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Stability of conjugated linoleic acid isomers in egg yolk lipids during frying

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Abstract

The stability of conjugated linoleic acids (CLA) was studied in egg yolk during storage and frying, using gas liquid chromatography (GLC) and silver ion high-performance liquid chromatography (Ag^+–HPLC). The eggs, containing 4.0% CLA per gramme of egg yolk, were stored in a refrigerator at 0–4 °C for 6 months, while the egg yolks were fried in a pan at 160–180 °C for 40 s. Either storage for 6 months or frying for 40 s did not significantly change the composition of CLA in egg yolk. However, the degradation of CLA was statistically significant, when the CLA mixture was fried under the same conditions at 160–180 °C for 40 s, suggesting that other components of egg yolk protected CLA from degradation. It is concluded that CLA is well preserved in egg before it is consumed.

Keywords: Conjugated linoleic acids; Egg; Frying; Storage; Stability

1. Introduction

Conjugated linoleic acids (CLA) have been a subject of extensive investigation for their anticarcinogenic (Scimeca, 1999), hypolipidemic (Yeung, Yang, Huang, Wang, & Chen, 2000), antiatherosclerotic (Lee, Kritchevsky, & Pariza, 1994; Nicolosi, Rogers, Kritchevsky, Scimeca, & Huth, 1997) and immune-enhancing (Cook, Miller, Park, & Pariza, 1993; Miller, Park, Pariza, & Cook, 1994) activities. CLA in food supply is quantitatively minor. It is estimated that CLA consumption in humans is about 0.5–1 g/day/person (McGuire, McGuire, Ritzenthaler, & Shultz, 1999). In addition to taking CLA supplements, feeding animals with a synthetic CLA mixture should be an alternative to enrich CLA in foods. Formulation of CLA in the diet of laying hens has led to incorporation of CLA into eggs and changes in yolk fatty acid composition (Raes et al., 2002; Aydin, Pariza, & Cook, 2001). We previously studied the incorporation pattern of 15 CLA isomers into the egg yolk lipids of hen in relation to that in the diet. It was found that the isomeric distribution pattern in the egg yolk lipids was different from that in the dietary fat (Yang, Huang, James, Lam, & Chen, 2002). The *trans*/*trans* CLA isomeres proved to be preferentially accumulated, although these isomers were quantitatively minor in both the diet and egg yolk. The c-9, t-11 and c-10, t-12 isomers were the two most abundant isomers and the incorporation of the former into the egg yolk was more preferred than the latter.

Stability of CLA in foods has not received much attention by either academics or industry. Although CLA has shown many beneficial effects, its decomposition must be prevented when CLA in foods is processed, stored and transported. In this regard, CLA in a pure form was reported to be rapidly decomposed to furan fatty acids when heated in air (Yurawecz, Hood, Mossoba, Roach, & Yu, 1995). Yang, Leung, Huang, and Chen (2000) examined the oxidative stability of individual CLA isomers in the form of free fatty acids, using a combination of gas–liquid chromatography (GLC) and silver ion high-performance liquid chromatography (Ag^+–HPLC) and found that CLA, as a whole, oxidized

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rapidly and more than 80% was degraded within 110 h in air at 50 °C. However, no study to date has addressed the stability of CLA in foods. The present study was carried out to further examine the stability of CLA in egg yolk during storage and frying.

2. Materials and methods

2.1. Egg

Eggs of 10 CSIRO hybrid white leghorns (*Gallus domesticus*) were collected as described previously (Yang et al., 2002). The hens were maintained on a diet that contained 2% CLA and only eggs on days 26–28 were saved for the present study. The fatty acid composition and CLA isomeric distribution in the fresh egg yolk were analyzed using the GLC method described by Chen, Chan, Kwan, and Zhang (1997), and Ag^+–HPLC method previously described by Sehat et al. (1998). It was found that CLA incorporated into eggs was 4.0 mg/g yolk. The remaining eggs were stored in a refrigerator (0–4 °C) for 6 months.

2.2. CLA and peanut oil

CLA mixture was obtained as a gift from Natural Lipids Ltd, AS, Norway. The GLC analysis showed that it contained 66.9% CLA. Peanut oil was obtained from Nuhua Peanut Oil Ltd, Shandong, China. The reason for choosing peanut oil as frying medium was that it contained an undetectable amount of CLA.

