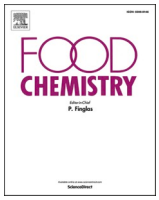
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Hydrogen-rich water consumption modifies the chemical, biochemical, nutritional, and bioactive properties of the goat's colostrum and mature milk

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ABSTRACT

This study aimed to investigate the effects of hydrogen-rich water (HRW) consumption in goats on the chemical composition, antioxidant activity, total phenolic content, in vitro antidiabetic activity (α-amylase and α-glucosidase inhibitions), free fatty acid profile, and volatile compounds of colostrum and mature milk. Goats were fed ad libitum with either normal water (NW) or HRW for 20–22 days before and 28 days after parturition. Colostrum and milk samples were collected from goats on the day of parturition, as well as on days 7, 14, 21, and 28 days. The milk fat content of goats fed with HRW was found to be higher compared to those fed with NW. On day 7, the total phenolic content was higher in the NW-fed milk compared to the HRW-fed milk. The ABTS radical scavenging activity of the HRW-fed colostrum was higher than NW. The inhibitory activity of α-amylase and α-glucosidase was higher in the HRW-fed milk. Some free fatty acids, including C2, C8, C10, C15, C18, and C20, increased in HRW-fed milk. Some volatile components were more abundant in the HRW-fed milk. The findings from this study may lead to new insights into the potential health benefits of milk from goats consuming HRW.

1. Introduction

Due to its functional properties, goat's milk has become the focus of interest as a healthy alternative for people with cow's milk allergy or intolerance (F. Wang et al., 2022; Y. Zhang, Usman, et al., 2024). Native to the Caucasus region and threatened with extinction, Georgian goats, also known as Gurcu, Tbilisi, and Caucasian goats, are one of the local dairy and meat breeds (Kuru et al., 2019). Colostrum, the first milk produced after birth, is rich in essential nutrients and bioactive components that ensure the survival of newborns (Blum & Hammon, 2000; Y. Sun et al., 2019). The antioxidant activity and mineral content of colostrum increase the resilience for the survival of newborn pups (Kim et al., 2009). The unique biological properties of colostrum make it an excellent material for developing nutritional supplements. These supplements have recently gained increasing popularity in the global market as potent immunostimulants (Mondeshka et al., 2022). It is known that colostrum turns into milk after the 5th day. The milk secreted by the mammary gland between the 1st and 5th day can be called goat milk (Mondeshka et al., 2022). During the transition from colostrum to normal milk, there may be gradual or sometimes changes in chemical composition and characteristics (Arain et al., 2008). The stage of the lactation period is significantly influenced by various factors such as age, breed, environmental stress, genetics, dry period, and calving season (Mehra et al., 2021). The chemical composition of goat milk is

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similar to cow milk and varies according to feed, breed, feeding conditions, and lactation stage (Park, 2017).

In goats, the type of feeding during pregnancy can influence diaper development, the mechanism of colostrum secretion, and, ultimately, the chemical composition of the colostrum (Zhou et al., 2023). In addition to animal nutrition, other parameters such as phytometabolites and microbial metabolism in the rumen can also influence the composition of milk (Mal et al., 2018). The type and quality of drinking water can also influence the chemical composition and properties of colostrum and milk. Goats require 1.43–3.5 L of water per kg per day (Parker & Brown, 2003). Many studies have investigated the effects of numerous drinking water parameters on the production and composition of milk of dairy goats, e.g., salt consumption (Costa et al., 2021; Nguyen et al., 2022), microbiological quality (Iancu, 2015) and water restriction under heat stress conditions (Alamer, 2009). Dietary enrichment with antioxidants, especially in ruminants, can help maintain the redox balance in the body. It has been emphasized that antioxidant-fortified feeds can increase the antioxidant content and immune system of animals and improve product quality for consumers (Zhang, Al-Wraikata, et al., 2024). Therefore, antioxidant-enriched feed has been proposed as an excellent strategy to combat oxidative stress in the animal body (Ebrahimi et al., 2024).

Molecular hydrogen (H_2), which is known to have a reducing property (Alwazeer et al., 2021), is a non-toxic, colorless, tasteless, and odorless gas with the food additive code E949 (Bulut et al., 2022). H_2 has more than 30 specific physical, chemical, and biological properties (Alwazeer et al., 2024). The physiological properties of H_2 include antioxidant, anti-stress, anti-inflammatory, redox regulator, and enhancer of internal antioxidant gene expression. There are several methods to administer H_2, including inhalation (Grepl et al., 2025), drinking hydrogen-rich water (HRW) (Aker et al., 2024), injecting hydrogen-rich saline (HRS), (G.-M. Li et al., 2013), taking hydrogen-rich baths (Asada et al., 2019) and using hydrogen-rich eye drops (Oharazawa et al., 2010).

Hydrogen-rich water (HRW) is a functional water with a dissolved hydrogen content of approximately 1.6 mg/L (under normal room conditions) (Alwazeer et al., 2022). HRW is an innovative functional beverage with many claimed benefits for humans, animals, and plants (Alwazeer, 2024a; Jafta et al., 2021). Due to the flammability of hydrogen and advanced containment solutions, its safe storage remains a critical issue (Patel et al., 2024). Therefore, new materials and methods for hydrogen storage are being developed to ensure safety and practicality in daily use (Patel et al., 2024). There is a general lack of understanding about the benefits and applications of HRW, which may hinder its acceptance (Aker et al., 2024). HRW can be obtained by various means, including electrolysis of water (LeBaron et al., 2022), injection of H_2 in water (Bulut et al., 2022), and the reaction of magnesium (Mg) with water (Çelebi et al., 2024; Q. Sun et al., 2012).

One of the methods for producing HRW is the electrolysis process (Asada et al., 2020). In recent years, numerous animal and clinical studies have demonstrated the health benefits of drinking HRW. HRW has been shown to be effective in relieving pain and improving the lives of women with premenstrual symptoms (Aker et al., 2024). Recently, our team found that supplementing HRW to goats improved kid survival and altered the metabolic profile during the peripartal period (Kuru, Makav, Kuru et al., 2024). In addition, HRW has been shown to ameliorate letrozole-induced polycystic ovaries in rats (Makav et al., 2023). Moreover, it has been reported that the external supply of oxygen or hydrogen can affect the production of IgM and IgG by B cells in broiler chickens (Shin et al., 2016). Furthermore, drinking HRW has improved both vitality and immune parameters in domestic mice by increasing available oxygen and hydrogen in active tissues (Sommer et al., 2007). Due to its antioxidant properties, HRW has been reported to increase antioxidant capacity and reduce oxidative stress markers in various animal studies (Yuan et al., 2024; Zhu et al., 2023). When HRW applications in livestock farming were examined, HRW in broiler chickens could increase poultry production by improving meat quality and antioxidant levels(Zhu et al., 2023). HRW improved growth and digestive efficiency in largemouth bass in aquaculture, highlighting its versatility across species (Yuan et al., 2024).

Although several studies have been conducted to investigate the compositional properties of goat milk during the lactation phase (Argüello et al., 2006; Delgado-Pertíñez et al., 2009; Sánchez-Macías et al., 2014), there are no studies evaluating the effects of drinking HRW on the composition of colostrum and milk. In this study, it was hypothesized that supplementing goats with water possessing antioxidant activity, i.e., HRW, may alter the composition and properties of milk. Therefore, the study aimed to investigate the effects of HRW supplementation on the composition, volatiles, free fatty acid profile, and bioactive properties of colostrum and mature goat milk during lactation.

2. Materials and methods

2.1. Ethical approval of the study

The study was conducted after the approval of the Local Ethics Committee for Animal Experiments of Kafkas University (approved under the number KAÜ-HADYEK/2022–123), Kars, Türkiye.

2.2. Location

The animal material for this study was provided by the A.R. Aksoy Education, Research and Application Farm of the Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye. The study area is located at an altitude of 1751 m and has coordinates 40°34′23“ N and 43°02`27” E.

2.3. Animal materials

Estrus synchronization was implemented in Gurcu goats to achieve synchronized parturition within 5–7 days during the breeding season. The study utilized 38 Gurcu goats aged between 3 and 4 years. Six Gurcu bucks were also included to facilitate mating during the estrus synchronization period.

2.4. Estrus synchronization

The estrus synchronization was achieved with an intravaginal sponge containing medroxyprogesterone acetate and equine chorionic gonadotropin (eCG). A sponge containing 60 mg medroxyprogesterone acetate was inserted into the vagina, and 400 IU eCG was administered intramuscularly on day 10. The sponges were removed on day 12, and the goats were mated by bucks 24 h later. The estrus was monitored for four days (Kuru et al., 2022).

2.5. Pregnancy examination

Pregnancy examination was performed by an apparatus, transrectal ultrasonography (5–7.5 MHz, Draminski iScan, Draminski, Poland) 35–37 days after mating of the goats, and pregnancy was diagnosed when the embryo became visible during the examination (Kuru et al., 2019) The pregnancy examination was repeated on day 60 for confirmation. Twenty of the 30 goats confirmed pregnant at the second pregnancy examination were included in the study.

