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Effects of calcium on physical properties and Fourier-transformed infrared spectra of raw and sterilized goat milk

Hawae, F., Pinkaew, S. and *Pakdeechanuan, P.

Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Pattani, Thailand, 94000

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Abstract

Calcium fortification induces instability of goat milk such as flocculation and sedimentation. The Fourier-transformed infrared (FTIR) spectra could be used as a tool for monitoring such as instability. The objective of this study was to investigate the changes in FTIR spectra, particle size and some properties of raw and sterilized goat milk when stimulated with calcium fortification. Either calcium citrate or calcium phosphate was fortified at 0, 25, 50, 75 and 100% of the recommended daily intake (RDI) per 200 mL of milk samples. For raw goat milk, calcium citrate lowered the pH of goat milk and increased the sediment content. Sterilization lowered the milk pH for both calcium types which can be even lowered when increasing the fortification level. The average particle sizes of raw and sterilized goat milk were 0.543 and 1.063 µm, respectively. The calcium phosphate-treated samples had larger particle sizes than the calcium citrate-treated samples, with particle sizes of 2.603 and 2.242 µm at 50% and 100% RDI, respectively. The sediment of calcium citrate-fortified raw goat milk was correlated with FTIR absorption values in lipid (3000-2800 cm^{-1}), protein (1700-1600, 1600-1500 cm^{-1}) and phosphate (1100-1000 cm^{-1}) regions with correlation coefficients of 0.89, -0.88 and 0.99, respectively. Such correlation was also found with the FTIR absorption values of the sediment of sterilized goat milk. Therefore, goat milk fortification with calcium particularly calcium citrate has resulted in altered FTIR absorption values and correlated to raw and sterilized goat milk's sediment.

1. Introduction

Goat milk is widely used in dairy products because of its high digestibility, hypoallergenic, and high nutritive values (Lou *et al*., 2018; Chen *et al*., 2019). Several studies found the lower content of αs1-casein in goat milk contributed to its hypoallergenic compared to cow milk (Bevilacqua *et al.,* 2001; Lara-Villoslada *et al*, 2004; Chen *et al*., 2018). These are the main reasons why goat milk is well accepted in terms of nutritional utilization over cow milk. The protein in goat milk is composed of 70% water-insoluble casein, 25% water-soluble whey protein, and 5% milk fat globule membrane protein (Chen *et al*., 2019). In the production of dairy products, the proteins in milk are prone to flocculation and precipitation, which has detrimental effects on the quality of goat milk products (Li *et al*., 2020). Any environmental change (temperature, pH, ionic strength) of milk would lead to a shift in the casein-mineral equilibrium, resulting in an increase or decrease in the concentration of free calcium ions, dissolution of the colloidal calcium phosphate from the micelle and release of specific casein from the micelle, and consequently causing the change of the micelle size (Luo *et al*., 2016). The occurrence of a considerable level of sedimentation (1% dry weight basis) (Deeth and Lewis, 2016) or gelation can mark the end of shelf-life and hence affect the market potential of UHT milk. The addition of calcium into milk induces changes in the mineral balance, especially an association of calcium, citrate and phosphate with the micellar phase (Philippe *et al*., 2003). Milk fortified with calcium salts causes multiple physicochemical changes, including increased concentration of ionic calcium (Ca^{2+}), reduced zeta potential and supersaturation of calcium which can destabilize milk proteins, particularly after heating (McKinnon *et al*., 2009; Lewis 2010; On-Nom *et al*., 2010). Moreover, fortification with calcium salts can cause organoleptic defects (Singh *et al*., 2007), sediment formation (Vyas and Tong, 2004) and fouling of heat exchangers during processing (Boumpa *et al*., 2008).

