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To cite this article: Awais Khan, Muhammad Nadeem, Muhammad Imran, Nabila Gulzar, Muhammad Haseeb Ahmad, Muhammad Tayyab, Muhammad Abdul Rahim & Chinaza Godswill Awuchi (2022) Impact of safflower oil derived conjugated linoleic acid supplementation on fatty acids profile, lipolysis and sensory properties of cheddar cheese, *International Journal of Food Properties*, 25:1, 2223-2236, DOI: [10.1080/10942912.2022.2130356](https://doi.org/10.1080/10942912.2022.2130356)

To link to this article: <https://doi.org/10.1080/10942912.2022.2130356>



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Published online: 03 Oct 2022.



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# Impact of safflower oil derived conjugated linoleic acid supplementation on fatty acids profile, lipolysis and sensory properties of cheddar cheese

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## ABSTRACT

Nutritionists recommend to consume 3 g Conjugated linoleic acid (CLA) to prevent cancers, cardiovascular diseases, diabetes, obesity and other life-style-related disorders. To fulfill the daily requirements of CLA, large proportions of dairy and meat products have to be included in the dietary plan that may have negative impacts such as excessive intake of fat, cholesterol, and lactose. Current investigation was aimed to determine the effect of safflower oil derived CLA on fatty acids composition, lipolysis and sensory properties of cheddar cheese. Safflower oil-derived conjugated linoleic acid (CLA) was added to cheddar cheese at four levels i.e. 1%, 2%, 3% and 4% concentrations (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> & T<sub>4</sub>), cheese without CLA supplement was used as control, vacuum packaged samples were ripened at 6–8°C for 90 days. Analysis of CLA supplement revealed that concentrations of  $\Delta 9c,11\ t-18:2$ ,  $\Delta 10\ t,12c-18:2$ ,  $\Delta 9\ t, 11c-18:2$  were 52.16, 44.37, 0.27 and 0.55 mg/100 g. In control, concentrations of  $\Delta 9c,11\ t-18:2$  and  $\Delta 10\ t,12c-18:2$  was 0.24% and 0.07% with a total CLA content of 0.31%. In T<sub>3</sub>, concentrations of  $\Delta 9c,11\ t-18:2$ ,  $\Delta 10\ t,12c-18:2$ ,  $\Delta 9c,11c-18:2$  and  $\Delta 9\ t, 11c-18:2$  were 1.12, 3.82, 1.63, 2.77 (total CLA 8.34mg/100g). In T<sub>4</sub>, concentrations of  $\Delta 9c,11\ t-18:2$ ,  $\Delta 10\ t,12c-18:2$ ,  $\Delta 9c,11c-18:2$  and  $\Delta 9\ t, 11c-18:2$ , were 1.47, 3.50, 3.25 and 3.44% (total CLA 11.67 mg/100 g). Supplementation of cheddar cheese at all four levels significantly increased the concentrations of lactic acid, citric acid, propionic and acetic acids. After 90 days of ripening, free fatty acids in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 0.14, 0.19, 0.23, 0.29 and 0.37% with no difference in composition and sensory properties. These results suggested that CLA content of cheddar cheese can be successfully increased with safflower oil-derived CLA supplement.

## ARTICLE HISTORY

Received 24 May 2022  
Revised 20 September 2022  
Accepted 25 September 2022

## KEYWORDS

CLA; Cheddar cheese;  
Lipolysis; Sensory properties

## Introduction

Conjugated linoleic acid (CLA) represents the isomeric form of linoleic acid in which two double bonds are separated by a methyl group.<sup>[1]</sup> The conjugation of double bond is usually at 9 and 11 or 10 and 12 carbon atoms in *cis* or *trans* form. CLA is perceived to have anti-obesity, anti-diabetic, cardio-protective and anti-carcinogenic effects.<sup>[2]</sup> CLA can be used as an effective bio-active compound to minimize/ prevent lifestyle and metabolic disorders such as, cardiovascular

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diseases, diabetes, cancers and obesity. Intake of 2 g CLA on a daily basis may be helpful to prevent carcinogenesis.<sup>[3]</sup> It has been found that  $\Delta^9c,11$  *t*-18:2 is the most significant form of CLA. CLA is reported in only meat and dairy products, in rumen, *Butyrivibrio fibrisolvens* converts linoleic acid to CLA. In meat and dairy products, concentration of CLA largely depends upon the feeding practices. In meat and milk of grazing animals, CLA content is considerably higher than non-grazing animals. CLA may not be present in milk and beef of animals confined to barns and fed on little or no lush green fodder. All over the world, huge variations exist in the feeding practices of ruminants. Studies have shown that CLA content of milk and meat may be considerably increased by feeding CLA rich sources to the dairy animals.<sup>[4]</sup>

CLA to the consumers. Production of CLA-enriched dairy products through manipulating the ration of dairy cows is not a sustainable method as milk processing industries collect milk from thousands of farms having different feeding practices and composition. Further, availability and affordability of CLA rich sources in the world do not allow to rely on feeding strategies to develop CLA enriched food products on a larger scale as availability and affordability of CLA rich sources is a serious issue. To produce CLA enriched milk, industries cannot afford on the naturally existing CLA. It is, therefore, extremely important to make the fortification strategies successful and improving health of consumers, consistent supply of CLA should be assured in foods. Production of synthetic CLA seems to be appropriate solution of the problem. Vegetable oils rich in linoleic acid may be used to produce synthetic CLA. Higher magnitude of linoleic acid is usually perceived to have higher amount of CLA.<sup>[5]</sup> Amount of linoleic acid is relatively higher in safflower oil as compared to vegetable oils. Oil content of the seed ranges from 35–40% which is higher than soybean and cottonseed.<sup>[6]</sup> Safflower is cultivated on thousands of hectares in the world and easily available. Fatty acids profile showed that linoleic acid (65–70%) is the major fatty acid, several phenolic compounds and antioxidant substances have been reported in safflower oil. Safflower oil may be used as a substrate to synthesize CLA ( $\Delta^9c,11$  *t*-18:2 and  $\Delta^{10}$  *t*,12*c*-18:2) by isomerization of C18:2 with urea.<sup>[7]</sup> In view of the need of consumers and industries, new ways of CLA supplementation should be discovered to develop functional foods.