2.6. Nutrition

The goats were fed daily with dry hay (0.6 kg/goat) and concentrates during gestation and lactation. Each goat received 0.5 kg/goat of concentrate with 16 % protein and 2700 kcal of metabolizable energy (Table 1). Drinking water was provided ad libitum to the goats during the entire study period.

Table 1
Characteristics of concentrate feed.

Ingredients	%	–
Corn	43.00	–
Barley	25.00	–
Cottonseed meal	16.50	–
Sunflower seed meal	5.00	–
Wheat bran	4.00	–
Molasses	4.00	–
Calcium carbonate	2.00	–
Salt	0.25	–
Vitamins and minerals	0.25	–
Nutrients	%	g/DM
Dry matter	89.0	–
Crude protein	16.0	17.87
Crude cellulose	10.0	6.28
Crude fat	3.2	3.52
Crude ash	9.0	6.04
Energy, kcal/kg ME	2700	3000
Vitamins	–	IU/kg
Vitamin A	–	15,000
Vitamin D	–	5000
Vitamin E	–	30,000
Minerals	%	–
Calcium	1.6	–
Phosphorus	0.4	–
Sodium	0.4	–
Trace minerals	–	mg
Manganese	–	50
Copper	–	10
Iron	–	50
Iodine	–	800
Zinc	–	50

2.7. Groups

Pregnant goats ($n = 20$) were divided into two groups 20–22 days before the expected parturition date. The goats were selected based on strict health criteria, including the absence of any history of abortion, dystocia, postpartum reproductive issues, mastitis, or metabolic diseases. Health status was confirmed through veterinary examinations, including physical assessments, conducted 30 days before the study. The goats were also balanced for age (range: 3–4 years), parity (2–3 births), live weight (35–45 kg), and the presence of multiple pregnancies to ensure homogeneity across groups.

Group 1 (G1, control, $n = 10$): The control group (G1) was provided with normal drinking water sourced from the same supply as the HRW but without hydrogen enrichment. The water was replaced at the same frequency (every 5–6 h) to maintain consistency between the groups. The temperature and cleanliness of the water were monitored regularly to ensure comparable conditions.

Group 2 (G2, HRW group, n = 10): Group 2 (G2) received hydrogen-rich water (HRW) ad libitum from 20 to 22 days before parturition until 28 days postpartum as a substitute for regular drinking water. The HRW was freshly prepared and supplied in troughs, ensuring unrestricted access for the animals. To maintain its optimal hydrogen concentration, the water was renewed every 5–6 h, with any remaining liquid completely removed before refilling (Kuru, Makav, Kuru et al., 2024). Additionally, troughs were examined daily to prevent contamination and maintain hygienic conditions. The goats in both G1 and G2 were housed in separate paddocks within the same farm to prevent intergroup interactions and potential external influences. To standardize environmental conditions and dietary intake, none of the animals were allowed to graze during the study period.

2.8. Preparation of hydrogen-rich water (HRW)

HRW was prepared by infusing an oxygen-hydrogen gas mixture (30/70, % vol) generated by an oxygen-hydrogen generator (HB-33+ model Epoch, Taiwan) into the drinking water. The hydrogen content in the HRW was continuously monitored using an SP60X redox probe (Consort, Belgium) (Bulut et al., 2022). The concentration of dissolved hydrogen in the HRW was approximately 1 mg/L (Bulut et al., 2022; Seo et al., 2012).

2.9. Colostrum/milk samples

Goat colostrum/milk samples were provided to the goats on the day of birth and on the 7th, 14th, 21st, and 28th days after birth. Colostrum and milk samples were stored at −80 °C until analysis. Colostrum samples were collected within three hours of the goats' birth, while milk samples were collected shortly before morning feeding on the sampling day. The teats were cleaned with warm water and dried with a paper towel before milking.

2.10. Chemical composition analysis

The titratable acidity (TA) of the colostrum/milk samples was measured using the alkali titration method, and the results were expressed as % lactic acid (AOAC, 1995). Milk pH was measured using a pH meter (Orion 4 Star pH meter, Thermo Fisher Scientific Inc. USA) (Hayaloglu et al., 2005). Fat, solid-not-fat components (SNF), protein, lactose, freezing point, density, and salt content of milk were determined using a semi-automatic analyzer (Milk Analyzer, MILKANA superior Plus) (Nasalean et al., 2015).

2.11. Total phenolic content

The total phenolic content (TPC) of the colostrum/milk samples was determined using the Folin-Ciocelteu's method as described in (İncili et al., 2023) with modifications. 0.8 mL of milk was placed in a test tube with 0.8 mL of Folin-Ciocalteu's reagent, which was diluted 1:1 (vol) with water. After vortexing, 0.8 mL of 2 % Na_2CO_3 was added to the solution. After incubation at room temperature for 2 h, the mixture was centrifuged at 4000 ×*g* for 10 min (Hettich 320R, Tuttlingrn, Germany), and the absorbance was measured at 760 nm. The gallic acid standard curve was constructed, and the results were expressed as mg Gallic Acid Equivalent (GAE)/L.

2.12. Antioxidant activity analysis (ferric reducing antioxidant power and $ABTS^{+\cdot}$ radical scavenging activity)

The FRAP method was applied for milk samples according to the method of (Benzie & Strain, 1996) with a few modifications. The FRAP reagent was prepared by mixing the following solutions in a ratio of 1:10:1 (vol): 20 mM ferric chloride ($FeCl_3$), 300 mM sodium acetate buffer (pH 3.6), and 10 mM TPTZ solution prepared in 40 mM HCl. The prepared reagent was kept in the dark to complete the reaction for 30 min. Various concentrations (1–100 μg/mL) of $FeSO_4.7H_2O$ were used to prepare a standard curve. For the analysis, 0.1 mL of colostrum/milk sample was mixed with 2.9 mL of FRAP reagent. An equal volume of pure water (0.1 mL) was used for the blank sample. All samples were prepared in duplicate and shaken for one minute. They were then incubated at 37 °C for 10 min in the dark, and after centrifugation at 4500 ×*g* for 10 min, the absorbance of the reaction mixture was measured at 593 nm in a UV spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) for each sample. The FRAP activity of the colostrum/milk samples was expressed as μM Fe(II)/L.

ABTS scavenging activity of colostrum/milk samples was measured according to the method (Hayaloglu & Demir, 2015). A 10 mL solution containing 6.62 mg potassium persulfate and 38.41 mg ABTS was prepared. The absorbance of the ABTS solution was adjusted to 0.70 ± 0.02 at 734 nm with ethanol. 0.1 mL of the milk sample was diluted 10-fold with water, then added to 2.4 mL of ABTS solution and shaken. After incubation in the dark for 10 min, the absorbance against ethanol was measured at 734 nm using a UV spectrophotometer (Shimadzu UV-

1800, Kyoto, Japan). The results were expressed in mg Trolox Equivalent (TE)/L.

2.13. α-Amylase and α-glucosidase inhibition activity

The α-amylase and α-glucosidase inhibitory activity was determined according to the method described by Cam et al. (2020) and Ayyash, Al-Nuaimi, et al. (2018) with some modifications.

For the α-amylase inhibition assay, 1.0 units/mL of α-amylase enzyme (from human saliva, Sigma) was prepared in 0.1 M potassium phosphate buffer (pH 6.8). A 100 μL of the α-amylase was mixed with 100 μL of the pepsin hydrolysate. The mixture was then incubated in a water bath at 37 °C for 5 min, and 250 μL of starch (1 %, *w/v*) was added to start the reaction. Next, 200 μL of DNS reagent consisting of 1 % 3,5-dinitro salicylic acid and 12 % sodium potassium tartrate was added to stop the reaction, and the mixture was kept at 100 °C for 15 min. After cooling, 2 mL of distilled water was added to the solution. The absorbance of the mixture was measured at 540 nm, and the α-amylase inhibitory activity was calculated using the following formula.

$$Inhibition\% = \left[1 - \frac{Abs\ hydrolysate - Abs\ blank}{Abs\ control}\right]$$

Abs hydrolysate: Absorbance in the presence of α-amylase and starch and hydrolysate.

Abs blank: Absorbance in the presence of starch.

Abs control: Absorbance in the presence of α-amylase and starch (no hydrolysate).

For the α-glucosidase inhibition assay, 1.0 unit/mL α-glucosidase enzyme was prepared in 0.1 M potassium phosphate buffer (pH 6.8). Then 100 μL α-glucosidase was mixed with 50 μL pepsin hydrolysate from goat milk. This mixture was incubated at 37 °C for 10 min, then 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside was put in the test tube. The solution was incubated at 37 °C for 30 min, and the reaction was ended by adding 2.5 mL of 0.1 M Na_2CO_3. The measurement was performed at 400 nm using a spectrophotometer, and the inhibition of α-glucosidase was calculated as:

$$Inhibition\% = \left[1 - \frac{Abs\ hydrolysate - Abs\ blank}{Abs\ control}\right]$$

Abs hydrolysate: Absorbance in the presence of α-glucosidase, *p*-nitrophenyl α-D-glucopyranoside, and hydrolysate.

Abs blank: Absorbance in the presence of α-glucosidase in the absence of hydrolysate.

Abs control: Absorbance in the presence of α-glucosidase and *p*-nitrophenyl α-D-glucopyranoside (no hydrolysate).

