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Effect of Conjugated Linoleic Acid on Fatty Acid Composition and Lipid Oxidation of Egg Yolk

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난황내 Conjugated Linoleic Acid가 지방산 조성과 지방산화에 미치는 효과

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Abstract

The effects of conjugated linoleic acid (CLA) in egg yolk on fatty acid composition and lipid oxidation during chilled storage (4℃) were investigated. CLA was synthesized according to the method of alkali isomerization using safflower seed oil (SSO). A total of 250 hens (200 days of age) were fed control diet (commercial formula feed for hen) or CLA-supplemented diet (1%, 2.5% and 5% CLA) or 5% SSO supplemented diet for 6 weeks, and eggs were collected for analysis of CLA, fatty acid compositions and lipid oxidation. Eggs from CLA-supplemented diets groups showed significantly ($p<0.05$) higher CLA content compared to those of control group. The contents of linoleic, palmitic, and myristic acid were increased as well as CLA content by feeding a CLA-supplemented diet. However, the contents of oleic and arachidonic acids in egg yolks were decreased by dietary CLA supplementation. The pH of egg yolk increased by the levels of CLA during storage. The contents of CLA were not significantly ($p>0.05$) changed during chilled storage for 28 days, whereas TBARS were significantly ($p<0.05$) increased. It is suggested that lipid oxidation of egg yolk might be affected by the levels of CLA in egg yolk due to changes in fatty acid compositions.

Key words : CLA, conjugated linoleic acid, egg yolk, lipid oxidation.

Introduction

Conjugated linoleic acid (CLA) collectively refers to a class of positional and geometric isomers of linoleic acid. CLA have been recognized as having anticarcinogenic and antioxidative properties in several animal models[1~4]. CLA has been shown to reduce the catabolic effects of immune stimulation in mice, rats and chickens without adversely affecting immune function[5, 6] and shown to enhance the growth and improve feed efficiency in rats[7].

CLA is produced in ruminants as a first intermediate in the biohydrogenation of dietary linoleic acid by the rumen bacteria *Butyrivibrio fibrisolvens*[8]. Consequently, foods derived from ruminant animals contain more CLA than the other foods from non-

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ruminant animals[9]. However, the CLA level of turkey lipid is similar to ruminant animals[9] and intestinal bacteria of rats also have the ability to synthesize CLA from linoleic acid[7].

Much efforts have been contributed to increased CLA content in various foods. Ha et al.[10] reported increased levels of CLA in grilled beef as compared to uncooked ground beef. Shantha et al.[11] observed an increase in CLA content of processed cheese when cheese was processed at 60 or 80 ℃. These findings indicated that CLA increases during processing, whereas Shantha et al.[12] reported unchanged CLA concentration during storage.

Undesirable changes in the fatty acid composition of foods are known to occur as a result of temperature treatments[13]. During storage, cooked beef oxidized rapidly, but oxidation did not affect CLA concentration[14].

Although oxidative reactions have been postulated to increase CLA concentrations by accelerating the formation of linoleic acid free radicals and a subsequent shift in double bonds to a conjugated system[10], oxidative reactions can also cause destruction of CLA by destruction of the conjugated double bond system[12].

Small amounts of CLA (0.6mg/g fat) is present in egg yolk[9]. Because CLA content in the egg is not substantially increased by a conventional feed, the only possible way to increase effectively CLA content is to use the chemically synthesized CLA as a diet additives. Increasing the CLA content and changing the fatty acids composition in egg yolk by dietary CLA supplementation may provide a value-added egg. When these beneficial fatty acids can be reconstructed in the special quality of egg, a value-added egg could be developed for human consumption. The purpose of this study was to determine if the constituents and fatty acid composition in egg yolk could be changed by feeding different levels of CLA. We also determined the effect of different contents of CLA in egg yolk on lipid oxidation during storage.

Materials and Methods

Samples

A total of 250 laying hens (Isa brown, 200 days age) were purchased from Institute Development of Livestock Production (Kyeongsangnamdo branch) and divided into 5 groups (50 hens/group and one hen/cage).

Each hen was housed in a wire cage (40×40×40 cm) that placed in a temperature (25℃) and humidity (70% RH) controlled room. Hen was subjected to an appropriate diet and water *ad libitum*. The light-dark cycle was a 12-12 hours. Eggs were collected by each week for analysis of CLA and fatty acid levels in the yolk during experimental period of 6 weeks. Eggs that were collected at feeding 6 weeks were stored for 28 days at 4℃ to investigate the changes in pH, CLA and fatty acid compositions of the egg yolks.