2.3. Frying

The change in CLA composition of eggs was monitored during frying. In brief, the yolk was separated from the white of these eggs. About 2 g crude yolks were saved for GLC and Ag^+–HPLC analysis. Other crude yolks (10.0 g) were fried in peanut oil in a pan for about 40 s; the temperature of peanut oil was 160–180 °C. After frying, the egg yolk was washed three times with chloroform/methanol (2:1 vol/vol) to remove the visible peanut oil. The egg yolk lipids were then homogenized and extracted using chloroform/methanol (2:1 vol/vol). The number of repeated experiments (n) was seven, in this experimental procedure.

The change in composition of pure CLA mixture during the frying was also monitored. The CLA mixture (1.0 g) and peanut oil (9.0 g) were mixed in 50 ml beakers, and then fried using the method described above ($n = 5$). The fatty acid compositions of the mixture prior to frying, and of the fried mixture were subjected to the GLC and Ag^+–HPLC analyses as described elsewhere below.

2.4. Fatty acid analysis

Total lipids in the egg yolks (130–150 mg each) or the CLA mixture samples (20 mg each), described above, were extracted using chloroform/methanol (2:1 vol/vol) containing heptadecanoic acid (17:0) as an internal standard, followed by acid-catalyzed methylation as described by Yang et al. (2002). In brief, total lipids were transesterified in 2 ml of 14% BF_3 in methanol under nitrogen at 90 °C for 2 min. Hexane (3 ml) and distilled water (1 ml) were added and mixed thoroughly. After the mixture was centrifuged at 2500 rpm for 10 min, the top hexane layer, containing fatty acid methyl esters (FAME), was saved and subjected to GLC and Ag^+–HPLC analyses. The number of analytical replicates was five.

The FAME mixtures were analyzed on a SP-2560 fused silica capillary column (100 m × 0.24 mm i.d., Supelco, Inc., Bellefonte, PA, USA) in a HP-5890 Series °C gas–liquid chromatograph equipped with a flame-ionization detector and an automated injector according to the method previously described (Chen et al., 1997). The column temperature was programmed from 180 to 220 °C at a rate of 2 °C/min and then held for 12 min. Injector and detector temperatures were set at 250 and 300 °C, respectively. Nitrogen was used as the carrier gas at a head pressure of 35 psi.

2.5. Ag^+–HPLC Analysis

The individual CLA methyl esters were separated using an HP-1100 HPLC equipped with a ternary pump delivery system as described by Sehat et al. (1998). In brief, 5 μl of the FAME mixture (5 μg/ml) in hexane were injected onto a silver-ion impregnated column (250 × 4.6 mm i.d., 5 μm, Chrompack, Bridgewater, NJ, USA) via a Rheodyne valve injector. Hexane, containing 0.1% acetonitrile, was chosen as a mobile phase at a flow rate of 1.0 ml/min. The separated individual CLA methyl esters were monitored at 233 nm. Only the CLA methyl esters were detected; the other FAME were not detectable because they have no absorption at 233 nm. Individual CLA methyl esters were identified according to the Ag^+–HPLC eluting pattern (Fig. 1) described by Sehat et al. (1998).

2.6. Statistics

Data were pooled from each experiment in which five analytical replicates were conducted. Data were expressed as mean ± standard deviation. Where applicable, analysis of variance (ANOVA) was used to statistically evaluate significant differences among the samples described above using Sigmastat (Jandel Scientific Software, San Rafael, CA, USA). Differences were considered significant when $p < 0.05$.