2.14. Free fatty acid profile

2.14.1. Preparation of free fatty acid samples

Two milliliters of colostrum/milk samples were mixed with 2.5 g Na_2SO_4. Then 5 mL diethyl ether and 300 μL 4 N H_2SO_4 were added and mixed for 1 min. The mixed samples were kept in the dark for one hour and then centrifuged at 2500 rpm at 0 °C for 5 min. 5 mL of hexane was added to the centrifuged samples, shaken for 3 min, then centrifuged at 3000 rpm 0 °C for 10 min, capped tightly, and allowed to stand for 2 h. While the mixture was waiting, one gram of aluminum oxide was placed in a syringe, and 5 mL of hexane/diethyl ether (1/1) was added and conditioned. Then, the upper phase of the samples was filtered through the syringe with alumina into 10 mL centrifuge tubes. The filtrate was again passed through aluminum oxide, and this time, the filtrate was eluted. Then, 5 mL of hexane/diethyl ether mixture was passed twice through aluminum oxide containing fatty acids. After filtration, the aluminum oxide was dried thoroughly and transferred to 15 mL centrifuge tubes. 2 mL of formic acid prepared in di-isopropyl was added and centrifuged at 2000 rpm for 10 min. At the end of centrifugation, the clear part was filled into 1.5 mL vials using a Pasteur pipette and injected into the GC–MS (Shimadzu, QP2010, Kyoto, Japan). All chromatographic conditions and calculation procedures were determined according to İncili et al. (2023). The results were given as % of peak area.

2.15. Volatile compound profile

Volatile compounds were determined using GC–MS, as described by Kanmaz et al. (2023). A 10 g colostrum/milk sample was filled into special vials (Supelco, Bellafonte, MA, USA) and extracted using the solid-phase microextraction (SPME) method. Briefly, the special vial was kept at 40 °C for 30 min, after which the fiber was immersed and held for the same duration to allow the components to be absorbed by the fiber. Volatile compounds were desorbed by injecting the special fiber into the Shimadzu GC-2010/QP-2010/MS (Shimadzu Corp.). Helium was used as the carrier gas at a flow rate of 1 mL/min. A capillary column (HP-5MS 60 m × 0.25 mm × 0.25 × μm, Agilent J&W GC columns, USA) was used, and the NIST and Wiley libraries were scanned to identify the compounds. The results were presented as % peak area.

2.16. Statistical analysis

The sample size was determined based on an effect size of 2.04 reported in a previous study (Saleem, 2019), with a power of 95 % and a significance level of 5 %. G*Power® program (Version 3.1.9.7, Franz Faul, Universitat Kiel, Germany) was used for power analysis.

Analyses of variance (two-way ANOVA) with Tukey's post hoc tests were performed with GraphPad Prism® (version 9.0, GraphPad Software Inc., San Diego, CA, USA). Two-way ANOVA was chosen to evaluate the main effects and interactions between the independent variables (group and time), as well as the group × time interaction. Tukey's multiple comparison test was used for pairwise comparisons of groups and days to control for Type I error inflation due to multiple comparisons. Statistical analysis was performed for the interactions between group, time, and group × time. The significance level was indicated as * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. The volatile compounds of the data were analyzed using both principal component analysis (PCA) and heat mapping to evaluate the relationship between the volatile compounds and the different water types ("HRW, control water") or "days". PCA was chosen to reduce the dimensionality of the data and identify patterns or trends, while heat map analysis was used to visualize the relationships and clustering of volatile compounds across different groups and days. PCA was performed using SPSS 22 software, and heat map analysis was conducted using R software (https://www.r-project.org) (X. Wang et al., 2019).

3. Results and discussion

3.1. Chemical composition

In the beginning, the titratable acidity (%) of colostrum was 0.496 ± 0.094 for HRW-fed goats and 0.499 ± 0.104 for NW-fed goats without significant difference ($P > 0.05$) (Fig. 1A). Colostrum samples (at day 0) from two water types (NW or HRW)-fed goats showed higher titratable acidity values than mature milk samples (at days 7, 14, 21 and 28) ($P < 0.0001$) (Fig. 1A). Studies have reported that titratable acidity (TA) values are high in colostrum, gradually decreasing throughout the lactation period, which is consistent with our findings (Güzeler et al., 2010; Mondeshka et al., 2022; Moreno-Indias et al., 2012; Sánchez-Macías et al., 2014). On the 0th day, the pH value of colostrum was 6.531 ± 0.109 for HRW-fed goats and 6.515 ± 0.052 for NW-fed goats, with pH values gradually increasing during lactation (Fig. 1B). There was no significant difference between the two groups ($P > 0.05$), but pH changed significantly with days of lactation ($P < 0.0001$). These results are consistent with the findings of previous studies, which also reported minor changes in pH after birth and non-significant effects of diet on pH

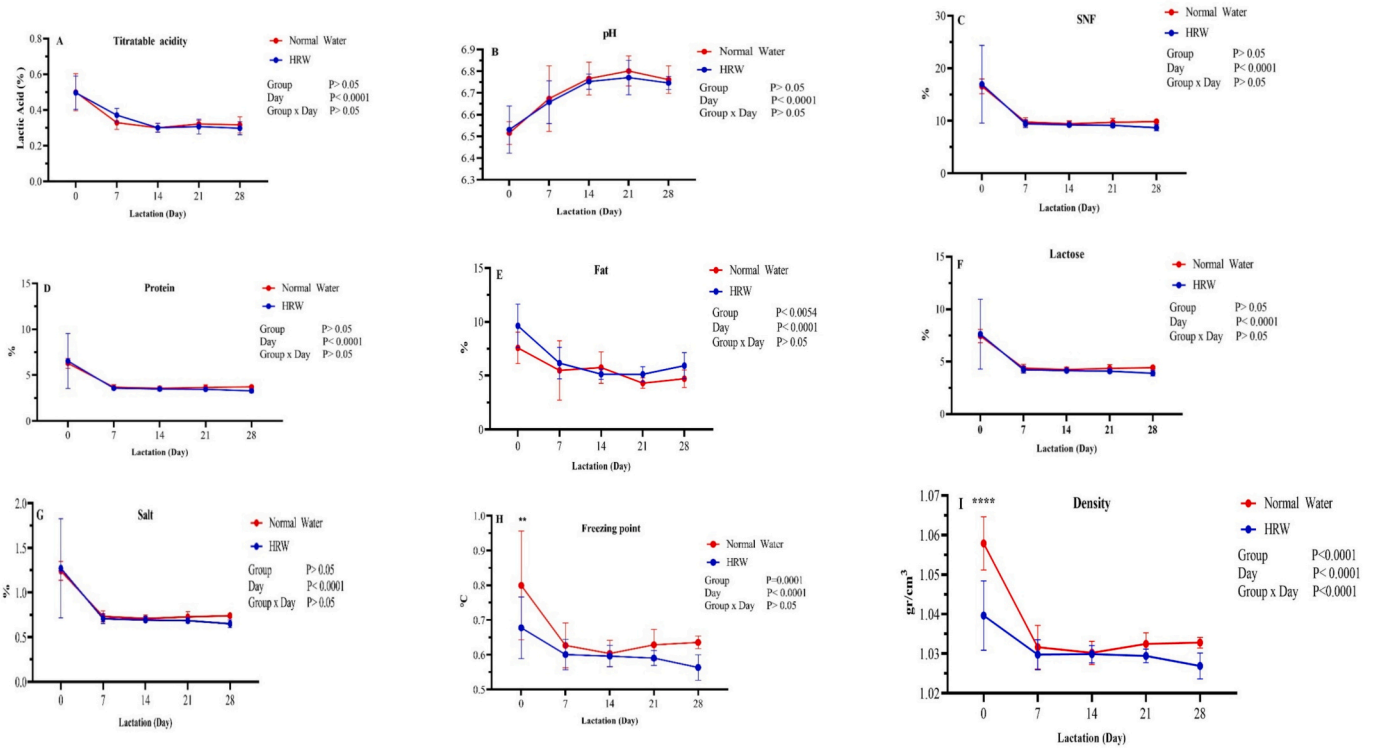


Fig. 1. Changes in some physicochemical and chemical parameters of colostrum/milk of goats fed with H_2-rich water and normal water (control) throughout the lactation period (0, 7, 14, 21, and 28). Titratable acidity (A); pH value (B); solid-not-fat (SNF); protein content (D); fat content (E); lactose content (F); salt content (G); freezing point value (H); and density value (I). Each value presents a mean ± SD. Control (normal water), HRW (hydrogen-rich water). Treatment groups were determined using two-way ANOVA with the *Tukey* post hoc test. *, indicates a statistical difference between HRW and normal water on the sampling day at $P < 0.05$. The degree of significance was denoted as follows: no sign at $P > 0.05$ for non-marked lactation days, ** $P < 0.01$, **** $P < 0.0001$.

value (Onjai-Uea et al., 2024; Ruiz et al., 2015).

At day 0, the SNF value (%) of colostrum was 16.960 ± 7.397 and 16.560 ± 1.400 for HRW- and NW-fed goats, respectively (Fig. 1C). A sharp decrease in SNF values occurred on day 7 (HRW at 9.438 ± 0.730 and normal water at 9.767 ± 0.811). These results are consistent with a previous study by (Sánchez-Macías et al., 2014), in which a gradual decrease in SNF levels was observed after the first postpartum day.

