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Lipid oxidation volatiles absent in milk after selected ultrasound processing

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ABSTRACT

Ultrasonic processing can suit a number of potential applications in the dairy industry. However, the impact of ultrasound treatment on milk stability during storage has not been fully explored under wider ranges of frequencies, specific energies and temperature applications. The effect of ultrasonication on lipid oxidation was investigated in various types of milk. Four batches of raw milk (up to 2 L) were sonicated at various frequencies (20, 400, 1000, 1600 and 2000 kHz), using different temperatures (4, 20, 45 and 63 °C), sonication times and ultrasound energy inputs up to 409 kJ/kg. Pasteurized skim milk was also sonicated at low and high frequency for comparison. In selected experiments, non-sonicated and sonicated samples were stored at 4 °C and were drawn periodically up to 14 days for SPME–GCMS analysis. The cavitational yield, characterized in all systems in water, was highest between 400 kHz and 1000 kHz. Volatile compounds from milk lipid oxidation were detected and exceeded their odor threshold values at 400 kHz and 1000 kHz at specific energies greater than 271 kJ/kg in raw milk. However, no oxidative volatile compounds were detected below 230 kJ/kg in batch systems at the tested frequencies under refrigerated conditions. Skim milk showed a lower energy threshold for oxidative volatile formation. The same oxidative volatiles were detected after various passes of milk through a 0.3 L flow cell enclosing a 20 kHz horn and operating above 90 kJ/kg. This study showed that lipid oxidation in milk can be controlled by decreasing the sonication time and the temperature in the system depending on the fat content in the sample among other factors.

1. Introduction

Ultrasound (US) processing is a relatively new field of endeavor for the dairy industry, and is finding applications either as an adjunct intervention in an existing milk processing line to improve process efficiency or as a method to modify the functionality of milk components for value addition in selected dairy product streams [1–3]. Some ultrasound applications including defoaming, degassing, de-fouling, milk homogenization, yogurt making, milk fat crystal control, and accelerated ice cream making have been reported to require specific energy inputs ranging between less than 0.1 and 230 kJ/kg [4–15]. Previous studies have deliberately considered low specific energy inputs to retain quality in milk systems [16] and scaled up ultrasonic processing applied to alter the functionality of dairy components [17]. Other potential ultrasonic interventions to milk processing including the development of enhanced cheese brining and enhanced lactose crystallization in cheese whey streams have been reported without clearly specifying the required energy inputs into the milk [18,19].

Ultrasound applied for defoaming in commercial operations that traditionally produce significant amounts of foam (stirring, homogenization or fermentation) uses 20 or 40 kHz. Such commercial defoaming applications have been shown to require specific energies below 0.1 kJ/kg to improve process efficiency and to reduce waste. Likewise, degassing of milk can be achieved with lower energy inputs [4]. The amount of fouling deposit in metal surfaces during heat processing of milk was shown to be reduced in an ultrasonic water bath with 40 kHz and 0.11 W/L or 233 kJ/kg during 5 h [5]. De-fouling and cleaning of ultrafiltration membranes was also demonstrated by using 50 kHz ultrasonics and 0.33 kJ/kg, enhancing whey ultrafiltration at 60 L/h [6].

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In addition, ultrasound homogenization of raw milk was achieved by applying 20 kHz (0.15 L) and 35–180 kJ/kg while reducing milk fat globules to the submicron range and matching conventional homogenization [7,8]. These conditions were also able to reduce yogurt making production time by 40% [7]. Additional positive effects in yogurt due to ultrasound are improvements of consistency and texture [7]. Milk pretreatment with 20 kHz ultrasound and 22 kJ/kg (based on temperature a measured increase) followed by milk inoculation has enhanced textural properties of yogurt (consistency, gel strength and viscosity) and water holding capacity [9–11]. More recently high frequency ultrasound has demonstrated to be practical for milk fat separation at frequency ranges between 400 kHz and 2000 kHz and specific energies of 9 kJ/kg for a 6 L ultrasonic batch reactor [12–14].

Others have shown that requirements for ultrasound at 20 kHz to control fat crystallization near room temperature are around 4–8 kJ/kg [20,21]. Similarly, ultrasound can enhance ice crystal fragmentation during freezing in ice cream and sorbet manufacturing with less than 0.5 kJ/kg between frequencies of 20 and 40 kHz [15].

Although there are limited industrial scale ultrasonic reactor designs for the many dairy applications detailed above, further development of the technology requires an understanding of the impact of ultrasound on milk quality changes, particularly milk oxidation triggered by free radicals formed during sonication and associated cavitation. Few studies have discussed the effect of sonication in promoting milk lipid oxidation with ultrasonic processing at low frequencies [22,23]. Other studies have evaluated the effects on oxidation on whey after ultrasound application in systems of various frequencies. Chouliara et al. [23] reported the presence of volatiles from oxidative reactions after ultrasound processing of raw, thermized, and pasteurized milk at 24 kHz and 15–25 °C at specific energies around 187 kJ/kg (or 16 min processing of 0.2 L milk at 200 W nominal power). Reiner et al. [22] also determined the presence of volatiles in commercially pasteurized homogenized milk processed at 24 kHz and 45 °C and specific energies greater than 240 kJ/kg (or 2.5 min processing of 0.2 L milk at 400 W measured power). Such high energy input levels, where temperature may increase more than 60 °C due to sonication, may be too high to realize any industrial uptake.