Food industries are attracting the customers through the development of functional foods.<sup>[8]</sup> Concentration of CLA in cheddar cheese depends upon its concentration in milk and processing conditions. Applications and acceptability of milk, butter, cream, ice cream and cheese can be increased by increasing functional value. Due to multiple food applications, unique flavor and texture, cheddar cheese is popular in several countries of the world. Fat content of cheddar cheese ranges from 28–30%, CLA content of cheddar cheese ranges from 0.3 to 0.5% and even some cases CLA is not present in cheddar cheese.<sup>[9]</sup> To fulfill the dietary requirements of CLA (1 g/day), about 200-gram cheddar has to be consumed which will deliver approximately 60-gram fat to the body. It is practically impossible to fulfill the dietary requirements of CLA from traditional dairy products. For the development of CLA rich food and dairy products, effect of processing technologies on CLA should be determined. CLA content of milk is considerably affected by the processing technologies, effect of conventional pasteurization, ultra-high temperature treatment, sterilization and microwave pasteurization on CLA content of milk is reported, it was found that conventional pasteurization led to decrease the concentration of CLA.<sup>[10]</sup> However, impact of cheddaring, pressing and ripening period on added CLA has not been reported in literature. In cheddar cheese, concentrations of proteins, minerals, bioactive compounds in more than milk therefore, cheese have higher functional value than milk. Therefore, consumption of functional foods derived is mounting across the globe.<sup>[11]</sup> Effect of safflower oil derived CLA on supplementation of cheddar cheese needs to be studied in detail. Supplementation of cheese with safflower oil derived CLA is not previously studied. This study aimed to determine the impact of safflower oil derived CLA on stability of CLA at different stages of processing and ripening, fatty acids composition, lipid oxidation and sensory analysis of cheddar cheese.

## Materials and methods

### Raw materials

Safflower seeds (after 15 days of harvesting) were obtained from an Agricultural Research Station, Punjab, Pakistan, oil was extracted by screw press at 20–25°C (yield 65.9%). For the analysis of raw materials and end products, chemicals were provided by Sigma Aldrich (St. Louis, MO, USA). Safflower oil was analyzed for free fatty acids (FFA), moisture content (MC), color (Lovibond Tintometer), iodine value (IV), saponification value (SV), unsaponifiable matter (USM) and refractive index (RI) following the standard methods of the American Oil Chemists Society.<sup>[12]</sup> Cow milk without standardization (fat 3.51% and protein 3.24) was used for the production of cheddar cheese and analyzed for fat, protein, mineral contents, solids not fat and total solids.

### Manufacture of conjugated linoleic acid (CLA) supplement from safflower oil

Method for the manufacture of CLA from safflower oil was optimized in our previous investigation<sup>[13]</sup> briefly, monounsaturated and saturated fatty acids were removed from saponified samples of safflower oil.<sup>[14]</sup> Liquor encompassing linoleic acids (LAs) were crystalized at 5°C for 12 hours by dissolving 120 g urea in 250 mL MeOH and LAs were recovered by vacuum filtration. The samples were acidified by bringing pH to <2 by using 200 mL HCl (with 6 N) and 200 ml deionized H<sub>2</sub>O. After acidification, 100 mL n-hexane was added to the solution for recovering of LAs and residual solution was mixed with 50 ml of H<sub>2</sub>SO<sub>4</sub> (6 N). C<sub>6</sub>H<sub>14</sub> (100 ml) was added for the purpose of re-extraction, both hexane portions were pooled and washed with deionized H<sub>2</sub>O (3 times). Hexane washed phase of samples were dried (by using sodium sulfate) and dried using rotary evaporator at a temperature of 35°C. CLA were purified from LAs, using urea.<sup>[4,13]</sup>

### Production of cheddar cheese and experimental plan

This study was organized in complete randomized design (CRD), all treatments were performed in triplicate. CLA supplement was added to the cow milk (fat 3.4%) at 1%, 2%, 3% and 4% (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>), cheese sample without CLA supplementation was used as control. Briefly, milk was pasteurized at 65°C for 30 min, pre-acidification was performed at 31°C through *Lactococcus lactis spp. lactis* and *Lactococcus lactis spp. cremoris* (DVS culture) for 45 min. Rennet (18 ml/100liter) and calcium chloride 35% (15 ml/100liter) were added and curd was cut after 30 min, vat temperature was then increased to 39°C in 45 min and cooking at this temperature for 40 min followed by cheddaring till pH 5.2, milling, salting (1.5%), pressing (3 bar for 16 hrs), vacuum packaging and ripening (5–6°C) for 90 days.<sup>[15]</sup>

### Cheddar cheese composition

Samples of cheddar cheese were analyzed for moisture, fat and protein contents at the frequency of 0, 45 and 90 days.

### Fatty acids profile

For the enumeration of fatty acids composition of CLA supplemented and cheddar cheese samples, standard procedure was adopted to extract fat from milk.<sup>[16]</sup> Extracted fat (50 mg) was taken in test tubes, methanolic hydrogen chloride 15% (Fluka) was mixed, capped and esters were by subjecting the tubes to 100°C for 60 min in a heating block. After cooling to room temperature, 2 ml each C<sub>6</sub>H<sub>14</sub> and deionized H<sub>2</sub>O were added to the contents of test tubes, followed by vortex at 1500rpm for 2 min and resting of 20 min. Hexane layer was harvested and dried over Na<sub>2</sub>SO<sub>4</sub>, 1 µl was injected to GC-MS (Agilent Technologies). Temperature of injector, FID was set 200 and 250°C, SP-2560 capillary

column 100 m x 0.25 id), keeping flow rate of He, H<sub>2</sub> and O<sub>2</sub> at 2 ml, 4 ml and 40 ml, respectively. FAME 37 (Supelco) and SLB-IL111 (Merck) were used as internal standards. Retention/ stability of CLA was checked in cheese milk, after pasteurization, after cheddaring (0 day), 45 and 90 days of ripening.<sup>[16]</sup>

### ***Determination of organic acids***

Grated cheese sample (10 g) was blended with H<sub>2</sub>PO<sub>4</sub> (40 ml), flasks were then vortex at 2000rpm for 2 min trailed by incubation in water bath at 65°C for 60 min and centrifugation at 6000rpm for 10 min. Supernatant was filtered using Whatman filter paper 1, put in vials and analyzed on HPLC (Perkin Elmer, Series 200) equipped with ShodexRSpak KC-118 model (8 mm × 300 mm i.d) ion exchange column. For the estimation of lactic acid, propionic acid, citric acid and acetic acid in CLA supplemented cheddar cheese samples and control, five standards for each organic acid were prepared and were quantified by calibration curve.<sup>[17]</sup>

