

RESEARCH ARTICLE

Impact of ultrasound processing on some milk-borne microorganisms and the components of camel milk

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ABSTRACT

Inactivation of pathogenic bacteria *Escherichia coli* O157: H7 and *Salmonella Typhimurium* in camel milk was investigated using ultrasound processing (900 W, 20 kHz, 100% power level). In addition, the effect of ultrasound treatment on raw camel milk components was studied to detect changes in fatty acid profile, lipid peroxides, protein fractions, and volatile compounds. Bacterial strains (10^6 CFU/ml) were added to pasteurized camel milk samples (70 ml) and transferred into a sterile aluminum container (30 mm x 120 mm, 100-ml total capacity) and then subjected to continuous ultrasound processing for 15 min in an ice water bath using a 13-mm diameter probe. The standard plate count (SPC) agar method and the in vivo imaging system (IVIS) were used to evaluate the viability of bioluminescence-transformed bacteria (*E. coli* O157: H7 and *S. Typhimurium*). The continuous ultrasound processing of camel milk resulted in significant ($P < 0.05$) reductions in *S. Typhimurium* and *E. coli* O157: H7. Relative to unsonicated raw camel milk, the *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and *trans*-10, *cis*-12 CLA contents were not affected ($P > 0.05$) by the ultrasound processing. The TBAR values, a marker of lipid peroxidation, and milk protein fractions were also similar ($P > 0.05$) between the sonicated and unsonicated raw camel milk. A total of 24 volatile compounds (VC) were identified including 8 aldehydes, 3 ketones, 5 acids, 5 esters, 2 aromatic hydrocarbonate, and 1 sulfo compound. Of these 24 VC, eleven VC increased ($P < 0.05$) and seven decreased ($P < 0.05$) after sonication. In conclusion, the results of this study showed that ultrasound processing of camel milk was efficient in inactivating subsets of milk-borne pathogens without detrimental effects on camel milk fatty acids, lipid peroxides, and protein fractions. However, there were some changes in milk VC which may affect the sensory quality of milk.

Keywords: Camel milk; Ultrasound processing; Pathogenic bacteria; Milk components

INTRODUCTION

The global camel population is estimated to be around 30 million heads to support the survival and transportation of desert dwellers (Faye et al., 2013). Camel milk has potential therapeutic characteristics (Hammam and Agriculture, 2019), in addition to its main role in the human diet, by acting as an antihypertensive (Ayyash et al., 2018), antidiabetic (Bussa et al., 2017), and anticarcinogenic manner (Habib et al., 2013). Nevertheless, raw camel milk harbors a wide range of pathogenic microorganisms, such as *E. coli* O157: H7, *S. Typhimurium* (Abeer et al., 2012), *Staphylococcus aureus*, *Coxiella burnetii*, *Helicobacter pylori* (Verraes et al., 2014), and *Bacillus cereus* (Omer and Eltinay, 2008).

Milk pasteurization was presented in the mid-nineteenth century as a certified technique to eliminate the most

dangerous and heat resistant milk pathogens such as *Coxiella burnetii* and *M. tuberculosis* (Jay, 1992). However, some pathogenic bacterial strains such as *E. coli* O157: H7, have the ability to survive and form biofilms inside the pasteurization equipment (Stopforth et al., 2003; Malek et al., 2012; Marchand et al., 2012). Additionally, non-thermal technologies, such as high hydrostatic pressure (HHP), pulsed electric field (PEF), ultrasound (US), cold plasma (CP), and ultraviolet (UV) have been investigated in the past few decades, aimed at inactivating microorganisms and undesirable enzymes without affecting the nutritional and sensory properties of foods (Lado et al., 2002).

The use of ultrasound treatment to inactivate bacterial cells was first reported in the late 1920s (Harvey and Loomis, 1929). Since then, this technology has gained a great interest in the food industry. The ultrasound technique is

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sound waves that have the ability to travel through liquid, gas, and solid materials with a frequency range greater than 20 kHz. In recent years, ultrasound treatment has been tested for enzymatic and microbial inactivation in different foods, such as fruit juices (Tiwari et al., 2008) milk and apple cider (D'amico et al., 2006). Cameron et al. (2009) reported the elimination of *E. coli* and *Pseudomonas fluorescens* from milk to zero levels using ultrasonication without any negative impacts on milk total fat, protein, and lactose content. In another study, D'amico et al. (2006) reported significant reductions in milk and apple cider microbial levels following ultrasound treatment. In liquid foods, ultrasound processing improves quality parameters, such as viscosity and homogenization, but it may also have an unfavorable impact on the appearance of off-flavors, degradation of specific food compounds and changes in the physicochemical parameters or structures of food components (Pingret et al., 2013). To our knowledge, no previous studies have looked at the effect of ultrasound treatment on camel milk pathogens and components. Therefore, the objectives of this study were to investigate the influence of ultrasound processing on the viability of *E. coli* O157: H7 and *S. Typhimurium* in camel milk, and the effects of this technology on fatty acid profile, lipid peroxides, protein fractions, and volatile compounds in camel milk.

