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EFFECT OF CONJUGATED LINOLEIC SUPPLEMENTATION ON FATTY ACID PROFILE, LIPID OXIDATION, AND SENSORY PROPERTIES OF PASTEURIZED MILK

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ABSTRACT
Analytical characterization of pasteurized milk enriched with 0%, 1%, 2%, 3% and 4% conjugated linoleic acid (CLA), control, T_1, T_2, T_3 and T_4 was performed. Experimental samples and control were filled in PET bottles, pasteurized at 65°C/30 min and stored at 2-3°C for 6 days. Supplementation of pasteurized milk with CLA supplement at all concentrations did not have any effect on milk composition. Total CLA content of control, T_1, T_2, T_3 and T_4 were 0.29, 0.56, 2.09, 2.91 and 4.15%. Free fatty acids, peroxide value and sensory characteristics of CLA supplemented milk samples and control were not different from each other. CLA supplement prepared from safflower oil may be used for increasing CLA in pasteurized milk without affecting the sensory properties.

Keywords: Conjugated linoleic acid; fatty acid profile; lipid oxidation; sensory properties

INTRODUCTION
The fatty acids in which two double bonds in carbon-hydrogen chain are separated from each other by a methyl group are referred as conjugated linoleic acid (CLA). Several isomeric forms of CLA have been discovered and these are present in *trans* and *cis* forms [1]. More than 400 fatty acids are present in milk, concentrations of saturated, monounsaturated and polyunsaturated fatty acids are about 66%, 30% and 4%, respectively [2]. Milk lipids are rich dietary sources of fat-soluble vitamins, β-carotene and essential fatty acids. In recent studies, therapeutic prospects of conjugated linoleic acid in prevention of some diseases is reported. Due to higher fat content, it is highly suitable for the production of butter and other fatty rich dairy products. Regular intake of CLA has been connected with prevention of several metabolic ailments such as diabetes, CVDs, cancers, obesity and inflammation etc. Unsaturated fatty acids are found in several vegetable oils and can be easily accessed and consumed however, beef and dairy products are the only sources of CLA [3]. To safeguard the body from metabolic diseases, it is recommended to consume 2g CLA/ day. Dairy products and beef contain approximately 30-50 mg/100 CLA, to fulfill the quota of 2g/day, huge intake of beef and dairy products is needed on regular basis. Consumption of beef and dairy products in excessive amounts may be harmful to the body therefore, transportation of CLA to human body via this method does not seem to be healthy and sustainable. Further, low fat versions of dairy products and lean beef may contain ever lower amount of CLA. It is worth mentioning that CLA is only present in the meat

and milk of grazing animals or lush green fodder is regularly included in the diet of animals [4]. Several under developed countries are facing acute shortage of good quality roughages for the feeding of livestock and cows are fed on poor quality feeds. Pakistan also faces acute shortage of fodder in summer and cows are fed on poor quality feed.

The presence of CLA in beef and milk of cows receiving poor feeds is highly questionable further, metabolic diseases are also highly prevalent in Pakistan and other under developed countries. In our previous investigations, CLA supplement has been produced from safflower oil. All over the world, pasteurized milk is one of the most commonly consumed dairy products, to develop sustainable strategies for the production of CLA enriched pasteurized milk, fatty acid composition, oxidative stability and sensory characteristics should be studied in detail. This investigation aimed to determine the impact of synthetic CLA supplementation on CLA isomers, fatty acid profile, oxidative stability and sensory characteristics of pasteurized milk.

MATERIALS AND METHODS

Raw Materials

Cow milk was used for the production of CLA enriched pasteurized milk and CLA was produced from safflower oil.

CLA Supplement

Linoleic acid of safflower oil were transformed to CLA by the method described by [5].

Experimental Plan

CLA was mixed to the unstandardized cow milk before pasteurization (in CRD) at 1%, 2%, 3% and 4% concentrations using distilled mono and diglycerides as emulsifier at 0.2% level, milk samples without CLA were kept as control, every treatment was run in triplicate. Experimental samples and control were filled in PET bottles (250ml capacity) and pasteurized at 65^{o}C/30 min and stored at 2-3^{o}C for 6 days.

Milk Composition

Fat, protein, lactose, ash contents and pH were measured at 0, 3 and 6 days of storage phase [6].

Fatty Acid Profile of CLA Supplement and Milk

Esters were prepared by reacting 50 mg fat 2ml methanolic sodium methoxide (0.5M) in a test tube then 2ml n-hexane was added and vortex at 1500 rpm for 2min followed by 15min rest and upper layer was used for injection in GC-MS (7890-B, Agilent Technologies) keeping temperatures of inlet and FID at 250 and 300oC with a split ratio of 1:40 using SP-2560 fused silica capillary column (100 m x 0.2mm) and FAME 37 standards [7].