At the beginning of lactation, the protein value (%) of colostrum was 6.539 ± 3.001 and 6.292 ± 0.543 for HRW- and NW-fed goats, respectively without significant difference ($P > 0.05$) (Fig. 1D). It has been reported that during the transition phase from colostrum to milk, some nutritional, metabolic, hormonal, and immunological changes occur affecting the composition and characteristics of colostrum (Arain et al., 2008; Sánchez-Macías et al., 2014). In addition, studies have reported that the protein content in colostrum is high and decreases during the lactation period (Argüello et al., 2006; Mondeshka et al., 2022; Zhou et al., 2023). The decrease in protein content at later stages, except in colostrum in goats, is probably due to the dependence on maternal amino acids necessary for fetal growth (Akkaya et al., 2020; Sadjadian et al., 2013).

In colostrum, the fat content (%) was 9.634 ± 2.014 and 7.586 ± 1.455 for HRW- and NW-fed goats, respectively. On day 28, it was 5.928 ± 1.213 and 4.717 ± 0.812 for HRW and NW, respectively (Fig. 1E). In general, fat content in colostrum/milk samples was higher in HRW-fed goats than in NW-fed goats ($P < 0.0054$). The fat content in both HRW and NW-fed goat milk samples decreased throughout lactation. The increase in fat content in the colostrum/milk of goats fed HRW can be attributed to the increase in blood triglycerides (Ebrahimi et al., 2024). Medium-chain triglycerides occur naturally in milk fat (Jadhav & Annapure, 2023). In the study conducted by Kuru, Makav, Boğa Kuru et al. (2024) in goats, the blood triglyceride level, which was the same as the HRW group control group during pregnancy, decreased in the HRW group after giving birth. Especially on the day of birth, the blood triglyceride level was the lowest (Kuru, Makav, Boğa Kuru et al., 2024). In the present study, the fat content in colostrum taken at birth in goats was the highest in the HRW group. It is thought that triglyceride, a medium-chain fat in the blood, passes to colostrum.

In different research conducted on improving the metabolic profiles of kids in the prepartum period in Georgian goats and increasing their health and survival rates, according to the analysis results of blood samples, group effect and time effect were found to be significant in triglyceride ($P < 0.001$, $P = 0.001$, respectively) and albumin ($P < 0.001$, $P = 0.002$, respectively) concentrations. In addition, in the same research, it was detected that triglyceride concentration decreased in both groups on the day of parturition and then increased in G2 (Control group) but decreased with HRW supplementation (Kuru, Makav, Boğa Kuru et al., 2024). Antioxidants have been reported to help improve lipid metabolism (Howard et al., 2002). One study showed that the addition of synthetic antioxidants (Agrado Plus) to the cows' feed did not affect fat content on day 21 of lactation (Boerman et al., 2014).

The lactose value (%) of the colostrum was 7.632 ± 3.329 and 7.452 ± 0.630 for HRW- and NW-fed goats, respectively (Fig. 1F). On day 28, it was 3.905 ± 0.259 and 4.443 ± 0.120 for HRW and NW, respectively. Lactose content decreased significantly throughout the lactation period ($P < 0.0001$). However, water type did not affect lactose content in goat colostrum/milk samples ($P > 0.05$). One study showed that adding synthetic antioxidants (Agrado Plus) to the cows' feed did not affect the lactose content at day 21 of lactation.

Salt content (%) in colostrum was 1.272 ± 0.555 and 1.242 ± 0.105 for HRW- and NW- fed goats, respectively. A sharp decrease in salt levels occurred on day 7 (HRW at 0.708 ± 0.055 and NW at 0.733 ± 0.061). On day 28, it was 0.651 ± 0.043 for HRW and 0.740 ± 0.020 for NW-fed goats (Fig. 1G). However, water type did not affect the salt content in the colostrum/milk samples ($P > 0.05$).

The freezing point value (°C) of colostrum was lower in HRW samples than in NW ($P < 0.01$). On day 7, there was a sharp decrease in freezing point values (HRW at 0.601 ± 0.044 and NW at 0.627 ± 0.065) (Fig. 1H). The freezing point value of colostrum samples of HRW-fed goats was lower than that of NW-fed goats ($P = 0.0001$) (Fig. 1H). The freezing point value decreased throughout the lactation period for both water types ($P < 0.0001$).

The density (g/mL) of colostrum was lower in HRW samples than in NW at 1.040 ± 0.009 and 1.058 ± 0.007 respectively ($P < 0.0001$) (Fig. 1I). A sharp drop in the density value occurred on day 7 (HRW at 1.030 ± 0.004 and NW at 1.032 ± 0.006) (Fig. 1I). On day 28, it was 1.027 ± 0.003 and 1.033 ± 0.001 for HRW and NW milk samples, respectively (Fig. 1I). The high-fat content of the HRW-fed goat colostrum samples can explain this. It is known that the fat density is lower than that of water. The density of milk/colostrum decreased over the lactation period for both water types ($P < 0.0001$, Fig. 1I).

3.2. Total phenolic content (TPC)

Results show that the type of water had no effect on the phenolic content in colostrum and milk on all days of lactation except day 7, where NW-fed goat milk had higher TPC value than HRW with 6.332 ± 1.451 and 4.960 ± 0.597 mg GAE/L, respectively (Fig. 2A). However, the TPC value was significant concerning group ($P = 0.0428$) and day ($P < 0.0001$). In addition, there was an interaction between group and day for TPC value ($P = 0.0121$). When TPC values of milk/colostrum samples were examined for each water type by day of lactation, colostrum samples (at day 0) of both NW- and HRW-fed goats had higher TPC values than milk samples (at days 7, 14, 21, and 28) ($P < 0.0001$) (Table S1, Fig. 2A).

One study showed that using a grape seed extract-enriched feed for sheep and goats increased the phenolic concentration in milk to a certain extent, with most phenols being lost via the urine (Leparmarai et al., 2019). In another study, the effect of an olive leaf-enriched diet on the phenolic composition of goat milk at day 30 showed that the milk obtained had a significantly higher total phenolic content than the milk of goats fed a standard diet ($P < 0.01$) (Ianni et al., 2021). While protein, lactose, and fat components of milk are synthesized in mammary gland epithelial cells, other components such as Na, Ca, Mg, and water are absorbed through the blood (Cai et al., 2018) Therefore, the content of blood serum and the factors affecting MGC (mammary gland cell) change the yield and composition of milk. Increased cellular ROS can damage the mitochondrial structure (Shokolenko et al., 2009) and increase the level of oxidative stress due to dysfunction of mitochondria (Song et al., 2021). In dairy cows, it has been shown that increased oxidative stress disrupts the organ function of the mammary gland and affects mammary epithelial cells (MEC) (Baratta et al., 2018; Hadaya et al., 2020). Although the effects of physiological nutritional factors on MGC have not been clearly elucidated, it has been reported that the osmotic pressure of mammary epithelial cells, blood flow rate, and oxidative stress of MGC affect the yield and composition of milk (Cai et al., 2018). Therefore, it is predicted that HRW, by its antioxidant activity, may regulate the oxidative status of goat MEC, allowing the allocation of resources to produce milk components in the mammary gland. The obtained results show the capacity of MEC to alleviate oxidative stress effects with HRW consumption.

3.3. Antioxidant activity

3.3.1. Ferric reducing antioxidant power (FRAP)

The FRAP value (μM Fe(II)/L) of the colostrum was 45.135 ± 13.831 and 49.102 ± 25.058 for HRW and NW, respectively. It decreased sharply on day 7 (HRW at 35.552 ± 8.991 and NW at 34.323 ± 6.240). On day 28, it was 29.458 ± 5.894 and 32.469 ± 6.273 for milk samples of HRW and NW, respectively (Fig. 2B). The type of water provided to the goats had no significant effect on the FRAP value of colostrum/milk ($P > 0.05$). In addition, there was no interaction between the group and day for the FRAP value ($P > 0.05$) (Fig. 2B). When the groups were compared by day, colostrum (day 0) from goats fed normal water showed significantly higher FRAP than milk samples from day 14 ($P = 0.0439$) and day 28 ($P = 0.0375$) (Table S1, Fig. 2B). It has been reported that lactation period can influence the antioxidant activities of goat milk (Alyaqoubi et al., 2014). Similar results were reported in a study investigating the changes in the antioxidant content of Malabari goat milk at different stages of lactation. The authors found that the milk's antioxidant capacity (FRAP) was higher in early lactation than in

