50.0 mL) of DNPH (20 mmol L^{-1}) was added, respectively. No significant increase of peak areas of aldehyde-DNPH adducts was observed when the mole ratio of mixed aldehydes versus DNPH (mol/mol) was above 1:2. These adducts were highly stable and did not show significant change with time. Therefore, the mole ratio of 1:2 was finally selected as the optimum mole ratio of mixed aldehydes versus DNPH.

The temperature and time are fundamental parameters for the derivatization reaction. Generally, increase of the temperature will accelerate the reaction rate. To obtain the highest reaction yield, derivatization temperature and time were studied respectively. A series of mixed 10 mmol L^{-1} standard solutions of aldehydes were incubated in the time range of 0-60 min with DNPH (20 mmol L^{-1}) at 25, 35, 45, 60 and 80 °C, respectively. As shown in Fig. 1A, the aldehyde-DNPH adducts (hexanal, heptanal, octaldehyde, nonanal, and decanal) decomposed gradually when the temperature was above 35 °C. On the other hand, the yield of aldehyde-DNPH adducts increased quickly when the reaction time was less than 40 min, and then increased slowly with the reaction time until 60 min (shown in Fig. 1B). At 25 °C and 35 °C, the yields of adducts varied slightly and the 25 °C was more easy to control. Consequently, the room temperature (25 °C) and the reaction time of 40 min were selected as the optimum derivatization conditions, which provided satisfactory detection sensitivity and adequate sample throughput.

Optimization of the HPLC conditions

The chromatographic behaviours of the five analytes were investigated with the following isocratic mobile phases: (a) methanol-water (45:55, v/v), (b) methanol-water (55:45, v/v), (c) methanol-water (65:35, v/v), (d) methanol-water (75:25, v/v), (e) methanol-0.1% phosphoric acid water solution (75:25, v/v), (f) methanol-0.1% acetic acid water solution (75:25, v/v), (g) methanol-0.1% formic acid water solution (75:25, v/v) and (h) methanol-0.05% phosphoric acid water solution (75:25, v/v), respectively. The total runtime was shortened with the increase of the concentration of methanol in mobile phase. The runtime was the shortest using mobile phases (d), but the peaks of hexanal and

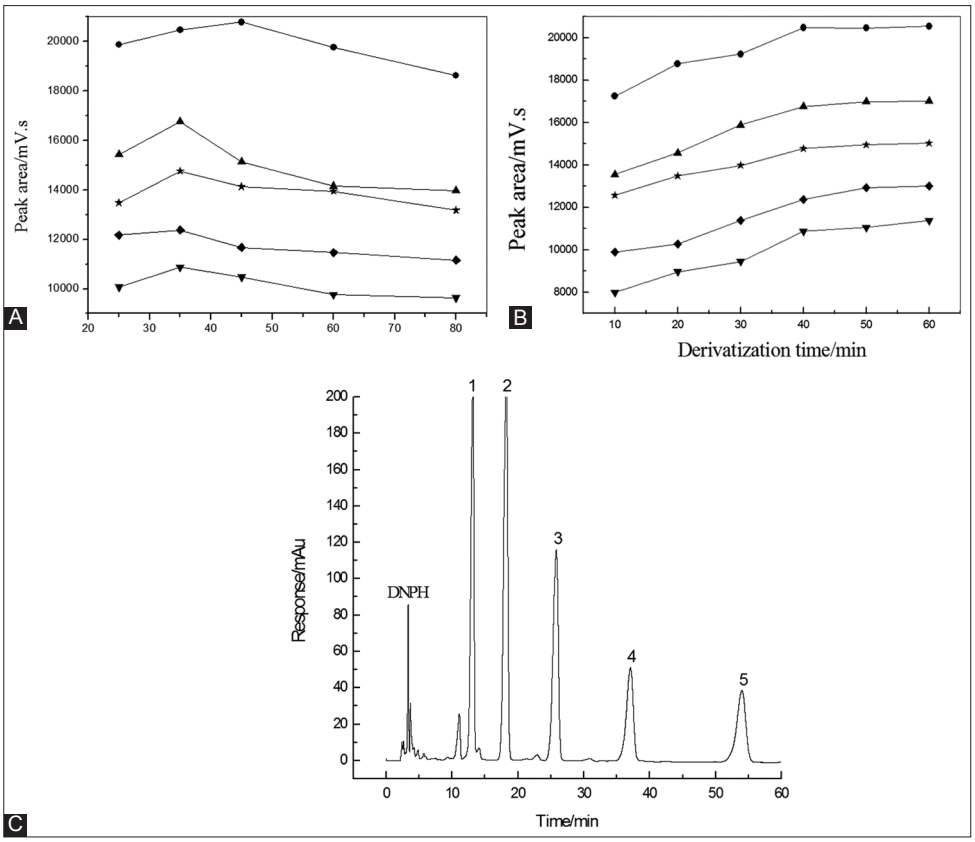

Fig 1. (A) Effects of the derivatization reaction temperature on peak areas of the aldehyde-DNPH adducts. Derivatization conditions: reaction time: 60 min; the ratio of mixed aldehydes to DNPH was 1:2 (Hexanal-DNPH (●); Heptanal-DNPH (▲); Octaldehyde-DNPH (★); Nonanal-DNPH (◆); Decanal-DNPH (▼)). (B) Effects of the derivatization reaction time on peak areas of the aldehyde-DNPH adducts. Derivatization conditions: frying temperature: 25 °C; the ratio of mixed aldehydes to DNPH was 1:2. (C) Typical HPLC chromatogram of a standard five analytes mixture solution under the optimum conditions. 1. Hexanal, 2. Heptanal, 3. Octaldehyde, 4. Nonanal, 5. Decanal. The separation conditions are described in the Section Optimization of the HPLC conditions.

heptanal were overlapped. Although the detection intensity of the analytes was stronger in mobile phases (a), (b), (c), the peaks of nonanal and decanal showed significant tailing. In mobile phases (e), (g) and (h), the five analytes had better resolutions and higher sensitivity, but still can not achieve a very good and complete base-line separation. Considering the increasing separation efficiency, decreasing migration time and improving chromatographic peak shapes, we selected mobile phase (f) for the further experiments.

