

Fatty Acid Composition and Fat Stability of Raw Milk and Pasteurized Milk from Laoshan Goats

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Abstract

In this study, the fatty acid profile and fat stability for seven consecutive days of raw milk and pasteurized milk from Laoshan goats have been evaluated by gas chromatography-mass spectrometry (GC-MS) after fatty acid methyl ester. The results showed that the concentrations of short chain fatty acids (SCFA) and saturated fatty acids (SFA) significantly increased by 47.36% and 11.68% after pasteurization respectively, while the concentrations of unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decreased by 26.08%, 26.45% and 22.15% respectively. The C10:0 (5.39%-8.57%), C12:0 (3.13%-5.28%), C14:0 (8.12%-11.87%), C16:0 (25.59%-28.53%), C18:0 (14.60-13.69%) and C18:1 (33.91-24.92%) are the most predominant fatty acids of Laoshan goat milk with significant differences. Moreover, the fat stability for seven consecutive days of raw milk and pasteurized milk was detected by sedimentation rate (R). The fat stability in pasteurized milk was more stable than that in raw milk, the sedimentation rate of raw milk and pasteurized milk consisted in a progressive decrease in the seven days by 82.99% and 79.77% respectively. What's more, significant difference was observed from 1st day to 4th day between raw milk and pasteurized milk, however, there was no significance from 5th to 7th. This is the first report to fully characterize the fatty acid contents and fat stability of Laoshan goat raw milk and its pasteurized milk and it provided a certain theoretical basis for the research and development of goat milk functional product.

Keywords: goat milk, fatty acid, GC-MS, stability of milk fat, sedimentation rate

1. Introduction

Caprine agriculture is an important part of the national economy in many countries especially in the Mediterranean and the Middle East (García et al., 2014), but few people drink goat milk in China because it has a unique flavor that people find intolerable. However, many studies have shown that goat milk has many nutritional benefits that surpass those of bovine milk; moreover, goat milk is a great source of easily digested nutrition. For example, there are more than 200 nutrients and biologically active substances in goat's milk with lower allergenicity (Durand et al., 2003). Furthermore, the epithelial growth factor (EGF), superoxide dismutase (SOD) and Vitamin E in goat's milk prevent skin aging and make skin smoother. Goat milk is suitable for lactose intolerant people because there is less lactose in goat milk than bovine milk (Frances, 2001).

Fat is one of the most important components in milk. The fat in goat milk includes more unsaturated fatty acids than cow's milk, which can reduce the risk of obesity and is closely related to human health. For instance, omega-3 and omega-6 fatty acids are known to benefit for cardiovascular disease (Simopoulos, 2008), and conjugated linoleic acid (CLA) has anti-carcinogenesis and anti-atherosclerosis activity while strengthening immunity (Benjamin & Spener, 2009). Other studies have shown that the flavor of goat milk is primarily influenced by short-chain fatty acids especially C8:0 and C10:0. Sumarmono et al. investigated the fatty acids profiles of fresh milk, yogurt and concentrated yogurt, the results showed that oleic acid (C18:1) was the most common fatty acid (Sumarmono et al., 2015). Not only pasteurization can kill all the pathogenic bacteria, but

also maintain the main nutrition and natural flavor of milk. The research revealed that pasteurization increased the content of trans fatty acids significantly (Herzallah et al., 2005). The study also found that with the treatment of high temperature short time (HTST) pasteurization (75 °C, 16 s) and ultra-high temperature (UHT) sterilization (140 °C, 4 s), the content of conjugated Linoleic Acid (CLA) decreased compared with raw cow milk, the reason may be that the hydrogen peroxide causes the decomposition of CLA and the temperature promotes the oxidation process (Costa et al., 2011). What's more, many factors can modulate the fatty acid composition including animal feed (Morales-Almaráz et al., 2011; Zervas & Tsiplakou, 2011).

