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Impact of additives on thermally-induced *trans* isomers in 9*c*,12*c* linoleic acid triacylglycerol

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ABSTRACT

Trilinolein, with or without additives, was placed in glass ampoules and subjected to thermal treatment at 180 °C or 240 °C for 8 h. Thermal treatment of trilinolein at 180 °C and 240 °C produced twice the amount of *trans* nonconjugated linoleic acids (NLAs) compared to conjugated linoleic acids (CLAs), and nitrogen stream reduced the amount of both *trans* NLA and CLA products. The presence of additives resulted in the suppression or induction of *trans* NLAs and CLAs, depending on the type of additive, the concentration of the additive, and the heating temperature. Our analysis indicates that TBHQ is an effective additive for reducing *trans* NLA formation and inducing *trans* CLA formation in frying oil. Glutathione and L-cysteine at 0.1% may also be used as additives for frying oil. At suitable concentrations, Fe^{3+} and Al^{3+} ions derived from oils can reduce *trans* NLAs and induce *trans* CLAs during frying.

1. Introduction

Edible oils have a high proportion of unsaturated fatty acids that can undergo *cis*,*trans* (*c*,*t*) isomerisation during refining and deep-fat frying, leading to the formation of *trans* fatty acids (TFAs). TFAs are generally defined as unsaturated fatty acids that contain nonconjugated double bonds in the *trans* configuration. Evidence suggests that excessive consumption of TFAs is associated with a higher risk of coronary heart disease (CHD) (Mensink, Zock, Kester, & Katan, 2003) and cardiovascular disease (Ganguly & Pierce, 2012; Gebauer et al., 2011). A 2% increase in TFA consumption has been reported to increase the risk of CHD by 23% (Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006). Some countries have attempted to increase consumer awareness of TFA content in processed oils by indicating TFA content on product labels. Additionally, there has been an increased effort to reduce the amount of TFAs present in processed oils due to their detrimental effects on health.

TFAs in most plant oils are mainly composed of *trans* linoleic acids (TLAs) (Hou, Wang, Wang, Xu, & Zhang, 2012; Li, Ha, Wang, Li, & Li, 2012), which isomerise from 9*c*,12*c* linoleic acid triacylglycerol. Recent interest in linoleic acid has prompted investigation into the formation chemistry of nonconjugated linoleic acids (NLAs). Our previous study showed that *trans* isomerisation of linoleic acids in soybean oils could be achieved at high temperatures, producing 9*t*,12*t*-NLA, 9*c*,12*t*-NLA, and 9*t*,12*c*-NLA as the predominant *trans* products (Li, Yuan, Li, Wang, & Ha, 2013). Another study provided insight into the chemistry of conjugated linoleic acids (CLAs) generated by the heating of edible oils (García-Martínez, Márquez-Ruiz, Fontecha, & Gordon, 2009). However, the identities and concentrations of the *cis*,*trans* (*c*,*t*), *trans*,*cis* (*t*,*c*), and *trans*,*trans* (*t*,*t*) CLA isomers have not been fully studied.

CLAs have gained considerable attention recently because of their health benefits (Wang, Jacome-Sosa, & Proctor, 2012; Yooheon & Yeonhwa, 2012). The two most active CLA isomers are 9*c*,11*t*-CLA and 10*t*,12*c*-CLA, which are the main CLA isomers in hydrogenated vegetable oils. These CLA isomers exhibit antitumour activity against a broad range of cancer cell types (Bergamo et al., 2013; Kelley, Hubbard, & Erickson, 2007). Beppu et al. (2006) studied the inhibitory effects of pure CLA isomers on human colon cancer cell lines and showed that 9*t*,11*t*-CLA has the strongest inhibitory effect, followed by 10*t*,12*c*-CLA and 9*c*,11*t*-CLA. The results of these studies point to the importance of quantifying the amounts of CLA isomers formed during the heating of oils.

Consumption of *trans* NLAs and CLAs, which are produced in edible oils during refining or frying, can either positively or adversely impact human health. Precise analytical methods are necessary to identify and quantify the NLAs and CLAs in oils in order to evaluate the impact of TLAs on human health. To date, several analytical approaches have been used to determine the various positional isomers of unsaturated fatty acids, including silver-ion thin-layer chromatography (Ag^+-TLC), infrared spectroscopy (IR),

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reversed phase high performance liquid chromatography (RP-HPLC), gas chromatography (GC), and gas chromatography–mass spectrometry (GC–MS). Characterisation of these isomer products can also be performed using a gas chromatography-flame ionisation detector (GC-FID), which may be a preferred method for such an analysis due to its accuracy, convenience, and cost (Antolín, Delange, & Canavaciolo, 2008).

Recent studies suggest that the formation of TFAs in edible oils increases in direct proportion to frying temperature and time (Chen et al., 2014; Yang, Yang, Nie, Xie, & Chen, 2011). Small amounts of TFAs are produced during the heating and frying of purified edible oils at 180 °C, while considerable accumulation of *trans* fatty acids occurs when oils are subjected to heating temperatures greater than 250 °C (Bansal, Zhou, Tan, Neo, & Lo, 2009). It has been reported that 9*c*,12*c* fatty acid molecules undergo chemical bond rotation, migration, and degradation, leading to the formation of NLAs, CLAs, and aldehydes (Christy, 2009; Christy, Xu, & Harrington Pde, 2009; Guillén & Uriarte, 2012). However, relatively little work has been devoted to characterising the role of various additives in the formation of *trans* NLAs and CLAs during deep-fat frying.

In this study, high temperature processing of 9*c*,12*c* linoleic acid triacylglycerol (trilinolein) was used to imitate the oil frying process. The objective of the study was to use GC-FID to investigate the effect of several additives on the formation of *trans* isomers during the heating of trilinolein. Results from the study may provide insight into the roles of these additives during deep-fat frying and may suggest ways to reduce the formation of *trans* NLAs and induce the formation of CLAs.