Analytical performance

To develop an accurate, valid and optimal chromatographic method, different HPLC parameters including mobile phase (methanol–water–phosphate, methanol-water-acetic acid or acetonitrile-water-glacial acetic acid), mobile phase pH value (3.0-8.0), category of column (Kromasil C18 column, 150×4.6 mm, 5 μm; Waters ODS-2 column 150×4.6 mm, 5 μm; YMC-Pack ODS-A column, 150×4.6 mm, 5 μm; or shim-pack ODS column 150×4.6 mm, 5 μm), column temperature (20, 30, 35, 40 or 45 °C), flow rate of mobile phase (0.8, 1.0 or 1.2 mL min^{-1}) were all examined and compared. Finally, an optimized HPLC condition was developed by comparing comprehensively the resolution, baseline, retention time and number of characteristic peaks in each chromatogram.

Through the experiments above, the optimum HPLC conditions for determining derivative products of hexanal (1.0 mmol L^{-1}), heptanal (1.0 mmol L^{-1}), octaldehyde (1.0 mmol L^{-1}), nonanal (1.0 mmol L^{-1}), and decanal (1.0 mmol L^{-1}) were mobile phase methanol-0.1% acetic acid (75:25, v/v), pH about 3.4, category of column Waters ODS-2 column, 150×4.6 mm, 5 μm, temperature 25 °C, and flow rate of mobile phase 1.0 mL min^{-1}. Fig. 1C shows the chromatogram of a standard five analytes mixture solution after derivatization under the optimum conditions. It was clear that a very good base-lined separation was achieved for the five analytes within 60 min.

Method validation

Appropriate method validation information concerning new analytical techniques for analyzing real samples is required by regulatory authorities. Validation of such methods include assessment of the stability of the

standard solutions, linearity, reproducibility, detection and quantification limits.

Stability of the solutions

The stability of standard solutions was determined by monitoring the peak area of standard mixture solutions and migration time with sample solutions over a period of one day (n=5). The results showed that the peak area and migration time of each analyte were almost unchanged (R.S.D.≤7.5%) and that no significant degradation was observed within the given period, indicating the solutions were stable at least in 24 h.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

To determine the linearity of the peak area response to the concentration for hexanal, heptanal, octaldehyde, nonanal, and decanal a series of concentration mixed standard solutions were tested. The response was linear over two orders of magnitude from 1.0×10^{-6} to 1.5×10^{-4} mol L^{-1}.

The detection limits are evaluated on the basis of a signal to noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of about three orders of magnitude with the detection limits ranging from 1.0×10^{-8} to 1.0×10^{-7} mol L^{-1} for the five analytes. Table 1 summarizes the regression equation, correlation coefficient, limit of quantification (LOQ) and limit of detection (LOD) according to the 3sb/m criterion [Dong et al., 2009], where m is the slope of the calibration curve and sb the standard deviation. The LODs of the analytes were low as 10^{-8} mol L^{-1}. In our case, LOQ was evaluated on the basis of a signal to noise ratio of 10. The LOQ were 1.07×10^{-7}, 6.5×10^{-8}, 2.12×10^{-7}, 3.76×10^{-7}, and 3.98×10^{-7} mol L^{-1} for hexanal, heptanal, octaldehyde, nonanal, and decanal.

Reproducibility

The reproducibility of the peak area and migration time was estimated by making repetitive injections of a standard mixture solution under the optimum conditions. The relative standard derivations (R.S.Ds) of the peak area and migration time were 4.7% and 5.3% for hexanal, 5.4% and 6.9% for heptanal, 4.1% and 5.5% for octaldehyde, 3.1% and 4.4% for nonanal, 4.9% and 5.5% for decanal, respectively (n=5).

Sample analysis

Aldehydes contents of different brands VOO after frying

In order to obtain the reliable results, we purchased all brands VOOs in local supermarket in Lanzhou, which were labeled as from VOO-1, VOO-2, VOO-3…and VOO-10 to carry out the experiments. The typical chromatograms of the VOO-1 and VOO-2 samples before and after frying were showed in Fig. 2. Fig. 2 (A) and (B) showed the chromatographs of VOO-1 and VOO-2 samples before frying. It is clear that there were no aldehydes. Fig. 2 (C) and (D) were the chromatographs of VOO-1 and VOO-2 samples after frying 3.0 min at 150 °C. It can be seen that some aldehydes have been determined obviously. By a standard addition method and comparison of the migration times of target analytes with those of the standards, some aldehydes have been determined in the tested oil samples. The same experiments were done for the other VOO samples. The similar experiments results had been achieved and listed in Table 2. According to the experiment results, we suggest that three aldehydes content of different brands of VOO is different in the same fried conditions, and the content of hexanal, octaldehyde, nonanal is 0.1-0.45 mmol L^{-1}, 0.11-0.89 mmol L^{-1}, 0.13-0.72 mmol L^{-1}, respectively, and the content of heptanal and decanal is substantially 0 mmol L^{-1}. The RSDs of aldehydes content in VOO samples were less than 5.9 % (Table 2) with this method, thus, we may safely infer the quality of VOO on the market. From the Table 2, we can see that these five aldehydes total content of VOO-1 is the lowest, thus, the quality of VOO-1 is more excellent.

Aldehydes contents of VOO at different temperature by different frying time

Generally, the degree of oils degradation depends tightly on frying time and temperature. In order to further validate the deterioration process of VOO frying, the VOO samples had been fried at 150 °C with frying time (3.0, 3.5, 4.0, and 5.0 min), and the chromatographs of VOO-1 sample are shown in Fig. 3. We can see that with the increased frying time the peak areas of the aldehydes increased obviously. The other VOO samples' results were similar to VOO-1 sample. The aldehydes content were listed in Table 3.

