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Effects of frying conditions on the formation of heterocyclic amines and *trans* fatty acids in grass carp (*Ctenopharyngodon idellus*)

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ABSTRACT

The effects of frying temperature and the number of frying cycles on the formation of heterocyclic amines (HAs) and *trans* fatty acids (TFAs) in grass carp were investigated. 9*t*-18:1 FAs was detected in all samples. The TFA contents of samples fried at 150–210 °C were not significantly different ($P > 0.05$). The content and number of different types of HAs increased with increasing frying temperature. 9H-pyrido[3,4-*b*]indole (Norharman), 1-methyl-9H-pyrido [3,4-*b*]indole (Harman), and 2-amino-3,7,8-trimethylimdazo[4,5-*f*]quinoxaline (7,8-diMeIQx) were detected in most of the tested samples. The differences in the surface colour (ΔE) increased with frying temperature, and ΔE of samples fried at 170 °C was significantly higher than that of 150 °C ($P < 0.05$). The analysis of different cycle times revealed that the TFA levels increased with an increase in the usage period of the frying fat, and 9*t*,12*t*-18:2 FAs was detected after the 40th frying cycle. As the number of frying cycles increased, the number of different types of HAs increased, seven types of HAs were detected after the 25th frying cycle.

1. Introduction

Frying is the most commonly used procedure for food preparation all over the world. Frying can not only confer good flavour and colour but also generate TFAs and HAs (Bansal, Zhou, Tan, Neo, & Lo, 2009; Janoszka, Blaszczyk, Damasiewicz-Bodzek, & Sajewicz, 2009). The design and operation of frying are important in determining the safety and quality of foods. The consumption of TFAs has some adverse effects. Excessive intake of TFAs may raise the level of cholesterol level in blood, and elevate the concentration of low density lipoprotein in the plasma. Grass carp is generally marketed as fresh. Its farmed production, consumption, and output rank first for freshwater fish. Frying is the most common method to cook fish. The nutritive value of fish can be affected by cooking (Weber, Bochi, Ribeiro, Victório, & Emanuelli, 2008). Most of the studies have been carried out on *cis* fatty acid profile, in particular the levels of omega-3 fatty acids (Larsen, Quek, & Eyres, 2010). However, few information of *trans* fatty acid profile of foodstuff was evaluated. The TFA composition of grass carp fried in vegetable oil, which is a popular fried food, remains unknown, more important is the need to explore the changes of TFAs under different conditions.

Protein-rich fish cooked at 150 °C would form mutagenic and carcinogenic compounds identified as HAs. The formation of HAs is very complicated. Earlier studies have shown that formation of HA is closely related to the cooking temperature, cooking time, cooking method, meat type, and chemical parameters, particularly the precursors. In several of the previous studies, the effects of cooking methods on the HAs content of various fish species (salmon, cod) were investigated (Johansson & Jägerstad, 1994; Pais, Salmon, Knize, & Felton, 1999), and it has been demonstrated that the content and type of HAs varied with different species and cooking condition. Yet, there is no information regarding the variation of HAs composition of fried grass carp.

Conventional deep fat frying is very common, locally the fried food is characterised by multiple frying over an extended period of time. During continuous deep-fat frying, the frying fats are used over an extended period of time, which causes their thermal decomposition and oxidation (Goburdhun, Jhaumeer-Laulloo, & Musruck, 2001). Information is scarce on the deleterious effects of different frying conditions employed to fried foods. A few studies have analysed the effects of the number of frying cycles on the formation of HAs. HA formation may be affected by the type, oxidation status, and antioxidant content of the frying fat (Johansson, Fredholm, Bjerne, & Jägerstad, 1995). However, the changes in the HAs in fish with the extended usage time of frying fat have not yet been reported.

Currently, lacking of management and standards about frying operations, awareness about the formation of HAs and TFAs of

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frying meat is deficiency. In processed meat, the presence and hazard of HAs and TFAs have become a major concern for both consumer and researchers. In the present study, a real approach to what happen in the fried food when foods are fried in frying oil and the formation of TFAs and HAs in grass carp under various frying conditions, including different frying temperatures and number of frying cycles, was investigated. In addition, we aimed to gain important insight into the mechanisms by which TFA and HA are formed in fried food and the role played by frying oil. The data from this study provided explicit information on the nutritive value of food after frying and could be used to identify conditions that minimise the HAs and TFAs formation in fried fish.

2. Materials and methods

2.1. Food samples

Supplier of grass carp (*Ctenopharyngodon idellus*) were obtained in the winter of 2012 from the local farmers' market in Nanjing Jiangsu. Selected specimens had an average weight of 2500 ± 50 g. The refined soybean oil used for frying was purchased from a local supermarket in Nanjing. The raw oil contained 0.59 ± 0.05 mg/g 9*t*-18:1. This oil was selected because it is the most frequently used oil in China.