Synthetic diets for laying hens

Basal diet identical to the National Research Council (NRC) standard rationals' specification for the diet of laying hens was synthesized with the diet components shown in Table 1. Treatment diets (1, 2.5 and 5 % CLA) were prepared by mixing an appropriate amount of the chemically-synthesized CLA with the basal diet by weight. A positive control diet (5 % safflower seed oil; SSO) was also prepared by mixing an appropriate amount of SSO with the basal diet by weight.

Each group of hens was adopted to the basal diet for a week (Table 1), and then subjected to one of either treatment diets

Table 1. Composition of the experimental basal diet for laying hens[1)]

Ingredient	%
Yellow corn	59.2
Wheat	5.0
Wheat bran	0.6
Soybean oil meal	15.4
Rapeseed oil meal	3.5
Limestone	9.8
Fat[2)]	3.0
Others[3)]	3.5

1) Diet contains amino acids and minerals identical to the NRC specifications for laying hen diet. Diet contains 2,950 ME kcal/kg, 20.5% protein and various amount of vitamins.
2) Fat was derived from the animals.
3) Primary calcium phosphate, NaCl, vitamin mixture, yeast mixture, lysine and methionine.

for 5 weeks: control (basal diet), 1% CLA, 2.5% CLA, 5% CLA or 5% SSO diet. All diets were freshly prepared and stored at the cold room temperature (5℃). Peroxide value of the diets containing CLA or SSO was not different from that of basal diet when determined before and after feeding.

Synthesis of CLA

CLA was chemically synthesized using SSO by alkaline isomerization method and purified by the low-temperature precipitation method described by Ha et al.[(2)]. The purified CLA was derivatized by 4% sulfuric acid in absolute methanol and analyzed by gas chromatography (GC).

Ethylene glycol, KOH, MeOH and Mn_3O_4 were purchased from Chunji Chemical Co. (Japan). Hexane (Burdick, USA) was HPLC grade. All other chemicals used were ACS grade.

Lipid analyses

Lipids were extracted with chloroform and methanol as described by Folch et al.[(15)]. Approximately 5 g of egg yolk and 50 ml Folch solution (chloroform : methanol= 2:1, v/v) added 50 μl of BHA (450 μg/ml) were homogenized using Polytron homogenizer (IKA Labortechnik T25-B, Malaysia) for 10 seconds. The homogenate was filtered with filter paper (Whatman No. 1). The residue and filter paper were blend with 50 ml of the Folch solution and then filtered again. About 25 ml water were added to the filtrate and centrifuged at 500×g for 10 min. The upper layer (methanol and water layer) was removed using an aspirator, and the bottom layer (chloroform-lipid extracts) was passed through anhydrous sodium sulfate(Na_2SO_4). The Na_2SO_4 was rinsed with 30 ml chloroform.

The extracts combined were concentrated using a rotarevaporator at 40℃ under nitrogen gas and stored at −40℃ for further analysis.

For fatty acid analysis, an aliquot of lipid extract (30mg) and 3ml of 4% H_2SO_4 in methanols were combined in a screw-capped test tube.

The test tube was located in boiling water for 20 min and then cooled at room temperature. The free fatty acid was conventionally extracted and quantitated by GC.

Fatty acid methyl esters were analyzed on a gas chromatography (Shimadzu GC-14A, Japan) with an on-column injector port and flame ionization detector. A Silar capillary column (30m×0.32mm×0.25 μm) was used for the separation of the fatty acid methyl esters. The gas chromatography oven temperature was 140℃, then increased at rate of 2℃/min to a final temperature of 230℃. The injector port and detector temperatures were set at 240 and 250℃, respectively. One μl of the fatty acid methyl ester solution was injected onto the split injection port (100:1 split ratio). Flow rates for He carrier gas was a 50 ml/min.

Lipid oxidation was determined by a

thiobarbituric acid assay using a modified method of Witte et al.[16]. Five g sample was added to 22.5ml 12% trichloroacetic acid, homogenized, and filtered (Whatman No. 1).

One ml of the resulting solution was added to 1ml of 20mM thiobarbituric acid and incubated at 25℃ for 24 hrs. The absorbance at 532nm was recorded and expressed as TBARS.

