Conjugated Linoleic Acid Concentration in Processed Cheese

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The conjugated linoleic acid (CLA) concentration of a variety of processed cheese products ranged between 3.2 to 8.9 mg/g fat. Processing cheddar cheese at temperatures of 80°C and 90°C under atmospheric conditions increased ($p < 0.05$) CLA content, while processing under nitrogen (70°C, 85°C) had no effect. Increasing concentrations of whey protein concentrate (WPC) and its low molecular weight (LMW) fraction from 0 to 6% increased CLA formation. Six percent WPC and LMW fraction produced a 35% and 19% increase in CLA concentration, respectively, compared to processed cheese. The high molecular weight fraction of WPC did not increase CLA concentration. These results suggest that processing conditions and whey components play a role in CLA formation in processed cheese.

KEY WORDS: Anticarcinogen, conjugated linoleic acid, free radicals, lipid oxidation, processed cheese, whey.

Conjugated linoleic acid (CLA), an isomer of linoleic acid, is a minor constituent of a number of foodstuffs, including dairy products, meats (1–6) and certain vegetable oils (7,8). CLA has also been detected in human tissues, blood and body fluids (3,9). Conjugated polyenoic acids have gained considerable attention in the past decade as free-radical markers in studying lipid peroxidation in brain (10) and red blood cells (11). More recently, Ha and coworkers (4,5,12) have reported that CLA has anticarcinogenic properties. In a series of experiments they observed that synthetically prepared CLA was effective in partially inhibiting initiation of mouse skin carcinogenesis by 7,12-dimethylbenz(a)anthracene (4) and of mouse forestomach neoplasia induced by benzo(a)pyrene (12). The *cis*-9, *trans*-11 (*c*-9, *t*-11) CLA isomer was found to be biologically active when incorporated into mouse forestomach phospholipids (12). Their observations also indicate that CLA could serve as an effective antioxidant (12).

Ha and coworkers (5) have reported increased levels of conjugated linoleic acid in processed cheeses, such as Cheese Whiz, as compared to natural cheddar cheese. Although the increased CLA concentration in processed cheese has been attributed to heat treatment and/or the presence of food additives, such as whey protein concentrates (6), no study has yet been conducted to directly show that processing conditions and/or food additives can increase the levels of CLA in processed cheese.

The present study deals with the effects of processing conditions and the addition of whey protein concentrate on the formation of conjugated linoleic acid during the preparation of processed cheese. Cheddar cheese was processed at different temperatures, under atmospheric conditions and in the presence of nitrogen. The effect of different forms and concentrations of whey protein concentrate (WPC) on the formation of CLA was studied. The CLA concentration in a variety of commercially available processed cheeses and cheese foods was also determined.

MATERIALS

Cheddar cheese and the different varieties of processed cheeses were purchased from a local grocery. Whey protein concentrate (WPC) was donated by Calpro Ingredients (Corona, CA). SPECTRAPOR dialysis membrane tubing (molecular weight cut-off 6,000–8,000) was obtained from Baxter Scientific Products (McGaw Park, IL) and DIAFLO ultrafiltration membranes (5000 molecular weight cut-off) were purchased from Amicon (Danvers, MA). Phosphatidylcholine was purchased from Sigma Chemical Company (St. Louis, MO). All chemicals and solvents were reagent-grade.

METHODS

Fractionation of the whey protein concentrate. A high molecular weight fraction (HMWF) of solubilized WPC was obtained by dialysis (25 mL; 0.4 g WPC/mL water) against 2.5 liters of water at 4°C. Dialysis water was changed every 8 hr, a total of three times. A low molecular weight (LMW) fraction of WPC was obtained by ultrafiltration (0.25 g WPC/mL water) at 4°C, in an Amicon ultrafiltration cell equipped with a 5,000 molecular weight cut-off membrane under 55 psi of nitrogen.

Preparation of processed cheese. The formulation used for processed cheese included 58% cheddar cheese (34% fat), 1.25% sodium tripolyphosphate, 0.6% sodium chloride and 40.15% water, resulting in a final fat concentration of 20%. Cheese and additives were mixed in a glass beaker, and the product was stirred at the specified temperature for 10 min. Cheese was processed under normal atmospheric conditions (70°C, 80°C, 90°C) or under nitrogen (70°C and 85°C).

To study the effect of WPC on CLA formation, four different concentrations (1.5%, 3.0%, 4.5%, 6.0%) of total whey protein concentrate (WPC), its HMW or LMW fraction was used. The WPC components replaced equivalent portions of water. HMW fraction was added at a protein concentration equal to WPC, and LMW fraction was added at a volume equal to solubilized WPC (0.25 g WPC/mL water). The protein content of the whey and the HMW fraction were determined by the Kjeldahl method (13). Processing with WPC and its fractions was carried out at 80°C under normal atmospheric conditions for 10 min. All samples were prepared from the same lot of cheddar cheese.