2. Materials and methods

2.1. Materials

Pure trilinolein standards (99%) were purchased from NU-CHEK Prep, Inc. (Elysian, MN, USA). Standards for a linoleic acid methyl ester (LAME) mixture (C18:2, 9*c*,12*c*; 9*c*,12*t*; 9*t*,12*c*; 9*t*,12*t*) were purchased from Sigma–Aldrich (St. Louis, MO). Standards for the 9*c*,11*t*, 10*t*,12*c*, and 9*t*,11*t* linoleic acid methyl esters were purchased from Matreya (State College, PA, USA). Vitamin E (V_E) was obtained from Sigma–Aldrich (St. Louis, MO). Butyl hydroxy anisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), $FeCl_3$, and $AlCl_3$ were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. L-glutathione and L-cysteine were obtained from Beijing Quanta Hengyi Technology Co., Ltd. Triundecanoin, used as an internal standard for GC-FID analysis, was obtained from NU-CHEK Prep. Isooctane, used as a chromatographic organic solvent, was obtained from Fisher Scientific (Fair Lawn, NJ). Silicone oil (viscosity 500 cSt), obtained from Dingye Industrial Co., Ltd. (Beijing, China), was used as the heating material.

2.2. Thermal processing

Each trilinolein sample (100 mg) was transferred to a 4 ml micro-glass ampoule. The ampoules in the absence (control) and presence of nitrogen (N_2, 5 ml/min, 5 min) were melted and sealed by a propane-oxygen flame, and then heated in a silicone oil bath. Heating experiments were carried out at 180 °C and 240 °C for 8 h in micro-glass ampoules with a length of 4 cm, an internal diameter of 1.5 mm, and a wall thickness of 1 mm. For incubation of trilinolein with an additive, the additive was added to the glass tube in advance. Temperature control during heating was accurate to ±2 °C. After incubation, the glass tubes were removed from the silicone oil bath and were cooled prior to subsequent analysis.

2.3. Preparation of fatty acid methyl esters

The heated glyceride samples were subjected to derivatisation according to the method described in the AOCS Official Method, Chapters 1–91 (AOCS, 1997). 100 mg of the heated sample was weighed into a stoppered centrifuge vial and mixed with 1 ml of triundecanoin (C11:0, dissolved in isooctane), the internal standard, and 0.05 ml of 2 mol l^{-1} methanolic KOH. The mixture was shaken well for 30 s, then centrifuged at 4000 rpm for 10 min. The supernatant layers were removed and dried by the addition of anhydrous magnesium sulphate.

2.4. GC-FID analysis

20 μl of the dried supernatant layer was diluted to 1 ml with isooctane and analysed using a GC-2010 chromatograph (Shimadzu, Kyoto, Japan) equipped with a CP-SIL 88 fused silica capillary column (100 m × 0.25 μm × 0.2 mm) and a flame ionisation detector. The initial temperature of 60 °C was maintained for 5 min and then increased to 160 °C at a rate of 25 °C min^{-1}. After a 5 min incubation at 160 °C, the temperature was again raised at a rate of 2 °C min^{-1} to achieve a final temperature of 225 °C. The sample was maintained at this final temperature for 15 min. The injection volume was 1 μl with a split ratio of 1:10, and helium (99.999%) was used as the carrier gas with a flow rate of 6.3 ml min^{-1}. The injector and interface temperatures were both 230 °C. Fatty acids were identified by retention time by comparing them to the retention times of known standards, using the calculation method of Li et al. (2013). The amount of *t*,*t*-CLAs in the samples, which include a mixture of 9*t*,11*t* and 10*t*,12*t*-CLAs, was calculated by comparing samples to a 9*t*,11*t*-CLA standard. Accurate quantification of the concentrations of the fatty acids was dependent on the absence of chemical interactions between the molecules.

2.5. Statistical analysis

Experiments were performed in triplicate according to a completely randomised design. Sigmaplot 12.0 software (Systat Software Inc., San Jose, CA, USA) was used for data plotting. Statistically significant differences between samples were determined by Duncan's multiple range tests using SPSS (Version 16.0, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effect of N_2 on heat-induced trans isomers at 180 °C and 240 °C

To elucidate the identities of the isomers produced by *trans* isomerisation of unsaturated fatty acids, trilinolein (C18:2-9*c*,12*c*, 100 mg) was incubated at 180 °C and 240 °C for 8 h. As shown in Table 1 and Fig. S1, the main products were 9*c*,12*t*-NLA and 9*t*,12*c*-NLA at nearly equal quantities, indicating that isomerisation of the 9,12 double bonds in 9*c*,12*c* fatty acids was equally probable. However, no 9*t*,12*t*-NLA was detected in trilinolein at 180 °C after 8 h, suggesting that while the heated trilinolein molecules readily isomerised to 9*c*,12*t*-NLA and 9*t*,12*c*-NLA, isomerisation to 9*t*,12*t*-NLA was unfavourable. These findings are in accordance with previous reports (Christy, 2009; Li et al., 2012). We noted that the quantity of 10*t*,12*c*-CLA was lower than that of 9*c*,11*t*-CLA after heating trilinolein at 180 °C and 240 °C for 8 h. A higher amount of *t*,*t*-CLAs, including 9*t*,11*t*-CLA and 10*t*,12*t*-CLA, was observed compared to that of 9*c*,11*t*-CLA and 10*t*,12*c*-CLA (Table 1), indicating that *t*,*t*-CLAs are the primary conjugated isomerisation products of heated trilinolein. Our results show that the transformation of