Dairy products often occur the phenomenons of fat floatin and aggregation during the process of production and storage. To restrain the bad phenomenons and improve the stability of milk fat, we often do by adding emulsifiers in the actual process. It is considered that the adsorption rate of the emulsifier must be large enough to stabilize the emulsion as soon as possible in the emulsification process (Jafari et al., 2008). Emulsion stability of milk from Ettawah Crossedbred goats changed after 30d of storage during frozen storage, the fat globule in goat milk only began to clustering, whereas the fat globule in cow milk already occurred coalescence (Nurliyani et al., 2015). The following order was derived for the stability of whey protein in milk: raw whole > HTST, homogenized, homogenized and pasteurized > skimmed and pasteurized, and skimmed UHT > homogenized UHT (Qi et al., 2015).

However, little work has been done to quantitate the fatty acids and fat stability of Chinese goat milk. The aim of this study was to evaluate the fatty acids compositions of both raw and pasteurized goat milk by gas chromatograph-mass spectrometry (GC-MS) and to analyze the stability changes via a 7-day sedimentation assay from Laoshan goat milk. To the best of our knowledge, this is the first report to quantitate the fatty acids and sedimentation rate of milk from Laoshan goat. Our findings have important implication for the dairy industry and provides a theoretical basis for application of pasteurization in goat milk industry.

2. Materials and Methods

2.1 Materials

In the current study, 50 Laoshan goat milk samples were collected at 10:00 AM from the Tai'an Three Hi Goat Farm in Shandong province. These 50 Laoshan goats, in the age of 38-41 months, were healthy, no acute mastitis visible clinical disease, and were at the peak lactation. All goats were well organized by utilizing the free stall bar raising technique, feeding the TMR silage diet, milking in central milking hall, clearing the fecal by labor. Whole milk samples were transported to the lab on ice and stored at -20 °C.

The GC-MS (QP2010) was purchased from Shimadzu. The digital thermostatic water bath (DK-98-IIA) was purchased from Jintan Jin Nan Instrument Factory. An electronic balance (AL104) was purchased from Mettler Toledo Instruments (Shanghai) Co., Ltd. A refrigerated centrifuge (TGL-18m) was from Jinan Longrui Trade Co., Ltd. An ultrasonic cleaner was purchased from Shanghai Kedao Ultrasonic Instrument Co., Ltd. A room temperature centrifuge (TG16-ws) was purchased from Changsha Xiangyi Centrifuge Instrument Co., Ltd. a visible spectrophotometer (V-1100D) was purchased from Shanghai Mapada Instruments Co., Ltd.

Hexane was purchased from Tianjin Guangfu Fine Chemical Research Institute. Potassium hydroxide was purchased from Tianjin kemi'ou Chemical Reagent Co., Ltd. Methanol was purchased from Shandong Yuwang Industrial Co., Ltd.

2.2 The Method of Analyzing Fatty Acid Composition

2.2.1 Fatty Acid Compositions of Laoshan Goat Milk

No spoiled goat's milk was selected, followed by melting at room temperature. Then Milk fat was centrifuged in a refrigerated centrifuge at 4 °C and 8000 r/min for 10 min—the fat accumulated in the upper layer (Palmquist & Jenkins, 2003). We used an alkali-catalyzed method to perform methyl esterification of the fatty acids in goat's milk (Talpur et al., 2009). Firstly, 0.2 g of milk fat was added into a 100 mL beaker, followed by the addition of 5 mL of hexane with 3 min of ultrasonic treatment to dissolve fat. Then 5 mL of methanol and 5 mL of methanol-potassium hydroxide solution (0.5 mol/L) were added, the mixture was placed in a blender shock with water bath at 55 °C for 25 min. Next, saturated brine was added to remove more impurities after heating. After the reaction, 300 µL of the supernatant was obtained, diluted by hexane (4 mL) and filtered with a 0.22 µm organic membrane.

2.2.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS was used for analysis (Talpur et al., 2009). The chromatographic column was a DB-5MS purchased from Aligent with a detector purchased from Shimadzu at 250 °C. The injection temperature was 230 °C; 1 µL

injection volume with helium carrier gas (99.9%) at 1.0 ml/min. The injection split ratio was 100:1 with an initial temperature of 60 °C that was maintained for 2 min. This then increased to 250 °C at 5 °C/min and held for 5 min. The temperature of the interface was 250 °C with electron ionization at 70 eV. The ion source temperature was 200 °C and was scanned from 45-500 m/z.

