Small Ruminant Research 160 (2018) 54–58

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Effects of feeding system, heat treatment and season on phenolic compounds and antioxidant capacity in goat milk, whey and cheese

Jorge L. Chávez-Servín, Héctor M. Andrade-Montemayor, Cecilia Velázquez Vázquez, Araceli Aguilera Barreyro, Teresa García-Gasca, Roberto A. Ferríz Martínez, Andrea M. Olvera Ramírez, Karina de la Torre-Carbot*

Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Av. de las Ciencias S/N, Juriquilla, Querétaro, Qro. CP 76320, Mexico

ARTICLE INFO

Keywords:
Phenolic compounds
Antioxidant capacity
Goat dairy products
Feeding systems

ABSTRACT

Phenolic compounds are present in goat milk and cheese. The composition of goat milk and its products may vary depending on factors such as season, feeding system and heat treatment. The aim of this work is to quantify total phenolic compounds (TPC) and antioxidant capacity in pasteurized and unpasteurized samples of milk, milk whey, and cheese from goats fed in two different systems (free-range grazing and permanent confinement), during dry and rainy seasons. TPC concentrations were highest in unpasteurized samples from dry season compared to pasteurized and rainy season: 132.4 ± 27.3, 76.5 ± 5.77 mg of gallic acid equivalent (GAE)/L for unpasteurized milk and milk whey, respectively, and 363.21 ± 52.97 mg GAE/Kg for cheese. Antioxidant capacity for dry season produce was significantly higher ($P < 0.05$) than rainy season produce. Free-range grazing was found to be a good option for producing a higher concentration of phenolic compounds and a higher antioxidant capacity.

1. Introduction

Goat milk and its derivatives are regaining prominence in the human diet due to their composition and recognized benefits for human health (Raynal-Ljutovac et al., 2008). Goat milk and its products are superior to cow's milk in a number of aspects: lower allergenicity of their proteins, greater digestibility and more bioactive components, among others. Because of these, goat milk has gained the image of being a healthy, functional product (Albenzio et al., 2012; Raynal-Ljutovac et al., 2008).

There have been a number of recent studies on phenolic compounds in foods, showing their benefits by reducing pathogenesis or severity of chronic disease, including cardiovascular disease (Lewandowska et al., 2016; Rangel-Huerta et al., 2015; Redan et al., 2016). But phenolic compound content in goat milk and dairy products like cheese and whey has so far been little studied (Hilario et al., 2010).

The feeding system used–free-range grazing or permanent confinement–has been found to affect the composition of goat milk, and may influence antioxidant activity, not only in the milk, but in milk products as well (Jordan et al., 2010; Keles et al., 2017). Other factors that can affect composition is the season of the year (rainy or dry) and the thermal treatment (pasteurization) process (Di et al., 2015; Hilario et al., 2010).

Data on goat milk phenolic content and antioxidant capacity can be used to improve goat milk quality and therefore it is important to assess the impact of each of these factors on the composition of goat milk and goat milk products. Accordingly, the aim of this study was to measure and compare the total phenolic compound (TPC) concentration and antioxidant capacity in pasteurized and unpasteurized samples of milk, cheese and whey from goats fed in permanent confinement and free-range grazing, during dry and rainy seasons.

2. Materials and methods

2.1. Study design

Healthy, multiparous goats, on their 2nd or 3rd lactation, from the Alpine breed were used, with an average weight of 50 ± 5 kg and an average milk production of 2.5 ± 0.4 kg/day. The milk samples were drawn after 60 days of lactation. The goats were raised in the state of Querétaro, in the municipality of El Marques, on the Amazcala Campus of the Autonomous University of Querétaro (UAQ). Animal welfare principles of the good practices manual for caprine milk production were followed (Kilkenny et al., 2017; SAGARPA, 2014). The project was

* Corresponding author.
E-mail address: karina.delatorre@uaq.mx (K. de la Torre-Carbot).

https://doi.org/10.1016/j.smallrumres.2018.01.011
Received 16 September 2017; Received in revised form 19 January 2018; Accepted 21 January 2018
Available online 31 January 2018
0921-4488/

reviewed and approved by the Bioethics Committee of the UAQ's School of Natural Sciences (FCN). To monitor animal health, a somatic cell count (SCC) was performed each week with an EKOMILK ™ device. An average SCC had a count of $260 \times 10^3 \pm 36 \times 10^3$ SCC/mL.