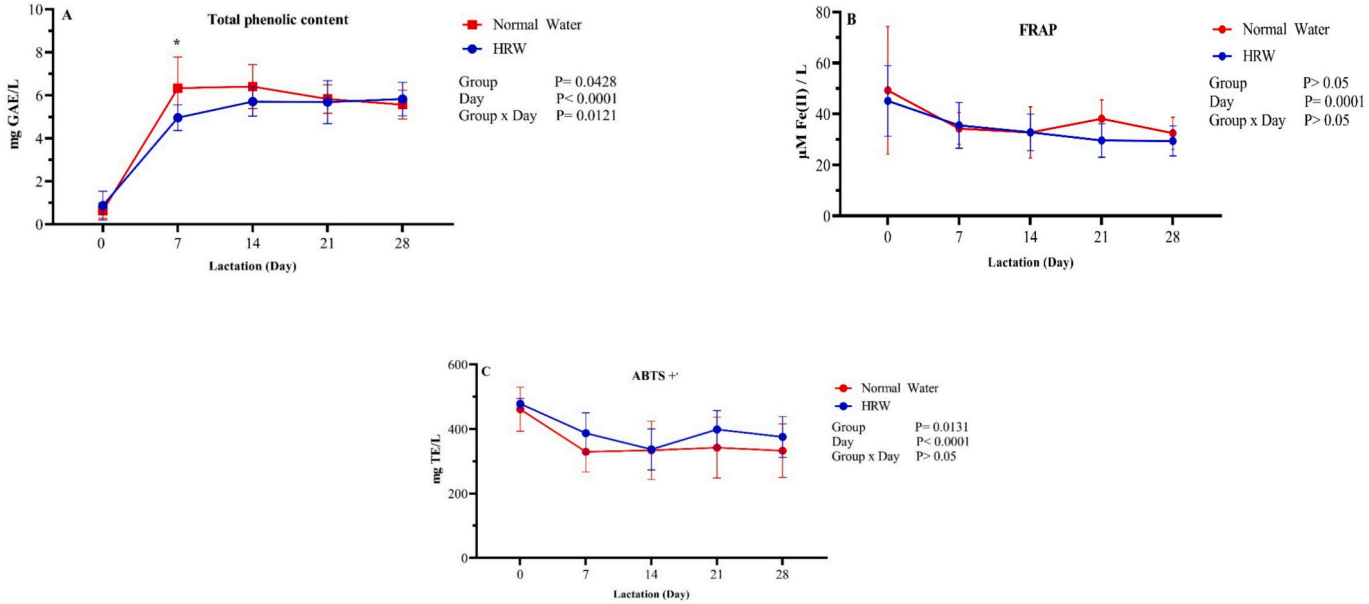


Fig. 2. Phenolic content and antioxidant activity of colostrum/milk of goats fed with H_2-rich water (HRW) and normal water (control) throughout the lactation period (0, 7, 14, 21, and 28 day). Changes in total phenolic content (A); ferric reducing antioxidant power (FRAP) (B); and $ABTS^{+\cdot}$ radical scavenging activity (C). Each value presents a mean ± SD. Treatment groups were determined using two-way ANOVA with the Tukey post hoc test. *, indicates a statistical difference between HRW and normal water on the sampling day at $P < 0.05$. The degree of significance was denoted as no sign when $P > 0.05$ at non-marked lactation days.

the middle and late lactation stages (Annie et al., 2019). In addition, one study found that the addition of saffron leaves (SP) to supplements improved the antioxidant status of milk (FRAP) in dairy goats (Ebrahimi et al., 2024). In another study, antioxidant supplementation (SOD, Zn, and Se) of buffalo feed did not alter the overall antioxidant activity of milk (Evangelista et al., 2022). Feeding iron lactate-enriched water to cows did not affect the oxidative stability of milk as measured by the thiobarbituric acid reactive substances (TBARS) assay (Mann et al., 2013). However, no studies have examined the effect of feeding HRW on the antioxidant activity of milk in animals.

3.3.2. $ABTS^{+\cdot}$ radical scavenging activity

Results show that the ABTS purification activity (mg TE/L) of colostrum was 478.025 ± 16.817 and 461.495 ± 68.652 for HRW- and NW-fed goats, respectively. A sharp drop in $ABTS^{+}$ occurred on day 7 (HRW at 387.128 ± 63.018 and NW at 329.366 ± 62.555). On day 28, it was 375.436 ± 63.107 and 332.902 ± 82.905 for HRW- and NW-fed goat milk samples, respectively (Fig. 2C). $ABTS^{+}$ scavenging values of HRW-fed goat colostrum/milk were higher than those of NW-fed goat samples ($P = 0.0131$). This value varied depending on the days of lactation ($P < 0.0001$). However, there was no group-by-day interaction for the ABTS scavenging activity value ($P > 0.05$) (Fig. 2C). When comparing the groups by days of lactation, colostrum (day 0) of HRW-fed goats had significantly higher $ABTS^{+}$ scavenging activity value than on day 14 ($P = 0.0007$) and day 28 ($P = 0.0426$). However, colostrum (day 0) of NW-fed goats showed a significantly higher $ABTS^{+}$ scavenging activity value than milk samples of other days (day 7, $P = 0.0020$; day 14, $P = 0.0034$; day 21, $P = 0.0085$; day 28, $P = 0.0030$) (Table S1, Fig. 2C). One study reported that there is a positive correlation between the antioxidant activity of milk and the use of natural antioxidants through the transfer of their active compounds to animal tissues (Sayiner et al., 2021) increasing total antioxidant capacity (Descalzo & Sancho, 2008). However, the FRAP method cannot detect this antioxidant capacity because it originates from thiol groups whose reduction potential is below the Fe^{3+}/Fe^{2+} half-value set (Benzie & Strain, 1996; Chen et al., 2003). However, ABTS reacts rapidly with most antioxidants and can be used in a wide pH range (Al-Saleh et al., 2014). Another study investigated the effects of oxygenated or hydrogenated water on growth performance, blood parameters, and antioxidant enzyme activity in broiler chickens. The authors found that adding both oxygenated and hydrogenated water significantly improved antioxidant activity (ABTS) (Shin et al., 2016).

Regarding the positive effect of HRW feeding on the ABTS value of milk, it was found that the use of HRW as a solvent in the extraction of phytochemicals from various agro-food wastes improved the extraction of phenolic compounds, flavonoids, anthocyanins, ascorbic acid and antioxidant activity (DPPH and ABTS) from various agro-food wastes (Alwazeer & Elnasanelkasim, 2023). The results of the above references suggest that drinking HRW after feeding the animals with forage or grass may improve the bioaccessibility and bioavailability of some phytochemicals in the digestive system (Alwazeer, 2024a). However, this hypothesis requires further studies to evaluate the influence of HRW drinking on the bioavailability and accessibility of nutrients. At this point, it should be recalled that the ABTS method measures the free radical scavenging activity of both hydrophilic and lipophilic antioxidants (Munteanu & Apetrei, 2021). Molecular hydrogen dissolves better in non-polar solvents and media than in polar ones (Alwazeer, 2024a, 2024b).

The bioactive compounds found in dairy products, especially peptides, provide various health benefits, including antioxidant, antimicrobial, ACE inhibitory, and antithrombotic effects. These bioactive peptides play a crucial role in mitigating the effects of free radicals such as hydroxyl radicals (^{-}OH), superoxide anions (O_2^{-}), hydroperoxyl radicals (^{-}OOH), and peroxyl radicals (ROO) (Singh & Gaur, 2024). It is known that molecular hydrogen (H_2) has a similar protective effect against free radicals (Alwazeer et al., 2024), decreases free radical formation, and reduces oxidative stress (Cumaoglu et al., 2024). Oxidative stress increases the incidence of postpartum diseases such as mastitis, decreasing milk quality (Castillo et al., 2013). Mastitis significantly changes enzymatic activity within the mammary gland, resulting in ROS accumulation and oxidative stress in the mammary tissue (Novac & Andrei, 2020). These effects have consequences on the antioxidant activity of milk (Andrei et al., 2016). Antioxidant enzymes are crucial for reducing lipid hydroperoxide and H_2O_2 levels, preventing lipid peroxidation, and preserving cell membrane integrity and function (Al-Hassan, 2018). Goat milk, rich in essential antioxidant enzymes, plays a vital role in supporting human cells (Aljuhani et al., 2015). However, antioxidant enzymes have been shown to affect milk bioactivity in goats during the postpartum period (Al-Hassan, 2018).

Previous studies in dairy goats have focused on the impact of various dietary intakes on some antioxidant enzyme activities, showing that such interventions can positively influence both enzymatic and non-enzymatic antioxidant defenses in blood and milk (Paraskevakis, 2015; Sharifi et al., 2017; Tsiplakou et al., 2018). Dietary intake can also affect milk proteins, vitamins, and lipids through oxidation, leading to a decrease in nutritional value and organoleptic properties (Smet et al., 2008). Consumption of hydrogen-rich water has been shown to reduce excessive free radical production and increase gene expression and the activity of antioxidant enzymes (Alwazeer et al., 2024; Sha et al., 2018).

In summary, consumption of HRW suggests that goats may increase antioxidant enzyme levels in their mammary glands and decrease oxidative stress, thus increasing antioxidant activity in goat milk.

3.4. α- Glucosidase and α-amylase inhibition activities

The α-amylase inhibitory activity of HRW- and NW-fed goat milk was highest in the colostrum (day 0) with 80.0 % and 49.58 %, respectively (Fig. 3A-B). The inhibitory activity of α-amylase was higher in HRW-fed goat milk than in NW-fed goat milk samples during lactation (day 0, $P < 0.001$; day 7, $P < 0.01$; day 14, $P < 0.0001$; day 21, $P < 0.001$; day 28, $P < 0.05$). During the lactation period, a decrease in α-amylase inhibitory activity was observed by day 28 in goat milk samples of HRW-fed goats and by day 14 in NW-fed goat samples (Fig. 3A).