*Corresponding author.
Email: *patcharin.p@psu.ac.th*

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These changes affect the stability of casein micelles together with other re-equilibrated constituents, bringing towards compromised heat stability of concentrated milk and its inability to withstand severe heat treatment leading to high fouling in heat exchangers and age-gelation of the final sterilized product (Markoska *et al*., 2019). There are several methods for measuring milk instability. However, most of those methods used chemical reagents and were time-consuming. FTIR is a rapid method which is used to quantify major components in food. It has been used for a wide range of applications such as predictors of stability during the processing and storage of cow milk (Grewal *et al*., 2017), predicting coagulation properties (Chessa *et al*., 2014) or cheese yield (Bittante *et al*., 2014) and protein structure of raw cow milk. FTIR provides valuable information on protein folding, denaturation, protein-protein interaction, and sub-unit assembly, which in turn helps better understand protein functionality (Luo *et al*., 2016). The characteristics of secondary structural features such as β-sheet, β-turns, α-helix, and random coils can be identified by the characteristic of peaks appearing at specific wavenumbers of FTIR. Furthermore, protein aggregation occurring because of intermolecular β-sheet formation with the involvement of hydrogen bonds results in the appearance of new bands (Eissa *et al*., 2006). The increase in the sediment during long-term storage of UHT milk was preceded by a rise in intermolecular β-sheet (1618, 1687 and 1696 cm $^{-1}$), without an expected decrease in the intramolecular β-sheet (1630 cm^{-1}) and α-helix structures (1653 cm^{-1}) of milk proteins. These overall changes in the structure of proteins were attributed to different heat and storage-induced structure modifications and interactions of proteins.

Previous studies have used the FTIR spectrum in goat milk samples such as determining the adulteration of cow milk to goat milk (Tarapoulouzi *et al*., 2020) investigating the adulteration of soy milk to goat milk (Windarsih *et al*., 2022), discriminating the milk samples from different types of goat breeds (Salleh *et al*., 2019) and others. The instability of goat milk is also important for the goat milk industry, therefore, using FTIR as a tool to monitor the instability has the advantage of being fast and applicable to the goat milk processing industry. This study aimed to investigate the changes in FTIR spectra, particle size, and some properties of goat milk as well as the correlation of FTIR spectra and sediment content upon calcium induction of raw and sterilized goat milk.

2. Materials and methods

2.1 Milk sample preparation

Raw fresh goat milk was collected from a farm in Pattani province, Thailand. It was bulk milk from 30 dairy goats, 75% crossbred Saanen (Thai native×Saanen), with 60±10 days in milk. The collected sample was packed in a polypropylene plastic bag, kept in a foam box (4±2°C) and transported to the lab within 1 hr. The chemical composition of milk was analyzed using the Association of Office Analytical Collaboration (AOAC) International (2000) method. The milk pH was 6.69±0.01 and chemical compositions per 100 g of fresh milk were 12.76±0.12 g of total solid, 3.52±0.04 g of protein, 4.43±0.06 g of fat and 5.08±0.35 g of lactose. The pH and milk compositions were matched with the Thai Agricultural Standard of Raw Goat Milk (TSA 6006-2008). Calcium citrate ($Ca_3(C_6H_5O_7)_2$) (A.R., Sigma-Aldrich, USA) and calcium phosphate ($Ca_3(PO_4)_2$) (A.R., HiMedia, India) were added to raw fresh goat milk at room temperature (25±2°C) at 0, 25, 50, 75 and 100% of Thai Recommended Daily Intakes (Thai RDI) per 200 mL with the Thai RDI value of calcium was 800 mg. For sterilized milk, the milk sample was heated to 55°C and homogenized at 2,500/500 psi with a two-stage pressure homogenizer (model PHB-100A, Thailand), then filled in the retort pouch of 200 mL each and sterilized at 112°C, F0 = 3 mins in a water spray retort.

2.2 Analytical procedure

2.2.1 pH

The pH was measured at room temperature (25°C) using a pH meter (pH Mettler, S20 evenEasy, ToledoTM).