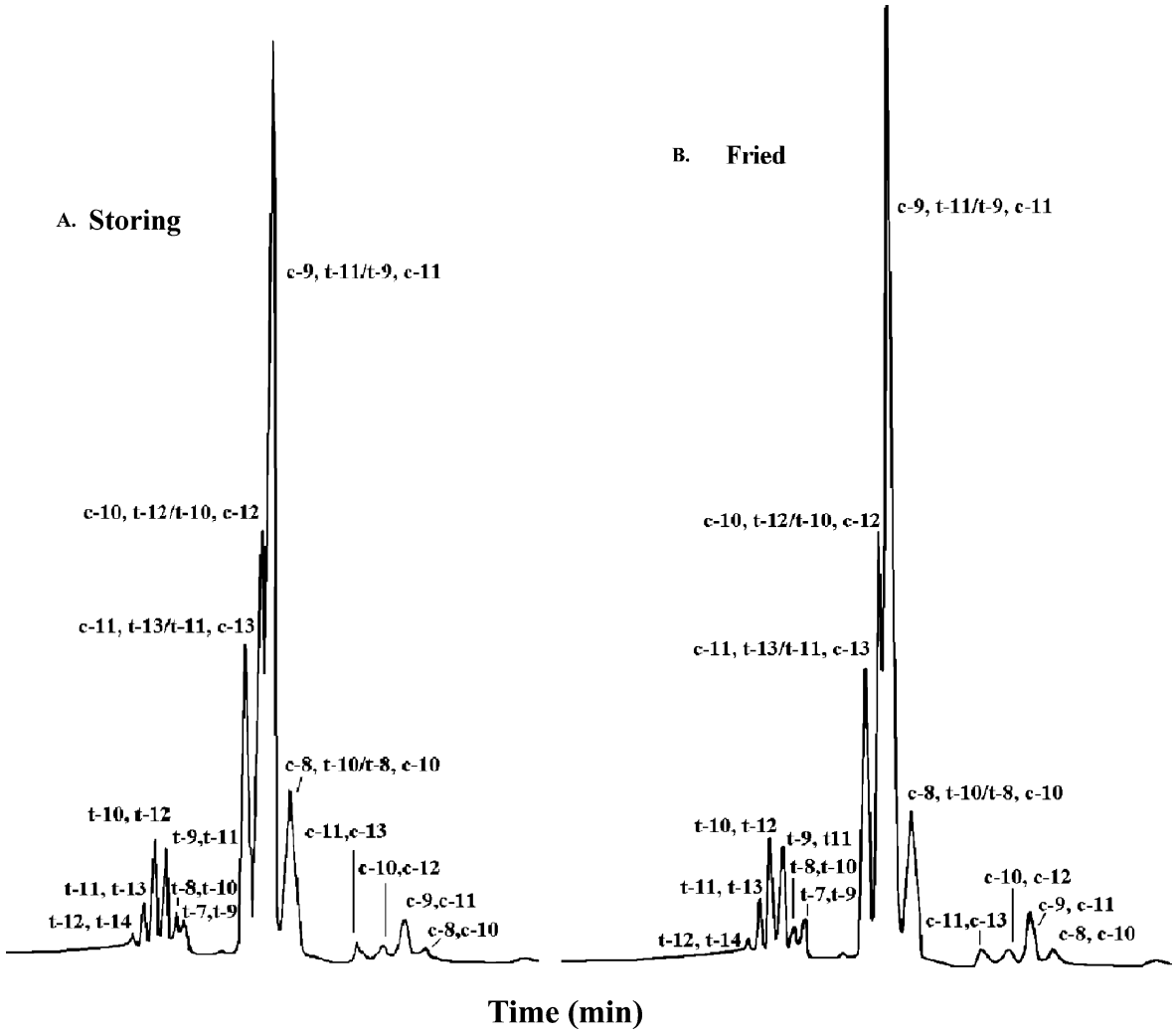

Fig. 1. Typical silver-ion high-performance liquid chromatograms of CLA, as a form of methyl esters, derived from egg yolk lipid, stored for 6 months (A) and fried for 40 s (B). *c*, *cis*, *t*, *trans*.

3. Results and discussion

The change in fatty acid composition of the pure CLA mixture was analyzed using GLC before and after frying (Table 1). Before frying, the mixture contained 9.9% CLA and the other fatty acids were mainly oleic acid (18:1 n-9) and linoleic acid (18:2 n-6), accounting for 40.5% and 32.6%, respectively, followed by 12.0% palmitic acid (16:0), 4.0% stearic acid (18:0) and 1.0% linolenic acid (18:3 n-3). When the CLA mixture was fried at 160–180 °C for 40 s, the amounts of 16:0, 18:0, 18:1 n-9, 18:2 n-6 and 18:3 n-3 remained unchanged but CLA as a whole was reduced to 9.1%.

Fatty acid composition of fresh egg yolk lipids, those stored at 0–4 °C for 6 months, and fried for 40 s in a pan at 160–180 °C are shown in Table 2. The CLA in yolk lipids of eggs stored for 6 months was not significantly different from that of the fresh egg yolk lipids, suggesting that the storage at 0–4 °C did not lead to any significant degradation of CLA in eggs. However, the CLA in egg yolk lipids was decreased from 4.3 to 3.9 mg/g after being fried for 40 s at 160–180 °C, but the difference

Table 1
Effect of frying on fatty acid composition of CLA mixture in peanut oil (% of total fatty acids)

	Control	Fried
CLA	9.9 ± 0.7^{b}	9.1 ± 0.2^{a}
16:0	12.0 ± 0.2	12.2 ± 0.1
18:0	4.0 ± 0.1	4.1 ± 0.1
18:1 n-9	40.5 ± 0.1	41.0 ± 0.1
18:2 n-6	32.6 ± 0.9	32.6 ± 0.1
18:3 n-3	1.0 ± 0.0	1.0 ± 0.0

The control represents an average of the samples prior to frying whereas the fried is an average of the samples that were heated in a pan at 160–180 °C for 40 s.