MATERIALS AND METHODS

Bacterial strains

Non-pathogenic gram-negative *Escherichia coli* O157: H7 (ATCC 43888) and *Salmonella Typhimurium* (ATCC 14028) were kindly provided by Dr. Jean Feugang, Department of Animal and Dairy Sciences, Mississippi State University. Both strains were cultured in Luria-Bertani (LB) broth (LB broth, Becton Dickinson, Sparks, MD, USA) at 37 °C with shaking (250 rpm) and transformed for bioluminescence emission through electroporation of pXen5-luxCDABE (Caliper life sciences, Hopkinton, MA, USA) containing ampicillin resistance gene into the target strains. Following culture in plates containing the LB agar medium with ampicillin (100 µg/ml), all plates were placed in the In Vivo Imaging System (IVIS Lumina XRMS Series III – PerkinElmer, Waltham, MA, USA) to visualize bacterial colonies that successfully integrated the transgene (pXen5-luxCDABE+AMP). These positive or bioluminescent clones were identified and selected to grow on LB broth for 18-24 hours at 150 rpm until stationary-phase.

Additionally, different *E. coli* O157: H7 (NCTC strain 12900) and *S. Typhimurium* (NCTC strain 12023) strains (The Global Bioresource Center, Manassas, Virginia, USA). Freeze-dried cells of both strains were activated according

to the manufacturer guideline. One milliliter of the stock culture of each strain was transferred into 10 ml tryptic soy broth (Difco Laboratories, Detroit, MI, USA), and then grown at 37 °C for 24 hours. Thereafter, 10 µl of loop inocula were transferred into 9 ml tryptic soy broth and incubated at 37 °C for at least 18 hours to achieve stationary-phase.

Bacterial concentrations were determined using the optical density measurements at 600 nm with a GENESYS 2 Spectrophotometer (Thermo Spectronic, New York, USA) as reported by (Gera and Doores, 2011). A conversion value of 0.01 optical density equal to 8.0×10^6 colony-forming unit/ml (CFU/ml) was used. Milk samples received initial inoculations of 10^6 CFU/ml (6 logs CFU/ml) of each bacterial strain.

Milk sample preparations

Raw camel milk samples were obtained in 250 ml sterile glass bottles (Frank and Friends Camel Dairy Farm, Morton-Pelahatchie, Mississippi, USA), and immediately transported in an ice-cooled box to the laboratory (Department of Animal and Dairy Sciences, Mississippi State University). Subsets of raw milk were pasteurized in the laboratory by heating 75 ml of raw camel milk in 100 ml glass tube at 65 °C for 30 min in a water bath, as previously reported by Sela et al. (2003). Pasteurized milk was subsequently cooled to 4 °C and serially diluted (ten-fold decimal from 10⁻¹ to 10⁻⁴) with sterile phosphate buffered saline (0.2 M PBS, pH7.5) as the diluent, using deionized water. The PBS (0.2 M) was prepared as described by Christian and Purdy (1962) and the pH was adjusted to 7.5 by the addition of 2M NaOH.

Ultrasound treatments

Raw and pasteurized camel milk samples were transferred into the sterile aluminum container (30 mm by 120 mm), which served as the treatment chamber, with a 100 ml total capacity. An ultrasonic processor (Ultrasonic Processor FS-900N, Hanchen Instrument, China), set at 900 W, 20 kHz (frequency is auto-tracking), with a 13-mm diameter probe was introduced into the aluminum container. To avoid contamination, the probe was immersed in 70% alcohol for 1 minute and left to air dry. The ultrasonic probe tip was immersed in the sample, in the center of the aluminum container, about 30 mm away from the aluminum container's bottom (Fig. 1). To determine the reduction of bioluminescent intensity and CFU measurements for non-bioluminescent bacteria, an aliquot (1 ml) of each strain culture was added in aseptic conditions to the pasteurized milk sample (70 ml) to yield an approximate 10^6 CFU/ml. A 10-ml sample was withdrawn after shaking and placed into 10-ml sterile vial before the sonication treatment; this was the 0-time sample (control). Milk samples were then

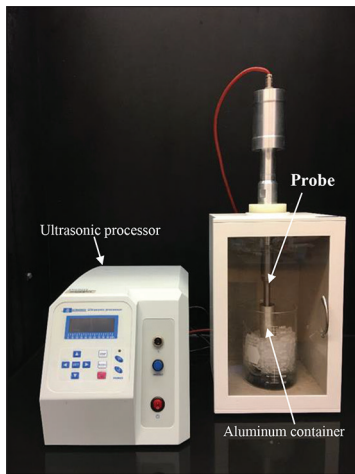


Fig 1. Ultrasound unit.

subjected to different ultrasonic treatment times (5, 10, 15 min) at 20 ± 3 °C.