2.3 The Method of Analyzing Milk Fat Stability

No spoiled goat's milk was selected, followed by melting at room temperature. The pasteurized milk was treated at 65 °C for 30 min. The samples were divided in two parts: taking 0.5 ml of the raw milk (treated milk) that was diluted 100-fold to measure the absorbance (A1) at 540 nm by a visible spectrophotometer (V-1100D); taking 5 ml of the raw milk (treated milk) that was centrifuged at 4000 r/min for 15 min, the clear liquid middle layer was diluted 100-fold, then analyzed similarly at 540 nm for a value of A2. The sedimentation rate ($R \leq 1$) was obtained by $A2/A1$ —higher R values imply more stable milk fat (Chen et al., 2012), using the principle of double refraction of fat globule.

2.4 Statistical Analyses

Samples were divided into untreated and pasteurized groups. Each group was analyzed for both fatty acid compositions and stability changes. The identification of fatty acids was assigned by the comparison of their retention time and spectrum with corresponding data from the reference compounds data by retrieving WILEY7 and NIST147 standard libraries in combination with artificial analysis diagram. The individual fatty acids data and 7-day stability changes data were presented as $\bar{x} \pm SD$; a one-way ANOVA evaluated the differences in fatty acid composition and stability changes using SPSS (12.0). Significance was defined at $P < 0.05$.

3. Results and Discussion

3.1 The Results and Analyses of Fatty Acid Composition in Raw Milk and Pasteurized Milk

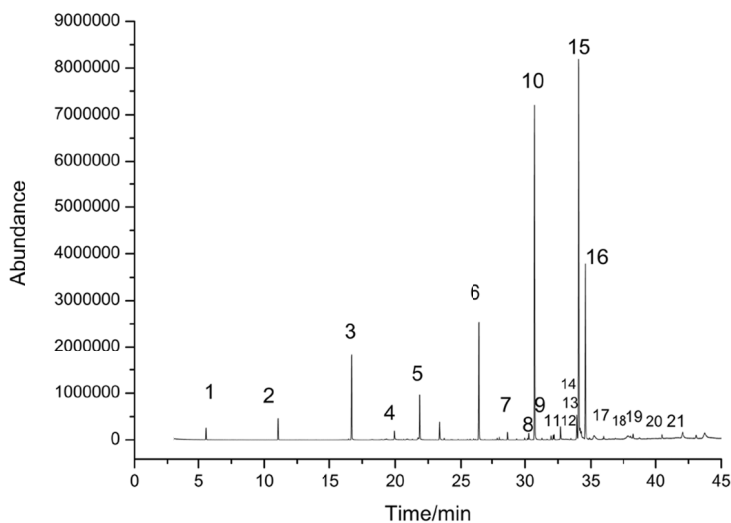


Figure 1. Total ionization chromatogram of fatty acids in goat raw milk

Note. The number from 1 to 21 represent for different peak appeared at different time.

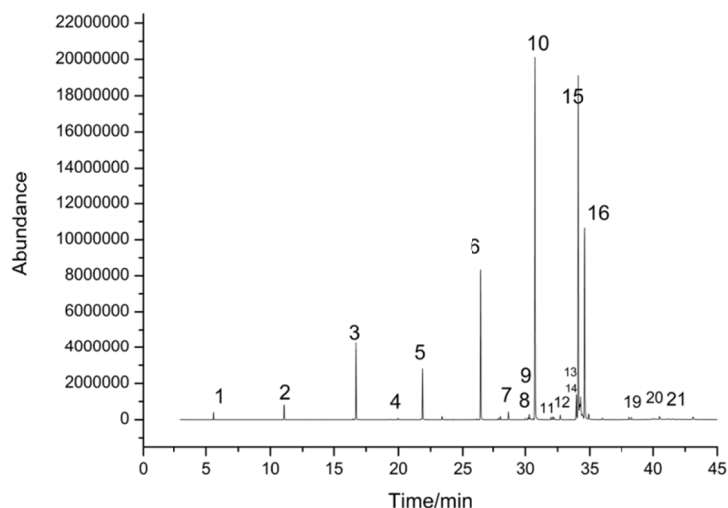


Figure 2. Total ionization chromatogram of fatty acids in pasteurized goat's milk

Note. The number from 1 to 21 represent for different peak appeared at different time.