The α-glucosidase inhibitory activity in HRW-fed goat milk was 69.28 %, while it was 49.26 % in NW-fed goat milk (Fig. 3B). The results showed that the ability of milk to inhibit α-glucosidase was affected by the type of water and the day of lactation. The results revealed that HRW-fed goat colostrum and milk samples had significantly higher α-glucosidase inhibitory activity than NW-fed goat samples on days 0 ($P < 0.0001$), 7 ($P < 0.001$) and 14 ($P < 0.05$) (Fig. 3B). The α-glucosidase inhibitory activity decreased as the day of lactation progressed, with the highest value observed in colostrum samples. When comparing the groups by day of lactation, HRW-fed goat colostrum (day 0) showed significantly higher α-glucosidase inhibition than on day 14 ($P = 0.0015$) and days 21 and 28 ($P < 0.0001$) (Table S1). However, there was no difference between colostrum samples (day 0) and milk samples (days 7, 14, 21, 28) regarding the α-glucosidase inhibitory activity of samples derived from NW-fed goats. There was also an interaction between group and day for the value of α-glucosidase inhibitory activity ($P = 0.0201$) (Fig. 3B).

In the treatment of type 2 diabetes mellitus, carbohydrate digestion is controlled by delaying postprandial blood glucose absorption through inhibition of the enzymes α-amylase and α-glucosidase (X. Li et al., 2022). The inhibitory activity of these enzymes can be used to evaluate the antidiabetic effect in vitro. The enzyme inhibitory effect (a-amylase and a-glucosidase) of milk is attributed to its bioactive compounds, such as vitamins, phenolic compounds, caseins, and serum proteins, which have the potential to exert antidiabetic effects (Harizi et al., 2023). Bioactive peptides, particularly from milk and dairy products, have been extensively studied for their beneficial effects on consumer health (Singh & Gaur, 2024). In a study investigating the α-amylase and α-glucosidase inhibitory activities of goat milk protein hydrolysates

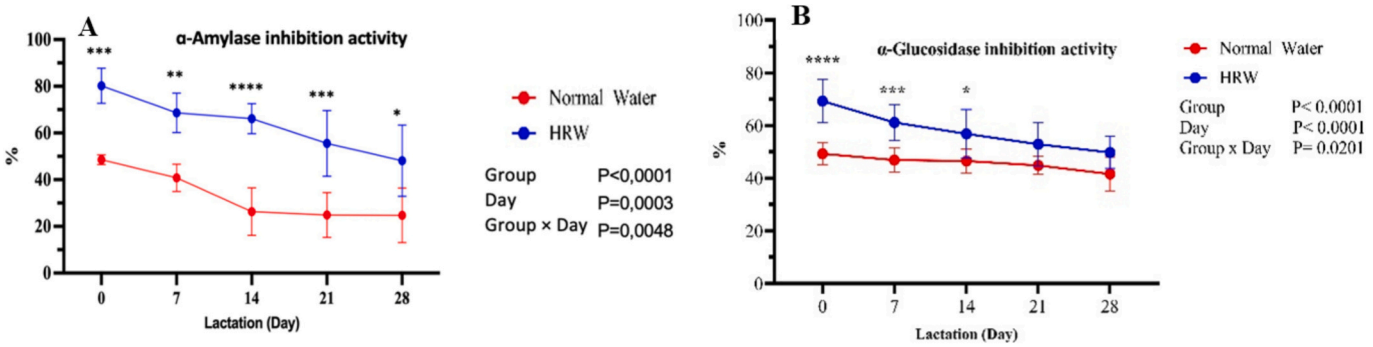


Fig. 3. Enzymatic activities of colostrum/milk of goats fed with H_2-rich water and normal water (control) throughout the lactation period (0, 7, 14, 21, and 28). α-amylase inhibition activity value (A) and α- glucosidase inhibition activity value (B). Each value presents a mean ± SD. Treatment groups were determined using two-way ANOVA with the Tukey post hoc test. *, indicates a statistical difference between HRW and normal water on the sampling day at $P < 0.05$. The degree of significance was denoted as no sign when $P > 0.05$ at non-marked lactation days, * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

prepared with different enzymes, it was reported that the hydrolysates exhibited potent antidiabetic activity and that this activity was influenced by factors such as concentration, enzyme type, and protein substrate specificity (Alwazeer, 2024a, 2024b). In their study, Ayyash, Al-Dhaheri, et al. (2018).

investigated the health benefits of fermented camel and bovine milk through in vitro analysis. It was reported that α-amylase inhibition increased in camel milk over 21 days, and α-glucosidase activity fluctuated between 30 and 40 % in both kinds of milk during storage. In another study, it was shown that the presence of hydrophobic amino acids, such as leucine (Leu) and proline (Pro), strongly affects α-glucosidase inhibitory activity (Liu et al., 2021). Peptides containing Pro at the N-terminus have been shown to play an important role in glycemic control (Nongonierma & FitzGerald, 2018). Siow and Gan (2016) emphasized in their studies that the inhibition of α-amylase activity by peptides is based on the interaction with aromatic amino acids. Additionally, Du et al. (2022) showed that hydrolysis products of goat milk whey proteins have α-glucosidase and α-amylase inhibitory activities. They showed that hydrolysate components containing a large amount of hydrophobic amino acids are effective in inhibiting α-glucosidase activity. Another study reported that in 9 adults with high blood glucose who drank 1.5 L of HRW daily for eight weeks, diabetes-related parameters improved, and oxidative damage decreased, which was attributed to the hydrogen concentration (Asada et al., 2020). This finding was explained by the fact that HRW protects cells from oxidative damage by scavenging reactive oxygen species (ROS) ($^{\bullet}O_2^-$ and $^{\bullet}OH$), which increase with the progression of diabetes. In our study, both α-amylase and α-glucosidase inhibitory activity were higher in HRW-fed goat milk samples. α-amylase breaks down starch into oligosaccharides, while α-glucosidase hydrolyzes oligosaccharides into glucose units. As discussed in the studies mentioned above, this mechanism can be inhibited by bioactive peptides, slowing the release of glucose into the bloodstream and regulating blood glucose levels. These inhibitory peptides interfere with the enzyme-substrate interactions by forming hydrogen bonds at the substrate binding sites. The inhibitory effect observed in our study for HRW-fed goat milk samples can be attributed to the bioactive peptides exclusively present in this group (Şahingil et al., 2024). These peptides probably hinder the ability of the enzymes to bind to hydrogen in the environment, resulting in a lower sensitivity of the polysaccharide chains to enzymatic hydrolysis. Furthermore, the significant inhibition of α-glucosidase activity observed in the present study may be due to the higher content of specific amino acids, namely Met, Trp, Tyr, and Cys, in HRW goat milk, as well as the presence of an N-terminal Pro-containing peptides found exclusively in HRW- milk (Data not shown).

3.5. Free fatty acid (FFA) profile

The PCA was performed on the percentage of free fatty acids in colostrum and milk samples collected on days 14 and 28 from 10 goats fed with NW or HRW. This analysis aimed to determine the effects of feeding NW and HRW on the free fatty acid profile of the milk. Fig. 4A shows the PCA results of goat milk samples determined by PC1 and PC6. The first and sixth cumulative variance contributions of PC1 and PC6 were 61 % and 1.6 %, respectively. Goat milk samples fed with NW and HRW are grouped and labeled with pink and green, respectively. In Fig. 4A, when the x-y distribution of component 1 (C1) and component 6 (C2) is evaluated, it can be seen that HRW feeding was generally on the right side, especially in the samples of day 14 and day 28. A hierarchical cluster heat map was created to explain the effects of feeding NW and HRW on the free fatty acid profile of the milk obtained from the goats on day 14 and day 28 (Fig. 4B). A total of 15 fatty acids were used as variables to create a heat map and color coding was used to represent the variable in the hierarchy. The classification of NW-fed and HRW-fed goat milk is indicated on the color scale as Control and HRW, respectively. The HRW-fed and NW-fed goat milk contains different amounts of short-, medium- and long-chain fatty acids, as shown in other studies (L. Wang et al., 2020). While C20 fatty acid predominates in the colostrum of HRW-fed goat milk, its amount is low in other samples, as shown in the heatmap diagram. The C2, C8, and C15 fatty acids were more abundant in HRW-fed goat milk than in others, while C4 and C6 fatty acids were more abundant in NW-fed goat milk than others. Although C18 fatty acid is present in some NW-fed goat milk, it was found in higher amounts in the HRW-fed milk.