2.2. Chemicals

All chemicals and reagents used were of either analytical or chromatographic grade. Seven *trans* fatty acid methyl ester (FAME) standards including 9*t*-14:1, 9*t*-16:1, 9*t*-18:1, 11*t*-18:1, 9*t*,12*t*-18:2, 11*t*-20:1, and 13*t*-22:1 FAME standards, were purchased from Tedia Co. (Fairfield, OH, USA). Twelve HA standards, namely IQ (2-amino-3-methulinidazo[4,5-*f*]quinoline), MeIQ (2-amino-3,4-dimethylinidazo[4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline),4,8-diMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline), 7,8-diMeIQx(2-amino-3,7,8-trimethylimdazo[4,5-*f*]quinoxaline), Harman (1-methyl-9H-pyrido[3,4-*b*]indole), Norharman (9H-pyrido[3,4-*b*]indole), Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine), Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-*b*]indole), AαC (2-amino-9H-pyrido[2,3-*b*]indole), and MeAαC (2-amino-3-methyl-9H-pyrido[2,3-*b*]indole), were purchased from Toronto Research Chemicals (Downsview, ON, Canada). The HPLC-grade solvents (including methanol, acetonitrile, and isooctane) were obtained from Tedia Co. (Fairfield, OH, USA). All other analytical-grade chemicals (such as ammonium acetate, sodium hydroxide, hydrochloric acid, and ammonia) were obtained from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China).

2.3. Sample preparation

Grass carp were transported to the laboratory alive within 30 min. Prior to the experiments the fish were kept in a pool under ambient temperature for 1 h and were exposed to natural photoperiod. The fish were killed by a blow on the head, and then eviscerated, then manually filleted at 4 °C. The fillets were immediately washed with tap water to remove any traces of blood and viscera. After that, the fillets were drained for 10 min at 0–4 °C and then minced to uniformity using a meat grinder, and round fish patties approximately 5.6 cm × 1.3 cm (diameter × thickness) were made using petri dishes; the fish patties exhibited an average mass of 45 g. The prepared patties were used for frying.

To study the effect of frying temperature on TFA and HA formation in fish patties, fish patties were fried in an open temperature-controlled fryer. The oil-to-fish ratio was 10:1 (*w*/*w*) (Ganbi, 2011), and the oil was heated to 150, 170, 190, or 210 °C. Fish patties were fried for a total of 10 min and were turned every 5 min. Two fish patties were fried at each temperature and three experimental replications were performed, the total number of samples was 24 (2 × 4 × 3). No salt or flour was added to the fish before or after cooking.

To investigate the number of frying cycles on the formation of TFA and HA in the fish patties, fish patties were placed into the fryer after the oil had reached 170 °C. The frying temperature was controlled at 170 ± 2 °C and monitored with a thermometer throughout the frying process. The samples were fried for 10 min and turned in every 5 min. In total, 50 frying cycles were performed, fresh samples taken for each cycle of frying, we cooked two patties at each frying cycle. The study was repeated three times. The total number of samples were 300 (2 × 50 × 3) and the total usage time of the oil was 500 min. Once a batch of fish patties finished frying, the next batch was started. The frying process was performed five cycles each day. After each day of frying, the oil was replenished with the original volume of fresh oil. Samples were collected after the 5th, 10th, 15th ... 50th frying cycles. No salt or flour was applied to the fish before or after cooking. Once fried, the fish patties were maintained at −20 °C under a nitrogen atmosphere until analysis.

2.4. Analysis of differences in the surface colour

The colour coordinates (L^*, a^*, and b^*) of the fried and raw fish patties (reference) were obtained using a colorimeter (Konica Minolta Co. Japan). The difference in the surface colour (ΔE) was calculated as follows (Solyakov & Skog, 2002):

$$\Delta E = [(L^* - L^*\text{reference})2 + (a^* - a^*\text{reference})2 + (b^* - b^*\text{reference})2]^{1/2}$$

where L^* corresponds to the lightness, a^* defines the red component, and b^* describes the brown component.

2.5. Determination of TFAs

The TFA profile was determined by gas chromatography. The grass carp lipid was extracted and measured using chloroform and methanol mixtures extracted at a ratio of 2:1 (*v*/*v*) (Folch, Lees, & Sloane Stanley, 1957). Methyl esters were prepared using 2 M KOH in methanol and isooctane according to the method described by Ichihara, Shibahara, Yamamoto, and Nakayama (1996) with minor modification. The extracted fat was dissolved in 4 mL of isooctane, and 0.2 mL of methanolic KOH was then added to the mixture. The mixture was vortexed for 2 min at room temperature, and the isooctane layer was then removed to obtain a volume of 5 mL. The isooctane layer was used for the GC analysis. The *trans* FAMEs were analysed using Chinese National Standard for the determination of *trans* fatty acids in foods (General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China (AQSIQ) & Standardisation Administration of the People's Republic of China (SAC), 2008). A SP-2560 column (100 m × 0.25 mm × 0.2 μm) (Supelco, USA) was used. The temperature was set to 60 °C, maintained at 60 °C for 5 min, increased from 60 to 165 °C at a rate of 5 °C/min, maintained at 165 °C for 1 min, increased from 165 to 225 °C at a rate of 2 °C/min, and maintained 225 °C for 17 min. Helium was used as the carrier gas at a flow rate of 1.3 mL/min, and the split ratio was 50:1. For the determination of recovery, 2 g raw fish patties was spiked with 0.2, 1, 5 mg/g of mixed *trans* FAME standard prior to extraction. The recovery rates for the different TFAs in the fish patties was between 92.52% and 99.27%.