In order to control the milk's temperature during treatment, the aluminum container was placed in an ice-water bath in order to maintain a constant temperature that did not exceed 23 °C. After subjecting the samples to the ultrasonic treatment, 1 ml of the sonicated milk was used immediately taken for bacterial analysis and approximately an additional 20 ml sample was collected and stored at -20 °C for components analysis. The experiments were carried out in triplicate for each sample.

Bacterial analysis

Total aerobic cell count

The total aerobic cell counts for the sonicated and unsonicated camel milk samples were measured using aerobic count plates (ACP) Petrifilm (3M, St. Paul, MN, USA). Briefly, the control and sonicated milk samples were serially diluted in PBS. Per the milk sample, one-milliliter of each dilution (10^{-1} to 10^{-4}) was plated in triplicate on ACP Petrifilm. The 3M Petrifilm plates were then incubated at 37 °C for 24 hours. All plates were read using an electronic counter 3M Petrifilm Plate Reader (3M Petrifilm Plate Reader Model 6499, St. Paul, MN, USA).

Bacterial growth evaluation

The standard plate count method was used to determine the viability of *E.coli* O157: H7 and *S. Typhimurium* in camel milk before and after sonication treatment. The control and sonicated camel milk samples that previously inoculated with *E.coli* O157: H7 and *S. Typhimurium* were 10-fold diluted and 100 µl of each dilution was spread on the surface of a selective media for each strain and plating triplicate for each dilution. The selective chromogenic medium Hicrome™ Salmonella Agar, improved (Sigma-Aldrich, St. Louis, USA) was used for *E.coli* O157: H7 and a selective medium Brilliant

Green Agar, modified (Sigma-Aldrich, St. Louis, USA) for *S. Typhimurium*. All bacterial plates were incubated at 37 °C for 24 hours and bacterial colonies were enumerating based on CFU/ml. Simultaneously, the viability of bioluminescence *E.coli* O157: H7 and *S. Typhimurium* was measured in photon per second (P/S) with an IVIS to visualize the survival of bacterial cells. All microbiological analyses were conducted in triplicate for each ultrasound experiment.

Components analyses

Analyses were performed before and after ultrasound treatment to evaluate effects on:

Fatty acids

The fatty acid profile of camel milk was analyzed with Gas Chromatography (GC - Shimadzu Scientific Instruments, Colombia, MD, USA) using the procedure outlined by Kramer et al. (1997). The GC temperature program and program settings were adjusted as described by AbuGhazaleh and Holmes (2007).

Lipid oxidation

The extent of oxidation in camel milk samples before and after sonication was assessed using the ThioBarbituric Acid Reactive Substances (TBARS) test which measures malondialdehyde, the secondary product of oxidation in the samples. The TBARS chemically react with lipid peroxidation components in the milk, resulting in colorimetric changes in samples that are spectrophotometrically measured at 532 nm, as described by Spanier and Taylor (1991).

Protein fractions

Protein fractions of the control and sonicated camel milk samples were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using X Cell Sure Lock Mini-Cell Electrophoresis System (Invitrogen, Carlsbad, CA, USA). Protein concentrations were measured using the Pierce Coomassie plus assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts among samples were mixed with the loading sample buffer (Nupage LDS - Thermo Fisher Scientific, Waltham, MA, USA), incubated at 70 °C for 10 min, centrifuged at $14,000 \times g$ for 20 min, and supernatants were loaded, together with a molecular weight marker (20 kDa to 118 kDa - Fisher's EZ-Run Pre-stained Protein Marker, USA), onto a 4-12% SDS-gradient gel electrophoresis (Bis-Tris Nupage Mini Gel - Thermo Fisher Scientific, Waltham, MA, USA). Following electrophoresis, the gels were stained with Coomassie brilliant blue R-250 staining solution (Bio-Rad, Hercules, CA, USA) for protein band visualization.

Volatile compounds

Extraction and concentration of volatile compounds in the control and sonicated milk samples were performed

by solid-phase microextraction (SPME) according to Thompson-Witrick et al. (2015). Briefly, a SPME fiber (50/30 μm DVB/Carboxen/PDMS, Supelco, Inc., Bellefonte, PA, USA) was exposed to the headspace above 10 mL of milk sample, and 30% w/v of salt in 20 mL headspace vials with Teflon-lined silicone septa (Chromacol, Fisher Scientific) for 30 minutes at 40 °C with an agitation speed of 250 rpm. Samples were equilibrated at 40 °C for 60 minutes prior to exposing the fiber. A MultiPurpose Sampler MPS XL (Gerstel, Linthicum, MD) SPME autosampler was used for the automation of extraction and injection. Volatile compounds were separated using a nonpolar Agilent-19091S column (Agilent; 30 m * 0.25 mm id * 0.25 μm film thickness) with He as the carrier gas at a flow rate of 2.0 mL/min (linear velocity 53.8 cm/sec). The GC oven temperature program was 35 °C held for 5 minutes and then increased to 225 °C at a rate of 6 °C/min. Once the final temperature of 225 °C was reached, it was maintained for 10 minutes. The MS was maintained at 200 °C and the sample mass was scanned in the range of 40 – 400 amu.