Lipid Oxidation

PV and FFA were measured by following the standard methods of AOCS [6].

Sensory Evaluation

Color, flavor and appearance of CLA enriched pasteurized milk samples was performed by 10 semi-trained judges using 9-point scale in a specialized sensory evaluation lab in separate booths having facility of data recording and palate rinsing [8].

Statistical Analysis

Anova technique was used for the analysis of data collected in CRD and analyzed by SAS 9.4.

RESULTS AND DISCUSSION

Milk Composition

Impact of CLA supplementation (1% to 4%) on fat, protein, lactose, ash contents and pH are revealed in Table 1. Supplementation of pasteurized milk with CLA at all concentrations did not modify the fat, protein, lactose, ash (p>0.05). From the regulatory and consumers perspectives, it is very important to follow the standards of identity and to maintain the milk composition. From industrial perspectives, percentage of protein in milk is very important for the nutritional and cheese making features as the yield of cheese is dependent on the protein content of milk [9]. From the commercial perspectives, lactose content of the milk has no commercial significance, however, determination of lactose in milk is used as important criterion for the estimation of milk quality for example, lactose should constitute minimum 46% solids-not-fat content [10]. CLA fortified milk may be easily adopted in the dairy industries as no specialized equipment or legislations are required for the manufacturing and marketing. Compositional attributes of CLA supplemented milk were not different from control milk except fat content [11]. In current investigation, effect of CLA supplementation on compositional attributes of milk was monitored for 6 days. Storage duration of 6 days did not reveal any significant effect on compositional attributes of milk. Chemical composition of pasteurized milk recorded in this investigation was not different from the literature [12]. Chemical composition of milk with elevated level of CLA is reported to have lower fat content with no difference in other major constituents [13,14].

Fatty Acids Composition of Pasteurized Milk

According to the recommendation of FDA, 3g CLA should be taken to prevent the above-mentioned metabolic disorders Shanta et al. 1994). To intake 3g CLA on daily basis, one must consume larger volumes of milk and meat that may lead to other medical problems. In the present investigation, CLA supplement was produced from safflower oil and it was used for the fortification of pasteurized milk. In CLA supplement, concentrations of two major isomers were as $\Delta 9c,11t$-18:2 and $\Delta 10t,12c$-18:2 was 52.41% and 44.55%. According to an early estimate extrapolated from animal trials, intake of 3g CLA/day is recommended [15]. Based on human metabolic rate, more recent guidelines suggest to intake 0.7 to 0.8g CLA/ day. Intake of 100ml CLA supplemented milk can fulfill the complete dietary requirements of CLA. In this investigation, pasteurized milk was fortified with 1% to 4% concentrations of CLA supplement and it was observed that concentrations of SCFA in control and all the treatments was at par with each other (p>0.05). Use of vegetable oils and their derived products can increase the unsaturated fatty acids (UFA) and functional value of dairy products, but for the researchers, it is technically challenging to deal with SCFA and lipid oxidation phenomenon as these products normally have lower concentrations of SCFA and oxidative stability than the normal dairy products. SCFA play a pivotal role in the production of typical dairy aroma of milk and dairy products, milk fat blended with vegetable oils had lower concentrations of SCFA. Concentration of CLA in milk can also be enhanced by manipulating the rations of cows, but it is difficult for the industries to collect, store, process this type of milk. Feed of cows was supplemented with 2% soybean oil and 1% fish oil; control group did not receive the supplementation of soybean oil and fish oil. Concentration of *cis*-9, *trans*-11 was 4.74g/100g fatty acids as compared to 0.52g/100g fatty acids in control [16]. Concentration of CLA in milk increased when cows were fed on calcium salts of unsaturated fatty acids [17]. Fish meal was included in the diet of cows to produce high CLA milk, resultant milk had higher concentration of CLA than control milk [18]. Supplementing the milk with synthetic CLA is convenient for the milk processing industries from processing and marketing viewpoints as no separate collection and storage facilities are required keeping the sources of variation minimum, it is possible to better control the quality of such products. Effect of time temperature combinations of pasteurization such as 85C/16 sec, 95C/5min, 63C/30min and 70-90C/5min is reported to have no effect on concentration of CLA [19]. Effect of storage interval of 6 days on CLA was evaluated. Concentrations of isomers of CLA in 6 days old pasteurized milk was not different from the initial values recorded at 0 day (p>0.05). Concentration of CLA gradually decreased during the storage phase of heat-treated milk [20]. In another investigation, it was reported that different processing methods altered the concentration of CLA isomers [21]. Effect of storage period of 6 days on CLAs of pasteurized milk was studied, after 60 days of storage at 25ºC, 36% CLA was lost.