The total ion chromatogram (TIC) of the fatty acids in raw goat's milk and pasteurized milk are presented in Figures 1 and 2, respectively. There were 21 fatty acids in raw milk and 19 fatty acids in pasteurized milk. In this study, the SFA:MUFA:UFA was 16.31:8.45:1 in raw milk and 22.18:8.02:1 in pasteurized milk. This is consistent with the previous findings (20:9:1) by Mattos and colleagues (Mattos et al., 2004). However, the World Health Organization (WHO) recommends that the ratio of SFA:MUFA:PUFA ideally be 1:1:1. Thus, fatty acids should be adjusted to optimize the nutrition of goat's milk and its products. In this study, palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) were also common fatty acids (> 10%) in both raw milk and pasteurized milk. Grouping of fatty acids were as follows: short-chain fatty acids (C4 to C10, SCFA), saturated fatty acids (SFA), and unsaturated fatty acids (UFA). Table 1 and Figure 3 illustrates that SCFA, SFA, and UFA were about 7.39%, 54.69%, and 37.92% in raw goat's milk, but 10.89%, 61.08% and 28.03% in pasteurized milk. In short, the concentration of unsaturated fatty acids in pasteurized milk was lower than that in raw milk—the saturated fatty acids were higher.

Table 1. The chemical components and their relative fatty acid contents in raw goat's milk and pasteurized goat's milk

Peak No.	Fatty acid type	Relative percentage/%		P
		Raw milk	Pasteurized milk	
1	Hexanoic acid C6:0	0.70±0.15	0.76±0.19	NS
2	Octanoic acid C8:0	1.30±0.23	1.56±0.33	NS
3	Decanoic acid C10:0	5.39±0.74	8.57±0.74	**
4	Undecanoic acid C11:0	0.21±0.13	0.24±0.05	NS
5	Dodecanoic acid C12:0	3.13±0.31	5.28±0.81	*
6	Tetradecanoic acid C14:0	8.12±1.03	11.87±1.11	*
7	Pentadecanoic acid C15:0	0.62±0.14	0.51±0.07	NS
8	7-Hexadecenoic acid C16:1cis-7	0.18±0.11	0.15±0.08	NS
9	9-Hexadecenoic acid C16:1cis-9	0.58±0.15	0.43±0.07	NS
10	Hexadecanoic acid C16:0	25.59±2.22	28.53±2.35	*
11	Cyclopropaneoctanoic acid	0.44±0.05	0.17±0.05	NS
12	Heptadecanoic acid C17:0	0.98±0.12	0.34±0.07	**
13	9,12-Octadecadienoic acid C18:2	1.87±0.19	1.42±0.32	NS
14	9,12,15-Octadecenoic acid C18:3	1.15±0.16	1.11±0.14	NS
15	9-Octadecenoic acid C18:1	33.91±2.46	24.92±2.43	**
16	Octadecanoic acid C18:0	14.60±0.73	13.69±0.92	NS
17	Nonadecanoic acid C19:0	0.15±0.09	ND	-
18	5,8,11,14-Eicosatetraenoic acid C20:4	0.23±0.08	ND	-
19	Eicosanoic acid C20:0	0.42±0.13	0.20±0.05	NS
20	Heneicosanoic acid C21:0	0.25±0.12	0.20±0.09	NS
21	Docosanoic acid C22:0	0.18±0.04	0.15±0.06	NS
	∑SCFA	7.39±0.07	10.89±0.10	**
	∑SFA	54.69±0.85	61.08±1.56	**
	∑UFA	37.92±1.58	28.03±1.44	**
	∑MUFA	34.67±0.69	25.50±0.78	**
	∑PUFA	3.25±0.09	2.53±0.13	**

Note. SCFA (short-chain saturated fatty acids): sum of C6:0+C8:0+C10:0;

SFA (saturated fatty acids): sum of C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C19:0+C20:0+C21:0+C22:0;

UFA (unsaturated fatty acids): sum of MUFA+PUFA;

MUFA (monounsaturated fatty acids): sum of 7-C16:1+9-C16:1+C18:1;

PUFA (polyunsaturated fatty acids): sum of C18:2+C18:3+C20:4;