The frying temperature was also investigated. The VOO samples had been fried 3.0 min at 150 °C, 160 °C, 180 °C,

Table 1: The regression equations, linearity, detection and quantification limits of hexanal, heptanal, octaldehyde, nonanal, decanal. (n=5)

Analytes	Concentration range (10^{-6} mol L^{-1})	Regression equation C (mol L^{-1}); Q (mAu)	Correlation coefficient	LOD (10^{-7} mol L^{-1})	LOQ (10^{-7} mol L^{-1})
Hexanal	1.0~100.0	Q=180.336C-8.56	0.9998	0.33	1.07
Heptanal	1.0~150.0	Q=185.125C+3.31	0.9998	0.21	0.65
Octaldehyde	1.0~100.0	Q=128.497C+8.35	0.9998	0.74	2.12
Nonanal	5.0~50.0	Q=57.245C-4.72	0.9991	1.24	3.76
Decanal	5.0~50.0	Q=45.960C-4.76	0.9993	1.38	3.98

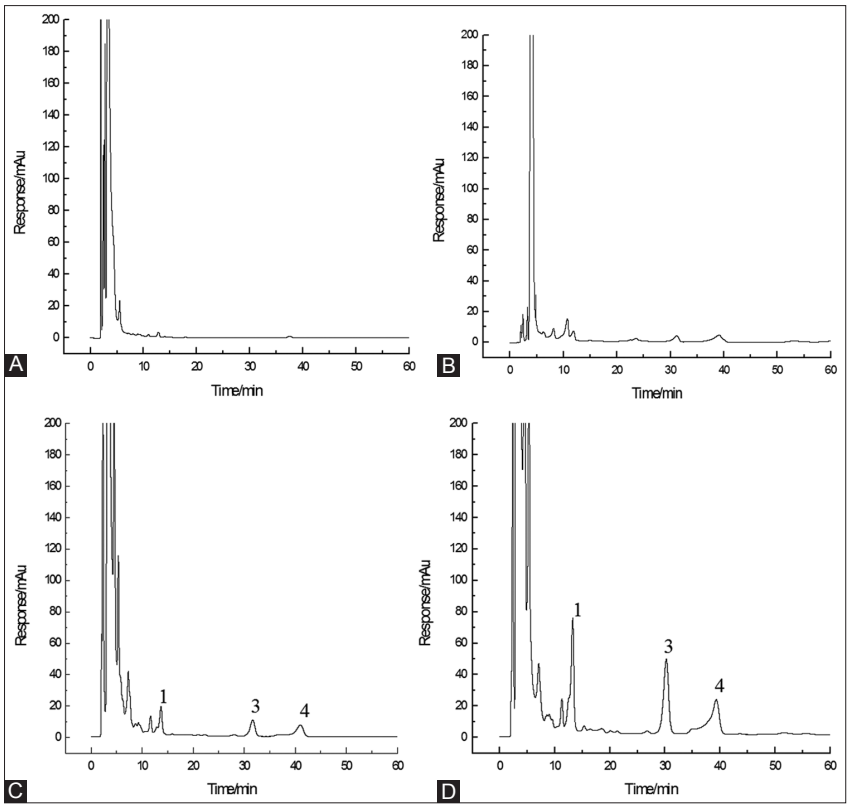

Fig 2. The HPLC chromatograms of VOO-1 and VOO-2 oil samples with (A) and (B) no frying, (C) and (D) frying 3.0 min at 150 °C. 1. Hexanal. 2. Heptanal, 3. Octaldehyde, 4. Nonanal, 5. Decanal. (The separation optimum conditions are same to Fig. 1C).

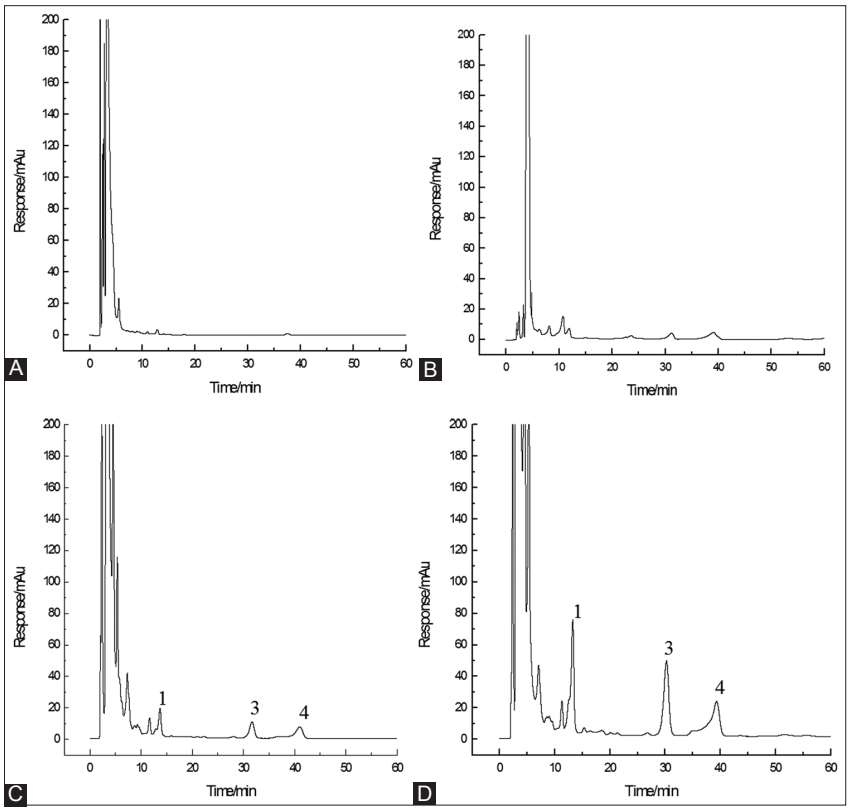

Fig 3. The HPLC chromatograms obtained from VOO-1 were fried at 150 °C with (A) 3 min, (B) 3.5 min, (C) 4.0 min, (D) 5.0 min. 1. Hexanal, 2. Heptanal, 3. Octaldehyde, 4. Nonanal, 5. Decanal. (The separation optimum conditions are same to Fig. 1C).