Lipid analysis. Fat from cheese, processed cheese and WPC were extracted by acid digestion followed by solvent extraction as described by de Jong and Badings (14). Fat content was determined by the Babcock method (13). Peroxide values were determined on extracted fat by thiosulfate titration (13). Conjugated linoleic acid (CLA) for use as reference in gas chromatographic analysis was prepared from linoleic acid (Sigma Chemical Co.) by the

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TABLE 1

Fat and Conjugated Linoleic Acid (CLA) Content in Commercially Available Cheese Products

Processed cheese (with brand names)	Total fat (%)	CLA content[a] (mg/g fat)	*c*-9,*t*-11 isomer[a] (mg/g fat)
Kroger Squeeze Cheese	18.0	8.92 ± 0.31	6.06 ± 0.16
Kraft Cheese Whiz	19.5	6.39 ± 0.05	3.07 ± 0.06
Kroger Nice & Cheesy	20.0	6.00 ± 0.36	3.78 ± 0.51
Kraft Velveeta	22.0	5.17 ± 0.18	2.50 ± 0.11
Unprocessed cheddar cheese	34.0	3.99 ± 0.21	2.24 ± 0.46
Kraft American Singles	25.0	3.19 ± 0.11	1.85 ± 0.25

[a]Values represent mean ± SD (n = 6), triplicate samples each injected two times in the GLC.

6.6% KOH method (# 957.13) of the Association of Official Analytical Chemists (13). Fatty acid methyl esters were prepared using boron trifluoride in methanol (15).

Gas-liquid chromatography (GLC) analysis of fatty acid methyl esters was carried out on a Hewlett-Packard (Palo Alto, CA) 5880A gas chromatograph, equipped with a flame-ionization detector (FID), a digital integrator and a Supelcowax-10 megabore column (60 m × 0.75 mm i.d., phase thickness 0.25 μm (Supelco Inc., Bellefonte, PA). GLC analysis was temperature-programmed from 75°C to 220°C at 20°C/min and held at 220°C for 40 min. Other parameters were: on-column injection; carrier gas (helium) flow rate, 2.5 mL/min; injection port temperature 250°C; detector temperature 250°C. CLA peaks were identified by comparison with retention times of the CLA reference standard and were further confirmed by mass spectral analysis on a gas chromatography/mass spectrometry (GC/MS) ion trap detector (ITD) system (16). The areas of the CLA peaks were calculated as mg/g of fat by using methyl docosanoate as an internal standard (17).

Statistical analysis. Processed cheese samples were prepared in triplicate and the fatty acid methyl esters of each sample were analyzed 2–3 times by GLC. Statistical calculations were conducted with MINITAB (State College, PA). The Student's t-test and one-way analysis of variance was done for a 5% level of significance.

RESULTS

Table 1 gives the fat content and the amount of CLA in commercially available cheddar and cheddar-based processed cheeses. CLA content ranged from 3.2 mg/g fat in processed cheese (Kraft American Singles) to 8.9 mg/g fat in cheese spread (Kroger Squeeze Cheese). Unprocessed cheddar cheese (CLA content 4.0 mg/g fat) had a value higher than that of the American Singles, but it was however lower than that of the other processed cheeses. The *c*-9,*t*-11 isomer was 56% of the total conjugated linoleic acid in unprocessed Cheddar cheese, and ranged between 48–68% of total CLA in other processed cheeses.

Table 2 shows the effect of the processing temperature on the CLA content of processed cheese. While processing cheese under atmospheric conditions at 70°C had little effect on CLA formation, increasing processing temperature to 80°C or 90°C produced a significant ($p < 0.05$) increase (4.99 ± 0.21 and 5.18 ± 0.21 mg CLA/g fat, respectively) as compared to unprocessed cheese (3.99 ± 0.20 mg/g fat). The concentration of the *c*-9,*t*-11 isomer rose from 2.24 ± 0.47 mg/g fat in plain cheese to 2.80 ± 0.18 in the cheese processed at 80°C under atmospheric conditions. However, the values were not significantly different ($p > 0.05$). Processing under nitrogen (70° or 85°C) did not increase CLA concentration. Lipid peroxides were not detected in any of the processed cheeses.

TABLE 2

Effect of Processing Temperature on the Conjugated Linoleic Acid (CLA) Content of Processed Cheese (CLA content of unprocessed cheese was 3.99[a] ± 0.20 mg/g fat.)[a]

Processing temperature (°C)	CLA content (mg/g fat)[b]		Peroxide value[c]
	Atmospheric conditions	Under nitrogen	
70	4.75[a] ± 0.21	4.53[a] ± 0.17	nd[d]
80	4.99[b] ± 0.20	—	nd
85	—	4.54[a] ± 0.25	nd
90	5.18[b] ± 0.21	—	nd

[a]Means different letters as superscripts (a,b) are significantly different ($p < 0.05$).