Volatile compounds were identified based upon their retention index values (RI) using nonpolar (DB-5) columns (30m x 0.25 mm i.d., 0.25 μm film; J&W, Folsom CA). The RI values were compared to literature values. Aliphatic hydrocarbon standards were analyzed in the same manner using a DB-5 column to calculate RI:

$$RI = 100N + 100n (tRa - tRn) / (tR (N+n) - tRN)$$

N is the carbon number of the lowest alkane and n is the difference between the carbon numbers of the two n-alkanes that are bracketed between the compounds; tRa, tRn, and tR (N+n) are the retention times of the unknown compound, the lower alkane, and the upper alkane.

Statistical analysis

The JMP predictive analytics software (Version pro 14.0) was used for statistical analyses. All data were analyzed with a one-way ANOVA, followed by Tukey's multiple comparison test at a significant level of $P < 0.05$.

RESULTS

Impact of ultrasound processing time on the viability of *E. coli* O157: H7 and *S. Typhimurium* in pasteurized camel milk

The survival curves of *E. coli* O157: H7 and *S. Typhimurium* in the mid-stationary phase exhibited almost the same inhibition pattern after 10 minutes of ultrasound processing. Complete inactivation of *E. coli* O157: H7 and 4.4 log reduction of *S. Typhimurium* were achieved after 15 minutes of ultrasound treatment (Fig. 2).

Enumeration and monitoring of surviving cells

The standard plate count results revealed that ultrasound processing of raw camel milk for 15 minutes resulted in a 2 log CFU/ml reduction ($P < 0.05$) in total aerobic bacteria compared to the control (Table 1). The initial bacterial population in raw camel milk was approximately 6 log CFU/ml. Additionally, sonicating camel milk for 15 minutes resulted in the total elimination of *E. coli* O157: H7 and a 4.4 log reduction in *S. Typhimurium* (Table 1). Bacterial bioluminescence emission in camel milk was monitored before and after ultrasound processing in 30 ml universal tubes using IVIS to monitor the survival of *S. Typhimurium* and *E. coli* O157: H7 (Fig. 3). The amount of bioluminescence in camel milk decreased after sonication from 4.55E+08 P/S for *E. coli* O157: H7 and 2.54E+09 P/S for *S. Typhimurium* before sonication to 1.36E+05 P/S and 3.84E+05 P/S after sonication, respectively. The results of the SPC method coincided with the photon measurements in the region of interest (ROI) which was taken concurrently using IVIS imager.

Impact of ultrasound processing on camel milk components

Fatty acid profile

The effect of sonication on the concentration of fatty acids are presented in Table 2. Except for a slight reduction

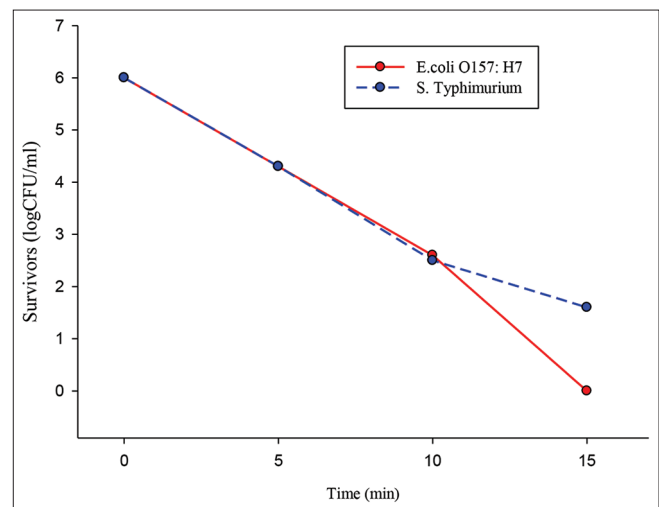


Fig 2. Survival curves of *E. coli* O157: H7 and *S. Typhimurium* treated with ultrasound waves at a different time period in pasteurized camel milk.

Table 1: Effect of sonication process on the total viable count in raw camel milk and on the surviving of *E. coli* O157: H7 and *S. Typhimurium* in pasteurized camel milk

Bacterial Strains	Control*	Sonication*	Log Reduction*
Total viable count	5.9	3.9	2.0
<i>E. coli</i> O157: H7	6.0	ND ^a	6.0
<i>S. Typhimurium</i>	6.0	1.6	4.4

*Count (log CFU/ml); ^aND, not detected (detection limit is < 1 CFU/ml)