### ***Lipolysis of cheddar cheese***

For the measurement of lipolysis in CLA supplemented cheese samples and control, cholesterol, peroxide value (PV) and Free fatty acids (FFA) were measured at 0, 45 and 90 days of ripening by following the standard methods of the American Oil Chemists Society<sup>[12]</sup>

### ***Sensory evaluation***

CLA supplemented cheese samples were tempered to 12°C for 30 min before sensory evaluation. Samples (50 g) were provided in individual sensory evaluation booths in a well-ventilated and illuminated sensory evaluation laboratory. Sensory evaluation was performed by a panel comprising of ten trained judges for color, flavor and texture using 9-point hedonic scale, distilled water was also provided in every sensory evaluation booth.<sup>[18]</sup>

### ***Statistical analysis***

Planning of this research work was done in CRD with a triplicate of each treatment. To determine the effect of CLA supplementation and storage duration of 90 days, two-way ANOVA was used, for the estimation of significant difference among the means, Duncan's Multiple Range (DMR) Test was used on a SAS 9.1 software.

## **Results and discussion**

### ***Chemical characteristics of safflower oil***

Free fatty acids, moisture content, iodine value, unsaponifiable matter, saponification value, refractive index (@40°C) in cold extracted safflower oil were 0.14%, 0.19%, 137.11 cg/100 g, 1.29%, 193.25 mgKOH/g and 1.4739. respectively. Results of safflower oil obtained in current investigation are not different from literature. Free fatty acids, moisture content, iodine value, unsaponifiable matter, saponification value and refractive index of safflower oil were 0.16%, 0.22%, 139.15 cg/100 g, 1.35% and 1.4759<sup>[19]</sup>

### ***Cheddar cheese composition***

Fat, protein, lactose, minerals, solids-not-fat and total solids content in cheese milk were 3.51, 3.24, 4.71, 0.72, 8.71 and 12.18%. In current investigation, cheddar cheese was supplemented with safflower

oil derived CLA supplement from 1 to 4% concentrations. Moisture, fat and protein contents of CLA supplemented cheddar cheese samples were similar to the control ( $p > .05$ ). From industrial application viewpoints, results of current investigation are highly encouraging as no alteration in standard cheese manufacturing process, equipment, packaging material and ripening temperature etc. are required. Chemical composition of CLA-enriched cheddar cheese was not different from standard cheese.<sup>[20]</sup> Fatty acids composition of some important cheese varieties was determined, amounts of CLA in Gouda, Tilsiter, Munster, Emmentaler, Greyzer, blue cheese, Gorgonzola, Brie and processed cheese were 0.40, 0.78, 0.62, 1.16, 1.24, 0.55, 0.69, 0.49 and 1.11 mg/100 g Butter with higher CLA content of CLA was produced by dry crystallization technique, composition of CLA enriched butter was not different from the standard butter.<sup>[20]</sup>

### **Fatty acids composition of CLA supplement**

Fatty acids composition of safflower oil showed that linoleic acid (74.61%) and oleic acid (11.66%) were the major fatty acids. Fatty acids composition of safflower oil recorded in this investigation is similar to literature.<sup>[21]</sup> Analysis of CLA supplement revealed that concentrations of  $\Delta 9c,11 t-18:2$ ,  $\Delta 10 t,12c-18:2$ ,  $\Delta 9c,11c-18:2$ ,  $\Delta 9 t, 11c-18:2$  were 52.16, 44.37, 0.27 and 0.55 mg/100 g (Table 2). In safflower oil derived CLA, conversion of linoleic acid to CLA was 22.58% and consistent to the findings of Gangidi et al.<sup>[22]</sup> Reasonable concentrations of  $\Delta 9c,11 t-18:2$  and  $\Delta 10 t,12c-18:2$  suggest that safflower oil-derived CLA can be used for the fortification of foods and dairy products. For the direct supplementation of milk, CLA oil (Clairinol G-80, Loders Crokiaan Channahon, IL) was analysed for CLA isomers, *cis-9/trans-11* isomer and *cis-10/trans12* isomer accounted for 33.04 and 33.68%.<sup>[23]</sup> Similar findings were reported Park et al.<sup>[24]</sup> Linoleic acid content of safflower oil is more than soybean, sunflower and canola oils; therefore, it is suitable to produce CLA. In an earlier investigation, it was reported that 8–10% CLA was produced from soybean oil while, the production of CLA from sunflower oil was zero.<sup>[25]</sup> Literature describes that  $\Delta 9c,11 t-18:2$  is safe for human use.<sup>[26]</sup> Soybean oil, canola oil, sunflower, cottonseed and safflower oils were used for the production of CLA using low-temperature crystallization technique, it was found that yield of CLA and its isomers were significantly higher in winterized vegetable oils than non-winterized vegetable oils. Highest yield (>30%) was obtained from winterized safflower oil.<sup>[27]</sup> Cottonseed oil was cooled to  $-30^{\circ}\text{C}$  and filtered, winterized version had higher concentrations of unsaturated fatty acids than native cottonseed oil.<sup>[28]</sup> C18:2 c9, c1, C18:2 c9, t11 and C18:2 t10, c12 in CLA oil used for the supplementation of Spanish dairy products were 0.43, 39.65 and 38.12% of the oil supplement.<sup>[29]</sup> FDA recommends to consume 3 g CLA on daily basis to prevent cancers and life style related disorders.