***P < 0.001; **P < 0.01; *P < 0.05; NS: no significant P > 0.05; and ND: not detected.

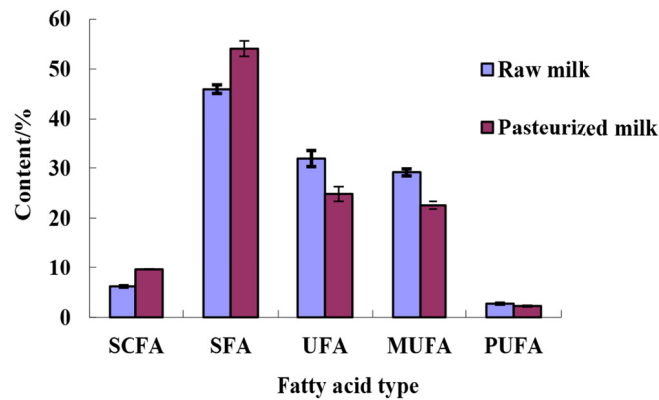


Figure 3. The content of SCFA, SFA, UFA, MUFA, and PUFA in Laoshan goat raw milk and pasteurized milk

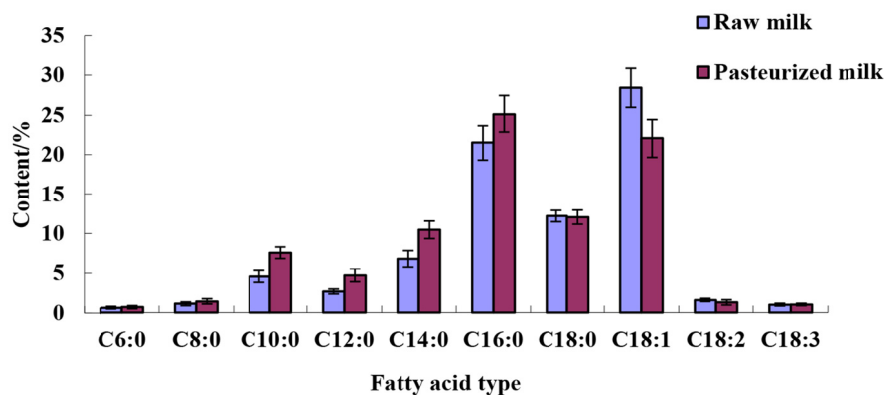


Figure 4. Comparisons of all fatty acids between Laoshan raw and pasteurized milk

3.1.1 Comparison of SCFA in Raw and Pasteurized Milk

The SCFA including C6:0, C8:0, and C10:0 were about 7.39% in the raw milk (Table 1, Figure 4), but this increased to 10.89% by 47.36% after pasteurization ($P < 0.01$), the long-chain fatty acids may change into short-chain and medium-chain fatty acids as a result of heating. The C6:0 and C8:0 contents were about 0.70% and 1.30% in raw milk and increased to 0.76% and 1.57% in pasteurized milk, respectively. There were no significant differences between them ($P > 0.05$). Nevertheless, there were more significant differences in C10:0 ($P < 0.01$)—the concentration increased from 5.39% to 8.57%. After pasteurization, the unique flavors of goat's milk are enhanced. However, SCFA is more easily digested than long-chain fatty acids and possess more health benefits including the regulation of intestinal flora (Chilliard et al., 2003). The amount of SCFA in Laoshan goat's milk is lower relative to that reported in the literature for other types of goat's milk (Talpur et al., 2009; Maroteau et al., 2014). Thus, Laoshan goat's milk may be more acceptable to the Chinese palate.

3.1.2 Comparison of SFA in Raw and Pasteurized Milk

The SFA in raw and pasteurized goat's milk accounted for 54.69% and 61.08% of the total fatty acids, respectively (Table 1, Figure 3). Significant differences can be observed between these two samples ($P < 0.01$). The concentrations of C12:0 and C14:0 were 3.13% and 8.12% in raw goat's milk and increased by 68.69% and 46.18% after pasteurization ($P < 0.05$). C16:0 was abundant in raw milk (25.59%), but 28.53% in pasteurized samples—an increase of 11.49% ($P < 0.05$). The C12:0, C14:0, C16:0, and C18:0 are the main energy sources. It has been reported that C12:0 has more risk to high cholesterol leukemia than palmitic acid. The contents of C12:0 and C14:0 in Laoshan raw goat's milk were lower than that reported in the literature (Cossignani et al., 2014). Moreover, the concentration of C18:0 was not affected significantly by pasteurization and decreased from 14.60% to 13.69% ($P > 0.05$). Fatty acids containing a cyclopropane ring in their structure (cyclopropane fatty acid) have been found in a wide variety of bacteria, a number of protozoa, and myriapoda, little is known about