Table 2: Determination results of there

Samples	Active ingredients	Original amount (10^{-3} mol L^{-1})	Added (10^{-3} mol L^{-1})	Found (10^{-3} mol L^{-1})	Recovery (%)	RSDs (%)
VOO-1	Hexanal	0.10	0.1	0.189	89	4.6
	Heptanal	0	0.1	0.091	91	3.2
	Octaldehyde	0.16	0.1	0.246	86	4.2
	Nonanal	0.18	0.1	0.298	118	4.8
	Decanal	0	0.1	0.105	105	5.9
VOO-2	Hexanal	0.38	0.1	0.491	111	3.5
	Heptanal	0	0.1	0.093	93	4.1
	Octaldehyde	0.31	0.1	0.412	102	4.6
	Nonanal	0.33	0.1	0.424	94	3.9
	Decanal	0	0.1	0.103	103	5.2
VOO-3	Hexanal	0.41	0.1	0.524	114	3.7
	Heptanal	0	0.1	0.092	92	4.3
	Octaldehyde	0.89	0.1	1.002	112	4.8
	Nonanal	0.35	0.1	0.442	92	4.5
	Decanal	0	0.1	0.104	104	5.8
VOO-4	Hexanal	0.45	0.1	0.543	93	3.8
	Heptanal	0	0.1	0.102	102	3.5
	Octaldehyde	0.45	0.1	0.552	102	4.1
	Nonanal	0.72	0.1	0.814	94	3.9
	Decanal	0.13	0.1	0.223	93	4.9
VOO-5	Hexanal	0.31	0.1	0.418	108	4.2
	Heptanal	0	0.1	0.103	103	4.1
	Octaldehyde	0.28	0.1	0.385	105	4.3
	Nonanal	0.44	0.1	0.544	104	3.9
	Decanal	0	0.1	0.102	102	4.2
VOO-6	Hexanal	0.26	0.1	0.368	108	3.3
	Heptanal	0	0.1	0.096	96	4.1
	Octaldehyde	0.21	0.1	0.312	102	3.6
	Nonanal	0.43	0.1	0.524	94	3.5
	Decanal	0.07	0.1	0.175	105	4.7
VOO-7	Hexanal	0.29	0.1	0.388	98	3.8
	Heptanal	0	0.1	0.104	104	4.2
	Octaldehyde	0.37	0.1	0.482	112	4.3
	Nonanal	0.41	0.1	0.514	104	4.7
	Decanal	0.05	0.1	0.153	96	5.2
VOO-8	Hexanal	0.43	0.1	0.528	98	3.5
	Heptanal	0.05	0.1	0.149	97	4.1
	Octaldehyde	0.23	0.1	0.322	92	4.6
	Nonanal	0.33	0.1	0.434	104	3.9
	Decanal	0.02	0.1	0.123	106	4.2
VOO-9	Hexanal	0.43	0.1	0.538	108	3.1
	Heptanal	0	0.1	0.093	93	3.9
	Octaldehyde	0.37	0.1	0.472	102	4.3
	Nonanal	0.55	0.1	0.642	92	4.9
	Decanal	0	0.1	0.103	103	5.1
VOO-10	Hexanal	0.37	0.1	0.473	103	3.3
	Heptanal	0.05	0.1	0.149	97	4.4
	Octaldehyde	0.11	0.1	0.212	102	4.8
	Nonanal	0.13	0.1	0.234	104	3.9
	Decanal	0	0.1	0.102	102	5.2

and boiling, and the chromatographs VOO-1 of sample are shown in Fig. 4. It is found that with the increased frying temperatures the peak areas of the aldehydes increased evidently. The other VOO samples' results were similar to VOO-1. The aldehydes content were listed in Table 4. From Fig. 3 and Fig. 4, VOOs were deterioration by the

Table 3: The aldehydes contents in the ten VOO samples under the different frying time at 100 ºC