A randomized experimental study with $2 \times 2 \times 2$ factorial arrangement was made. First, goats were allocated into two treatments (n = 20): Group 1: goats were confined and fed a controlled diet designed according to their needs (National Research Council, 2007), consisting of 1 kg of concentrate (88% dry matter (DM)), 1.5 kg of alfalfa hay (85% DM) and 1.5 kg of corn silage (40% DM) (White corn: *Zea mays* L.). Group 2: free-range grazing from 9:00 a.m. to 2:00 p.m. on 12 ha of thorny deciduous forest land, with a predominance of shrubs such as *Prosopis laevigata*, *Acacias* spp, *Celtis* spp, *Opuntia* spp and other types of cactus, and grasses, with 450 mm of precipitation and temperate climate. The diet was supplemented each day with preparations in the following proportion: 0.8 kg of concentrate (88% DM) 0.75 kg of alfalfa hay (85% DM) and 0.5 kg of corn silage (40% DM). The composition of the concentrate in DM was as follows: 33.27% ground corn (White corn: *Zea mays* L.), 33.0% barley, 11.17% soybean meal, 10.08% dry destiller grain (DDG), 10.30% corn gluten meal (60% CP), and 2.16% mineral and vitamin premix™. Nutrient contents of foods were: concentrate 1.9 Mcal ENl/kg/DM and 19.26% CP, alfalfa hay: 1.35 Mcal ENl/kg/DM and 18% CP and corn silage: 1.45 Mcal ENl/kg/DM and 6% CP.

For both groups of goats, milk was drawn during two seasons of the year (rainy and dry). For each season, half of the milk samples were pasteurized and half were left unpasteurized, same milk samples were used to produce cheese and whey. This produced a total of 8 classes of milk, 8 classes of whey, and 8 classes of cheese ($2 \times 2 \times 2$). Four subsamples were drawn of each of these categories: a total of 96 samples all together. TPC concentration and antioxidant capacity were then evaluated in an aqueous extract obtained from the milk, whey and cheese.

2.2. Milking, pasteurization and cheese making process

The goats were milked once a day (7–9 a.m.). After cleaning the udders, a mobile milker was used (J. Delgado FLACO™ brand) to extract the milk, with a 38–40 kpa vacuum and a pulse ratio of 70–30. Pasteurization (for pasteurized samples) was carried out in a stainless-steel double-bottom pasteurizer with automatic temperature control, stirred at a temperature of 63–65 °C for 30 min, and subsequently cooled to 26 °C. Lactic acid culture–*Lactobacillus* and Mesofilos MA400 (2 g/100 l)–was added to both pasteurized and unpasteurized milk samples at 30 °C, and organically-derived rennet (Cuamex ™) was added in a solution with water at 28 °C (1.5 ml/L). The curd was added in small grains and 1/3 of the serum (whey) was later removed, with water added at 40 °C, and that temperature was maintained for 30 min. The mix was then salted in 10% brine for 12 h, molded and allowed to stand for 24 h at room temperature.

2.3. Analysis of samples

Samples of goat milk and whey were taken in 12 ml screw-cap plastic tubes, and sampling of goat cheese was done in Ziploc-type plastic bags. Samples were covered with aluminum foil to protect them from light. They were refrigerated during transport and then stored in a deep freezer at −40 °C until further analysis. All samples were prepared at room temperature and in low-light conditions. The phenolic compounds in milk, whey and cheese were extracted by a previously developed method (Hilario et al., 2010; Vazquez et al., 2015) and subsequently quantified using the Folin-Ciocalteu method (Singleton and Rossi, 1965).