cyclopropane fatty acid in mammal (Sledzinski et al., 2013). Interestingly, cyclopropanoic acid was detected in goat's milk account for 0.44%, nevertheless, decreased down to 0.17% in pasteurized milk. Fatty acid containing cyclopropanerings could display biological activity. For instance, 2-hexyl-cyclopropanedecanoic acid increased human cyclooxygenase activity (Liang et al., 2011). The eicosanoic acid (C20:0), heneicosanoic acid (C21:0), and docosanoic acid (C22:0) decreased from 0.42% to 0.20%, 0.25% to 0.20%, and 0.18% to 0.15%, respectively ($P > 0.05$). The C17:0 was decreased from 0.98% to 0.34% ($P < 0.01$). The C19:0 was not detected in pasteurized milk, but was present in raw goat's milk (0.15%).

3.1.3 Comparison of Unsaturated Fatty Acids in Raw Milk and Pasteurized Milk

Unsaturated fatty acid plays a key role in human health including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The concentration of UFA was about 37.92% and 28.03%, respectively, in raw milk and pasteurized milk—a drop of 26.08% ($P < 0.01$). The MUFA in raw milk including C16:1cis-7, C16:1cis-9, and C18:1 were 34.67%, but this decreased to 25.50% in pasteurized milk ($P < 0.01$). The PUFA included arachidonic acid (C20:4), linoleic acid (C18:2), and linolenic acid (C18:3)—they were 3.25% and 2.53% in raw and pasteurized milk, respectively ($P < 0.01$).

The C18:1 is the main indicator of fatty acids in milk. It is a neutral fatty acid that has no effect on the concentration of serum cholesterol. The content of C18:1 in Laoshan goat raw milk accounted for 33.91%, which is much higher than that in pasteurized milk (24.92%)—the concentrations were very significant ($P < 0.01$). Furthermore, the concentration of C18:1 was higher than palmitic acid (33.91% versus 25.59%) in raw milk but lower in pasteurized milk (24.92% versus 28.53%). This implies that unpasteurized milk has a lower risk of causing cardiovascular disease than pasteurized milk.

The C18:2, C18:3 and C20:4 are essential fatty acid that decrease the concentration of serum cholesterol—they cannot be synthesized in the human body. They also have a major influence on childhood development. The content of C18:2 and C18:3 were 1.87% and 1.15% in raw milk, but 1.42% and 1.11% in pasteurized milk. No significant differences were found in C18:2 and C18:3 between the two milk types. The concentration of C20:4 was 0.23%, however, it was not detected in pasteurized milk.

3.2 Stability of Milk Fat in Raw and Pasteurized Milk

Distribution of fat globule in goat's milk appeared homogen, the size of fat globule in goat's milk showed uniform and smaller than the bovine milk fat globule (Nurliyani et al., 2015). Smaller fat globules are usually better dispersed and provide a more homogeneous mixture of fat in milk (Attaie & Richter, 2000). Temperature also determines the physical state of oil droplets in O/W emulsions, which influences the emulsion properties such as viscosity and stability (Nurliyani et al., 2015).

Table 2. Changes in the sedimentation rate of milk fat in raw goat's milk and pasteurized goat's milk for 7 days