Statistical Analysis

Analyses of all samples were conducted in triplicate. The effects of supplemented CLA levels of diet and storage time on the content of CLA or fatty acid compositions and lipid oxidation of egg yolk were analyzed using ANOVA with SAS[17] at 5% level of significance.

Results and Discussion

Fig. 1 shows the CLA content in total fatty acids of egg yolk after feeding 6 weeks with the synthetic diets. The observed wide variations in CLA content within treatments was most likely due to different levels of CLA in diets. There was no significant ($p>0.05$) differences in CLA content between control and 5% SSO treatment. However, the egg yolks from all three CLA supplemented diets showed significantly ($p<0.05$) higher CLA content compared to control. It is possible that feed could influence CLA accumulation in egg yolk.

Chin et al.[7] reported that intestinal bacteria of rats had the ability to synthesize CLA from linoleic acid. The CLA level of turkey lipid was similar to ruminant animals[9]. Laying hens subjected to the control diet did not have the ability to accumulate CLA in egg yolk, and the hens subjected to CLA-supplemented diets increased effectively CLA content in egg yolk. These results imply that dietary CLA is the only source to elevete CLA in eggs.

According to several reports[7,9,10,18], the amount of $C_{18:2}$ fatty acids, as CLA precursors, decreased as CLA content increased. Lin et al.[18] showed that the oleic acid had a positive relationship with CLA content in fermented diary products. However, these findings are not confirmed in this

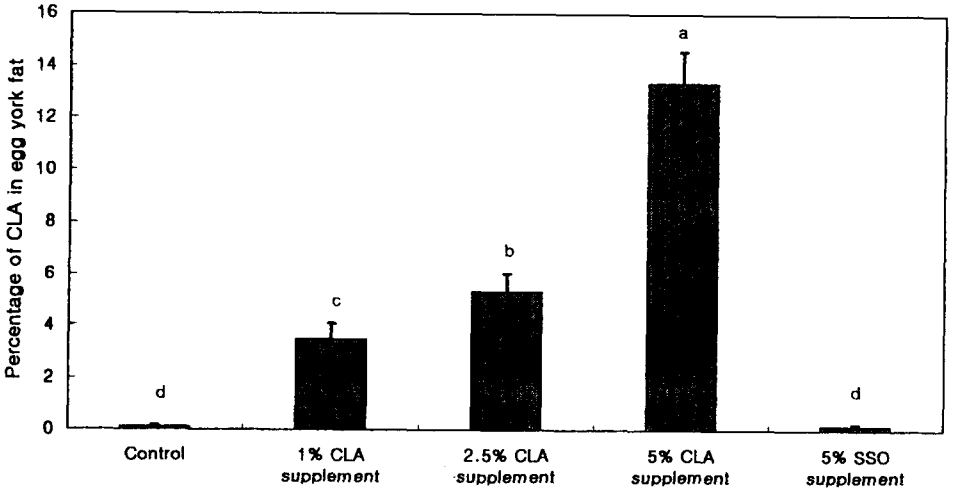

Fig. 1. Effect of dietary CLA supplementation for 6 weeks on CLA accumulation in egg yolk fat. Different letters denote a difference ($p<0.05$) in means within the diets.

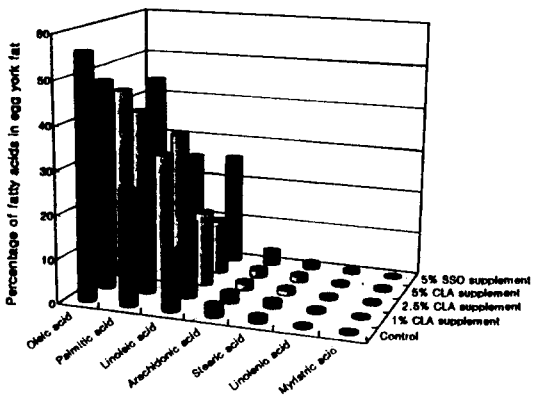

Fig. 2. Effect of dietary CLA supplementation for 6 weeks on the fatty acids composition in egg yolk fat.

study. Fig. 2 shows the fatty acids composition of egg yolk from different diets. The linoleic acid level of control was significantly ($p<0.05$) lower than that of the others (1% and 2.5% CLA or SSO-supplemented diets). Conversely, the content of oleic acid decreased as supplemented CLA content of diet increased. While the arachidonic acid level in egg yolk was decreased with increasing of CLA level of diets, the contents of myristic and palmitic acid were increased. These results suggested that the content of linoleic acid in egg yolk could be increased as well as the content of CLA if laying hens were fed a CLA-supplemented diet.