**Table 1.** Effect of CLA supplementation on composition of cheddar cheese.

Treatments	Moisture%	Fat%	Protein%
Control	40.13 ± 0.15	32.42 ± 0.04	26.74 ± 0.09
T <sub>1</sub>	40.88 ± 0.11	32.35 ± 0.13	26.57 ± 0.13
T <sub>2</sub>	40.25 ± 0.19	32.87 ± 0.02	26.92 ± 0.12
T <sub>3</sub>	40.66 ± 0.24	32.46 ± 0.03	27.15 ± 0.02
T <sub>4</sub>	40.78 ± 0.35	32.21 ± 0.07	26.84 ± 0.13

<sup>1</sup>All the means shown in Table 1 under the columns of moisture, fat and protein are statistically non-significant ( $p > 0.05$ )

CLA = conjugated linoleic acid

Control: without supplementation of CLA

T<sub>1</sub>: 1% CLA Supplementation

T<sub>2</sub>: 2% CLA Supplementation

T<sub>3</sub>:3% CLA Supplementation

T<sub>4</sub>: 3% CLA Supplementation

**Table 2.** Fatty acid profile of milk, safflower oil and CLA supplement (mg/100 g).

Fatty acid	Milk	Safflower oil	CLA Supplement
C4:0	2.55 ± 0.05	–	–
C6:0	2.89 ± 0.09	–	–
C8:0	2.44 ± 0.02	–	–
C10:0	2.71 ± 0.03	–	–
C12:0	2.97 ± 0.11	–	–
C14:0	12.39 ± 0.21 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	–
C16:0	27.43 ± 0.29 <sup>a</sup>	5.94 ± 0.14 <sup>b</sup>	–
C18:0	8.19 ± 0.24 <sup>a</sup>	2.47 ± 0.02 <sup>b</sup>	–
C18:1	24.64 ± 0.33 <sup>a</sup>	11.66 ± 0.22 <sup>b</sup>	–
C18:2	1.98 ± 0.02 <sup>b</sup>	74.61 ± 0.76 <sup>a</sup>	–
C18:3	0.51 ± 0.02 <sup>a</sup>	0.13 ± 0.02 <sup>b</sup>	–
C20:1	0.24 ± 0.04 <sup>b</sup>	0.45 ± 0.02 <sup>a</sup>	–
C24:1	0.07 ± 0.01 <sup>b</sup>	0.19 ± 0.03 <sup>a</sup>	–
Δ9c,11 <i>t</i> -18:2	0.25 ± 0.02 <sup>b</sup>	–	52.16 ± 0.39 <sup>a</sup>
Δ10 <i>t</i> ,12c-18:2	0.06 ± 0.01 <sup>b</sup>	–	44.37 ± 0.25 <sup>a</sup>
Δ9c,11c-18:2	–	–	0.27 ± 0.01
Δ9 <i>t</i> , 11c-18:2	–	–	0.55 ± 0.06

Means mentioned in Table 2 are product of triplicate of every treatment and triplicate analysis (3x3 = 9).

Means carrying a different in letter in a row are statistically significant ( $p < 0.05$ )

### Fatty acids composition of cheddar cheese

Linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid) is converted to CLA (*cis*-9,*trans*-11-

conjugated diene) by isomerization. CLA has anti-cancer, cardio-protective, anti-diabetic, anti-obesity effects. Nutritionists recommend to regularly consume 3 g CLA/ day to prevent lifestyle related disorders.<sup>[30]</sup> From nutritional prospects and functional food development viewpoints, results of current investigation are highly promising. In current investigation, CLA supplement was added to cheese milk and concentrations of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were almost times increased in cheese curd. During curd formation, magnitudes of fat, protein and some minerals are about ten times concentrated therefore, cheese is a tremendous source of good quality proteins, fats and calcium etc.<sup>[31]</sup> At zero-day, total CLA content in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and control were 2.4, 5, 8.34, 11.67 and 0.31%, respectively ( $p > .05$ ). GC-MS analysis showed the presence of four isomers of CLA in experimental samples; Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2, Δ9 *t*, 11c-18:2 while, Δ9c,11 *t*-18:2 and Δ10 *t*,12c-18:2 was recorded in control sample. In control sample, concentrations of Δ9c,11 *t*-18:2 and Δ10 *t*,12c-18:2 were 0.24% and 0.07% with a total CLA content of 0.31% (Table 3). Supplementation of cheddar cheese with safflower oil CLA supplement at all four levels significantly increased CLA content. In T<sub>1</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 0.28, 0.98, 0.41 and 0.73 mg/100 g. In T<sub>2</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 0.82, 1.92, 0.81 and 1.45 mg/100 g. In T<sub>3</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 1.12, 2.82, 1.63 and 2.77 mg/100 g. In T<sub>4</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 1.47, 3.51, 3.25 and 3.44 mg/100 g. CLA content of milk and dairy products can be increased by feeding management however, production of CLA enriched milk and value-added dairy products on large scale is not sustainable by this way. Sustainable commercial production of CLA rich dairy products can only be achieved by using CLA oil/supplement. Vegetable oils may be directly added to increase the concentration of C18:1, C18:2, C18:3 however, use of vegetable oils may lower the consumer's acceptability. Further, dairy products added with vegetable oils have lower concentrations of short-chain fatty acids and poor oxidative stability. In USA, average per day consumption of cheese is about 43.9 g.<sup>[32]</sup> CLA enriched cheddar cheese will provide 1.08, 2.25, 3.75, 5.25 g and 0.13 g CLA at T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and control levels. Daily requirements of CLA can be obtained from one slice of T<sub>3</sub> and T<sub>4</sub>. In current investigation, effect of ripening/ storage phase of 90 days was evaluated on CLA content of cheddar cheese, it was recorded that concentration of CLA was significantly influenced by the storage

