

Trans Fatty Acid Content in Chinese Fried Dough Used Repeated Frying Oil

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาปริมาณกรดไขมันชนิดทรานส์ด้วยวิธีแอตเทนนูเอตเต็ดโททัลรีเฟลกชันฟูเรียทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี ของปาท่องโก๋ที่ใช้น้ำมันทอดซ้ำ เนื่องจากเป็นอาหารทอดที่เป็นที่รู้จักกันกว้างขวางและมักพบการใช้น้ำมันทอดซ้ำการเตรียมปาท่องโก๋ทำตามสูตรข้างฉลากของแป้งปาท่องโก๋สำเร็จรูป โดยการทอดแต่ละครั้งใช้ส่วนผสมทั้งหมดรวม 80 กรัม แบ่งออกเป็นปาท่องโก๋ 10 ชิ้น ทอดในน้ำมันปาล์ม 6,000 กรัม อุณหภูมิ 170 องศาเซลเซียส เป็นเวลา 3 นาที ระยะห่างของการทอดแต่ละครั้งคือ 10 นาที ทอดต่อเนื่องไปจนครบ 5 ชั่วโมงโดยไม่มีการเติมน้ำมันใหม่ลงไป มีการเก็บตัวอย่างเพื่อนำไปวิเคราะห์ทุก 1 ชั่วโมงรวมทั้งการทอดในครั้งแรกด้วย รวมทั้งสิ้น 6 ครั้ง ผลการวิเคราะห์พบว่าปริมาณกรดไขมันชนิดทรานส์ในหน่วยร้อยละของไขมันทั้งหมดที่การทอดครั้งแรกจนถึงที่ชั่วโมงที่ 5 มีค่า 0.07 ± 0.04 , 0.17 ± 0.00 , 0.20 ± 0.11 , 0.27 ± 0.05 , 0.31 ± 0.01 และ 0.37 ± 0.04 ตามลำดับ และระยะเวลาที่ใช้ทอดมีผลต่อปริมาณกรดไขมันชนิดทรานส์โดยพบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณกรดไขมันชนิดทรานส์ของการทอดครั้งแรกกับการทอดที่ชั่วโมงที่ 4 และ 5 รวมทั้งการทอดที่ชั่วโมงที่ 1 กับการทอดที่ชั่วโมงที่ 5 ดังนั้นผู้บริโภคควรหลีกเลี่ยงการรับประทานอาหารทอดที่ใช้น้ำมันทอดซ้ำ

คำสำคัญ : กรดไขมันชนิดทรานส์ ปาท่องโก๋ อาหารทอด น้ำมันทอดซ้ำ

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Abstract

This study aimed to study *trans* fatty acid (TFA) content in Chinese fried dough (or Pa-tong-ko in Thai) which cooked with repeated frying oil by using the attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR). Chinese fried dough, one of well-known fried foods in Thailand, was chosen to be a representative of fried food in this study because the repeated frying oil is commonly found in its cooking. Chinese fried dough was cooked following the instant Patonggo mix powder package label. 80 g dough was prepared and was separated into 10 equal pieces for a single frying. Then they were fried with 6,000 g palm oil, 170 °C temperature for 3 minutes. Therefore ten pieces of dough were fried every 10 minutes continually until it reaches 5 hours without renew oil added. The samples were collected every 1 hour including the first fried ones. So after 5 hours frying, there were total 6 times collected. The results showed that the amount of *trans* fatty acid (expressed as % of total fat) at first time frying till hr5 was 0.07 ± 0.05 , 0.17 ± 0.00 , 0.20 ± 0.11 , 0.27 ± 0.05 , 0.31 ± 0.01 and 0.37 ± 0.04 respectively. Moreover, frying time affects *trans* fatty acid content in fried food because the significant difference of *trans* fatty acid content (% of total fat) at each time frying was found following first time frying and hr4, first time frying and hr5, and hr1 and hr5 as well. Therefore, consumers should avoid consuming food that used repeated frying oil.

Keywords : *Trans* fatty acid, Chinese fried dough, fried food, repeated frying oil

Introduction

Nowadays *trans* fatty acid (TFA), which is unsaturated fatty acid by trans configuration, is increasingly studied due to their negative effects on human health. Some studies showed the increase of plasma concentration of low density lipoprotein (LDL) cholesterol and the plasma ratio of total cholesterol to high density lipoprotein (HDL) cholesterol compared with saturated fats from *trans* fatty acid intake, and the studies also found the decreasing of concentration of HDL cholesterol as well (Mensink and Katan, 1990; Zock and Katan, 1992; Aro et al., 1997). Increase of coronary heart disease (CHD) is the most important phenomenon since 2% energy increase from *trans* fatty acid containing food which is equivalent to 4 g of 2,000 kcal standard diet, may cause 23% CHD increased risk (Mozaffarian et al., 2006). Moreover, the intake of *trans* fatty acid may involve with an

increased inflammatory marker, such as C-reactive protein and interleukin-6 (Lopez-Garcia et al., 2005; Remig et al., 2010). Therefore TFA contents were widely concerned by many countries about reducing these contained in food.