As the proposed CLA formation mechanism in ruminant animals or fermented dairy products, oleic acid isomers are common intermediates during microbial biohydrogenation, oxidation, or isomerization of linoleic acid[7,9,10,18]. It has been suggested that the microbial metabolic reactions could contribute to the positive relationship between CLA and oleic acid isomers contents[18]. This postulation may be possible in foods from ruminants but may be not possible in egg. It was showed that intestinal bacteria of laying hens did not have the ability to synthesize CLA from linoleic acid (Fig. 1). Therefore, dietary CLA supplementation may contribute to the positive relationship between linoleic acid and CLA content in egg yolk. Fatty acid composition in egg yolk affected by CLA was agreed with the results from Cook et al.[5] and Miller et al.[6] that CLA reduced tissue arachidonic acid levels.

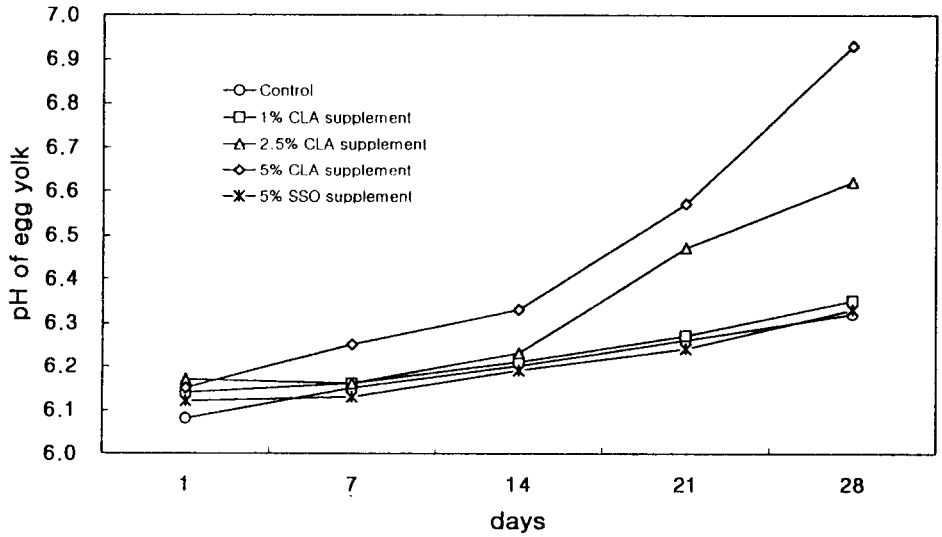

Fig. 3. Changes in pH of egg yolk from different diets during chilled(4℃) storage.

Fig. 3 shows the changes in pH of egg yolk during 28 days storage at 4℃. In general, the pH of foods is known to affect quality characteristics such as microbial growth, lipid oxidation or water-holding capacity. There were no significant ($p>0.05$) differences in pH between samples after storage of 7 days, whereas pH of eggs from 2.5% or 5% CLA-supplemented diets was significantly ($p<0.05$) higher than that of the others after storage of 28 days. When compared pH increasing patterns, the higher CLA content showed the faster increases in pH. It is possible that the content of CLA could influence pH of egg yolk during storage. More research is needed to determine if changes in pH during storage are due to the levels of CLA in egg yolk.

The content of CLA (Fig. 4) and oxidative deterioration (as measured by TBARS; Fig. 5) of the eggs was followed during chilled storage. In general, there were no significant ($p>0.05$) changes in CLA content during 28 days storage. No significant ($p>0.05$) increases in TBARS were observed over the first 7 days of storage, after which TBARS formation became more rapid with the exception of eggs from CLA-supplemented diets. According to Ha et al.[10] and Shantha et al.[14], oxidative reactions could influence CLA concentrations by either causing the formation of linoleic acid radicals, which in turn could be converted to CLA by hydrogen donors, or causing the oxidative destruction of the conjugated double-bond system of CLA. Shantha et al.[14] reported that the CLA concentrations in beef patties cooked by frying (60℃), baking (80℃) and broiling (60℃) showed significantly increased levels of CLA during refrigerated storage for 7 days. They also reported that oxidation did not affect CLA concentrations[12]. In this study, no changes in CLA contents with increasing TBARS during 28 days chilled storage were observed.