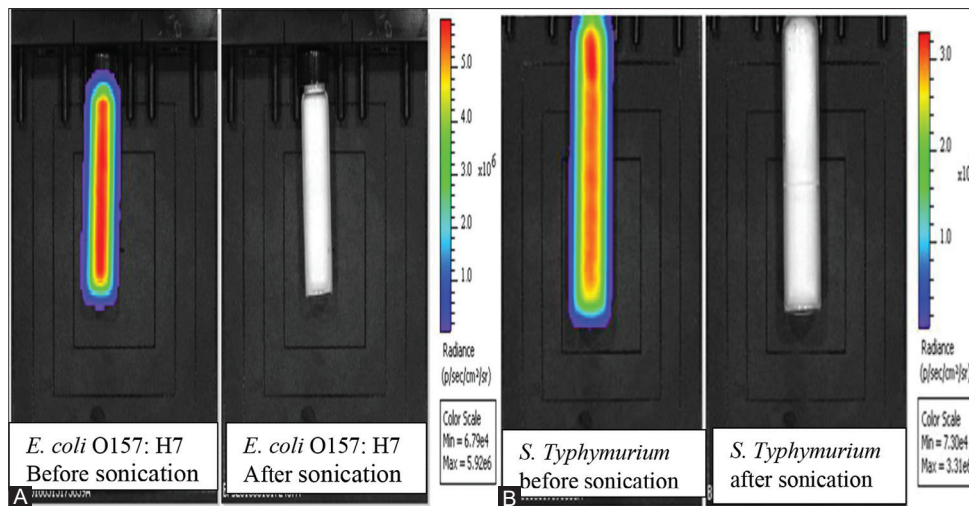


Fig 3. Quantification of bioluminescent *E. coli* O157: H7 (A) and *S. Typhimurium* (B) in pasteurized camel milk before and after sonication.

Table 2: Average fatty acids profile (g/100g fatty acids) for raw and sonicated camel milk

Fatty acid	Raw milk	Sonicated	SEM	P-value
C6:0	0.32	0.36	0.022	0.24
C8:0	0.32	0.37	0.024	0.28
C10:0	0.23	0.25	0.011	0.36
C12:0	0.86	0.88	0.017	0.57
C14:0	9.55	9.59	0.036	0.52
C14:1	0.79	0.80	0.009	0.57
C16:0	22.88	22.71	0.065	0.17
C16:1	5.16	5.15	0.009	0.81
C18:0	17.58	17.57	0.183	0.95
C18:1 <i>trans</i>	5.83	5.57	0.015	0.01
C18:1c9	20.80	20.60	0.068	0.13
C18:1c11	0.43	0.42	0.003	0.17
C18:2 t9t12	0.11	0.11	0.0007	0.12
C18:2 c9c12	3.74	3.74	0.009	0.79
C18:3n6	0.38	0.38	0.002	0.17
C18:3n3	0.53	0.53	0.001	0.38
C20:1n9	0.07	0.06	0.0004	0.02
C20:4n6	0.20	0.20	0.001	0.84
C20:5n3 (EPA)	0.01	0.01	0.005	0.55
C22:5n3	0.09	0.11	0.016	0.41
C22:6n3 (DHA)	0.01	0.04	0.008	0.08

($P < 0.05$) in C18:1 *trans* and C20:1n9, no significant changes were detected between the control and the sonicated milk samples. Two conjugated linoleic acids (CLA) (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) were evaluated as well and no significant changes ($P > 0.05$) were detected between the control and the sonicated milk samples (Fig. 4).

Lipid oxidation

The effect of treatments on camel milk TBARS values is presented in Fig. 5. No significant differences ($P > 0.05$) in TBARS values were observed between the control and the sonicated milk samples.

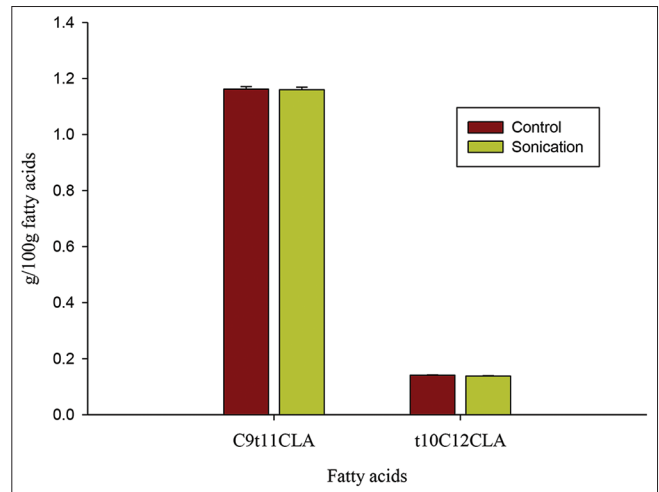


Fig 4. Effect of sonication on *cis*-9, *trans*-11, CLA and *trans*-10, *cis*-12 CLA in raw camel milk.

Milk protein

Representative gel electrophoreses of ultrasonicated and control camel milk samples are presented in Fig. 6. In lane 1, it is possible to observe four protein bands with apparent molecular weights of 15, 35, 37, and 88 kDa, respectively. The sonicated camel milk (lane 2) had the same bands in addition to a new band with a molecular weight of 65 kDa. The SDS-PAGE profile of camel milk samples showed no significant differences ($P > 0.05$) in protein molecular weight between the control and sonicated samples (Fig. 6).