2.2.2 Dried sediment percentage

The sediment was measured by a centrifugation method (Katsiari *et al*., 2002). The milk sample was well shaken and approximately 40 mL was accurately weighed, then poured into a calibrated tube and centrifuged at 1,540×*g* for 10 mins. Subsequently, the supernatant was discarded and the sediment was dried in a hot air oven (model UNB 500 Mermert, German) at 105°C to get a constant weight (weight of dried pellet). The percentage of dry weight was calculated based on the following equation:

% Dry Sediment = (weight of dried pellet × 100)/weight of sample

2.2.3 Milk particle diameter

The particle size distribution of milk was analyzed by laser particle size diffraction (Beckman coulter, USA). The skim milk was separated from the milk sample by the centrifuging process and then diluted at a ratio of 1:100 (Wang *et al*., 2017). The refractive index for casein micelles in milk and the dispersant was set at 1.57 and 1.34, respectively. Each sample was measured

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in triplicates.

2.2.4 Fourier-transformed infrared spectra

FTIR spectra were acquired in duplicate within the range of 4000-400 cm^{-1} using a Bruker FTIR spectrometer (Bruker, Tensor27, USA) with a resolution of 4 cm^{-1} and an average of 16 scans for each spectrum as described by Grewal *et al*. (2017). Approximately 0.5 mL of milk sample was added into an attenuated total reflectance (ATR) cell. A background spectrum was scanned at the beginning of the measurements with DI water using the same instrumental conditions as for the sample spectra acquisition. The amide I region (1700-1600 cm^{-1}) was used for calculating the second derivative of the spectra (Markoska *et al*., 2019). Spectral of dried sediment samples were also acquired to estimate the composition of sediment fraction. At the beginning of the measurement process, the background spectrum was scanned with a blank diamond ATR cell.

2.3 Statistical analysis

The data were presented as mean ± SD and all data were analyzed based on the analysis of variance (ANOVA). Significant differences between means were analyzed by using Duncan's multiple range test (Steel and Torrie, 1980) at a 5% probability level ($p<0.05$). The Pearson correlation (r) was calculated to define the statistical relationship between the dried sediment of calcium-fortified milk and the FTIR absorbance.

3. Results and Discussion

3.1 pH

The type of calcium affected the pH in both raw and sterilized milk samples (Table 1). Calcium citrate-fortified raw goat milk resulted in a decrease in milk pH, whereas a similar result was found in the report of Pawlos *et al*. (2022). The addition of cations induced a displacement of calcium and consequently increased ionic calcium which could interact with inorganic phosphate and citrate. Then, the displacements of equilibrium between $H_2PO_4^-$ and HPO_4^{2-} and $citrate^{2-}$ and $citrate^{3-}$ occurred. As a result of these processes, displacements were responsible for H^+ liberation, hence decreasing the pH (Philippe *et al*., 2005). Increasing calcium citrate and calcium phosphate from 0% RDI to 100% RDI resulted in a decrease in milk pH. The pH of milk fortified with calcium citrate was lower than when it was fortified with calcium phosphate. Sterilization reduced the pH of the milk in both calcium citrate-added and calcium phosphate-added treatments. This occurred due to the high temperature induced in transferring calcium and phosphate from the micelles to the colloidal phase, thus causing the Ca^{2+} to increase and change of milk pH (On-Nom *et al*., 2010). Another reason might be due to the presence of lactose in milk breaking down into formic acid through isomerization and degradation (Raynal-Ljutovac *et al.* 2007; Chandrapala *et al*., 2010). The addition of calcium citrate in sterilized goat milk from 0% to 100% RDI resulted in a decrease in milk pH from 6.41 to 6.35 ($p<0.05$) and the calcium phosphate addition from 0% to 100% RDI decreased the pH of milk from 6.42 to 6.36 ($p<0.05$).