Lipid Oxidation

Lipid oxidation is one of the major reasons of quality degradation, and it is more thought-provoking in functional dairy products having a greater number of UFA. In this study, the concern of lipid oxidation was overcome using lower temperature storage i.e. 2-3ºC. Effect of storage temperature on chemical reactions taking place is well documented in literature [2]. As compared to other solid fats, milk fat is not resilient to auto-oxidation, objectionable flavors are quickly developed [22]. Development of oxidative rancidity deteriorates the delicate flavor of milk and dairy products, milk with flavor defects had lower consumer acceptability. For the measurement of oxidative deterioration in CLA supplemented pasteurized milk, free fatty acids (FFA) and peroxide value (PV) were used. In milk, FFA are generated due to the lipolytic activities of indigenous and exogenous lipases. In free form, fatty acids can induce bad/undesirable flavor to pasteurized milk [23].

Among the dairy products, pasteurized milk is the most used product which is used all over the world. Therefore, it should have a typical dairy flavor without any taste/ flavor defects [24]. Free fatty acids are not only implicated in the generation of objection flavor in pasteurized, their role as a catalyst of lipid oxidation is also recently discovered [25]. Supplementation of pasteurized milk with CLA supplement at the four concentrations had no effect ($p>0.05$) on free fatty acids (Table 4). FFA content of CLA supplement was 0.08%. Storage phase of 6 days did not show any significant impact on the production of FFA in CLA supplemented milk samples and control.

For the estimation of oxidation status and expected shelf life of foods, peroxide value is regarded as empirical method. For the estimation of oxidative stability of milk fat, peroxide value was used an indicator of lipid oxidation [5]. Analysis of CLA supplemented milk samples and control after 3 days of storage revealed a non-significant impact on peroxide value. In the storage phase of 6 days, peroxide value steadily increased however, PV of all experimental sample and control was not different from each other. After 6 days of storage phase, CLA supplemented breast meat samples had lower oxidative stability than non-supplemented samples [22, 26].

Sensory Evaluation

A panel of ten trained judges did not report any significant variation in color, flavor and appearance scores of pasteurized milk. Determination frequencies of 3 and 6 days indicated a non-significant impact of storage on color, flavor and appearance of CLA supplemented milk samples. Milk with elevated level of CLA was subjected to sensory evaluation, trained judges were unable to find difference in flavor of normal and high CLA milk [27].

Conclusion

Gas chromatographic analysis of CLA supplement revealed the presence of seven isomers with 22.58% conversion rate from linoleic acid to CLA. Addition of CLA supplement from 1 to 4% had a non-significant effect on milk composition. Total CLA content of T_3 and T_4 was 2.91% and 4.15% as compared to 0.29% in control. FFA, PV and sensory properties were not affected by the addition of CLA supplement up to 4% till 6 days of storage. Concentration of CLA may be increased in milk by supplementing safflower oil based CLA supplement.

Table 1 Milk Composition

Treatments	Days	Fat%	Protein%	Lactose%	Ash%	pH
Control Without CLA Supplement	0 3 6	4.18±0.04 4.16±0.05 4.15±0.02	3.22±0.2 3.21±0.01 3.17±0.03	4.68±0.06 4.66±0.07 4.62±0.09	0.74±0.01 0.73±0.01 0.72±0.02	6.67±0.05 6.65±0.08 6.61±0.06
T_1 1% CLA Supplement	0 3 6	4.13±0.07 4.19±0.04 4.15±0.09	3.21±0.01 3.19±0.04 3.16±0.02	4.65±0.02 4.63±0.09 4.59±0.10	0.75±0.01 0.75±0.03 0.71±0.05	6.65±0.10 6.64±0.05 6.59±0.01
T_2 2% CLA Supplement	0 3 6	4.20±0.06 4.17±0.02 4.12±0.04	3.18±0.16 3.17±0.08 3.15±0.03	4.64±0.05 4.62±0.08 4.60±0.12	0.72±0.01 0.72±0.03 0.69±0.01	6.63±0.02 6.62±0.01 6.58±0.04
T_3 3% CLA Supplement	0 3 6	4.10±0.15 4.14±0.03 4.16±0.08	3.17±0.02 3.20±0.07 3.13±0.06	4.63±0.15 4.60±0.14 4.58±0.03	0.74±0.06 0.72±0.02 0.71±0.01	6.75±0.05 6.71±0.09 6.67±0.03
T_4 4% CLA Supplement	0 3 6	4.15±0.09 4.18±0.01 4.16±0.12	3.16±0.09 3.12±0.08 3.18±0.03	4.66±0.04 4.63±0.10 4.60±0.16	0.73±0.07 0.74±0.04 0.72±0.01	6.72±0.17 6.68±0.02 6.62±0.01

Means shown in an individual column are not statistically affected by the addition of CLA ($p>0.05$)