On day 0 (colostrum), the fatty acid profile of HRW-fed goats was similar to that of the NW-fed control group, indicating that HRW had no significant effect in the early stages. However, by days 14 and 28, differences between the milk of HRW- and NW-fed goats became more pronounced. Over time, the milk of HRW-fed goats showed an increase in short-chain fatty acids (C2, C8, C10) and a decrease in long-chain fatty acids (C18, C18:1, C18:2, C18:3). While control samples tend to maintain relatively stable levels of both short-chain and long-chain fatty acids over time. Medium-chain fatty acids, such as C8 (caprylic) and C10 (capric), provide a rapid energy source without being stored as body fat due to their fast metabolism (da Silva & Rial, 2024). Therefore, they are often included in the diets of infants, athletes, malabsorption patients, and elderly individuals with myasthenia to provide quick energy (Jia et al., 2023). In the present study, these fatty acids were found in higher amounts in the milk of HRW-fed goats. The decrease in long-chain fatty acids (C18, C18:1, C18:2, C18:3) in HRW-fed goat milk may create a healthier lipid profile, potentially reducing the risk of cardiovascular disease. This change could improve the n-6/n-3 ratio, positively affecting heart health (Farková et al., 2024). The metabolic effects of HRW may have contributed to this process by promoting the increase of

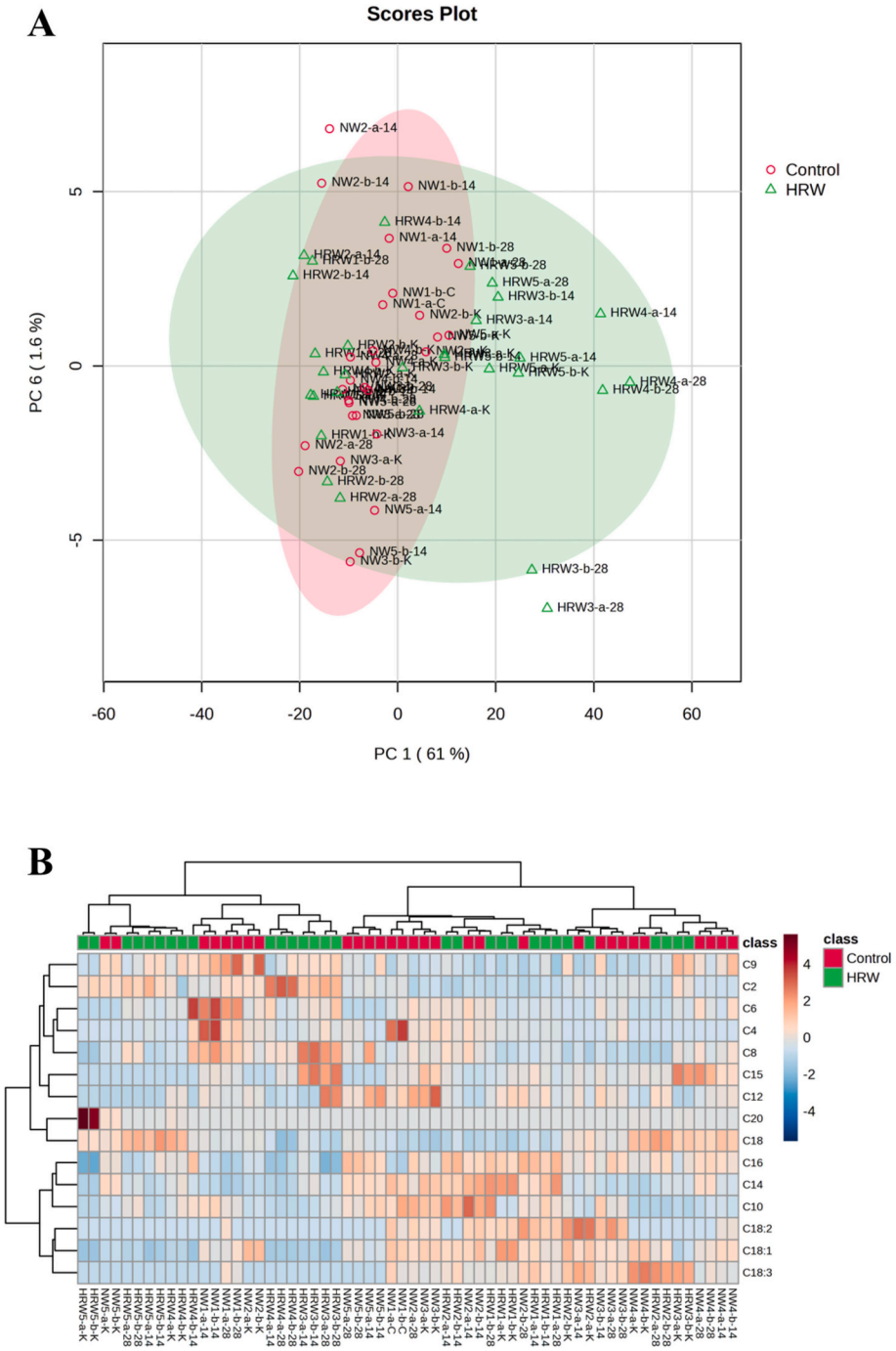


Fig. 4. Free fatty acid profile of colostrum/milk of goats fed with H_2-rich water and normal water (control) throughout the lactation period (0, 7, 14, 21, and 28). Principal component analysis of free fatty acids of goat milk samples (A). The heatmap plot shows the relative abundances of free fatty acids in goat milk samples (variables clustered on the horizontal axis) on different days (B); the phylogenetic tree is mainly concerned with the correlation between free fatty acids in days; the color scale bar represents the normalized peak area value. A deeper red indicates more abundance, a deeper blue indicates less abundance. NW: normal water (control), HRW: hydrogen-rich water. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

short- and medium-chain fatty acids.

Fatty acids influence the nutritional quality and physicochemical properties of dairy products, shaping their sensory characteristics, while unsaturated fatty acids, especially conjugated linoleic acid (CLA) and alpha-linolenic acid (ALA), provide health benefits by preventing cardiovascular diseases and offering antioxidant and anticancer effects. Medium- and short-chain fatty acids are beneficial in reducing the risk of metabolic syndrome and cardiovascular diseases while positively affecting cancer prevention and gut health (Guo et al., 2025). The fluctuations in the fatty acid profile among different goat milk and colostrum samples from the same group (HRW and control) may be linked to the physiological conditions of each animal. Additionally, the variation in milk fat profile can also be associated with the fact that milk fat is the most energy-rich component of milk, and its level is influenced by various factors such as dietary fiber, plant and seafood oils, and overall diet (Lanier & Corl, 2015).

In a study examining the effects of genetic and growth factors such as breed, management, stage of lactation, and age of the animal on milk composition in hairy goats, it was reported that the highest amounts of fatty acids are C16, C18:1, and C18 (Gharibi et al., 2020). (L. Wang et al.,

2020) performed a comparative lipidomic analysis of different milk types by UHPLC-Q-TOF-MS and found that the highest fatty acids in goat milk were C16, C18, C14, and C10. In another study comparing the fatty acids of Greek mountain sheep and goat milk, it was found that the proportion of short-chain fatty acids and fatty acids such as C6, C8, C10, C18, and C20 was higher in goat milk (Basdagianni et al., 2019). A study examining the fatty acid profiles of goat milk in different lactation periods reported that single-chain fatty acids and C15 and C17 fatty acids were higher than in human milk (Liao et al., 2024). Studies have generally reported that feeding dairy animals affects the fatty acid composition of milk. Acetate and β-hydroxybutyrate, precursors for fatty acid synthesis in ruminants, are produced by microorganisms in the rumen and transported to the mammary tissue via the blood. In animals with restricted feed, the concentration of these precursors can decrease, negatively affecting milk fat synthesis (Fox et al., 2015). Feeding with HRW can influence the production of fatty acid precursors through rumen fermentation. Although short-term HRW intake does not significantly alter the composition of the microbiota depending on the application time, it has been reported to change metabolic function before any compositional changes occur (Akita et al., 2023; Kaji et al., 2015).

Specifically, HRW can modify the intestinal environment to favor bacteria that produce more short-chain fatty acids. This effect is attributed to the suppression of ROS production by hydrogen, which helps restore the anaerobic environment in the intestine, thereby enhancing the metabolic function of obligate anaerobic bacteria to produce more short-chain fatty acids (Akita et al., 2023).

3.6. Volatile compound profile

PCA was performed on the percentage of volatile compounds in 10 goats fed with either NW or HRW. Fig. 5A shows the results of the PCA of goat milk samples identified by PC1 and PC2. The aim was to determine how the volatile profiles of HRW-fed and NW-fed goat milk change during day 28 of lactation. Fig. 5A shows the score and loading plots obtained from the PCA of the milk samples defined by the two principal components (PC1 and PC2). The cumulative variance contributions of PC1 and PC2 were 74.4 % and 10.5 %, respectively, and explained 84.9 % of the variance of the volatiles present in all goat milk samples. The distribution map showed the relationship of volatile compounds between different water types at day 28 of lactation by distinguishing some of the HRW- and NW-fed goat milk samples (Fig. 5B). The HRW- and NW-fed goat milk samples are grouped, with the samples marked in green representing the control group (NW) and the samples marked in pink representing HRW. When evaluating the x-y distribution of component 1 (C1) and component 2 (C2) in Fig. 5A, it can be seen that HRW-fed goat samples were mostly separated in this graph. When the x-y data of C1 and C2 are evaluated in Fig. 5A, the values are distributed with the values of C1 and C2 in the negative and positive directions.