The lack of changes in CLA contents during storage could be due to the greater stability of CLA compared to polyunsaturated fatty acids[14]. On the other hands, it is also possible that CLA in tissue could inhibit lipid oxidation during storage. There

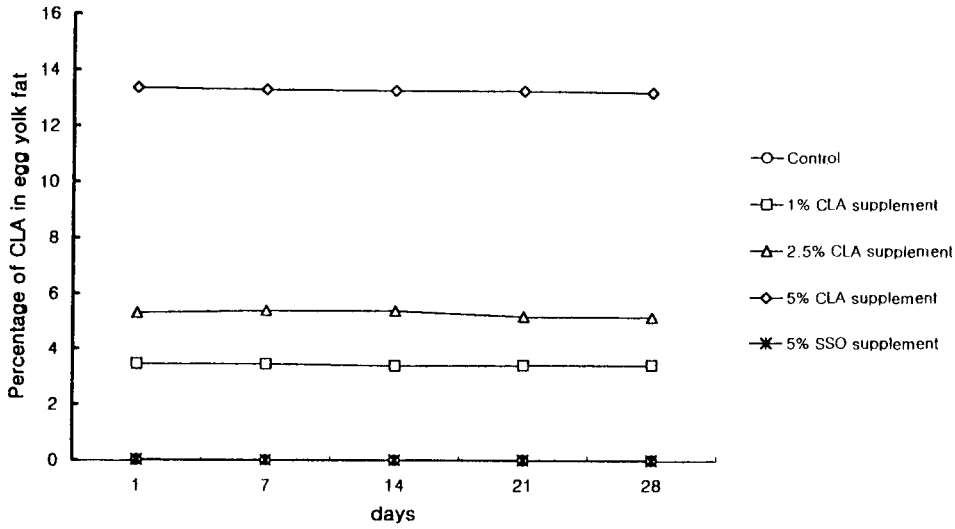

Fig. 4. Effect of storage on the content of conjugated linoleic acid(CLA) in egg yolk from different diets.

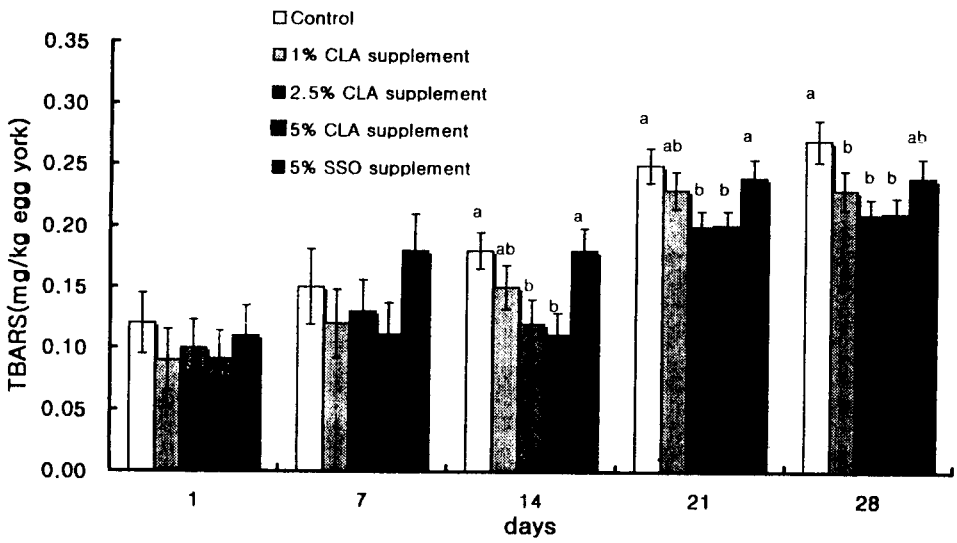

Fig. 5. Thiobarbituric acid reactive substances(TBARS) in stored(4℃) egg from different diets. Different letters denote a difference ($p<0.05$) in means within the diets.

were significant ($p<0.05$) differences in TBARS between CLA-supplemented groups (2.5% and 5% CLA supplemented diets) and control after storage 28 days. These results suggested that lipid oxidation of egg yolk could be affected by the levels of CLA. Because CLA is a stable component [12], it was postulated that high levels of CLA in fatty acid compositions could reduce the formation of fatty acids free radicals and a subsequent reduce oxidation reactions.