Samples	Analytes	Aldehydes contents (10^{-3} mol L^{-1})			
		Frying temperature 100 ºC			
		3.0 min	5.0 min	8.0 min	10.0 min
VOO-1	Hexanal	0.10	0.67	1.03	2.73
	Heptanal	0	0.05	0.10	0.25
	Octaldehyde	0.16	0.09	0.34	1.07
	Nonanal	0.18	0.72	1.38	1.81
	Decanal	0	0	0.10	0.15
VOO-2	Hexanal	0.38	2.35	3.78	11.71
	Heptanal	0	0.07	0.12	0.28
	Octaldehyde	0.31	0.19	0.73	1.86
	Nonanal	0.33	1.12	2.26	3.21
	Decanal	0	0	0.15	0.17
VOO-3	Hexanal	0.41	2.78	4.12	10.92
	Heptanal	0	0.07	0.13	0.34
	Octaldehyde	0.89	0.54	1.72	4.98
	Nonanal	0.35	1.44	2.67	3.61
	Decanal	0	0.05	0.10	0.15
VOO-4	Hexanal	0.45	2.81	4.34	11.44
	Heptanal	0	0.10	0.15	0.27
	Octaldehyde	0.45	0.26	0.92	2.28
	Nonanal	0.72	3.01	5.13	7.31
	Decanal	0.13	0.21	0.42	0.93
VOO-5	Hexanal	0.31	1.78	3.06	9.97
	Heptanal	0	0.05	0.13	0.29
	Octaldehyde	0.28	0.15	0.55	1.68
	Nonanal	0.44	1.77	3.52	4.63
	Decanal	0	0.05	0.10	0.30
VOO-6	Hexanal	0.26	1.82	2.57	8.7 8
	Heptanal	0	0.08	0.19	0.31
	Octaldehyde	0.21	0.12	0.45	1.47
	Nonanal	0.43	1.87	3.64	4.31
	Decanal	0.07	0.15	0.25	0.32
VOO-7	Hexanal	0.29	1.74	2.87	7.68
	Heptanal	0	0.06	0.14	0.24
	Octaldehyde	0.37	0.19	0.76	1.62
	Nonanal	0.41	1.53	3.17	4.02
	Decanal	0.05	0.13	0.22	0.34
VOO-8	Hexanal	0.43	2.58	4.46	11.25
	Heptanal	0.05	0.14	0.25	0.35
	Octaldehyde	0.23	0.12	0.49	1.41
	Nonanal	0.33	1.32	2.64	3.47
	Decanal	0.02	0.05	0.13	0.26
VOO-9	Hexanal	0.43	2.55	4.31	11.24
	Heptanal	0	0.08	0.13	0.32
	Octaldehyde	0.37	0.18	0.72	1.64
	Nonanal	0.55	2.47	4.92	5.64
	Decanal	0	0.05	0.16	0.23
VOO-10	Hexanal	0.32	1.92	3.24	9.61
	Heptanal	0.05	0.11	0.24	0.31
	Octaldehyde	0.14	0.07	0.28	0.98
	Nonanal	0.12	0.50	0.96	1.23
	Decanal	0	0.07	0.15	0.27

Table 4: The aldehydes contents in the VOO samples under frying 3.0 min at different frying temperature

Samples	Analytes	Aldehydes contents (10^{-3} mol L^{-1})			
		Frying time 3.0 min			
		100 (ºC)	150 (ºC)	180 (ºC)	Boiling
VOO-1	Hexanal	0.10	0.20	1.12	2.89
	Heptanal	0	0	0.10	0.31
	Octaldehyde	0.16	0.17	0.19	0.37
	Nonanal	0.18	0.37	1.76	3.16
	Decanal	0	0.10	0	0.30
VOO-2	Hexanal	0.38	0.78	3.74	10.98
	Heptanal	0	0.05	0.13	0.19
	Octaldehyde	0.31	0.33	0.35	0.65
	Nonanal	0.33	0.67	3.29	10.33
	Decanal	0	0.10	0.13	0.26
VOO-3	Hexanal	0.41	0.84	4.13	11.45
	Heptanal	0	0	0.10	0.23
	Octaldehyde	0.89	0.83	0.85	1.82
	Nonanal	0.35	0.71	3.53	10.74
	Decanal	0	0.05	0.12	0.24
VOO-4	Hexanal	0.45	0.91	4.48	12.17
	Heptanal	0	0.05	0.24	0.35
	Octaldehyde	0.45	0.44	0.52	0.94
	Nonanal	0.72	1.47	7.13	15.34
	Decanal	0.13	0.28	0.47	0.96
VOO-5	Hexanal	0.31	0.63	3.14	9.98
	Heptanal	0	0	0.15	0.27
	Octaldehyde	0.28	0.25	0.31	0.56
	Nonanal	0.44	0.87	4.45	12.27
	Decanal	0	0.05	0.10	0.20
VOO-6	Hexanal	0.26	0.52	2.64	6.97
	Heptanal	0	0.05	0.15	0.32
	Octaldehyde	0.21	0.22	0.23	0.45
	Nonanal	0.43	0.86	4.32	11.77
	Decanal	0.07	0.14	0.28	0.39
VOO-7	Hexanal	0.29	0.61	2.93	7.37
	Heptanal	0	0.05	0.10	0.23
	Octaldehyde	0.37	0.35	0.37	0.76
	Nonanal	0.41	0.84	4.13	10.94
	Decanal	0.05	0.10	0.15	0.30
VOO-8	Hexanal	0.43	0.45	4.32	11.38
	Heptanal	0.05	0.10	0.15	0.25
	Octaldehyde	0.23	0.25	0.26	0.45
	Nonanal	0.33	0.67	3.34	9.77
	Decanal	0.02	0.10	0.12	0.24
VOO-9	Hexanal	0.43	0.88	4.37	11.88
	Heptanal	0	0.12	0.17	0.30
	Octaldehyde	0.37	0.35	0.38	0.74
	Nonanal	0.55	1.12	5.54	13.28
	Decanal	0	0.10	0.12	0.28
VOO-10	Hexanal	0.37	0.75	3.77	10.63
	Heptanal	0.05	0.10	0.15	0.25
	Octaldehyde	0.11	0.13	0.12	0.24
	Nonanal	0.13	0.28	1.31	3.57
	Decanal	0	0.03	0.10	0.15