Data are expressed as means ± SD, $n = 5$ samples.

Means in the same row with unlike superscripts were significantly different ($p < 0.05$).

Table 2
Effect of storage and frying on fatty acid composition of egg yolk lipids (mg/g yolk)

	Fresh	Storage	Fried
CLA	4.0 ± 0.3	4.3 ± 0.4	3.9 ± 0.5
16:0	28.4 ± 0.9	26.8 ± 2.6	27.3 ± 3.0
16:1 n-9	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
16:1 n-7	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
18:0	18.0 ± 0.5[a]	15.9 ± 1.5[b]	15.5 ± 1.9[b]
18:1 n-9	30.5 ± 1.5[b]	34.4 ± 4.1[a]	36.4 ± 4.2[a]
18:1 n-7	1.5 ± 0.2	1.4 ± 0.2	1.4 ± 0.1
18:2 n-6	13.7 ± 0.9[b]	15.2 ± 1.6[a]	16.0 ± 1.5[a]
18:3 n-3	1.5 ± 0.1[b]	1.9 ± 0.2[a]	2.0 ± 0.3[a]
20:2 n-6	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.0
20:3 n-6	0.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.3
20:4 n-6	2.5 ± 0.1	2.0 ± 0.4	2.0 ± 0.7
22:4 n-6	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:5 n-6	1.6 ± 0.3	1.4 ± 0.1	1.5 ± 0.1
22:5 n-3	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
22:6 n-3	1.3 ± 0.1	1.5 ± 0.4	1.4 ± 0.7
FA/yolk	105 ± 5.1[b]	106.7 ± 7.7[b]	114 ± 8.0[a]

The Fresh refers to the egg yolks that were analyzed within the two weeks after collection; the Storage refers to the samples that were stored for 6 months at 0–4 °C while the Fried refers to the samples that were fried in peanut oil for 40 s at 160–180 °C.

Data are expressed as means ± SD, $n = 7$ samples.

Means in the same row with unlike superscripts were significantly different ($p < 0.05$).

Table 3
Effect of frying on composition of CLA isomers in peanut oil (% of total CLA)

CLA isomers	Control	Fried
t,t-12,14 CLA	1.1 ± 0.3	1.0 ± 0.4
t,t-11,13 CLA	1.5 ± 0.4	1.8 ± 0.6
t,t-10,12 CLA	2.8 ± 0.7	3.0 ± 0.8
t,t-9,11 CLA	2.8 ± 0.6	2.9 ± 0.8
t,t-8,10 CLA	1.0 ± 0.3	1.3 ± 0.5
t,t-7,9 CLA	1.3 ± 0.3	1.3 ± 0.3
Total t,t CLA	10.3 ± 2.5	11.3 ± 3.3
c,t/t,c-11,13 CLA	20.6 ± 0.8	20.8 ± 0.7
c,t/t,c-10,12 CLA	25.9 ± 0.8	24.9 ± 1.6
c,t/t,c-9,11 CLA	20.6 ± 0.6	19.6 ± 1.3
c,t/t,c-8,10 CLA	16.3 ± 0.5	16.3 ± 0.6
Total c,t/t,c-CLA	83.4 ± 2.7	81.5 ± 4.0
c,c-11,13 CLA	1.5 ± 0.3	1.9 ± 0.5
c,c-10,12 CLA	2.1 ± 0.1	2.3 ± 0.3
c,c-9,11 CLA	1.7 ± 0.0	1.8 ± 0.1
c,c-8,10 CLA	1.0 ± 0.1	1.2 ± 0.3
Total c,c-CLA	6.4 ± 0.3	7.2 ± 1.0

The Control refers to the CLA mixture prior to frying whereas the Fried refers to the samples that were heated in a pan at 160–180 °C for 40 s.

Data are expressed as means ± SD, $n = 5$ samples.

Means in the same row with unlike superscripts were significantly different ($p < 0.05$).