There are 2 major sources of *trans* fatty acid (Mozaffarian et al., 2006; Remig et al., 2010), the first is natural source being produced by bacteria in ruminants stomach (biohydrogenation). The second is mostly found in partially hydrogenated vegetable oil during partial hydrogenation, and refining process. In addition, frying causes physical and chemical changes in food such as oxidation, pyrolysis, polymerization, hydrolysis and isomerization (Ariyapitipan, 2008). Therefore, *trans* fatty acid content in fried food might be increased because of *trans* isomerization that made configuration changed from *cis* to *trans* form. Moreover, high levels of *trans* fatty acid might be dramatically increased while using repeated frying oil (Ariyapitipan, 2008).

The official methods for analysis of *trans* fatty acid content are gas chromatography (GC) and infrared spectroscopy (IR). GC method consists of several steps such as extracting samples, preparing fatty acid methyl esters (FAME) and internal standard that take a long time for separation (up to 1.5 hours) (Kim et al., 2007; Mossoba et al., 2009) while IR method is a rapid, easy and accuracy analytical method to determine the total *trans* fatty acid content. The IR method is based on the absorbance of isolated *trans* fatty acid double bond at wavelength number 966 cm^{-1} (Kim et al., 2007).

Chinese fried dough (or Pa-tong-ko in Thai), one of the most well-known fried food in Thailand, was chosen to be a representative of fried food in this study. The reason was that because most of the shops selling Chinese fried dough in Thailand tend to use repeated frying oil for frying the dough (Wanauwat, Kodcha and Pattanapraisorn, 2011).

The purposes of this study

To study *trans* fatty acid (TFA) content in Chinese fried dough (or Pa-tong-ko in Thai) which the repeated frying oil used without renew oil added.

Materials and Methods

Chemicals : Trielaidin [1,2,3, tris (*trans*-9-octadecanoate)] and Triolein [1,2,3, tris (*cis*-9-octadecanoate)] with more than 99% purity were purchased from Sigma-Aldrich (St. Louis, MO, USA) as fatty acid standards. *n*-Hexane was obtained from Mallinckrodt chemicals (Phillipsburg, NJ, USA) as fat extracted solvent.

Sample preparation : Chinese fried dough in 80 g amount (10 pieces) were made up from ingredient mixture containing instant Patonggo mix powder, yeast, vegetable oil and water. Then the dough was fried with 6,000 g palm oil every 10 minutes continually repeated until 5 hours by temperature controllable fryer with frying condition as 170°C and 3 minutes. Samples were collected at first time frying and the end of each hour frying. Afterwards, samples were kept in polyethylene bag at -20 °C until fat extraction and *trans* fatty acid determination.

Standard calibration curve preparation : Trielaidin and triolein were mixed to get five standards covering 0.5% to 50% *trans* fatty acid level, according to 2000.10 AOAC method in 2000 (AOAC, 2000).

Fat extraction : Ultrasonic extraction with *n*-hexane as solvent and the appropriate condition; 60 °C, 40% ultrasonic intensity level for 120 minutes reported by Soonpan, 2010 was used. The round bottom flask containing 5 g of Chinese fried dough and 30 ml *n*-hexane was placed in ultrasonic bath under the above mentioned condition. After extraction, the extract was filled through Whatman no.42 filter paper. The filtrate was evaporated by rotary evaporator and dried in vacuum desiccator for 60 minutes.

***Trans* fatty acid content determination :** After fat extraction, the attenuated total reflection fourier transform infrared spectrometer (Perkin Elma Spectrum One ATR-FTIR, USA) was used to determine *trans* fatty acid content. The parameter was set up according to the manufacturer's recommendation for using a zinc selenide ATR cell which maintained at 65±2 °C for fully melted sample and other parameters were set as 1050-900 cm⁻¹ spectral range, 4 cm⁻¹ resolution and 64 scans.

The single-beam spectrum collected of air was used as reference background while the test sample was collected against that of the reference background and converted into absorbance. The height of the negative second derivative of the *trans* band was used for calculations of *trans* fatty acid content since the sensitive and accuracy improved. After each sample analysis, the ATR cell was cleaned by acetone and each repeated test sample was scanned for 3 times.

Calculations : *Trans* fatty acid content was calculated by using the linear regression equation computed from standard calibration curve being plotted between peak height of standards and % of *trans* fatty acid of total fat. The sample's peak height *trans* band was replaced into the equation to get *trans* fatty acid content as % of total fat. Mean and standard deviation were reported.