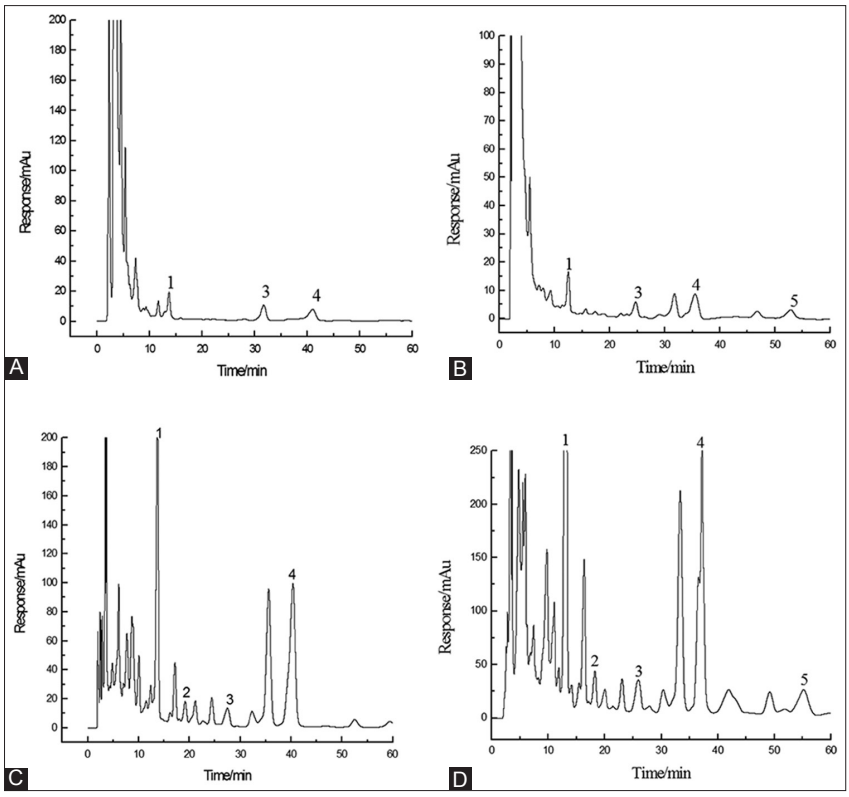

Fig 4. The HPLC chromatograms obtained from VOO-1 were fried 3 min at (A) 150 °C, (B) 160 °C, (C) 180 °C, (D) Boiling. 1. Hexanal, 2. Heptanal, 3. Octaldehyde, 4. Nonanal, 5. Decanal. (The separation optimum conditions are same to Fig. 1C).

extension of frying time and temperature. As we can see from Table 3 and Table 4, it is clear that when the frying time was over 3.0 min and frying temperature over 150 °C, the aldehydes concentrations were beyond reported safety values in the literature (100.0 mg/mL) (Andrikopoulos et al. 2003).

On the other hand, in order to confirm the safe frying condition of VOO, the same experiment were done for frying 0-3.0 min at 150 °C, and frying 3.0 min at 100-150 °C. The frying time was less than 3.0 min and the frying temperature was under 150 °C, the content of aldehydes was below 10.0 μg/mL. According to the experiment results, we suggest that the frying process should be done with the shorter time at the lower temperature.

Contrast the aldehydes content of different fried conditions for VOO fried foods

The oil uptake of food ranges in percentages from 4% to 14% of total weight, depending on the type of food and the frying medium (Elias et al. 2008). Thus, the quality of oils has a major influence on the quality of the final product. According to above experiments, aldehydes appeared evidently in VOO under different fried conditions. In order to demonstrate the aldehydes content in fried foods, the VOO fried foods were detected by the method.

Since the fried potato complies with the dietary traditions of people all over the world, the potato is chose to study further. Because the frying time of potato was sure in fried process, the frying temperature was investigated. The potato had been cut into strips and fried 3.0 min at 150 °C, 160 °C, 180 °C, and boiling by different VOO samples, and the chromatographs of fried potato by VOO-1 were shown in Fig. 5. It is found that although the great taste and colors of potato was presented at 180 °C and boiling, however, the frying temperature over 180 °C, the great aldehydes would produce in fried potatoes. As we can see from Table 5, when the frying temperature over 150 °C at fried 3 min, the aldehydes concentrations were gradually increased. Therefore, the lower frying temperature is the prerequisite and foundation of the healthy diet. The other VOO samples were also used to fry potato, and the similar VOO-1 results were obtained and listed in Table 5.

CONCLUSION

A significant experiment result which the aliphatic monoaldehydes would appear in virgin olive oil after frying was obtained. If the frying time was over 3.0 min and frying temperature over 150 °C, the great aldehydes would produce in VOO samples, and the concentrations of aldehydes would beyond the safety range. If the frying

Table 5: The aldehydes contents in the VOO-containing potato samples under frying 3.0 min at different frying temperatures

Potato frying by samples	Analytes	Aldehydes contents (10^{-3} mol L^{-1})			
		Frying time 3.0 min			
		150 (°C)	160 (°C)	180 (°C)	Boiling
VOO-1	Hexanal	0.10	0.31	0.73	2.73
	Heptanal	0	0	0.10	0.10
	Octaldehyde	0.16	0	0	0.64
	Nonanal	0.18	0.17	0.19	0.97
	Decanal	0	0	0	0.10
VOO-2	Hexanal	0.38	1.17	2.85	10.24
	Heptanal	0	0	0	0.12
	Octaldehyde	0.31	0	0	1.27
	Nonanal	0.33	0.35	0.34	1.82
	Decanal	0	0	0	0.12
VOO-3	Hexanal	0.41	1.25	3.28	11.47
	Heptanal	0	0	0.12	0.13
	Octaldehyde	0.89	0.12	0.24	3.56
	Nonanal	0.35	0.36	0.34	1.92
	Decanal	0	0	0	0.15
VOO-4	Hexanal	0.45	1.35	3.38	12.13
	Heptanal	0	0	0	0.13
	Octaldehyde	0.45	0.05	0.10	1.38
	Nonanal	0.72	0.73	0.74	3.91
	Decanal	0.13	0.15	0.22	0.35
VOO-5	Hexanal	0.31	0.95	2.46	9.13
	Heptanal	0	0	0.13	0.13
	Octaldehyde	0.28	0	0	1.12
	Nonanal	0.44	0.45	0.44	2.20
	Decanal	0	0	0	0.15
VOO-6	Hexanal	0.26	0.78	1.68	8.25
	Heptanal	0	0	0.10	0.12
	Octaldehyde	0.21	0	0	0.85
	Nonanal	0.43	0.44	0.45	2.58
	Decanal	0.07	0.10	0.15	0.23
VOO-7	Hexanal	0.29	0.88	2.72	7.58
	Heptanal	0	0	0.14	0.15
	Octaldehyde	0.37	0	0	1.48
	Nonanal	0.41	0.41	0.42	2.46
	Decanal	0.05	0.05	0.08	0.13
VOO-8	Hexanal	0.43	1.31	3.23	11.94
	Heptanal	0.05	0.05	0.17	0.20
	Octaldehyde	0.23	0	0	0.92
	Nonanal	0.33	0.33	0.34	1.86
	Decanal	0.02	0.02	0.05	0.10
VOO-9	Hexanal	0.43	1.73	3.45	11.98
	Heptanal	0	0.05	0.13	0.25
	Octaldehyde	0.37	0	0	1.45
	Nonanal	0.55	0.53	0.55	2.75
	Decanal	0	0	0.08	0.14
VOO-10	Hexanal	0.37	1.15	2.96	9.93
	Heptanal	0.05	0.05	0.10	0.15
	Octaldehyde	0.11	0	0	0.45
	Nonanal	0.13	0.15	0.14	0.78
	Decanal	0	0	0.10	0.15