[b]Values represent mean ± SD (n = 6), triplicate samples each injected two times in the GLC.

[c]Refers to cheese processed under both nitrogen and atmospheric conditions.

[d]Not detected.

Table 3 shows the effect of varying concentrations of WPC and its HMW and LMW fractions on the CLA content of cheese processed at 80°C. CLA content increased with increasing concentrations of total whey protein concentrate (WPC) and its LMW fraction from 0 to 6%, although significant increases ($p < 0.05$) compared to processed cheese with no additives were only observed at concentrations >4.5%. Six percent WPC or its LMW fraction produced a 35% and 19% increase in CLA concentration, respectively, compared to processed cheese with no additives, and a 68% and 48% increase in CLA concentration, respectively, compared to unprocessed cheese. The HMW fraction did not increase CLA concentration compared to processed cheese without additives. Although there was an increase in the total CLA in processed cheese, when WPC or its LMW fraction was used as

TABLE 3

Effect of Different Concentrations of the Total Whey Protein Concentrate (WPC), Its High Molecular Weight Fraction (HMWF) or Its Low Molecular Weight Fraction (LMWF) on the Conjugated Linoleic Acid (CLA) Content of Processed Cheese (Total CLA content in processed cheese without additives was 4.99 ± 0.21,[a] while the *c*-9,*t*-11 isomer content was 2.24 ± 0.46[a] mg/g fat.)

Whey component	Percentage of whey component added			
	6%	4.5%	3.0%	1.5%
	Total CLA content (mg/g fat)[a]			
WPC	6.72 ± 0.25	6.51 ± 0.45	5.38 ± 0.83	5.19 ± 0.65
HMWF	5.02 ± 0.39	4.83 ± 0.27	4.74 ± 0.44	4.63 ± 0.29
LMWF	5.92 ± 0.40	5.31 ± 0.16	5.11 ± 0.34	4.84 ± 0.24
	c-9,*t*-11 isomer content (mg/g fat)[a]			
WPC	2.89 ± 0.49	2.84 ± 0.26	2.78 ± 0.36	2.63 ± 0.20
HMWF	2.51 ± 0.15	2.57 ± .08	2.42 ± 0.19	2.54 ± 0.35
LMWF	2.86 ± 0.14	2.75 ± 0.22	2.71 ± 0.29	2.40 ± 0.09

[a]Values represent mean ± SD (n = 6), triplicate samples each injected two times in the GLC.

additive, the concentration of the *c*-9, *t*-11 isomer alone showed no significant increase.

DISCUSSION

Conjugated linoleic acid was found at higher concentrations in all cheddar-based processed cheeses than in unprocessed cheddar cheese, except for the American cheese (Table 1). It is not known whether differences in CLA concentrations observed between the processed and unprocessed cheeses were due to processing conditions, additives or storage. In addition, the CLA content of milk fat undergoes seasonal variations (1,2). Riel (2) observed that conjugated dienoic content of milkfat was twice as high in summer (1.46%) as in winter A(0.78%). Thus, the variation in CLA concentrations in processed cheese observed in this study could be due to high CLA concentrations in the original milk and thus in the cheese used in the product.

Ha and coworkers (5) postulated a mechanism for the formation of CLA, in which linoleic acid is first converted to a linoleic acid radical followed by addition of hydrogen to form a conjugated double bond system. Several factors in cheese processing could influence the formation of CLA by this mechanism. These include temperature, which could increase the formation of linoleic acid radicals, atmospheric conditions, which could influence the conversion of the linoleic acid radical to either conjugated isomers or lipid peroxides, and hydrogen donors, which could aid in the formation of conjugated double bonds.

High processing temperature (≥80°C) increased the formation of CLA in processed cheese (Table 2). However, no increase in CLA formation was observed with increasing temperature when the cheese was processed under nitrogen instead of atmospheric conditions. These data indicate that both processing temperature and the presence of air play a role in the formation of CLA. Oxygen radicals, such as the hydroxyl radical, are known to initiate lipid oxidation by causing the formation of lipid free radicals (18). The presence of air during processing could result in the formation of oxygen radicals, which could increase the formation of the linoleic acid radical thereby increasing the concentration of CLA. The ability of increased temperature to increase CLA formation in the presence of air could be explained by the increased formation of linoleic and/or oxygen radicals at higher temperatures. Lipid peroxides were not detected when the cheese was processed under either atmospheric conditions or nitrogen. This indicates that any linoleic acid radicals formed during processing were primarily being converted to CLA instead of lipid peroxides.