Statistical analysis : The effect of using repeated frying oil on *trans* fatty acid content in Chinese fried dough was calculated by using the following statistics; analysis of variance between groups (two way-ANOVA) and Tukey HSD test.

Results

Following figure 1, *trans* fatty acid content was expressed as % of total fat (mean $\pm$ standard error of triplicate extraction). Number of frying parameter was chosen to explain the effect of using repeated frying oil on *trans* fatty acid content. By using two-way ANOVA, the number of frying was affected *trans* fatty acid content (p -value=0.000). While using Tukey HSD test the significant difference of *trans* fatty acid content (% of total fat) between hr0 (first time frying) and hr4, hr0 and hr5 and hr1 and hr5 was found (p -value=0.005, 0.000 and 0.022 respectively). Moreover, weights of Chinese fried dough before and after frying were observed (Table 1).

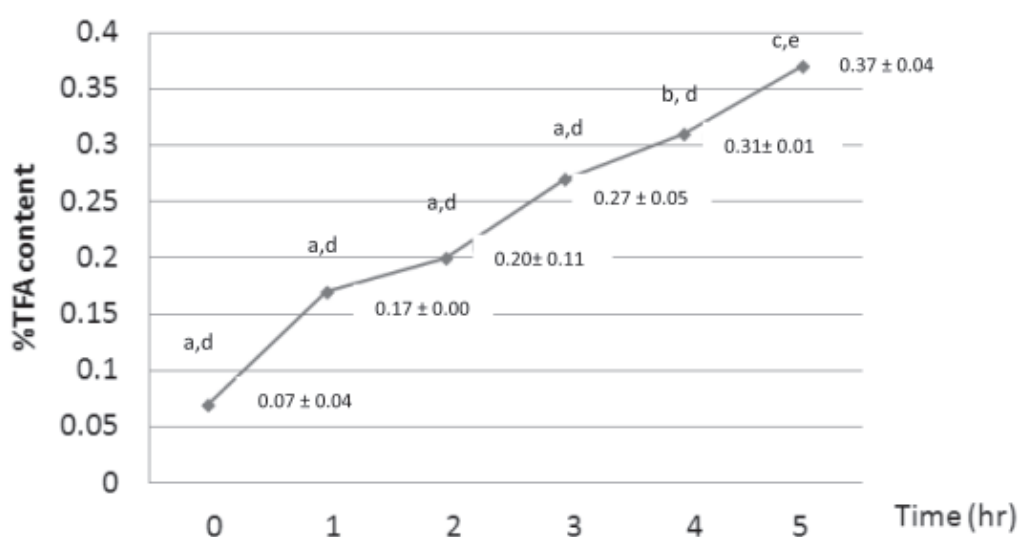


Figure 1 Shows the amounts of *trans* fatty acid in Chinese fried dough after frying at 170°C for 3 min between hr 0 (first time frying) and hr 5. The values represent the mean $\pm$ standard error of triplicate extraction. The values at the time points not sharing a common letter are significantly different ($P < 0.05$).

Table 1 Weights of Chinese fried dough

Time	Weight of dough (g)	Weight of Chinese fried dough (g)	Weight of each Chinese fried dough piece (g)	Different weight of Chinese fried dough before and after frying (g)
hr0*	81.18	88.32	8.83	7.14
hr1	80.65	87.72	8.77	7.07
hr2	81.24	88.07	8.81	6.83
hr3	80.25	86.18	8.62	5.93
hr4	79.78	85.77	8.56	5.99
hr5	80.60	86.24	8.62	5.64

* hr0 = first time frying

Discussion

In this study, conditions of sample preparation were set to resemble to the peddler's normal behavior of using repeated frying oil without renew oil added, and frying condition was adapted from the research of Wananuwat et al. (2011) that collected information from vendors. On the other hand, interval time between each frying was controlled to make the variance as low as possible.

Effect of using repeated frying oil on *trans* fatty acid content occurred in fried food was observed. The result showed the increase of *trans* fatty acid content (% of total fat) from hr0 (first time frying) to hr5 as 0.07 ± 0.04 , 0.17 ± 0.00 , 0.20 ± 0.11 , 0.27 ± 0.05 , 0.31 ± 0.01 and 0.37 ± 0.04 respectively. This is similar to a previous study of Tsuzki et al (2008) that reported *trans* fatty acid content increased gradually when 1.0 g of representative oil containing triolein, trilinolein and trilinolenin was fried at 180°C . These results support the hypothesis that frying process may lead to the occurrence of *trans* fatty acid in fried food because when oil is heated during processing, *cis* double bonds occurring naturally can change to *trans* isomerization (Wagner, Auer, and Elmadfa, 1999), together with lipid oxidation. Now, several countries are increasingly concerned about frying oil temperature. Many European countries have determined that the frying oil temperature must not exceed 180°C . In France, it was established that oil commercially used in frying must contain 3% alpha-linolenic acid at most

(Fox, 2001; Wolff, 2002). Moreover, the increased weight of Chinese fried dough after frying reflected more total fat that people received in case high consumption.