temperature was over 180 °C, the abundance aldehydes would even appear in foods by VOO frying. Therefore, the VOO should better be used in the lower temperature, and the frying time should as possible as short. The results supply the helpful demonstration for the right eating of VOO. The assay results listed above indicate

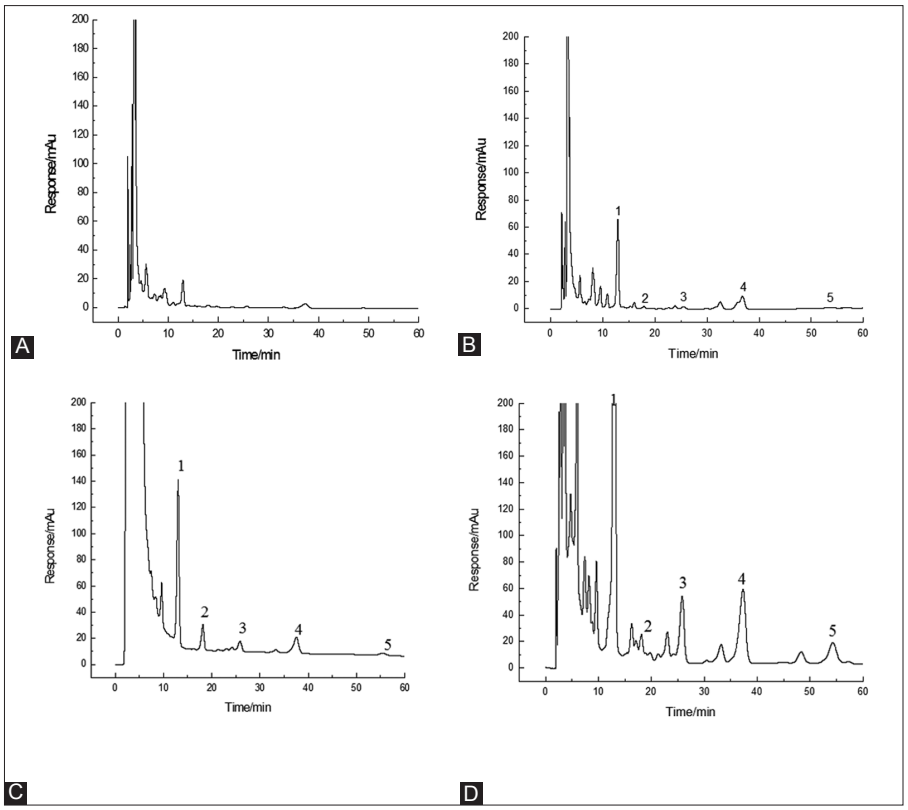

Fig 5. The HPLC chromatograms obtained from VOO-1 fried potato 3 min at (A) 150 °C, (B) 160 °C, (C) 180 °C, (D) Boiling. 1. Hexanal, 2. Heptanal, 3. Octaldehyde, 4. Nonanal, 5. Decanal. (The separation optimum conditions are same to Fig. 1C).

that the aldehydes, especially highly toxic and highly active aldehydes exist in almost all over-fried VOOs. The HPLC method has been developed for the simultaneous analysis of five aliphatic aldehydes in fried VOO and fried potato samples to identify frying effect for different olive oil using a derivatization reagent DNPH, which is an easily accessible common laboratory chemical without laboratory synthesis. Compared with other instruments detection or physicochemical index discriminate, this proposed analytical method could be attractive for the determination of the aldehydes in practical samples.

ACKNOWLEDGE

This work is supported by the National Natural Science Foundation of China (Nos. 21675163, 21405161), and the top priority program of "One-Three-Five" Strategic Planning of Lanzhou Institute of Chemical Physics, CAS.

CONFLICT OF INTEREST

Zhongyang Wang, Yaming Sun, Hui Li, Shuqing Dong, Liang Zhao declare that they all have no conflict of interest

REFERENCES

Al-Rimawi, F. 2014. Development and validation of a simple reversed-phase HPLC-UV method for determination of oleuropein in olive leaves. J. Food Drug Anal. 3: 285-289.

Alonso-Salces, R. M., M. V. Holland and C. Guillou. 2011. H-1-NMR fingerprinting to evaluate the stability of olive oil. Food Control. 12: 2041-2046.

Andrikopoulos, N. K., G. Boskou, G. V. Z. Dedoussis, A. Chiou, V. A. Tzamtzis and A. Papathanasiou. 2003. Quality assessment of frying oils and fats from 63 restaurants in Athens, Greece. Food Serv. Technol. 3: 49-59.

Andrikopoulos, N. K., G. V. Z. Dedoussis, A. Falirea, N. Kalogeropoulos and H. S. Hatzinikola. 2002. Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. Int. J. Food Sci. Nutr. 4: 351-363.