Addition of whey protein concentrate (WPC) at concentrations normally found in processed cheeses (19) resulted in an increase in CLA concentration (Table 3). The CLA content of the WPC (7% fat) was less than 0.075% of the total fatty acid methyl esters (data not shown). Therefore, the maximum amount of CLA contributed by the WPC (6%) was 0.016 mg/g fat, whereas the total increase in the CLA content of processed cheese containing 6% WPC was about 1.73 mg/g fat. This indicates that the observed increase in CLA was not solely due to fat contributed by WPC.

Addition of the HMW or LMW fractions of WPC to the processed cheese indicated that only the LMW fraction increased the concentration of CLA. Components in WPC and the LMW fraction that could increase the formation of CLA include lipid oxidation catalysts (which could increase the formation of the linoleic acid radical) and hydrogen donors. Previous studies in our laboratory have shown that a LMW fraction of whey is not capable of catalyzing the oxidation of phosphatidylcholine liposomes (20). In fact, the LMW fraction was found to inhibit lipid oxidation catalysts, such as iron, lipoxidase, hemoglobin and photoactivated riboflavin. The ability of the LMW fraction to inhibit nonmetal lipid oxidation catalysts suggests that the antioxidant activity is not due to chelation but instead to inactivation of free radicals by acting as a hydrogen donor. These data suggest that the ability of the whey LMW fraction to increase the formation of CLA in processed cheese could be due to interactions between

the LMW components and the linoleic acid radicals resulting in the formation of CLA. Studies are currently underway to identify the LMW components responsible for the observed increase in CLA formation.

The increased level of CLA in processed cheese is caused by a combination of both processing temperature and the presence of whey protein concentrate. The components in whey protein concentrate that increased CLA formation were primarily low molecular weight compounds, indicating that whey ultrafiltration permeate could be used to increase the concentration of CLA in processed cheese. While processing conditions and additives were capable of increasing total CLA concentrations, they did not significantly increase the concentration of the *c*-9,*t*-11 isomer, which is thought to be the biologically active isomer (12). Additional research is underway to determine the ability of other food additives to increase the concentration of CLA in processed cheese.

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REFERENCES

1. Parodi, P.W., *J. Dairy Sci. 60*:1550 (1977).
2. Riel, R.R., *Ibid. 46*:102 (1963).
3. Fogerty, A.C., G.L. Ford and D. Svoronos, *Nutrition Reports International 38(5)*:937 (1988).
4. Ha, Y.L., N.K. Grimm and M.W. Pariza, *Carcinogenesis 8(12)*: 1881 (1987).
5. Ha, Y.L., N.K. Grimm and M.W. Pariza, *J. Agric. Food Chem. 37(1)*:75 (1989).
6. Aneja, R.P., and T.N. Murthi, *Nature 350*:280 (1991).
7. Ackman, R.G., C.A. Eaton, J.C. Sipos and N.F. Crewe, *Can. Inst. Food Sci. Technol. J. 14(2)*:103 (1981).
8. Brown, H.G., and H.E. Snyder, *J. Am. Oil Chem. Soc. 59*:280 (1982).
9. Dormandy, T.L., and D.G. Wickens, *Lipids 45*:353 (1987).
10. Rehncrona, S., D.S. Smith, B. Akesson, E. Westerberg and B.K. Siesjo, *J. Neurochem. 34*:1630 (1980).
11. Dodge, J.T., and G.B. Phillipis, *J. Lipid Res. 7*:387 (1966).
12. Ha, Y.L., J. Storkson and M.W. Pariza, *Cancer Res. 50*:1097 (1990).
13. *AOAC, Official Methods of Analysis,* 15th edn., Association of Official Analytical Chemists, Washington, DC, 1990.
14. de Jong, C., and H.T. Badings, *J. High Resolution Chromatogr. 13*:94 (1990).
15. Ackman, R.G., W.M.N. Ratnayake and E.J. Macpherson, *J. Am. Oil Chem. Soc. 66*:1162 (1989).
16. Ratnayake, W.M.N., A. Timmins, T. Ohshima and R.G. Ackman, *Lipids 21*:518 (1986).
17. Shantha, N.C., and R.G. Ackman, *J. Chromatogr. 533*:1 (1990).
18. Kanner, J., J.B. German and J.E. Kinsella, *CRC Crit. Rev. Food Sci. and Nutr. 25*:317 (1987).
19. Caric, M., and M. Kalab, in *Cheese: Chemistry, Physics, and Microbiology,* Vol. 2, edited by P.F. Fox, Elsevier Applied Science, London, England, 1987, pp. 339-383.
20. Colbert, L.B., and E.A. Decker, *J. Food Sci. 56*:1248.

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