Table 2 Fatty Acid Profile of CLA Supplemented Pasteurized Milk

Fatty Acid/ CLA Isomer	Control	T_1	T_2	T_3	T_4
C4:0	1.90±0.01a	1.90±0.01a	1.85±0.02a	1.83±0.01a	1.82±0.08a
C6:0	2.21±0.02a	2.21±0.04a	2.17±0.01a	2.15±0.04a	2.14±0.06a
C8:0	2.41±0.05a	2.38±0.07a	2.37±0.09a	2.33±0.09a	2.31±0.04a
C10:0	2.69±0.02a	2.66±0.10a	2.63±0.06a	2.60±0.04a	2.55±0.03b
C12:0	2.95±0.01a	2.93±0.08a	2.90±0.03a	2.87±0.02a	2.77±0.15b
C14:0	12.29±0.41a	12.16±0.14a	12.10±0.27a	11.95±0.32b	11.55±0.65b
C16:0	27.19±0.35a	27.05±0.29a	26.43±0.55b	26.29±0.25b	25.71±0.74c
C18:0	8.11±0.09a	8.04±0.22a	7.93±0.41a	7.81±0.18a	7.19±0.04b
C18:1	24.51±0.15a	24.32±0.36a	24.13±0.76a	23.51±0.64b	23.17±0.18b
C18:2	1.95±0.01g	2.65±0.06f	3.62±0.02e	4.15±0.16d	5.61±0.06b
C18:3	0.49±0.06a	0.40±0.02b	0.41±0.01b	0.39±0.04a	0.37±0.02b
Δ9*c*,11*t*-18:2	0.23±0.03e	0.25±0.01e	0.76±0.03d	1.03±0.06c	1.42±0.04a
Δ10*t*,12*c*-18:2	0.06±0.01d	0.08±0.01d	0.71±0.05c	0.91±0.12b	1.24±0.07ba
Δ9*c*,11*c*-18:2	----	0.03±0.01d	0.09±0.01c	0.14±0.02b	0.23±0.01a
Δ9t, 11c-18:2	----	0.06±0.01d	0.13±0.02c	0.22±0.02b	0.35±0.02a
Δ10c,12*t*-18:2	----	0.04±0.01d	0.11±0.03c	0.13±0.02c	0.25±0.03a
Δ8,9,11,10,12c-c18:2	----	0.03±0.01c	0.09±0.01b	0.10±0.01b	0.17±0.01a
Δ8,9,11,10,12t-t18:2	----	0.07±0.02d	0.20±0.02c	0.38±0.03b	0.49±0.02a

Means shown in an individual is statistically affected by the addition of CLA, if it contains a different ($p<0.05$)

Table 3 Oxidative Stability of CLA Supplemented Pasteurized Milk

Treatments	Days	Free Fatty Acids%	Peroxide Value (MeqO_2/Kg)
Control	0 3 6	0.08±0.01 0.08±0.02 0.09±0.01	0.22±0.04b 0.29±0.02b 0.51±0.07a
T_1	0 3 6	0.08±0.01 0.09±0.01 0.12±0.02	0.22±0.04b 0.31±0.02b 0.44±0.04a
T_2	0 3 6	0.08±0.01 0.07±0.01 0.08±0.02	0.22±0.04b 0.32±0.05b 0.49±0.01a
T_3	0 3 6	0.08±0.01 0.08±0.03 0.09±0.02	0.22±0.04b 0.35±0.04b 0.49±0.06a
T_4	0 3 6	0.08±0.01 0.08±0.02 0.09±0.01	0.22±0.04b 0.36±0.01b 0.55±0.06a

Means shown in an individual is statistically affected by the addition of CLA, if it contains a different ($p<0.05$)

Table 4 Sensory Properties of CLA Supplemented Pasteurized Milk

Treatments	Days	Color	Flavor	Appearance
Control	0	8.32±0.05	8.21±0.06	8.24±0.03
	3	8.27±0.03	8.17±0.09	8.22±0.04
	6	8.29±0.03	8.14±0.05	8.13±0.04
T_1	0	8.30±0.09	8.16±0.04	8.20±0.09
	3	8.24±0.12	8.12±0.01	8.17±0.06
	6	8.22±0.05	8.16±0.05	8.20±0.09
T_2	0	8.25±0.10	8.15±0.08	8.14±0.08
	3	8.22±0.04	8.12±0.06	8.13±0.10
	6	8.12±0.10	8.08±0.08	8.09±0.02
T_3	0	8.17±0.02	8.16±0.09	8.18±0.02
	3	8.14±0.16	8.13±0.12	8.13±0.01
	6	8.13±0.10	8.18±0.16	8.17±0.15
T_4	0	8.23±0.05	8.11±0.05	8.19±0.13
	3	8.21±0.12	8.08±0.01	8.16±0.15
	6	8.11±0.14	8.05±0.04	8.15±0.05

Means shown in an individual is statistically affected by the addition of CLA, if it contains a different ($p<0.05$)

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