Briefly, for obtaining phenolic compounds in milk and whey, samples were thawed in a water bath at 35°–40 °C and then homogenized. Then 8 ml of fluid goat milk or milk whey was measured and transferred to a 25 ml volumetric flask; where it was dissolved with 10 ml of methanol-water (1:1, v/v); and protein precipitation was carried out with 500 µl of Carrez I, 500 µl of Carrez II and 5 ml of acetonitrile. The solution was complemented to 25 ml with methanol-water (1:1, v/v). After complete clot protein precipitation, the resulting solution was placed in a centrifuge tube and centrifuged at 7800 × g and 5 °C for 15 min. The liquid phase was separated and subsequently used for quantification of total phenolic compounds.

For the extraction of TPC in goat cheese the samples were thawed in a water bath at 35°–40 °C. Once thawed, 10 g previously ground, the samples were weighed, and 10 ml of methanol were added. Sample was vortexed for 40 min, and subsequently centrifuged at 3000 × g for 10 min at 5 °C. The extract was obtained as the supernatant, in which TPC were later quantified.

All samples were prepared at room temperature and in low-light conditions.

2.3.1. Determination of TPC and antioxidant capacity

TPCs were quantified using the Folin-Ciocalteu method (Vazquez et al., 2015). A calibration curve was prepared using gallic acid (GA) as standard with concentrations of 0.005–0.12 mg/mL. GA is conventionally used as standard reference, because it is a compound cheaply available in highly purified form, and is endowed with average reactivity. TPC content is typically expressed in Gallic Acid Equivalents (GAE) (Miniati, 2007). The equation of the line used was $y = 4.3008x + 0.0007$, with a $R^2 = 0.9998$. Values were reported in mg of GA equivalents (GAE) per liter for milk and whey, and mg GAE/Kg for cheese. The antioxidant capacity was determined using de Ferric Reducing Ability of a Plasma (FRAP) method (Benzie and Strain, 1996). A calibration curve was prepared using ascorbic acid (AA) at concentrations of 2.20–9.62 mg EAA/mL. The equation of the line used was $y = 0.0194x-0.0143$, with an $R^2 = 0.9998$. The antioxidant capacity was also determined using the 2,2-diphenyl-1-picrylhydrazyl, DPPH method (Brand-Williams et al., 1995). A calibration curve was prepared using AA at concentrations of 2.20–39.62 mg EAA/mL. The equation of the line used was $y = -0.0139x + 0.5685$, with an $R^2 = 0.9998$. Values were reported in mg EAA/L for milk or whey and mg EAA/Kg for cheese.

2.4. Statistical analysis

The study was carried out on a completely randomized design basis with a $2 \times 2 \times 2$ factorial arrangement: 2 feeding systems (free-range grazing or permanent confinement), 2 seasons of the year (rainy or dry), and 2 thermal treatments (pasteurized or unpasteurized). The differences between these groups were evaluated using ANOVA and *post-hoc* Tukey HSD tests. We used a 95% confidence interval and a significance level of $P < 0.05$.

3. Results and discussions

All variables– feeding system, season of year and pasteurization–had an impact on the studied variables: antioxidant capacity and, especially, TPC concentration.

TPC concentration was found to be 34.69% higher ($P < 0.05$) in milk from free-range goats than in milk from confined goats. These results are consistent with the results of other previously published studies which concluded that it is possible to improve the quality, achieve changes in the composition of and increase the concentration of phenolic compounds in milk by making some modifications to the goats' feeding system, by adding high content phenolic compounds foods (Jordan et al., 2010; Keles et al., 2017), increasing consumption of fresh forages (Di et al., 2015), or with the free-range grazing system (Hilario et al., 2010). TPC concentration was also significantly higher ($P < 0.05$) in cheese made from unpasteurized milk than in pasteurized milk. Antioxidant capacity, measured by the FRAP assay, was significantly higher in unpasteurized whey and cheese ($P < 0.05$) than