Volatile compounds

A total of 24 VC were identified using a combination of retention index and mass spectral matching against the NIST library standards (Table 3). The total number of VC identified in the camel milk prior to sonication was 13. Of these 13 compounds, seven compounds

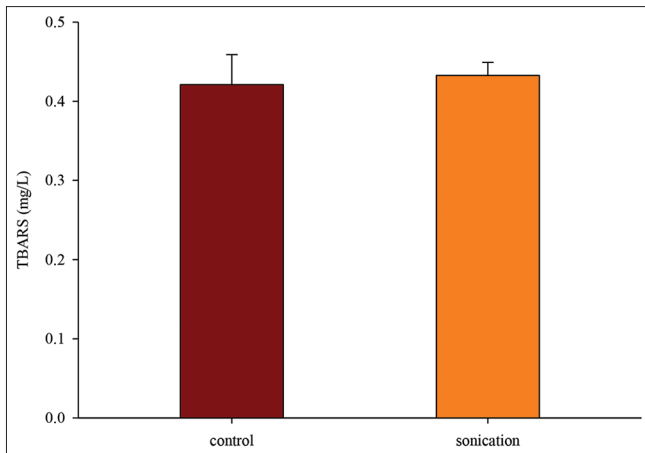


Fig 5. Effect of sonication on TBARS in raw camel milk.

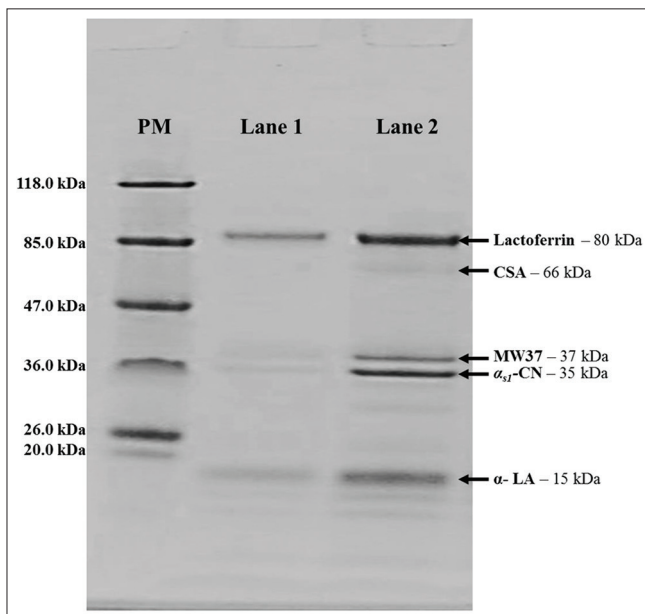


Fig 6. Effect of sonication process on protein fractions of raw camel milk (lane 2) compared to the non-sonicated (control) raw camel milk (lane 1). PM = protein marker.

(Nonanal, decanal, undecanal, 2- undecanal, dodecanal, β -hydroxydodecanoic acid, and 2- heptadecanone) disappeared after sonication, two compounds (methyl salicylate, 2-pentadecanone) decreased ($P < 0.05$) and four were not affected ($P > 0.05$). Sonication also resulted in the formation of eleven VC that were not detected in milk before sonication and these compounds were (O-cymene, γ -Terpinene, 4-nonenal, methyl caprate (octanoate), octanoic acid, β -phenethyl acetate, methyl caprate (decanoate), ethyl caprate (decanoate), γ -dodecalactone, 2-pentadecanone, Z-7-tetradecanoic acid, and palmitoleic acid). Additionally, Sonication had no effect ($P > 0.05$) on heptanal, oxime-methoxy-phenyl, 4-methylbenzaldehyde and 9-headecenoic acid (Table 3).

Table 3: Volatile compounds of camel milk prior to and following sonication treatment

Compound Name	Control	Sonication	P-value
Heptanal	1.23E+08 ^a	1.78E+07	0.286
Oxime- methoxy-phenyl	3.13E+07	2.83E+07	0.5239
O-Cymene	ND ^b	6.15E+06	<0.0001
γ -Terpinene?	ND	1.30E+07	0.0446
4-Methylbenzaldehyde	1.16E+07	7.28E+06	0.2952
4-Nonenal	ND	2.92E+07	0.0017
Nonanal	1.34E+08	ND	0.009
Methyl caprylate (octanoate)	ND	1.37E+07	<0.0001
Octanoic acid	ND	1.73E+07	0.0001
Methyl salicylate	1.72E+07	5.88E+06	0.0008
Decanal	2.08E+07	ND	0.0053
β -Phenethyl acetate	ND	4.42E+06	0.0016
Undecanal	1.47E+07	ND	0.0064
Methyl caprate (decanoate)	ND	1.80E+06	0.0309
2-Undecanal	1.06E+08	ND	0.0043
Ethyl caprate (decanoate)	ND	3.82E+06	0.0069
Dodecanal	1.21E+07	ND	0.0034
γ -Dodecalactone	ND	7.17E+06	0.0069
2-Pentadecanone	1.05E+07	3.43E+06	0.0285
β -Hydroxydodecanoic acid	7.57E+06	ND	0.0017
Z-7-Tetradecanoic acid	ND	1.50E+07	0.0271
2-Heptadecanone	4.60E+06	ND	0.0597
Palmitoleic acid	ND	2.09E+07	0.0132
9-Hexadecenoic acid	1.52E+07	3.05E+06	0.2249