In conclusion, it was found that laying hens did not have the ability to accumulate CLA in egg yolk. Therefore, to produce eggs accumulated CLA, the dietary CLA supplementation was recommended. The contents of linoleic, palmitic, and myristic acid could be increased as well as CLA content in egg yolk if laying hens fed a CLA-supplemented diet.

However, the contents of oleic and arachidonic acid in egg yolk could be decreased by dietary CLA supplementation. It was also observed that pH of egg yolk sharply increased by the levels of CLA during storage at 4℃. The contents of CLA were not significantly changed by chilled storage for 28 days, whereas TBARS were significantly increased during storage. It was assumed that lipid oxidation of egg yolk might be affected by the levels of CLA in egg yolk due to changes in fatty acid compositions. Further research, under strictly controlled conditions, is necessary to explain the relationship between CLA and lipid oxidation in egg yolk.

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References

1. Ha, Y. L., Grimm, N. K. and Pariza, M. W. : Anticarcinigens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis*, **8**, 1881

(1987).
2. Ha., Y. L., Storkson, J. M. and Pariza, M. W. : Inhibitation of benzo(a)pyrene-induced mouse forestomach neoplasis by conjugated dienoic derivatives of linoleic acid. *Cancer Res.*, **50**, 1097 (1990).
3. Ip, C., Chin, S. F., Scimeca, J. A. and Pariza, M. W.: Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Res.*, **51**, 6118 (1991).
4. Ip, C., Singh, M., Thompson, H. J. and Scimeca, J. A.: Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.*, **54**, 1212 (1994).
5. Cook, M. E., Miller, C. C., Park, Y. and Pariza, M. W. 1993. Immune modulation by altered nutrient metabolism: nutritional control of immune induced growth depression. *Poult. Sci.*, **72**, 1301 (1993).
6. Miller, C. C., Park, Y., Pariza, M. W. and Cook, M. E. : Feeding conjugated linoleic acid to animals partially overcomes catabolic response due to endotoxin injection. *Biochem. Biophys. Res. Commun.*, **198**, 1107 (1994).
7. Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E. and Pariza, M. W. : Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J. Nutrition*, **124**, 2344 (1994).
8. Kepler, C. R., Hirons, K. P., McNeil, J. J. and Tove, S. B. : Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J. Biol. Chem.*, **241**, 1350 (1966).
9. Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. : Dietary Sources of Conjugated Dienoic Isomers of Linoleic Acid, a Newly Recognized Class of Anticarcinogens. *J. Food Composition and Analysis*, **5**, 185 (1992).
10. Ha, Y. L., Grimm, N. K. and Pariza. M. W. : Newly Recognized Anticarcinogenic Fatty acids : Identification and Quantification in Natural and Processed Cheeses. *J. Agric. Food Chem.*, **37**, 75 (1989).
11. Shantha, N. C., Decker, E. A. and Ustanol, Z. : Conjugated linoleic acid concentration in processed cheese. *J. Am. Oil Chem. Soc.*, **69**(5), 425 (1992).
12. Shantha, N. C., Ram, L. N., O'Leary, J., Hicks, C. L. and Decker, E. A. : Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. *J. Food Sci.*, **60** (4), 695 (1995).
13. Kamal, M., Youssef, E. and Rashwan, M. R. A. : Changes in lipid fractions, phospholipid fractions and fatty acid composition in Hubbard chicken tissue during boiling and frying. *Fleischwirtschaft*, **69**(3), 377 (1989).
14. Shantha, N. C., Crum, A. D. and Decker, E. A. : Evaluation of conjugated linoleic acid concentrations in cooked beef. *J. Agric. Food Chem.*, **42**, 1757 (1994).
15. Folch, J., Lees, M. and Sloane-Stanley, G. H. : A simple method for the isolation and purification of total lipid from animal tissues. *J. Biol. Chem.*, **226**, 497 (1957).
16. Witte, V. C., Krause, G. F. and Bailey, M. E. : A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.*, **35**, 582 (1970).
17. SAS : SAS/STAT Software for PC. Release 6. 11, SAS Institute, Cary, NC, USA (1996)
18. Lin, H., Boylston, T. D., Chang, M. J., Luedecke, L. O. and Shultz, T. D. : Survey of the conjugated linoleic acid

contents of dairy products. *J. Dairy Sci.*, **78**, 2358 (1995).

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