The collapse of cavitation bubbles resulting from localized pressure gradients, caused by a series of compression and rarefaction cycles during ultrasound processing, is responsible for the localized generation of hot spots, which lead to temperature increase in the liquid [24,25]. Extreme temperature and shear conditions created by cavitation result in the generation of hydroxyl and hydrogen radicals [26], which can promote lipid oxidation. Similar results on raw milk sonicated at 20 kHz and 400 W nominal power were also reported by Marchesini et al. [27], who also found that the addition of CO_2 prior to sonication appeared to decrease the formation of oxidation products as well as the detection of a burnt off-flavor.

Given that high rates of hydroxyl radical formation have been detected between 400 and 800 kHz, this is of concern for newly developed applications, such as milk fat separation in the high frequency range [28]. Torkamani et al. [29] recently explored the formation of lipid oxidative volatile compounds after sonication of fresh Cheddar whey at 37 °C and found no impact from sonication at 20, 400, 1000, and 2000 kHz (specific energies up to 390 kJ/kg).

The current study explores the possible formation of oxidation volatile compounds from cooled, untreated and heat treated raw milk as well as pasteurized skim milk subjected to a wide range of frequencies (20–2000 kHz) using various types of transducers and reactor chambers. The sonication vessels were characterized in terms of sound intensity and by following the formation of free radicals in water.

2. Materials and methods

2.1. Sample preparation and handling

Full fat raw milk (4.1 ± 0.3% w/w fat) was obtained locally from a dairy plant (Bega cheese, Melbourne, Australia): first in the month of December (milk A) and second one year later on two instances in December (milk B) and in March (milk C). The milk collected between December and March is representative of the mid-lactation cycle. The milk A used during December trials for the first year was aliquoted into three lots of samples (A1, A2 and A3), two of which were further treated such that: sample A1 represented untreated raw milk kept at 4 °C, sample A2 represented milk pasteurized (3000 LPH Pasteurizer; GEA VT 20 FC, Thomastown, VIC, Australia) at 72 °C for 15 s, and the sample A3 represented pasteurized milk that was homogenized (at 45 °C and 140 MPa and 200 MPa at first and second pass, respectively; Homogenizer, Invensys APV, Clayton, VIC, Australia). A fourth set of raw milk (milk D; 4.0 ± 0.3% w/w fat) was also sampled in December (in the following year) from an experimental dairy farm (Department of Primary Industries Ellinbank, VIC, Australia). Pasteurized skim milk (milk E, 0.1 ± 0.3% w/w fat, Parmalat Australia Ltd, South Brisbane, QLD, Australia) was obtained from a local supermarket.

2.2. US treatment of milk and experimental design

A stainless steel reactor vessel (250 × 180 × 80 mm) able to carry volumes ranging between 0.2 L and 2 L was designed to fit the following transducer horns and plates: (a) a 20 kHz sonotrode (450 W Branson Digital Sonifier, Branson Ultrasonic Corporation, Connecticut, USA) submerged near the middle of the fluid (Fig. 1a); (b) high frequency transducers were placed at the bottom of the reactor with vertical upwards direction of the ultrasound waves (Fig. 1b). Each horizontal transducer was either square in shape (active area 100 × 100 mm, 400 kHz, 1000 kHz, or 2000 kHz; Sonosys Ultraschallsysteme GmbH, Neuenbuerg, Germany) or round (with an active area 79 mm^2, 1600 kHz; nebulizer type, APC International Inc., Mackayville, PA, USA). In all cases, except for the 1600 kHz nebulizer, the power input level could be tuned from the generator. The reactor was placed in a temperature controlled water bath to minimize temperature increase (max. 5 °C after 5 min and 20 °C after 20 min) at selected operating temperatures (4 °C, 20 °C, 45 °C or 63 °C). A 20 kHz horn-transducer (UP-I1000hd, Hielscher Ultrasonics GmbH, Teltow, Germany) was used to process milk in a 300 mL custom-made continuous flow stainless steel reactor operating at 750 mL/min. The 20 kHz horn-transducer (cross-sectional area 310 mm^2) was made of titanium and was operated for 15 min with 101 kW/m^2 net power via radial vibration. In this case, energy delivery into the milk sample was maximized by minimizing the sample volume.

The designs of five experiments, performed in triplicate (unless otherwise specified), to explore the appearance of volatile compounds from lipid oxidative reactions in raw, treated, and skim milk, is included in the following (Table 1):

- *Experiment 1:* Explored the appearance of volatile compounds in 1 L non-homogenized and homogenized raw milk A at 4 °C or 63 °C after 5 min sonication at two frequencies (20 and 1600 kHz) with transducers operating at maximum power.
- *Experiment 2:* Consisted of comparing three US treatments of 1 L of non-homogenized raw milk B at 4 °C at frequencies 20 kHz, 400 kHz, and 1600 kHz using the same specific energy. The choice of 4 °C in this case is justified by the potential for applying high frequency ultrasound for milk fat separation at low