^aThe data is representative of the area underneath the curve, ^bND: not detected within the sample

DISCUSSION

The conventional heat treatment or pasteurization of camel milk has been reported to significantly impact milk composition and destroy protective proteins and immunoglobulins (Hattem et al., 2011). In recent years, the increased consumers' interest in nutritional and high-quality, healthy food has led to the development of nonthermal treatment methods to reduce microbial contamination (Cappozzo et al., 2015).

E. coli O157: H7 is an important foodborne pathogen that causes severe illness such as hemorrhagic colitis by producing a powerful toxin (Wells et al., 1991). Outbreaks of *E. coli* O157: H7 associated with pasteurized milk have been reported (Goh et al., 2002). Alternatively, an outbreak of *Salmonella Typhimurium* infection has been reported due to contamination after pasteurization (Olsen et al., 2004). The results of the current study showed that sonication time enhanced the effect of ultrasound treatment on microbial inhibition. Our results are in accordance with Li et al. (2016) who also reported that longer sonication time led to greater reductions in the surviving population of *E. coli* and *Staphylococcus aureus* in a 0.85% saline solution. Additionally, Shamila-Syuhada et al. (2016) reported that *E. coli* and *S. Typhimurium* were equally susceptible to the

ultrasound treatment in UHT milk and the inactivation rate of bacterial cells increased as the exposure time was increased from 5 to 15 minutes.

In addition to the SPC method, the effect of sonication on the inoculated bacterial strains was also monitored by measuring the bioluminescence emission before and after sonication treatment. Bioluminescence imaging technology is a new powerful tool that can be applied to monitor and track the growth of bioluminescence bacteria in different hosts (Contag et al., 1998) and milk (Maye et al., 2016). Previous studies reported strong correlations between the traditional bacterial count method and the bioluminescence signal (Maye et al., 2016). Consistent with the observed log reductions with the PCA, The amounts of bioluminescence for both tested strains in this study were also reduced following sonication further demonstrating the viability of bioluminescent imaging as a tool to monitor bacterial survival in milk.

In this study, we investigated the total aerobic bacteria population in raw camel milk for their susceptibility to ultrasound treatment. The 2 log reduction following sonication was consistent with the findings of Herceg et al. (2012a). These authors reported 2 and 2.2 log reductions, respectively; while (D'amico et al., 2006) reported 2.6 log reduction in total bacteria in raw bovine milk after only 6 minutes of sonication treatment. The differences between studies may be attributed to the different processing conditions among these studies. For instance, the duration, frequency, and intensity of ultrasound processing, ultrasonic probe size and position, and the amplitude of ultrasound waves, the initial concentration, and the strains of the bacterial load and their growth phase could affect the outcomes of these different studies (Herceg et al., 2012a; Herceg et al., 2012b).

Previous studies (Li et al., 2016) reported that Gram-negative bacteria are more sensitive to ultrasound processing than Gram-positive bacteria. However, a study (Scherba et al., 1991) reported no significant relationship between the Gram-status of the bacterial culture and the ultrasonic inactivation. In the present study, we used two Gram-negative bacterial strains and *E. coli* appeared more sensitive to ultrasound treatment than *S. Typhimurium*. The lethality effect of ultrasound treatment is multifactorial, including mechanical damage to cell membrane, intracellular pore formation, cell membrane retreating and disruption, the release of cytoplasm contents, and free radicals production that result in DNA degradation (Golmohamadi et al., 2013). Furthermore, it has been reported that the efficiency of ultrasound treatment is influenced by factors such as the size of bacterial cells, the suspending medium, and the microbial strain tested (Lee et al., 1989).

Data presented in Table 2 imply that the ultrasound processing of camel milk had no explicit impact on the all detected fatty acids except for C18:1 *trans* and C20:1n9. In reviewing the literature, no data was found on the association between ultrasonication and its impact on bovine or camel milk fatty acid profiles. In addition, two CLA isomers naturally found in dairy products derived from ruminants were evaluated. The *cis*-9, *trans*-11 CLA, which has been linked to positive health impacts such as anti-carcinogenic, anti-diabetic, anti-atherogenic, and immune system enhancement and the *trans*-10, *cis*-12 CLA that has been linked to body fat reductions (Belury, 2002). Our results did not show significant differences in the levels of CLAs between sonicated and control samples. Herzallah et al. (2005) reported that pasteurization at 85 ± 1.0 °C for 16 sec or at 95 ± 1.0 °C for 5 minutes had no significant effect on bovine milk CLA content. However, Rodríguez-Alcalá et al. (2014), reported that sterilization of raw cow milk resulted in a rearrangement of *cis*-9, *trans*-11 CLA to *trans*-9, *trans*-11 CLA. Additionally, when Herzallah et al. (2005) heated the milk in a microwave at 95.8 ± 1.0 °C for 5 minutes, milk CLA significantly decreased. The changes in CLA formation in these studies were attributed to the heating of milk, which therefore may explain the no change in CLA formation in our study.