Table 4
Effect of storage and frying on composition of CLA isomers (% of total CLA)

CLA isomers	Fresh	Storage	Fried
t,t-12,14 CLA	0.8 ± 0.5	0.8 ± 0.3	0.7 ± 0.6
t,t-11,13 CLA	1.7 ± 0.5	1.3 ± 0.3	1.3 ± 0.8
t,t-10,12 CLA	3.1 ± 0.5	3.7 ± 1.0	3.5 ± 2.2
t,t-9,11 CLA	4.2 ± 1.6	4.0 ± 1.0	4.1 ± 2.2
t,t-8,10 CLA	1.3 ± 0.4	1.1 ± 0.3	1.1 ± 0.8
t,t-7,9 CLA	1.2 ± 0.4	1.2 ± 0.2	1.3 ± 0.5
Total t,t CLA	12.3 ± 3.0	12.2 ± 3.2	12.0 ± 7.1
c,t/t,c-11,13 CLA	12.9 ± 1.2	12.0 ± 1.2	12.4 ± 1.8
c,t/t,c-10,12 CLA	16.0 ± 1.6	16.7 ± 1.7	16.6 ± 1.5
c,t/t,c-9,11 CLA	41.2 ± 2.4	42.3 ± 2.8	42.2 ± 4.0
c,t/t,c-8,10 CLA	11.1 ± 0.7	10.9 ± 0.5	10.9 ± 1.3
Total c,t/t,c-CLA	81.2 ± 3.0	81.9 ± 3.5	82.0 ± 7.3
c,c-11,13 CLA	1.8 ± 0.6	0.5 ± 0.3	0.6 ± 0.4
c,c-10,12 CLA	1.5 ± 0.4	1.5 ± 0.4	1.7 ± 0.6
c,c-9,11 CLA	2.5 ± 0.3	2.9 ± 0.3	2.3 ± 0.9
c,c-8,10 CLA	0.9 ± 0.7	1.0 ± 0.5	1.2 ± 1.1
Total c,c-CLA	6.6 ± 0.9	5.9 ± 0.9	5.9 ± 1.4

The Fresh refers to the egg yolks that were analyzed within the two weeks after collection; the Storage refers to the samples that were stored for 6 months at 0–4 °C while the Fried refers to the samples that were fried in peanut oil for 40 s at 160–180 °C.

Data are expressed as means ± SD, $n = 7$ samples.

Means in the same row with unlike superscripts were significantly different ($p < 0.05$).

found was statistically insignificant. When compared with the values of fresh eggs, storage for 6 months led to a decrease in stearic acid (18:0) but increases in oleic acid (18:1 n-9) and linoleic acid (18:2 n-6). No significant difference was observed between the egg yolks fried and that those were stored for 6 months (Table 2).

Frying the CLA mixture in peanut oil for 40 s at 160–180 °C did not lead to any significant change in CLA isomeric distribution (Table 3). No differences in percentage composition of CLA isomers were observed between the egg yolks of the fresh and those stored for 6 months on those that were fried for 40 s at 160–180 °C (Table 4).

The main finding of the present study was that traditional preparation of fried eggs in a hot pan did not degrade very much of the CLA. These results clearly demonstrate that CLA, incorporated into egg yolk lipids, was stable after being fried for 40 s at 160–180 °C, which is typical of practice in making fried egg. This is in agreement with the previous study, that showed that separation of egg yolk from egg white by boiling eggs at 100 °C for 5 min did not lead to any significant change in CLA content of eggs (Yang et al., 2002). However, the CLA mixture in peanut oil was unstable under the same frying conditions. This is in agreement with a previous observation (Yang et al., 2000) that CLA, as a form of free fatty acids, is extremely unstable in air and its oxidative degradation rate is similar to that of docosahexaenoic acid (22:6 n-3). In the same study, CLA became more stable if it was incorporated into triacylglycerols. Thus, the CLA was stable in egg yolk lipids because it was present in the forms of phospholipids and triacylglycerols, whereas CLA was relative unstable in peanut oil, because it existed as a form of free fatty acid. It is also known that phospholipids and proteins can function as antioxidants under certain con-

ditions (Chen & Nawar, 1991). In this regard, yolk phospholipids and proteins were probably protective to CLA during the frying.

The present study showed that storage at 0–4 °C did not reduce very much of the CLA in egg yolks. The amount of CLA incorporated into egg yolk after being stored for 6 months was similar to that in the fresh egg. The rate of oxidative degradation of CLA is determined by many factors, including activation energy, oxidation–reduction potential, temperature and oxygen availability. We have found that CLA, either in the form of free fatty acids or incorporated into triacylglycerols, shows little or no degradation when it is stored in a refrigerator of 4 °C. In addition, oxygen penetration would be a limiting factors in oxidation of CLA in whole egg.

4. Conclusion

CLA has been shown to possess many beneficial effects associated with its consumption. Formulation of CLA in feeds of hens has been used to enrich CLA in eggs. The present study examined the stability of CLA isomers in egg yolks during storage and frying. It was concluded that CLA was stable in eggs during storage for a period of 6 months at 0–4 °C and no significant degradation of CLA occurred in egg yolk when it was fried for 40 s at 160–180 °C.

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