**Table 3.** Stability of fatty acids and CLA Isomers during 90 days of ripening (mg/100 g).

Fatty Acid/ CLA Isomer	Control		T <sub>1</sub>		T <sub>2</sub>		T <sub>3</sub>		T <sub>4</sub>	
	0-Day	90-Days	0-Day	90-Days	0-Day	90-Days	0-Day	90-Days	0-Day	90-Days
C4:0	1.92 ± 0.05 <sup>a</sup>	1.80 ± 0.01 <sup>b</sup>	1.91 ± 0.02 <sup>a</sup>	1.79 ± 0.01 <sup>b</sup>	1.88 ± 0.05 <sup>a</sup>	1.75 ± 0.02 <sup>b</sup>	1.86 ± 0.03 <sup>a</sup>	1.63 ± 0.01 <sup>c</sup>	1.84 ± 0.07 <sup>a</sup>	1.75 ± 0.08 <sup>b</sup>
C6:0	2.25 ± 0.09 <sup>a</sup>	2.11 ± 0.02 <sup>b</sup>	2.22 ± 0.06 <sup>a</sup>	2.13 ± 0.04 <sup>b</sup>	2.20 ± 0.02 <sup>a</sup>	2.11 ± 0.01 <sup>b</sup>	2.18 ± 0.02 <sup>a</sup>	2.05 ± 0.04 <sup>b</sup>	2.16 ± 0.02 <sup>a</sup>	2.04 ± 0.06 <sup>b</sup>
C8:0	2.44 ± 0.02 <sup>a</sup>	2.31 ± 0.05 <sup>b</sup>	2.41 ± 0.07 <sup>a</sup>	2.29 ± 0.07 <sup>b</sup>	2.39 ± 0.08 <sup>a</sup>	2.27 ± 0.09 <sup>b</sup>	2.36 ± 0.01 <sup>a</sup>	2.23 ± 0.09 <sup>b</sup>	2.34 ± 0.10 <sup>a</sup>	2.21 ± 0.04 <sup>b</sup>
C10:0	2.71 ± 0.03 <sup>a</sup>	2.62 ± 0.02 <sup>b</sup>	2.67 ± 0.10 <sup>a</sup>	2.56 ± 0.10 <sup>b</sup>	2.65 ± 0.04 <sup>b</sup>	2.53 ± 0.06 <sup>a</sup>	2.62 ± 0.07 <sup>b</sup>	2.51 ± 0.04 <sup>b</sup>	2.61 ± 0.01 <sup>a</sup>	2.50 ± 0.03 <sup>b</sup>
C12:0	2.97 ± 0.11 <sup>a</sup>	2.82 ± 0.01 <sup>b</sup>	2.94 ± 0.03 <sup>a</sup>	2.93 ± 0.08 <sup>a</sup>	2.91 ± 0.07 <sup>a</sup>	2.90 ± 0.03 <sup>a</sup>	2.88 ± 0.11 <sup>a</sup>	2.87 ± 0.02 <sup>a</sup>	2.85 ± 0.13 <sup>a</sup>	2.77 ± 0.15 <sup>b</sup>
C14:0	10.39 ± 0.21 <sup>a</sup>	9.29 ± 0.41 <sup>b</sup>	10.25 ± 0.16 <sup>a</sup>	10.16 ± 0.14 <sup>a</sup>	10.14 ± 0.17 <sup>a</sup>	10.08 ± 0.27 <sup>a</sup>	10.04 ± 0.16 <sup>a</sup>	9.95 ± 0.32 <sup>b</sup>	9.84 ± 0.19 <sup>b</sup>	9.55 ± 0.65 <sup>b</sup>
C16:0	25.43 ± 0.29 <sup>a</sup>	24.19 ± 0.35 <sup>b</sup>	25.13 ± 0.41 <sup>a</sup>	24.15 ± 0.29 <sup>b</sup>	25.88 ± 0.37 <sup>a</sup>	24.43 ± 0.55 <sup>b</sup>	25.62 ± 0.44 <sup>b</sup>	24.19 ± 0.25 <sup>c</sup>	25.33 ± 0.46 <sup>b</sup>	24.13 ± 0.74 <sup>c</sup>
C18:0	7.19 ± 0.24 <sup>c</sup>	6.78 ± 0.09 <sup>f</sup>	7.09 ± 0.04 <sup>d</sup>	6.88 ± 0.22 <sup>e</sup>	7.01 ± 0.13 <sup>d</sup>	6.81 ± 0.41 <sup>e</sup>	7.94 ± 0.13 <sup>a</sup>	7.51 ± 0.18 <sup>b</sup>	7.79 ± 0.21 <sup>a</sup>	7.16 ± 0.04 <sup>c</sup>
C18:1	24.64 ± 0.33 <sup>a</sup>	23.51 ± 0.15 <sup>b</sup>	24.47 ± 0.17 <sup>a</sup>	23.32 ± 0.36 <sup>b</sup>	24.28 ± 0.26 <sup>a</sup>	23.13 ± 0.76 <sup>b</sup>	24.11 ± 0.46 <sup>a</sup>	23.51 ± 0.64 <sup>b</sup>	23.29 ± 0.43 <sup>b</sup>	22.17 ± 0.18 <sup>c</sup>
C18:3	0.51 ± 0.02 <sup>a</sup>	0.41 ± 0.06 <sup>b</sup>	0.48 ± 0.01 <sup>a</sup>	0.39 ± 0.02 <sup>b</sup>	0.49 ± 0.03 <sup>a</sup>	0.33 ± 0.01 <sup>c</sup>	0.48 ± 0.06 <sup>a</sup>	0.38 ± 0.04 <sup>b</sup>	0.46 ± 0.03 <sup>a</sup>	0.37 ± 0.02 <sup>b</sup>
Δ9C,11 <i>t</i> -18:2	0.24 ± 0.04 <sup>g</sup>	0.16 ± 0.03 <sup>h</sup>	0.28 ± 0.01 <sup>g</sup>	0.18 ± 0.01 <sup>h</sup>	0.82 ± 0.01 <sup>e</sup>	0.66 ± 0.03 <sup>f</sup>	1.12 ± 0.03 <sup>c</sup>	1.03 ± 0.06 <sup>d</sup>	1.47 ± 0.11 <sup>a</sup>	1.32 ± 0.04 <sup>b</sup>
Δ10 <i>t</i> ,12 <i>c</i> -18:2	0.07 ± 0.01 <sup>h</sup>	0.04 ± 0.01 <sup>i</sup>	0.98 ± 0.01 <sup>f</sup>	0.85 ± 0.01 <sup>g</sup>	1.92 ± 0.05 <sup>d</sup>	1.80 ± 0.05 <sup>e</sup>	2.82 ± 0.07 <sup>b</sup>	2.64 ± 0.12 <sup>c</sup>	3.51 ± 0.09 <sup>a</sup>	3.31 ± 0.07 <sup>b</sup>
Δ9 <i>c</i> ,11 <i>c</i> -18:2	–	–	0.41 ± 0.01 <sup>f</sup>	0.27 ± 0.01 <sup>g</sup>	0.81 ± 0.01 <sup>d</sup>	0.69 ± 0.01 <sup>e</sup>	1.63 ± 0.03 <sup>b</sup>	1.36 ± 0.02 <sup>c</sup>	3.25 ± 0.03 <sup>a</sup>	2.85 ± 0.01 <sup>b</sup>
Δ9 <i>t</i> , 11 <i>c</i> -18:2	–	–	0.73 ± 0.01 <sup>g</sup>	0.58 ± 0.01 <sup>h</sup>	1.45 ± 0.02 <sup>e</sup>	1.21 ± 0.02 <sup>f</sup>	2.77 ± 0.02 <sup>c</sup>	2.33 ± 0.02 <sup>d</sup>	3.44 ± 0.07 <sup>a</sup>	2.94 ± 0.02 <sup>b</sup>