Now, Thai Food and Drug Administration (FDA) has already set punishment of using repeated frying oil which contained polar substance more than 25% (w/w), (announcement of Ministry of public health 347 edition, 2012) but the amount of *trans* fatty acid on food labels has not yet been regulated.

Moreover, the increase in Chinese fried dough weights in this study have shown that oil absorption by food during the frying process occurred. These can consequently increase not only total fat but also *trans* fat levels in fried foods. Therefore, the consumers should concern about sources and health effects of *trans* fatty acid occurred in fried food.

Conclusion

The result of present study indicated that using repeated frying oil caused the increase of *trans* fatty acid content in fried food which may be harmful to consumers. However, this is only primary information that is implied from only one kind of fried foods. Further study on this particular field is needed in the near future.

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References

- AOAC. (2000). *Announcement of Ministry of public health 347 edition (2012) : production method of food used repeated frying oil.*
- Ariyapitipan, T. (2008). Health hazards of repeatedly used deep frying oils. *J NutrAssoc*, 43(1). Retrieved from http://www.nutritionthailand.or.th/scripts/natmagazine_content.asp?nMAGID=25&nStoryID=252.
- Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M. (1997). Stearic acid, *trans* fatty acids, and dairy fat : effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. *Am J Clin Nutr* 65: 1419-1426.

- Association of Official Analytical Chemists (AOAC). (2000). *Official Method of Analysis of the Association of official analytical chemists*. 17th ed. Washington D.C. : The Association of Official Analytical Chemists.
- Fox R. (2001). *Regulation in the European Union*. In : ROSSELL JB (Ed), *Frying : improvement quality*. New York : CRC Press.
- Kim, Y., Himmelsbach, D.S., and Kays, S.E. (2007). ATR-fouriertransform mid-infrared spectroscopy for determination of *trans* fatty acids in ground cereal products without oil extraction. *J Agric Food Chem* 55 : 4327-4333.
- Lopez-Garcia, E., Schulze, M.B., Meigs, J.B., Mason, J.E., Rifai, N., Stampfer, M.J., Willert, W.C., and Hu, F.B. (2005). Consumption of *trans* fatty acid is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* 135(3) : 562-566.
- Mensink RPM, Katan MB. (1990). Effect of dietary *trans*-fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* 323 : 439-445.
- Mossoba, M.M., Seiler, A.J., Kramer, J.K.G., Milosevic, V., Milosevic, M., Azizian, H., et al. (2009). Nutrition labeling: rapid determination of total *trans* fats by using internal reflection infrared spectroscopy and a second derivative procedure. *J Am Oil ChemSoc* 86 : 1037-1045.
- Mozaffarian, D., Katan, M.B., Ascherio, A., Stampfer, M.J., and Willett, W.C. (2006). *Trans* fatty acid and cardiovascular disease. *N Engl J Med* 354 : 1601-1613.
- Remig, V., Franklin, B., Margolis, S., Kostas, G., Nece, T., and Street, J.C. (2010). *Trans* fats in America : a review of their use, consumption, health implications, and regulation. *J Am Diet Assoc* 110(4) : 585-592.
- Soonpan, P. (2010). *Analysis of trans fatty acid content in deep fried foods, milk and dairy products by attenuated total reflection-fourier transform infrared spectroscopy*. Master's Thesis. Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.
- Tsuzuki, W., Nagata, R., Yunoki, R., Nakajima, M., and Nagata, T. (2008). *cis/trans*-Isomerisation of triolein, trilinolein and trilinolenin induced by heat treatment. *J Food Chem* 108 : 75-80.

- Wagner K-H, Auer E, Elmadfa. (1999). Content of *trans fatty acid* in margarines, plant oils, fried products and chocolate spreads in Austria. *Eur Food Res Technol* 210 : 237-241.
- Wananuwat N., Kodcha S., and Pattanapraison P. (2011). *Safety Chinese fried braed frying*. Retrieved from http://www.rdi.ku.ac.th/Techno__ku60/res-48/index48.html.
- Wolff RL. (2002). Are *transisomers* of alpha-linolenic acid of nutritional significance for certain populations. *Lipids* 37 : 1147- 1148.
- Zock PL, Katan MB. (1992). Hydrogenation alternatives : Effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *J Lipid Res* 33 : 399-410.

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