Day	Raw milk			Pasteurized milk			P
	A1	A2	R	A1	A2	R	
1st	1.241±0.005	0.183±0.005	0.147±0.006	1.208±0.007	0.209±0.002	0.173±0.002	*
2nd	1.064±0.005	0.131±0.008	0.123±0.002	1.068±0.004	0.156±0.006	0.146±0.002	**
3rd	0.947±0.001	0.072±0.008	0.076±0.002	0.928±0.007	0.087±0.004	0.094±0.003	*
4th	0.792±0.010	0.036±0.003	0.045±0.004	0.841±0.002	0.061±0.002	0.072±0.002	*
5th	0.723±0.005	0.045±0.004	0.053±0.002	0.819±0.008	0.046±0.002	0.056±0.002	NS
6th	0.689±0.006	0.020±0.007	0.030±0.002	0.756±0.005	0.033±0.005	0.043±0.003	NS
7th	0.692±0.004	0.017±0.0004	0.025±0.002	0.743±0.007	0.026±0.003	0.035±0.002	NS
P	-	-	NS	-	-	NS	-

Note. NS: $P > 0.05$, not significant; * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

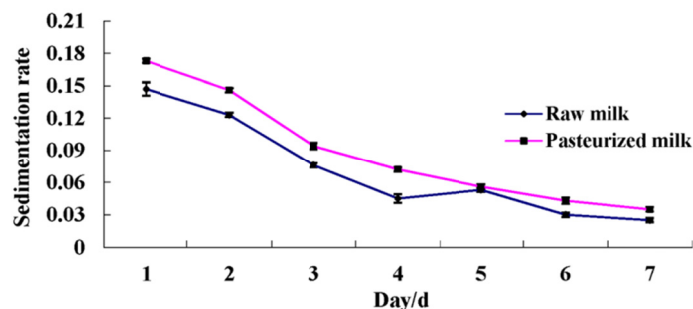


Figure 5. Changes in the sedimentation rate of fat in raw and pasteurized milk over 7 days

The fat stability changes in raw and pasteurized milk are shown in Table 2. There were no significant differences for both raw goat's milk and pasteurized goat's milk in the 7-days changes of fat stability ($P > 0.05$). Significant differences can be found between raw milk and pasteurized milk on the 1st, 2nd, 3rd and 4th day ($P < 0.05$, $P < 0.01$, $P < 0.05$, $P < 0.05$). However, no significant differences were observed on the 5th, 6th, 7th day ($P > 0.05$). Figure 5 illustrates that the stability drops over seven days both in raw milk and pasteurized milk by 82.99% (from 0.147 to 0.025%) and 79.77% (from 0.173 to 0.035%), while on the fifth day, the sedimentation rate had a slight increase by 0.18% compared with the fourth day (0.045%) in raw milk. On the 1st, 2nd, 3rd, and 4th day, the sedimentation rate increased by 17.69%, 18.70%, 23.68% and 60.00% compared with that of raw milk. Over seven days, the pasteurized milk was more stable than raw milk because the heat may cause the size of the fat granule to become smaller with a more uniform distribution (Raikos et al., 2009). To sum up, pasteurization can enhance the stability of goat's milk fat.

4. Conclusions

We detected 21 fatty acids in Laoshan goat's milk including both unsaturated and saturated fatty acids. The primary fatty acids were C16:0 (25.59%), C18:0 (14.60%), and C18:1 (33.91%). The amount of UFA relative to total was 37.92%, but the content of PUFA (3.25%) was lower, which plays a key role in human health. Thus, pasteurization in addition to the base diet can improve the composition of fatty acids in goat's milk. The content of SFA increased while UFA decreased in pasteurized milk, and the contents of SCFA, SFA and UFA in pasteurized goat's milk changed significantly compared with raw goat's milk ($P < 0.01$). Furthermore, this approach offers a simple and rapid method to detect the composition of fatty acids in milk which can handle more than eight samples in a day. This method minimizes the heavy workload and pollution, while remarkable enhancing the efficiency of treatment.

We noted that the stability of milk fat increased after pasteurization by 17.69%. The significant differences happened between raw goat's milk and pasteurized goat's milk on the 1st, 2nd, 3rd, and 4th day ($P < 0.05$, $P < 0.01$, $P < 0.05$, $P < 0.05$). During the seven days, the stability of milk fat had no significant changes both in raw goat's milk and pasteurized goat's milk ($P > 0.05$). This research provided a theoretical basis for the application of pasteurization in the goat industry.

We quantitated the composition of fatty acids and the stability of fat in Laoshan goat raw and pasteurized milk. These findings will have value for the dairy industry and will help the field to develop better quality goat's milk and provide a theoretical foundation to better utilize goat's milk and develop new products from goat's milk.

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