Means carrying a different in letter in a row are statistically significant ( $p < 0.05$ )

duration of 90 days. After 90 days of ripening of T<sub>1</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 0.18, 0.85, 0.27 and 0.58 mg/100 g. After 90 days of ripening of T<sub>2</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 0.66, 1.80, 0.69 and 1.21 mg/100 g. After 90 days of ripening of T<sub>3</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 1.03, 2.64, 1.36 and 2.33 mg/100 g. After 90 days of ripening of T<sub>4</sub>, magnitudes of Δ9c,11 *t*-18:2, Δ10 *t*,12c-18:2, Δ9c,11c-18:2 and Δ9 *t*, 11c-18:2 were 1.32, 3.31, 2.85 and 2.94 mg/100 g. After 90 days of ripening of control, magnitudes of Δ9c,11 *t*-18:2 and Δ10 *t*,12c-18:2, were 0.16 and 0.04 mg/100 g. After 90 days of ripening, total CLA content in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 0.2, 1.88, 4.36, 7.36 and 10.42 mg/100 g. Pandit et al.<sup>[33]</sup> reported that concentration of CLA in cheddar cheese slightly increased after six months of ripening ( $p > .05$ ). CLA oil was added to cheese so that consumers obtain 2.5gCLA/day by consuming 125 g cheese. At day one, C18:2 c9, c12, C18:2 c9, t11, C18:2 t10, c12 were 0.63, 36.81, 34.79%. After 10 weeks of storage, C18:2 c9, c12, C18:2 c9, t11, C18:2 t10, c12 were 0.82, 34.59 and 32.86%. Amount of *cis*-9,*trans*-11, *trans*-7, *cis*-9 and *trans*-11,*cis*-13 in Brazilian Prato cheese were 0.85, 0.73 and 0.27 mg/100 g.<sup>[34]</sup> Fatty acids composition of synthetic CLA (Bioriginal Food & Science Corp., Saskatoon, SK) showed 60% CLA, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 were present in 53 and 44% concentrations.<sup>[35]</sup> Effect of refrigeration storage on CLA isomer of milk and dairy products is described in literature, short-term refrigeration had no effect on concentration of CLA isomers of dairy products.<sup>[29]</sup>

### Organic acids

During cheese ripening, a series of complex, chemical, microbiological and physical events change the chemistry of principal components of cheese. Extensive research work has been done to evaluate the biochemical and microbiological parameters which lead to the conversion of a chalky curd to flavorful cheese. Milk composition, water activity, pH, mineral contents, salt, microbial enzymes and microflora have a great deal of impact on ripening of cheese. Acceptability of cheddar cheese largely depends upon the flavor produced in the ripening phase, flavor profile of cheddar cheese is highly complicated and varies from one variety to the other. Organic acids, sulfur compounds, ketones, alcohols, ketones and phenolic compounds play major role in the flavor profile of cheese.<sup>[36]</sup> During cheese ripening, organic acids are produced by the hydrolysis of fat, bacterial activities or by the added acids. Total aroma intensity of Reggiano cheese was strongly correlated to the organic acids.<sup>[37]</sup> Organic acids are also extremely important for controlling the undesirable bacteria in cheese. Principle purpose of starter culture addition in cheese is to produce lactic acid, it can efficiently inhibit the coliforms, production of lactic acid is extremely important for the proper texture, flavor and good keeping quality.<sup>[38]</sup> Acetone, diacetyl, bacterial enzymes and bacteriocins are released as a result of autolysis of cells in cheese which is the further metabolized for the production of organic acids.<sup>[39]</sup> As described in symposium on flavor in dairy foods and meat, mechanism of formation of organic acids is: unsaturated fatty acids are converted to hydroperoxides by oxidation which are then converted to aldehydes which are further converted to organic acids and alcohols. However, production of organic acids in CLA supplemented cheese should be investigated in detail. In current investigation, impact of CLA supplementation on production of lactic acid, citric acid, propionic acid and acetic acid was monitored for 90 days. CLA supplemented cheddar cheese samples had higher concentrations of lactic acid, citric acid, propionic acid and acetic acid than control cheddar cheese (Table 4). Concentrations of lactic acid, citric acid, propionic acid and acetic acid went on increasing throughout the ripening period. After 90 days of ripening, concentration of lactic acid in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 3489, 3714, 4019, 4133 and 4532 ppm ( $p < .05$ ). After 90 days of ripening, concentration of citric acid in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 233, 335, 611, 993 and 1854 ppm ( $p < .05$ ). After 90 days of ripening, concentration of propionic acid in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 145, 354, 722, 1156 and 1794 ppm ( $p < .05$ ). After 90 days of ripening, concentration of acetic acid in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 17433, 19794, 24311, 28219 and 35749 ppm ( $p < .05$ ). Hard cheese samples produced from milk of sheep, cow and goat were produced to determine the fatty acids profile and CLA content. Sheep milk had significantly higher

**Table 4.** Effect of CLA supplementation on production of organic acids in cheddar cheese (ppm).

Treatments	Ripening Days	Lactic Acid	Citric Acid	Propionic Acid	Acetic Acid
Control	45	3489 ± 0.35 <sup>i</sup>	181 ± 0.11 <sup>j</sup>	94 ± 0.07 <sup>i</sup>	15784 ± 1.95 <sup>j</sup>
	90	4329 ± 0.55 <sup>f</sup>	233 ± 0.15 <sup>h</sup>	145 ± 0.26 <sup>h</sup>	17433 ± 1.60 <sup>i</sup>
T <sub>1</sub>	45	3714 ± 0.92 <sup>h</sup>	193 ± 0.61 <sup>i</sup>	144 ± 0.16 <sup>h</sup>	18519.1.73 <sup>h</sup>
	90	4471 ± 1.23 <sup>d</sup>	335 ± 0.46 <sup>g</sup>	354 ± 0.18 <sup>g</sup>	19794 ± 1.43 <sup>g</sup>
T <sub>2</sub>	45	4019 ± 0.73 <sup>g</sup>	379 ± 1.14 <sup>f</sup>	488 ± 0.28 <sup>f</sup>	20691 ± 2.40 <sup>f</sup>
	90	4893 ± 0.75 <sup>c</sup>	611 ± 1.51 <sup>e</sup>	722 ± 0.21 <sup>e</sup>	24311 ± 1.91 <sup>e</sup>
T <sub>3</sub>	45	4133 ± 1.25 <sup>e</sup>	719 ± 0.32 <sup>d</sup>	812 ± 0.62 <sup>d</sup>	22713 ± 0.93 <sup>d</sup>
	90	5146 ± 0.8 <sup>b</sup>	993 ± 0.08 <sup>c</sup>	1156 ± 0.49 <sup>c</sup>	28219 ± 1.36 <sup>c</sup>
T <sub>4</sub>	45	4532 ± 0.64 <sup>d</sup>	1094 ± 0.26 <sup>b</sup>	1256 ± 0.88 <sup>b</sup>	29154 ± 1.75 <sup>b</sup>
	90	5973 ± 0.94 <sup>a</sup>	1854 ± 0.1.29 <sup>a</sup>	1794 ± 1.49 <sup>a</sup>	35749 ± 2.15 <sup>a</sup>

