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Comparison of DNA quality in raw and reconstituted milk during sterilization

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ABSTRACT

Responses to milk sterilization are usually evaluated only in terms of physicochemical properties and microbial safety, thus undervaluing the importance of DNA quality in an authentication process by methods based on PCR. Because DNA is a heat-sensitive molecule, we hypothesized that the heating process may impair the detection or quantification of DNA in raw fresh milk (FM) or reconstituted milk (RM), and that differences in DNA quality might exist between FM and RM. We thus investigated the effects of sterilization on the quality of DNA extracted from FM or RM; differences in DNA quality between FM and RM were also evaluated. The quality of extracted DNA from FM or RM was assessed by the specific detection of FM or RM composition in goat milk mixtures using primers targeting the bovine *12S* gene, as well as by monitoring DNA yield, purity, ratio of mitochondrial (mt) to nuclear (n) DNA, and the level of DNA degradation. Polymerase chain reaction readily detected both untreated and heat-treated FM or RM in cow-goat milk mixtures, and gave a good sensitivity threshold (0.1%) under all sterilization conditions. The DNA yield and mtDNA:nDNA ratio of FM and RM varied significantly during the sterilization process. These results demonstrated that the sterilization altered the quantification of DNA in FM or RM during sterilization, but DNA could still be readily detected in sterilized FM or RM by PCR. Furthermore, we noted significant differences in DNA integrity, yield, and mtDNA:nDNA ratio between FM and RM during sterilization, which may have potential as a means to distinguish FM and RM.
Key words: milk, sterilization, DNA quality, PCR

INTRODUCTION

It has been widely recognized that mislabeling of food products and ingredient substitutions are major issues in nutritional quality and food safety (Wong and Hanner, 2008; Cawthorn et al., 2013; Everstine et al., 2013). With the increase in global trade of food products, accurate and speedy authentication is essential for correct labeling to ensure food safety. The use of PCR authentication has become one of the prospective standards recently. Such PCR-based monitoring approaches are limited, however, by the relatively low content and extractability of DNA of adequate quality, both of which depend upon the initial sample's purity and subsequent degree of DNA degradation (Spaniolas et al., 2008).

Nevertheless, manufacturing of food products usually involves heat treatment, and DNA quality is often deteriorated by food processing, result in the degradation of DNA into small fragments (Novak et al., 2007). For example, Chen et al. (2005) reported that the size of DNA in a soybean product was reduced after sterilization. Studies on meat processing revealed that the DNA fragment size was progressively degraded into smaller fragments with increasing duration of heating and temperature (Guoli et al., 1999; Sakalar et al., 2012; Musto et al., 2014).

In terms of dairy products, to ensure total microbiological safety and extend shelf life of milk, sterilization is most often achieved through UHT processing, from about 135 to 145°C for just a few seconds, or by retort processing, in which liquid milk is heated only to a temperature of about 110 to 120°C but for several minutes. In this context, sterilization can become the crucial aspect in an authentication process by PCR-based methods. At present, studies about the effects of heating process on DNA quality in milk are mainly focused on sensitivity analysis (López-Calleja et al., 2004, 2005; Liao et al., 2017b); the degradation of DNA in milk during sterilization has not been evaluated.

Until recently, sterilized milk products were produced from raw fresh (**FM**); however, the number of commercially available sterilized milk products derived from reconstituted milk (**RM**) has increased in recent years. Therefore, monitoring the changes of DNA quality in FM and RM during sterilization is an important initial step for optimizing any DNA-based analytical

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protocols. Considering the results obtained from previous studies (López-Calleja et al., 2004, 2005; Liao et al., 2017b) and the lack of reports on DNA quality from sterilized FM and RM, the primary aim of the current study was to investigate the effects of sterilization on the quality of DNA extracted from FM and RM; we also evaluated differences in DNA quality between FM and RM.