3.2 Dried sediment percentage

The sedimentation of 1% dry weight can indicate the end of shelf-life and affect the market potential of sterilized milk (Deeth and Lewis, 2016). Increasing the calcium addition has resulted in an increase in the percentage of dried sediment (Table 2) for both types of calcium. In raw goat milk, the sediment content increased significantly ($p<0.05$) from 0.25 to 0.71 g/100 g and 0.25 to 0.39 g/100 g when added with calcium citrate and calcium phosphate at 25% RDI. The results obtained were found to be even higher when calcium was added up to 100% RDI, whereby the sediment increased to 1.69 and 1.86 g/100 g respectively. The calcium salt increases the binding of calcium ions to free inorganic phosphate and citrate, forming calcium phosphate and calcium citrate complex in micelles and destabilizing goat milk protein (Huang et al., 2022). For the sterilized milk, high temperature caused increasing milk sediment due to the denaturation and coalescence of protein. In this study, with an addition of 25% RDI, the sediment content of sterilized milk fortified with calcium citrate or calcium phosphate has increased from 0.35 to 2.06 g/100g and 0.57 g/100 g, respectively. Meanwhile, adding calcium citrate and calcium phosphate at 100% RDI, the sterilized milk contained 3.89 and 2.16 g/100 g of dry sediment, respectively. It was found that the

Table 1. Effect of calcium on pH of raw and sterilized goat milk.

Type of milk	Type of calcium	Level				
		0% RDI	25% RDI	50% RDI	75% RDI	100% RDI
Raw milk	CC	6.77±0.01^{a}	6.49±0.01^{c}	6.51±0.01^{c}	6.49±0.01^{c}	6.46±0.02^{d}
	CP	6.77±0.01^{a}	6.77±0.01^{a}	6.76±0.01^{ab}	6.76±0.01^{ab}	6.74±0.01^{b}
Sterilized milk	CC	6.41±0.02^{a}	6.37±0.01^{b}	6.37±0.01^{b}	6.36±0.01^{c}	6.35±0.01^{c}
	CP	6.42±0.02^{a}	6.41±0.01^{a}	6.41±0.01^{a}	6.36±0.01^{c}	6.36±0.01^{c}

Values are presented as mean±SD. Values with different superscripts within the same column of the same type of milk are statistically significantly different ($p<0.05$). CC: Calcium citrate, CP: calcium phosphate

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Table 2. Dried sediment percentage of raw and sterilized calcium-fortified goat milk (g/100 g).

Type of milk	Type of calcium	Level				
		0% RDI	25% RDI	50% RDI	75% RDI	100% RDI
Raw milk	CC	0.25±0.03^{h}	0.71±0.03^{f}	0.90±0.02^{e}	1.27±0.05^{c}	1.69±0.05^{b}
	CP	0.25±0.03^{h}	0.39±0.04^{g}	1.09±0.02^{d}	1.60±0.02^{b}	1.86±0.14^{a}
Sterilized milk	CC	0.35±0.08^{g}	2.06±0.10^{d}	3.01±0.11^{c}	3.58±0.19^{b}	3.89±0.10^{a}
	CP	0.35±0.08^{g}	0.57±0.03^{fg}	0.87±0.01^{de}	0.80±0.05^{ef}	2.16±0.05^{d}

Values are presented as mean±SD. Values with different superscripts within the same column of the same type of milk are statistically significantly different (p<0.05). CC: Calcium citrate, CP: calcium phosphate

sterilized goat milk with added calcium citrate had more precipitation than calcium phosphate. The sediment most probably from the reaction of citrate anion binds to the denatured protein (Boumpa *et al*., 2008).