<sup>4</sup>If in one column, means are expressed with a different letter, it indicates significant variation ( $p < 0.05$ )

organic acids, polyunsaturated fatty acids and CLA.<sup>[40]</sup> Impact of fatty acids composition on CLA in cheese samples was studied for six months, sheep milk showed highest concentrations of CLA and polyunsaturated fatty acids.<sup>[41]</sup> Concentration of organic acids increased in cheddar cheese with the progression of ripening period.<sup>[21]</sup>

### Lipolysis

In ripening phase of cheddar cheese (six to twelve months), several biochemical changes take place that led to the production of desired textural and flavor properties. Biochemical events in cheese ripening may be broadly classified to metabolism of residual lactose, proteolysis and lipolysis. Lipases of bacterial and milk origins cause hydrolysis of the triglyceride molecules which cause the release of short-chain fatty acids and free fatty acids which can significantly contribute in flavor of several varieties of cheese.<sup>[42]</sup> In excessive amounts FFA can the acceleration of lipid oxidation.<sup>[43]</sup> Controlled lipolysis plays a vital role in ripening of Cheddar and Gouda cheese however, excessive lipolysis may lead to the rancidity and off-flavor.<sup>[38]</sup> Concentrations of free fatty acids in Parmesan, Cheddar and Swiss cheese were 136, 492 and 427 mg/kg cheese.<sup>[44]</sup> In current investigation, supplementation of cheddar cheese from 1 to 4% concentrations had a non-significant effect on free fatty acids (Table 5). Both ripening period and CLA supplementation had pronounced effects on production of FFAs ( $p < .05$ ). After 45 and 90 days of ripening period, highest FFA values were recorded in T<sub>4</sub> followed by T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub> and control. Determination frequencies of 45 and 90 days showed the same increasing trend. After 45 days of ripening, FFA of control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 0.11, 0.15, 0.18, 0.22 and 0.25% ( $p < .05$ ). After 90 days of ripening, FFA of control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 0.14, 0.19, 0.26, 0.29 and

**Table 5.** Effect of CLA supplementation on lipolysis of cheddar cheese.

Treatments	Days	Cholesterol	Free Fatty Acids%	Peroxide Value (MeqO <sub>2</sub> /Kg)
Control	0	165 ± 0.27 <sup>a</sup>	0.08 ± 0.01 <sup>h</sup>	0.25 ± 0.03 <sup>b</sup>
	45	160 ± 0.13 <sup>b</sup>	0.11 ± 0.02 <sup>g</sup>	0.28 ± 0.01 <sup>b</sup>
	90	142 ± 0.19 <sup>g</sup>	0.14 ± 0.01 <sup>f</sup>	0.48 ± 0.05 <sup>a</sup>
T <sub>1</sub>	0	164 ± 0.07 <sup>a</sup>	0.08 ± 0.01 <sup>h</sup>	0.25 ± 0.03 <sup>b</sup>
	45	155 ± 0.09 <sup>c</sup>	0.15 ± 0.01 <sup>f</sup>	0.26 ± 0.04 <sup>b</sup>
	90	132 ± 0.16 <sup>h</sup>	0.19 ± 0.02 <sup>e</sup>	0.43 ± 0.07 <sup>a</sup>
T <sub>2</sub>	0	166 ± 0.15 <sup>a</sup>	0.08 ± 0.01 <sup>g</sup>	0.25 ± 0.03 <sup>b</sup>
	45	149 ± 0.12 <sup>d</sup>	0.18 ± 0.01 <sup>f</sup>	0.29 ± 0.02 <sup>b</sup>
	90	124 ± 0.21 <sup>i</sup>	0.23 ± 0.02 <sup>d</sup>	0.46 ± 0.01 <sup>a</sup>
T <sub>3</sub>	0	163 ± 0.05 <sup>a</sup>	0.08 ± 0.01 <sup>h</sup>	0.25 ± 0.03 <sup>b</sup>
	45	144 ± 0.03 <sup>e</sup>	0.26 ± 0.03 <sup>c</sup>	0.27 ± 0.02 <sup>b</sup>
	90	116 ± 0.02 <sup>j</sup>	0.29 ± 0.02 <sup>b</sup>	0.49 ± 0.06 <sup>a</sup>
T <sub>4</sub>	0	162 ± 0.04 <sup>a</sup>	0.08 ± 0.01 <sup>h</sup>	0.25 ± 0.03 <sup>b</sup>
	45	135 ± 0.02 <sup>f</sup>	0.25 ± 0.02 <sup>c</sup>	0.26 ± 0.01 <sup>b</sup>
	90	110 ± 0.06 <sup>k</sup>	0.37 ± 0.01 <sup>a</sup>	0.53 ± 0.02 <sup>a</sup>

<sup>5</sup>Means carrying a different in letter in a column are statistically different ( $p < 0.05$ )