Bravo, A., J. C. Herrera, E. Scherer, Y. Ju-Nam, H. Rubsam, J. Madrid, C. Zufall and R. Rangel-Aldao. 2008. Formation of alpha-dicarbonyl compounds in beer during storage of Pilsner. J. Agric. Food Chem. 11: 4134-4144.

Casal, S., R. Malheiro, A. Sendas, B. P. P. Oliveira and J. A. Pereira. 2010. Olive oil stability under deep-frying conditions. Food Chem. Toxicol. 10: 2972-2979.

Conde, C., S. Delrot and H. Geros. 2008. Physiological, biochemical and molecular changes occurring during olive development and ripening. J. Plant Physiol. 15: 1545-1562.

Eggink, M., M. Wijtmans, R. Ekkebus, H. Lingeman, I. J. P. de Esch, J. Kool, W. M. A. Niessen and H. Irth. 2008. Development of a selective ESI-MS derivatization reagent: Synthesis and optimization for the analysis of aldehydes in biological mixtures.

Anal. Chem. 23: 9042-9051.

Elias, R. J., V. F. Laurie, S. E. Ebeler, J. W. Wong and A. L. Waterhouse. 2008. Analysis of selected carbonyl oxidation products in wine by liquid chromatography with diode array detection. Anal. Chim. Acta. 1: 104-110.

Endo, Y., C. M. Li, M. Tagiri-Endo and K. Fujimoto. 2001. A modified method for the estimation of total carbonyl compounds in heated and frying oils using 2-propanol as a solvent. J. Am. Oil Chem. Soc. 10: 1021-1024.

Frankel, E. N. 2010. Chemistry of extra virgin olive oil: Adulteration, oxidative stability, and antioxidants. J. Agric. Food Chem. 10: 5991-6006.

Giuffre, A. M., M. Capocasale, C. Zappia and M. Poiana. 2017. Influence of high temperature and duration of heating on the sunflower seed oil properties for food use and bio-diesel production. J. Oleo Sci. 11: 1193-1205.

Giuffre, A. M., C. Zappia and M. Capocasale. 2017. Effects of high temperatures and duration of heating on olive oil properties for food use and biodiesel production. J. Am. Oil Chem. Soc. 6: 819-830.

Guillen, M. D. and P. S. Uriarte. 2011. A very simple, fast, and non-destructive approach to predict the time at which edible oils submitted to high temperature reach the established limits of safety. Food Chem. 2: 802-806.

Guillen, M. D. and P. S. Uriarte. 2012. Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated α, β unsaturated aldehydes. Food Chem. 3: 915-926.

Inoue, T., K. Ando and K. Kikugawa. 1998. Specific determination of malonaldehyde by N-methyl-2-phenylindole or thiobarbituric acid. J. Am. Oil Chem. Soc. 5: 597-600.

Kataoka, H., A. Sumida, N. Nishihata and M. Makita. 1995. Determination of aliphatic-aldehydes as their thiazolidine derivatives in foods by gas-chromatography with flame photometric detection. J. Chromatogr. A. 2: 303-311.

Katragadda, H. R., A. Fullana, S. Sidhu and A. A. Carbonell-Barrachina. 2010. Emissions of volatile aldehydes from heated cooking oils. Food Chem. 1: 59-65.

Lioumbas, J. S., A. Zamanis and T. D. Karapantsios. 2013. Towards a wicking rapid test for rejection assessment of reused fried oils: Results and analysis for extra virgin olive oil. J. Food Eng. 2: 260-270.

Miyashita, K., K. Kanda and T. Takagi. 1991. A simple and quick determination of aldehydes in autoxidized vegetable and fish oils. J. Am. Oil Chem. Soc. 10: 748-751.

Pereira-Caro, G., B. Sarria, A. Madrona, J. L. Espartero, M. E. Escuderos, L. Bravo and R. Mateos. 2012. Digestive stability of hydroxytyrosol, hydroxytyrosyl acetate and alkyl hydroxytyrosyl ethers. Int. J. Food Sci. Nutr. 6: 703-707.

Rada-Mendoza, M., M. L. Sanz, A. Olano and M. Villamiel. 2004. Formation of hydroxymethylfurfural and furosine during the storage of jams and fruit-based infant foods. Food Chem. 4: 605-609.

Ramirez-Jimenez, A., B. Garcia-Villanova and E. Guerra-Hernandez. 2000. Hydroxymethylfurfural and methylfurfural content of selected bakery products. Food Res. Int. 10: 833-838.

Rui, X., X. Xu, H. Wu, W. Li, X. H. Chen, M. Jiang and M. S. Dong. 2014. A survey of equol contents in Chinese stinky tofu with emphasis on the effects of cooking methods. Int. J. Food Sci. Nutr. 6: 667-672.

Seppanen, C. M. and A. S. Csallany. 2002. Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature. J. Am. Oil Chem. Soc. 10: 1033-1038.

Uriarte, P. S. and M. D. Guillen. 2010. Formation of toxic alkylbenzenes in edible oils submitted to frying temperature influence of oil composition in main components and heating time. Food Res. Int. 8: 2161-2170.

You, J. M., T. Yan, H. X. Zhao, Z. W. Sun, L. Xia, Y. R. Suo and Y. L. Li. 2009. A sensitive fluorescence reagent for the determination of aldehydes from alcoholic beverage using high-performance liquid chromatography with fluorescence detection and mass spectrometric identification. Anal. Chim. Acta. 1: 95-104.

Yukawa, N., H. Takamura and T. Matoba. 1993. Determination of total carbonyl-compounds in aqueous-media. J. Am. Oil Chem. Soc. 9: 881-884.

Zhang, J. B., M. J. Li, W. L. Li, Q. C. Chu and J. N. Ye. 2011. A novel capillary electrophoretic method for determining aliphatic aldehydes in food samples using 2-thiobarbituric acid derivatization. Electrophoresis. 6-7: 705-711.