3.3 Average particle size diameter

Six samples were replicated to measure the particle size of the following treatments; raw goat milk, sterilized goat milk, sterilized goat milk fortified with calcium citrate to 50% or 100% RDI and sterilized goat milk fortified with calcium phosphate to 50% or 100% RDI. Figure 1 shows the particle size diffraction in terms of % differential volume which was calculated to particle size in each treatment. The average particle size of raw goat milk and sterilized goat milk were 0.543 and 1.063 µm, respectively. Sterilization caused milk casein to lose its stability, while the calcium phosphate was dissociated, hence causing the free calcium ion to induce the aggregation of casein proteins (Grewal, Chandrapala, Donkor, Apostolopoulos and Vasiljevic, 2017). In addition, whey protein was denatured by sterilization and the whey monomers then formed polymers through disulfide bonds, and β-lactoglobulin and κ-casein formed casein-whey protein aggregation by thiol-disulfide bond exchange reactions (Lin *et al*., 2018).

The addition of Ca compounds induced the increase in particle size (Figure 1). Sterilized goat milk with added 50% RDI of calcium citrate had an average particle size of 1.417 µm which was 33% larger than that of non-fortified milk. The calcium phosphate-treated sample had larger particle sizes than the calcium citrate-treated samples with particle sizes of 2.603 µm and 2.242 µm, at 50% and 100% RDI, respectively.

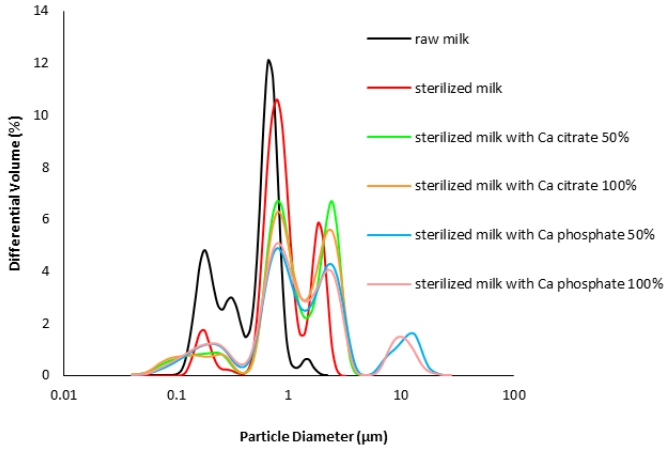

Figure 1. Particle diameter of raw, sterilized and calcium-fortified sterilized goat milk.

3.4 Fourier-transformed infrared spectra

Figure 2 shows the FTIR peak between 3500 and 3000 cm-1 corresponding to hydrogen bonding (O-H stretching). According to the report by Grewal, Chandrapala, Donkor, Apostolopoulos and Vasiljevic (2017) the peaks between 3000 and 2800 cm^{-1} show the C-H stretch and peaks between 1800 and 1700 cm^{-1} show the C=O stretching vibrations of ester linkage pertaining to triacylglycerols. The peaks between 1700 and 1500 cm^{-1} region have two main parts, namely amideI (1700-1600 cm^{-1}) corresponding to C=O stretching vibrations of the peptide bonds and amideII (1600-1500 cm^{-1}) attributed to C-N stretching vibrations in combination with N-H bending, respectively. The peaks between 1200 and 900 cm^{-1} embody the characteristic peaks of various C-O vibrations in carbohydrates (Zhou *et al*., 2006) and the bands around 995 and 987 cm^{-1} indicated changes in stretching vibration of $-PO_3^{2-}$ moiety of the serine-phosphate residue (Grewal *et al*., 2018).

The FTIR spectra analyzed the correlation coefficient using Pearson's correlation (r) to determine the statistical relationship between sediment content and absorbance of calcium-fortified milk (Table 3). When the calcium citrate was added to raw milk, it was found that there was a decrease in the FTIR absorption value in the protein region (C=O) and an increase in FTIR absorbance in lipid, protein (C-N) and phosphate region associated with increased sediment content. The sediment of raw goat milk fortified with calcium citrate was found to be correlated with absorbance in the lipid (3000-2800 cm^{-1}), protein (1700-1600, 1600-1500 cm^{-1}) and phosphate (1100-1000 cm^{-1}) exhibiting a correlation coefficient of 0.89, -0.88 and 0.99, respectively. Zhao *et al*. (2021) reported that the addition of calcium salt could destroy the goat milk casein. The peak at 1075 cm^{-1} has been related to the presence of covalently bonded phosphate groups to the casein in milk (Jaiswal *et al*., 2015), while greater intensity for this peak may indicate