0.37%. Ullah et al.<sup>[45]</sup> modified the fatty acids composition of milk by supplementing with chia (*Salvia hispanica*) oil, milk with modified fatty acids composition was transformed to cheddar cheese. FFA in experimental samples were significantly higher than the control sample of cheddar cheese. Flavor profile of 45 days old chia supplemented cheddar cheese was similar to 90 days old control cheese. Concentrations of unsaturated fatty acids were increased in milk fat using low melting point crystallization, cheddar cheese was produced from various fractions of milk fat, it had more free fatty acids than control cheese.<sup>[46]</sup> Increased number of cardiovascular diseases, increased knowledge of nutrition, controversies regarding the intake of cholesterol and changing life styles led the consumer to avoid fat rich dairy products. On average basis, cholesterol content of milk, cream, butter, ice cream and cheddar cheese are 15, 140, 220, 110 and 170 mg/100 g.<sup>[47]</sup> CLA supplementation at all four concentrations had no effect on cholesterol content of cheddar cheese. Cholesterol in all treatments and control decreased in the entire ripening phase, estimation intervals of 45 and 90 days showed the same trend. Decline in cholesterol content was dependent upon level of CLA supplementation and storage period. After the completion of ripening phase, concentrations of cholesterol in control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 142, 132, 124, 116 and 110 mg/100 g. During the development of functional dairy products with elevated levels of unsaturated fatty acids, it is technically challenging to develop oxidative stable products. With an exception of ripened cheeses, lipid oxidation is an issue of dairy products, cheese has lower oxidation-reduction potential therefore, lipid oxidation is normally not an issue in ripened cheeses.<sup>[48]</sup> For the estimation of oxidative stability of vegetable fats, accelerated oxidation methods such active oxygen method and Rancimat techniques are normally used however, due to delicate nature and complexity of dairy products, these techniques are normally not applied to determine oxidative stability.<sup>[49,50]</sup> Peroxide value is traditionally used to measure the oxidation of dairy products.<sup>[51]</sup> After 90 days of ripening, peroxide value of control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 0.48, 0.43, 0.46, 0.49 and 0.53 (MeqO<sub>2</sub>/kg). These values are relatively less than the values of 1.99, 3.99, and 10.64Meq/kg reported for palm olein, sesame oil, and sunflower oil in a different study.<sup>[52]</sup> Free fatty acids of butter did not increase during the storage phase of three months.<sup>[53]</sup> *Trans* free margarine produced from blends of palm oil, palm kernel oil and chia oil blends were stored at refrigeration temperature for 180 days, free fatty acids of margarine did not significantly increase during the storage period.<sup>[47]</sup> After 6 days of storage, free fatty acids of CLA rich milk were similar to initial values recorded at zero day.<sup>[53]</sup> Oxidative stability of isomers of CLA was investigated and was in the order of *t,t*-CLA > *c,t*-CLA > *c,c*-CLA.<sup>[54]</sup> *Trans* double is more as compared to *cis* double bond.<sup>[55]</sup>

### Sensory evaluation

Supplementation of cheddar cheese with CLA supplement had no effect on color, flavor and texture of cheddar cheese, as described in the Table 6. After 90 days of ripening, color, flavor and texture scores of control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were not different from each other. After 90 days, flavor score of control T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was 8.15, 8.10, 8.11, 8.12 and 8.07. Flavor score of 90 days old cheddar cheese

**Table 6.** Sensory properties of CLA supplemented cheddar cheese.

Treatments	Days	Color	Flavor	Texture
Control	45	7.27 ± 0.03b	7.17 ± 0.09b	7.22 ± 0.04 <sup>b</sup>
	90	8.20 ± 0.07 <sup>a</sup>	8.15 ± 0.02 <sup>a</sup>	8.19 ± 0.07 <sup>a</sup>
T <sub>1</sub>	45	7.24 ± 0.12 <sup>b</sup>	7.25 ± 0.01 <sup>b</sup>	7.27 ± 0.06 <sup>b</sup>
	90	8.18 ± 0.06 <sup>a</sup>	8.10 ± 0.15 <sup>a</sup>	8.16 ± 0.05 <sup>a</sup>
T <sub>2</sub>	45	7.22 ± 0.04 <sup>b</sup>	7.12 ± 0.06 <sup>b</sup>	7.21 ± 0.10 <sup>b</sup>
	90	8.19 ± 0.13 <sup>a</sup>	8.11 ± 0.03 <sup>a</sup>	8.08 ± 0.03 <sup>a</sup>
T <sub>3</sub>	45	7.14 ± 0.16 <sup>b</sup>	7.13 ± 0.12 <sup>b</sup>	7.19 ± 0.01 <sup>b</sup>
	90	8.13 ± 0.11 <sup>a</sup>	8.12 ± 0.18 <sup>a</sup>	8.10 ± 0.11 <sup>a</sup>
T <sub>4</sub>	45	7.21 ± 0.12 <sup>b</sup>	7.23 ± 0.01 <sup>b</sup>	7.16 ± 0.15 <sup>b</sup>
	90	8.16 ± 0.15 <sup>a</sup>	8.07 ± 0.03 <sup>a</sup>	8.12 ± 0.04 <sup>a</sup>

If in a column, mean is expressed with a different letter, it shows statistically significant difference ( $p < 0.05$ )

supplemented with vegetable oil was similar to normal cheddar cheese.<sup>[45]</sup> Similar results were reported.<sup>[46]</sup> From industrial aspects and functional food development viewpoints, these results are highly encouraging.

## Conclusion

In T3, T4 and control, total CLA content were 8.34, 11.67 and 0.31 mg/100 g, respectively. After 90 days of ripening, flavor score of control, T3 and T4 were 90.5, 90.2 and 89.3% of the total score. It can be concluded that safflower oil derived CLA can be used to raise CLA content of cheddar cheese.

## Acknowledgments

The authors are highly obliged to the Library Department, University of Veterinary and Animal Sciences (UVAS), and IT Department, Higher Education Commission (HEC, Islamabad), for access to journals, books, and valuable database. The research was completed after utilizing the available resources in the Department and University. The authors are also grateful to Kampala International University, Kampala, Uganda, for helping in providing resources for literature collection.

## Author's contribution

Awais Khan performed the methods and investigation. Muhammad Nadeem conceptualization, funding acquisition and provided supervision in analysis while Muhammad Imran, Nabila Gulzar, Muhammad Haseeb Ahmad, Muhammad Tayyab, Muhammad Abdul Rahim and Chinaza Godswill Awuchi supported for original drafting of the manuscript

## Consent for publication

All authors agreed for publication of this manuscript.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This research work was funded by Higher Education Commission of Pakistan under National Research Program for Universities (Grant No. NRPU/6978).

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## Data availability statement

The dataset supporting the conclusions of this article is included within the article.

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