MATERIALS AND METHODS

Sample Collection and Experiment Design

Authentic samples of pooled FM from cow were obtained from the collection tank of local dairy farm. Samples were transported on ice packs (to maintain an optimum low temperature) to the laboratory and processed immediately or stored at −80°C until used. Milk powder was produced by using the FM described above, according to the method of Martin et al. (2008). Then, the powder was reconstituted in distilled water to yield RM of the same overall composition as the source FM. A total of 30 FM and 30 RM samples were prepared and sterilized by autoclaving according to a previous study with some modifications (Corredig and Dalgleish, 1996). Briefly, each milk sample was transferred into 50-mL capped glass centrifuge tubes and sterilized at 121°C for 0 (raw control), 1, 5, 10 and 15 min, respectively (Table 1).

Six FM and 6 RM samples from each treatment were used for DNA extraction and quality evaluation; each sample was tested in duplicate. In addition, to determine whether the DNA from different processed FM and RM gave rise to comparable sensitivity in adulteration detection, various proportions (0.1, 0.5, 1, 10, and 30%) of FM or RM in goat milk were prepared for DNA extraction and further PCR analysis; each sample was also tested in duplicate. The goat milk samples were also sourced from the collection tank of local dairy farm and their species was authenticated.

DNA Extraction and Quality Evaluation

DNA Extraction. The DNA was extracted from milk according to our previous work (Liu et al., 2014), with slight changes. Briefly, 10 mL of milk was centrifuged for 10 min at 4,150 × *g*. The top and middle layers were removed, leaving the bottom sediment, which was washed twice with 600 μL of PBS (pH 7.4, 4.8 μg of NaCl, 0.12 μg of KCl, 0.864 μg of Na_2HPO_4, 0.144 μg of KH_2PO_4, and 600 μL of double distilled H_2O). After the washing step, the sediment was mixed with 350 μL of extraction buffer (pH 8.0, 100 m*M* Tris Cl, 100 m*M* NaCl, and 5 m*M* EDTA), 50 μL of 20% (wt/vol) SDS, and 10 μL of proteinase K (20 mg/mL), and incubated at 56°C for 4 h. The mixture was extracted with an equal volume of Tris-phenol, phenol: chloroform:isoamyl alcohol (volume ratio of 25:24:1), and chloroform:isoamyl alcohol (volume ratio of 24:1). Finally, DNA was precipitated with ice-cold absolute ethanol and washed once with ethanol:water (volume ratio of 7:3). Twenty-five microliters of Tris-EDTA (pH = 8.0, 1 m*M* Tris-Cl and 0.5 m*M* EDTA) was added to dissolve DNA.

DNA Yield, Purity, and Integrity. The DNA yield (expressed as ng/mL of milk) was calculated using a UV/visible spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) and the absorption (A) ratio at 260 and 280 nm (A_{260}/A_{280}) was taken as a purity index. The level of degradation of extracted DNA was examined by agarose gel electrophoresis, visualized using UV light, and digitally photographed.

PCR Amplification. The cow-specific primer *12SBT-REV* was selected for specific detection of FM or RM compositions in goat milk mixtures by amplifying a 346-bp fragment in the *12S* gene. The sequence of the primer was adopted from López-Calleja et al. (2005): 5′-CTAGAGGAGCCTGTTCTATAATCGATAA-3′ and 5′-AAATAGGGTTAGATGCACTGAATCCAT-3′. The PCR reaction consisted of 3.4 μL of 2× TaqMan Universal PCR Master Mix (CWBIO, Beijing, China), 0.3 μL of assay-specific forward and reverse primers, 1 μL of the template DNA, in a total volume of 10 μL with DNase-/DNA-free water (CWBIO). The PCR protocol was 95°C for 10 min followed by 30 cycles of 94°C for 30 s, 63°C annealing for 30s, and 72°C for 30 s, and a final extension at 72°C for 10 min using an Aritik PCR System (Thermo Fisher Scientific Inc., Waltham, MA). The amplification products were resolved by electrophoresis agarose gel and visualized under UV light and digitally photographed.

Real-Time PCR Amplification. To determine the relative quantity of mitochondrial (**mt**)DNA products, *12S*, a gene specific in the mitochondrial genome, and bovine *HBB*, a housekeeping gene acting as the nuclear

Table 1. Sterilization times and endpoint core temperatures (mean ± SD) of autoclaving sterilization

Sterilization time (min)	Endpoint core temperature (°C)
0	Raw control
1	66.23 ± 0.25
5	76.00 ± 0.50
10	84.70 ± 0.35
15	94.23 ± 0.25