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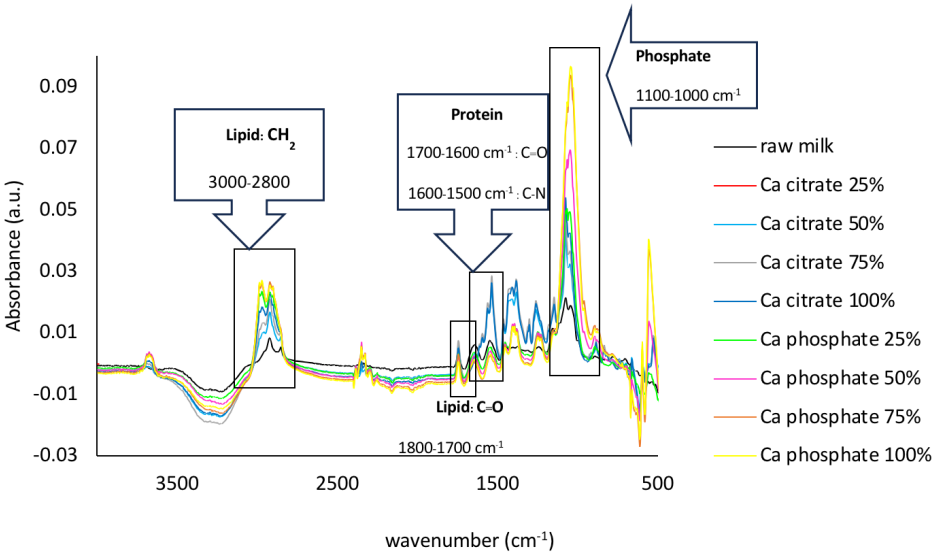

Figure 2. FTIR spectra of raw goat milk and calcium-fortified goat milk.

Table 3. Coefficient correlation of sediment and FTIR absorbance of calcium-fortified goat milk.

Type of milk	Type of calcium	Lipid		Protein			Phosphate
		CH_2 asymmetric	CH_2 symmetric	C=O	C=O	C-N	PO_3^{2-}
Raw milk	CC	0.89*	0.59	0.12	-0.88*	0.90*	0.99*
	CP	0.52	0.42	-0.15	-0.57	-0.5	0.67*
Sterilized milk	CC	-0.39	-0.48	-0.75*	-0.72*	-0.64*	-0.13
	CP	-0.16	-0.13	-0.1	0.54	-0.87*	-0.29

*correlation (Pearson's correlation) is significant at the 0.05 value level. CC: Calcium citrate, CP: calcium phosphate.

an association of addition of $CaPO_4$ with the micelle. On the other hand, the IR absorbance of both types of calcium-enriched sterilized goat milk at the phosphate region was not correlated with the sediment contents.

4. Conclusion

The addition of calcium citrate and calcium phosphate decreased milk pH and increased the sediment content of raw and sterilized goat milk. The particle size of calcium phosphate-fortified sterilized goat milk was larger than that fortified with calcium citrate. The sediment of calcium citrate-fortified raw goat milk was found to be correlated with FTIR absorption values in lipid (3000-2800 cm^{-1}), protein (1700-1600, 1600-1500 cm^{-1}) and phosphate (1100-1000 cm^{-1}) regions. The FTIR absorbance was also related to the sediment content of fortified sterilized goat milk. Thus, changes in FTIR spectra could be used as a tool to predict the instability of goat milk.

Conflict of interest

The authors declare no conflicts of interest.

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