Lipid oxidation refers to the oxidative degradation of lipids from free radicals, which in milk leads to the formation of undesirable off-flavor and flavorful secondary oxidation products such as 4-hydroxynonenal (HNE), hexanal, and malondialdehyde (MDA) (Akoh, 2005). The thiobarbituric acid reactive substances (TBARS) is a routine test to measure the MDA in milk samples. In the current study, the ultrasonication is expected to create intracellular cavitation that results in temperature increased and free radical generation, such as hydroxyl and hydrogen radicals promoting lipid oxidation (Makino et al., 1983). The TBARS values were similar between sonicated and control milk samples suggesting little oxidation during the ultrasound treatment. Juliano et al. (2014) concluded that concomitant decreases of sonication temperature and time are keys to control lipid oxidation in milk. Additionally, a study reported that milk proteins, in particular, casein and lactoferrin (LF) could inhibit lipid oxidation. The greater LF content in camel milk than in bovine milk (2.44 times greater; Park (2009) along with the low milk temperature during the ultrasound treatment (20 ± 3 °C) in our study may explain the lack of ultrasound effect on TBARS.

SDS-PAGE results for camel milk samples showed two bands belonging to whey proteins. The first band with a molecular weight of 15 kDa may correspond to α -LA in agreement with El-Agamy et al. (1997) who recognized α -LA at a molecular weight of 15 kDa. The second observed band

of 88 kDa was defined as the camel lactoferrin (LF). In a previous study, a similar protein of 80 kDa was characterized as a camel milk lactoferrin by Redwan and Tabll (2007). These authors also revealed the presence of two camel milk casein fractions of 35 kDa and 37 kDa likely corresponding to α_{s1} -CN and MW37, respectively. Ochirkhuyag et al. (1997) stated that the molecular mass of α_{s1} -CN of dromedary (*Camelus dromedarius*) is 35 kDa compared to 34 kDa for camel (*Camelus bactrianus*). When camel milk samples were sonicated for 15 minutes, no changes in casein or whey protein electrophoretic patterns were noticed. This finding is in agreement with Yanjun et al. (2014) who reported no significant changes in the molecular weight of ultrasonicated reconstituted milk protein concentrate samples. However, the intensity of all detected bands of sonicated samples increased in this study. In addition, the band of approximately 65 kDa was attributed to the camel serum albumin or CSA that is consistent with Farah (1986) who reported comparable CSA molecular weight (66 kDa). Further investigations are needed to interpret the band intensity changes and the appearance of a new band after the ultrasound processing of raw camel milk.

Gas chromatography coupled with a mass spectrometer (GC-MS) was used for the identification of the volatile and semi-volatile compounds within camel milk prior to and following sonication treatment. SPME has been used as a viable extraction technique for volatile and semi-volatile compounds in fruit-flavored malt beverages and fermented milk (Dan et al., 2017). The current study found that a number of VC were detected in camel milk following ultrasound processing. These findings are in agreement with Riener et al. (2009), who showed that 14 volatiles generated by ultrasound treatment of pasteurized bovine milk for 15 minutes. The increase in VC formation in Riener's study was attributed to the increase in temperature consistent with the findings of others (Pereda et al., 2008). However, a study (Vazquez-Landaverde et al., 2005) reported no effect of thermal processing on VC in raw and pasteurized milk. In our study, milk temperature was maintained low (20 ± 3 °C) during ultrasound treatment and therefore, any increase in VC would be attributed to the sonication reactions. The mechanism(s) by which sonication increases the formation of VC in milk is not well-known but probably can be attributed to fatty acids oxidation (Vazquez-Landaverde et al., 2006) consistent with the observed reductions in some fatty acids (C18:1 trans, C18:1c9, C20:1n9, and C22:6n3) in this study.

CONCLUSION

This study set out to determine the effect of nonthermal ultrasound processing on camel milk microflora including

some pathogenic strains and on the main components of camel milk. This technique was effective to inactivate *E. coli* O157: H7 and *S. Typhimurium* from camel milk. However, the total aerobic microorganisms count reduced by only 2 logs. Furthermore, camel milk fatty acids, protein fractions, and lipid peroxides were not affected significantly by ultrasound treatment. However, the formation of VC increased after ultrasound processing. Further tests are needed to evaluate the impacts of the proposed ultrasound technique on camel milk sensory properties.

Authors' contributions

Amer AbuGhazaleh and Jean Feugang helped with experimental design, writing and editing the manuscript. Katherine Witrick and Benny Park helped with sample analyses. Namariq Dhahir carried out the experiments, performed the statistical analysis, and helped draft the manuscript.

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