A hierarchical clustering heat map was performed to explain the effect of feeding with NW and HRW on volatile compounds in the milk collected from the 28th day of lactation (Fig. 5B). The heat map results are consistent with the PCA mentioned above (Fig. 5B). A total of 76 volatile compounds (acids, alkenes, esters, aldehydes and ketones) was used as variables to create the heat map and color coding was used to represent the variable in the hierarchy. The classification of goats fed with NW and HRW is represented in green and purple on their respective color scales. While volatile compounds were detected in the milk of goats fed with HRW, these compounds were either absent or found in lower amounts in the milk of goats fed with NRW. Among these compounds are terpenes such as limonene, styrene, isoborneol, α-pinene, sabinene, and α-humulene; acids such as propionic acid, lauric acid, 2-ethylhexanoic acid, and isobutyric acid; esters such as butanoic acid methyl ester, ethyl acetate, and butanoic acid methyl ester; and aldehydes such as hexanal, heptanal, and nonanal. Volatile components such as acids and terpenes were more abundant in goat milk-fed HRW. In a study identifying volatile components in human and goat milk at different lactation periods, decanoic acid, lauric acid, caprylic acid, and hexanoic acid, which are the most abundant compounds formed by the breakdown of fatty acids, were identified (Liao et al., 2024). In the present study, the number and amount of acids were higher in goat milk fed with HRW. A study identifying volatile components in the milk of pasture and stall goats identified 41 compounds. It was reported that terpenes were predominant in the milk of pastured goats and that the volatile components were influenced by the feeding regime (Balivo et al., 2023). A total of 51 volatile compounds were detected in the ohmic-heated sheep milk, with acids, esters, and hydrocarbons being the most abundant components, respectively (Balthazar et al., 2024). Fatty acids in milk contribute to the characteristic flavor and texture of raw milk by promoting the formation of various volatile compounds under the influence of enzymes. In addition to fats, the chemical reactions of carbohydrates and amino acids also contribute to forming volatile compounds (Liao et al., 2024). Feeding goats hydrogen-rich water may have affected fatty acid and amino acid biosynthesis, leading to different volatile compounds.

4. Conclusion

In this study, for the first time, the effect of feeding Gurcu goats with HRW on the composition, potential antioxidant activity, in vitro antidiabetic activity, free fatty acid composition, and volatile components of colostrum and milk were investigated. The present study showed that goat milk becomes more functional when goats drink HRW by improving the nutritional characteristics of colostrum and milk passed on to the baby. Consumption of HRW is a natural, effective, and promising strategy to support a healthy antioxidant balance, increase nutritional qualities, and enhance the health benefits of goat milk for human consumption.

When evaluating these results in relation to goat and dairy farms, it can be assumed that this study may help farmers make better decisions regarding product quality, as well as animal health and nutrition. It could also enable society to respond more appropriately to the demand for healthy food. Specifically, by integrating HRW into drinking water systems on goat farms, the bioactive and nutritional properties of colostrum and mature milk, crucial for both animal nutrition and the dairy sector, will be sustainably enhanced. Goat and dairy farms intending to adopt this strategy should consistently plan their production processes and incorporate suitable equipment to fully realize the benefits of hydrogen water. Consequently, implementing HRW as a drinking water source and integrating it into milk quality and health monitoring systems can serve as effective approaches to enhance the bioactive and nutritional properties of dairy products.

One of the limitations of this study is the practical applicability of hydrogen-rich water, which may pose challenges since hydrogen gas has low solubility in water under normal conditions and tends to escape quickly, complicating its stability and the determination of the correct dosage in hydrogen-rich water (HRW). This issue presents difficulties for conducting large-scale animal trials. To address these usability concerns, new technology is needed to ensure consistent hydrogen delivery, be portable and user-friendly, and allow precise control over dosage. The small number of animals included in the study and the short duration of feeding HRW form other study limitations. Further studies should investigate a larger number of animals with longer HRW feeding duration. The mechanisms by which HRW affects milk characteristics and other factors related to animal parturition, such as milk hormones, signaling types, and hormone levels in the mammary gland, as well as animal stress hormones, mobility, number of births, and abortions, should also be investigated. In addition, it would be valuable to determine how the HRW affects the conversion properties of the products prepared from the milk obtained.

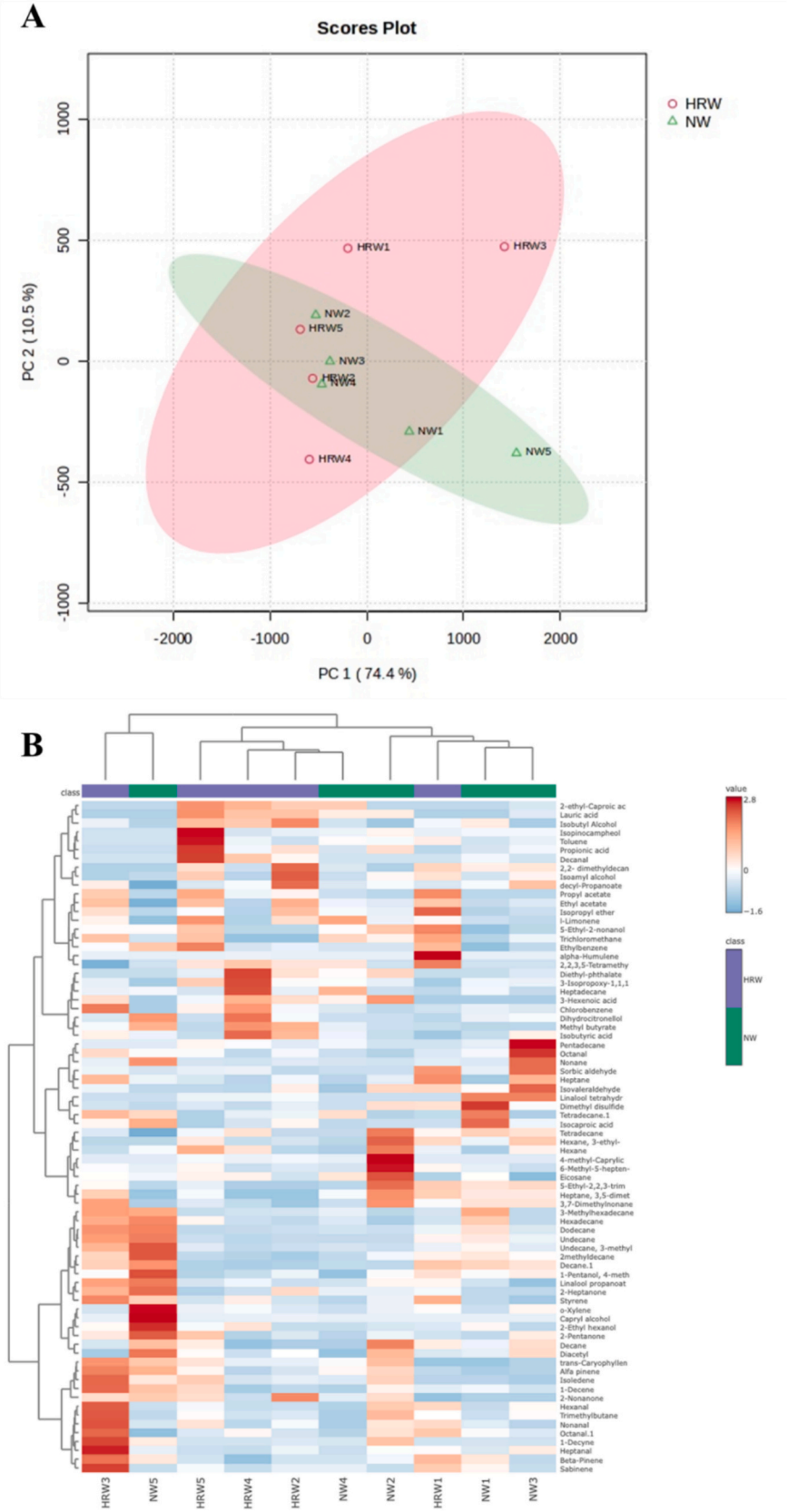


Fig. 5. Principal component analysis (PCA) of volatile compounds (A; x, component1; y, component2; B; component1; y, component3; C; component1; y, component4) of colostrum/milk of goats fed with H_2-rich water and normal water (control) throughout the lactation period (0, 7, 14, 21, and 28) (A). Heatmap of the volatile compounds present at 28th in the goat milk (B). The heatmap plot indicates the relative abundance of volatile organic compounds in different milk samples (variables clustered on the horizontal axis); the color intensity is proportional to the relative abundance of volatile compounds. NW: Normal water (control), HRW: Hydrogen-rich water.

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CRediT authorship contribution statement

Hilal Kanmaz: Writing – original draft, Visualization, Investigation, Formal analysis. **Didem Şahingil:** Investigation, Funding acquisition, Formal analysis. **Duried Alwazeer:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Menekşe Bulut:** Resources, Conceptualization. **Mushap Kuru:** Resources, Project administration, Methodology, Investigation, Conceptualization. **Mustafa Makav:** Validation, Methodology, Conceptualization. **Buket Boğa Kuru:** Investigation, Formal analysis. **Fikret Bektaşoğlu:** Investigation, Formal analysis. **Ali Adnan Hayaloğlu:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.143855.

Data availability

All data can be shared if required.

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