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Phenolic contents of pasteurised and ozonated camel milk

AJLOUNI Said, RHEE Michelle, RANADHEERA Chaminda Senaka & DUNN Timothy

School of Agriculture and Food, Faculty of Veterinary and Agriculture Sciences,
The University of Melbourne, Australia

Corresponding author:

Prof Said Ajlouni
School of Agriculture and Food, FVAS,
The University of Melbourne. Parkville, Victoria 3010, Australia
said@unimelb.edu.au

Abstract

Due to the negative impact of heat treatment on milk quality, this study examined ozonation as an alternative pasteurisation technique to maintain quality, acceptable shelf life, and high nutritional value of camel milk.
Total polyphenol content (TPC) reported as Gallic Acid Equivalent (GAE) in Australian raw camel milk was 12.09±0.4 mg GAE /100 ml. Ozonation for 5 and 10 min at room temperature significantly ($P<0.05$) reduced the TPC by 0.66 mg GAE/100 ml and 0.72 GAE mg/100 ml, respectively. Similarly, the TPC in pasteurised milk was significant ($p<0.05$) decreased by 2.62 mg GAE/100 ml. Similar to the effect of ozonation on TPC, the antioxidant activities in camel milk ozonated for 10 min were significantly ($P<0.0$) reduced by 0.55 mg TE/100 ml and 4.04 mg TE/100ml using DPPH and ABTS methods, respectively. Results showed also that pasteurisation reduced the antioxidant activities by 3.57 mg TE/100 ml (DPPH method), and 5.40 mg TE/100 ml (ABTS method). HPLC and titration methods demonstrated that vitamin C content in raw camel milk were 3.02±0.21mg/100 ml and 3.29±0.03mg/100 ml, respectively. Ozonation for 10 minutes had the greatest effect on the vitamin C content, and caused 76.80% reduction.

Introduction

Camel breeding in Australia is minimal when compared to the breeding of other domesticated animals. However, Australia has the largest feral camel population in the world. Around 1 million feral dromedary camel (*Camelus dromedaries*) are spread over central Australia and are considered to be the biggest pest in Australia (Zeng & Gerritsen, 2013; Edwards et al., 2004). These camels are believed to be causing negative environmental impacts on the dessert terrain and are also responsible for economical and infrastructural damage (Zeng & Gerritsen, 2013). For hundreds of years camels in the Middle East, Asia, and Africa have been commercially exploited. In Australia, it is being considered in recent years as part of an integrated management strategy to commercially harvest these feral camels. This strategy will help reducing the wild camel population and the costs associated with conservation management (Zeng & Gerritsen, 2013). Additionally, the economic significance of camel products, in general, especially milk, is growing locally and globally (Antunac, 2015). Over the last decade, there has been an increase in camel milk production, due to increasing demand (Nagy et al., 2015). Camel milk still attracts a premium cost, when compared with cow milk due to the current low production in comparison with the

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huge consumers' demands. A camel is able to produce 3 to 10 liters of milk daily during lactation. However, the number of milking has not been specified in the literature (Antunac, 2015; Al haj & Al Kanhal, 2010).

It should be noted that research on commercialisation of camel products has not yet been elucidated in the literature. Nevertheless, Helbig (2016), a journalist for *The Courier-Mail*, published an article where a new business has started the first stages of implementing a camel farm located in Clarendon, Queensland (Australia). The first heard consisted of 33 feral camels brought up from South Australia, and around 80 feral camels which were caught in central Australia. The business is working to scale up, where the investors, the Australian Wild Camel Corporation and SNC Agri, are helping to make a 4500-head outback camel dairy. This would make it the biggest camel dairy farm in the world.

It is anticipated that Camel milk will become one of the major foods that can gain attention in the Australian market as a functional food with a potential to become the next super food to hit our shelves (Antunac, 2015). However, processing camel milk through the traditional method of heat pasteurisation may affect its nutritional and functional properties. Consequently, developing an alternative method to preserve camel milk quality and its nutritional value is essential. Ozonation is an emerging process that uses ozone gas for microbial inactivation at low temperatures (Cavalcante et al., 2013; Varga & Szigeti, 2016; Khadre et al., 2001). This investigation was conducted with the anticipation that ozonation could be a better alternative than heat pasteurisation to maintain safe and more nutritious camel milk.

Materials and Methods

Fresh camel milk (10 liters) was collected at random from a local camel farm located at Kyabram, Victoria, Australia on the 26th of May and the 1st of August 2016. The analyses were performed in two trials. The collected milk (10 liters) was subdivided into six 300 ml sub-samples per treatment per trial. A total of 12 replicates were performed for each treatment. Each analysis was done in duplicates. Ozonation for 5 and 10 minutes at room temperature, and batch pasteurisation (63^{0}C for 30 minutes) were conducted. Raw milk was used as a control. The treated milk samples were then subjected to microbial and chemical analyses. However, results from chemical analyses will be addressed in this manuscript.

Determination of total polyphenol content by Folin-Ciocalteu assay

Milk samples were diluted with 70% methanol to 1:1 ratio before analyses. The total polyphenol analysis was done following the Folin-Coicalteu method (Singleton et al., 1965) with some modifications. The diluted milk sample (100µl) was mixed with 900µl Folin-Ciocalteu phenol reagent and 4 ml of sodium carbonate (Na_2CO_3, 7.5%), and incubated in the dark at room temperature for 15 minutes. The solution was then centrifuged for 5 mins at 9000 rpm at room temperature. The supernatant was used to determine the absorbance reading at a wavelength of 765 nm, and TPC was reported as Gallic acid equivalence (GAE) mg/100ml camel milk.

Determination of antioxidant activity via the DPPH assay:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution was prepared by dissolving 10 mg of DPPH in 10 ml methanol following the method of Martysiak-Żurowska & Wenta (2012). This DPPH stock solution was diluted with methanol until there was an absorbance reading no greater than 1.0 (working solution). The milk sample (0.2 ml) was mixed with 1ml DPPH working solution, and then left for 10 minutes at room temperature. This was then centrifuged for 5 minutes at 9000 rpm at room temperature. The absorbance was measured at 515 nm for each sample, and the scavenging rate was reported as Trolox equivalent mg/100 ml (mg TE/100 ml).

Determination of antioxidant activity via ABTS:

Milk samples were diluted 1:5 using methanol (70%) following the procedures of Turoli et al. (2004). Of the diluted milk sample (0.1 ml) was mixed with ABTS working solution (1ml) and left at room temperature for 10 mins. The solution was then centrifuged for 5 min at 9000 rpm. The absorbance was measured at 734 nm, where the scavenging activity was reported as mg TE/ 100 ml milk.

Determination of vitamin C content in camel milk

a. Ascorbic acid concentration via HPLC

AA was determined using HPLC following the method of Abramovich, Friel & Hossain (2013) and Romeu-Nadal et al. (2006) with some modifications. The modifications involved the precipitation of milk proteins, and the elimination of turbidity using Carrez solution.

b. Ascorbic acid concentration via titration method

Vitamin C concentration was determined using the modified AOAC official titrimetric method 967.21 (1968). The Dichloroindophenol (DCIP) titration solution was prepared by dissolving 2,6-dichloroindophenol (25 mg) and sodium bicarbonate ($NaHCO_3$, 21 mg) in deionised water (100 ml).

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Results and Discussion

Total polyphenol contents in raw, pasteurised and ozonated camel milk

Data obtained from the two trials (n=12) revealed that ozonation of camel milk for 5 and 10 minutes caused significant ($p<0.05$) reduction in total polyphenol content (TPC) estimated as Gallic acid equivalence (GAE) when compared with the control (Fig. 1). The same data showed also that batch pasteurisation caused significant ($P<0.05$) reduction in TPCs. However, decline in the TPCs after 5 minutes (0.66 mg GAE/100 ml) and 10 minutes (0.72 mg GAE/100 ml) ozonation were significantly smaller than that in the pasteurised milk (2.62 mg GAE/100 ml) (Fig. 1).

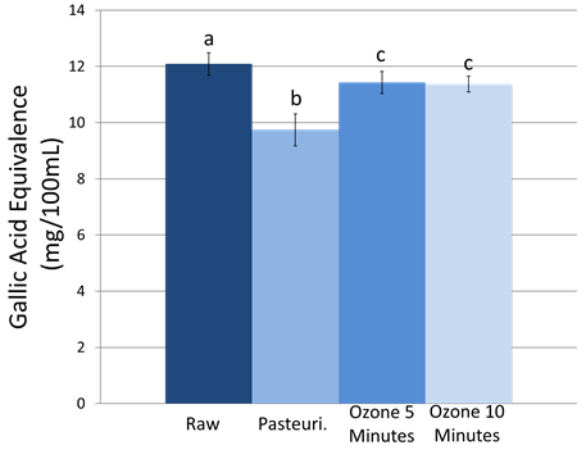

Figure 1. Total polyphenol content (mg GAE/100ml) in raw, pasteurised, and ozonated camel milk.

Antioxidant activity of raw, pasteurised ozonated and camel milk

Raw camel milk showed the highest antioxidants activity for both DPPH and ABTS, with and antioxidant activity of 8.71 (Fig. 2) and 20.41 mg TE/100 ml, (Fig 3.) respectively. The DPPH assay demonstrated reduction in the antioxidant activities after 5 and 10 minutes ozonation were only 0.55 mg TE/100 ml and 0.68 mg TE/100 ml, respectively, when compared with the control (Fig. 2).

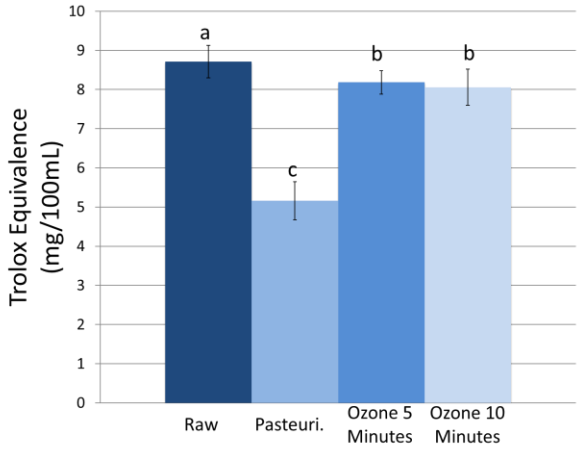

Figure 2. Antioxidant activity in raw, pasteurised, and ozonated camel milk measured *via* the DPPH method

However, along with this, there was a larger and significant ($p<0.05$) reduction in batch pasteurisation treatment, where up to 3.57 mg TE/100 ml was destroyed. The reduction in antioxidant activity *via* pasteurisation indicated greater loss in camel milk quality. However, the smaller reduction in DPPH activity *via* ozonation compared with pasteurisation (Fig. 2) might indicate that ozonation is a better treatment than heat treatment in order to maintain antioxidant activity in camel milk.

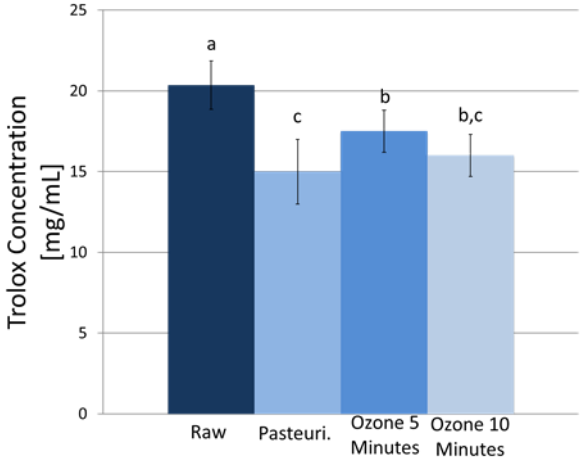

Figure 3. Antioxidant activity in raw, pasteurised, and ozonated camel milk measured *via* ABTS

Vitamin C contents in treated camel milk measured using HPLC and titration methods

Both HPLC & titration methods revealed greater decline in camel milk vitamin C content after ozonation, when compared with pasteurisation. For example, analyses of Vit C contents *via* titration of ozonated (5 & 10 minutes), and pasteurised camel milk samples showed decline by 2.08, 2.52, and 0.77 mg/100 ml milk, respectively (Fig. 4). Similarly, the HPLC assay of treated camel milk showed 0.78, 1.81, and 0.56 mg/100 ml reduction in ozonated (5 & 10 min) and pasteurised samples, respectively (Fig. 5). These data revealed that pasteurisation of camel milk had less severe effect on milk vitamin C degradation in comparison with ozonation.

Conclusion

In conclusion, ozonation of camel milk caused less damage to phenolic compounds, and more significant reduction in the vitamin C content when compared with pasteurization. It is recommended that sensory tasting and specific antioxidant compound such as lactoferrin, vitamin E and vitamin should be included in future studies.

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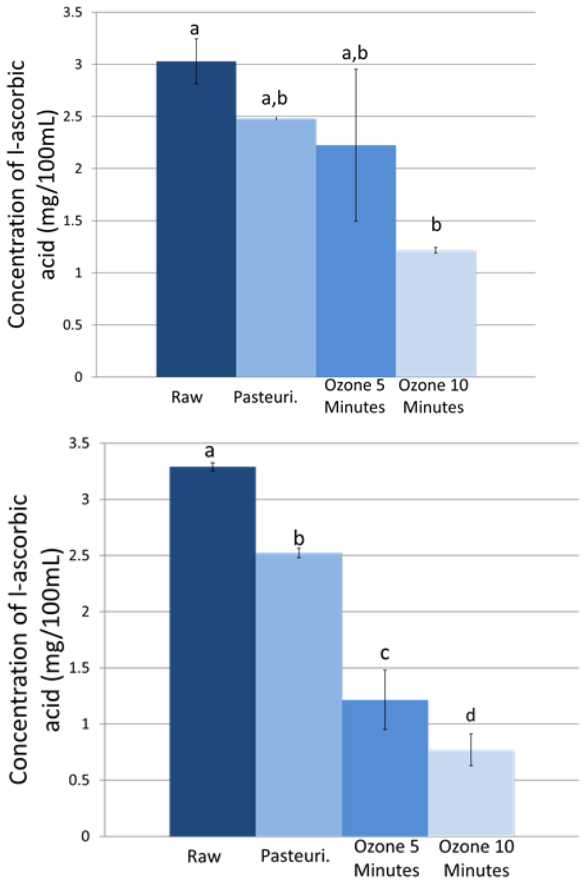

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Figure 4: Vitamin C concentration determined by titration method

Figure 5: Vitamin C concentration (mg/100 ml) in raw, pasteurised, and ozonated camel milk determined *via* HPLC

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