Traditional goatskin churn (Chekoua): quality of fermented goat milk flavoured with leaves of Phoenician juniper stored under ambient conditions

Abstract

Goat milk possesses several nutraceutical and functional properties. This study focused on the physicochemical and bacteriological properties of a local, traditionally fermented raw goat milk from Southern Algeria supplemented with the leaves of the Phoenician juniper.

Analysis of the goat milk sample supplemented with juniper leaves was conducted against a control sample (without juniper leaves) and stored in a traditional goatskin churn (Chekoua) at ambient temperature. Some physicochemical parameters were tracked, namely pH, titratable acidity, temperature, lactose content, and total dry matter. The microbiological analysis included the following parameters: the total aerobic mesophilic flora, fungal flora, total and thermotolerant coliforms, coagulase-positive staphylococci, faecal streptococci, lactic acid bacteria, and the pathogenic bacteria *Salmonella spp* and *Listeria monocytogenes*.

The obtained results showed an evolution of the physicochemical parameters during five days of conservation by increasing the titratable acidity, which proved to be significant for the test sample supplemented with *J. phoenicea* leaves compared to the control sample. The microbiological quality was characterized by the absence of pathogenic species, and a reduction of bacterial contaminants (probably due both to the effect of milk fermentation and the addition of juniper leaves as an aromatic plant endowed with antimicrobial properties).

The study results showed that both goatskin and Phoenician juniper leaves can be used to improve the microbiological and sensory quality of fermented goat milk stored in the open air by the inhabitants of Southern Algeria. Such an artisanal practice should be maintained as a regional tradition. Further studies on fermented goat milk and its dairy by-products are recommended.

Keywords: Goat milk, *J. phoenicea* L leaves, physicochemical properties, traditional knowledge, goatskin churn (Chekoua)

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Introduction

In Algeria, goat milk has long been marginalized. It is mainly manufactured by families in mountainous regions (rural communities) and consumed raw or fermented [1]. Goat breeding represents only 13% of livestock located in difficult regions (mountains, steppe, Sahara) [2]. Goats are raised mainly for their milk, meat, and hair. For populations living in extreme areas such as mountains, goat breeding represents a means to achieve food security. In Algeria - a developing country where agriculture is a main component of the national economy and provides an income for some of the Algerian working population^[3], Algerian livestock farming is characterized by extensive practices and production systems, poorly developed forage crops, and local breeds^[4].

Due to its acrid taste, goat milk is not always appreciated by consumers. However, when processed, especially into cheese, it is more digestible and very popular^[5]. Many different raw goat milk products for human consumption are available, including goat's cheese (cheese made from raw goat milk is prohibited in many countries as microbial loads are higher), liquid milk (which can be fortified with vitamins and minerals), and desserts (goat milk ice cream and flavoured yoghurt)^[6]. Many scientific studies show that dairy products traditionally prepared from raw milk have typical flavours and nutritional qualities that are increasingly sought after by consumers. Their value is mainly reflected in their production and consumption rates ^[7]. Many Algerian families – particularly those in rural areas -are reliant on the consumption of milk and milk products as a main source of calcium and proteins. The milk products are traditionally prepared from raw milk using mainly traditional processing and preservation methods, depending on the breed and the species raised, and the traditional practices of each region^[8].

Yet, the characterization of milk consumption and the diversification of milk products in Algerian traditions remain largely unknown and unstudied. The current study aimed to follow some physicochemical and microbiological parameters of fermented goat milk flavoured with leaves of Phoenician juniper stored in a traditional goatskin churn under ambient conditions against a controlled sample (without juniper leaves), a traditional product of the southwest region of Algeria.

Materials and methods

Sampling of raw goat milk

The raw goat milk of the Arabia Horra goat breed was obtained from a farm located in Bechar province (South-west of Algeria). A sufficient volume of the milk was distributed in two traditional goatskin churns (Chekoua)

(Fig. 1). A mass of 30g of *J. phoenicea* L leaves was added to the test sample. The second sample, without juniper leaves, was considered as a control.

Figure 1: Raw goat milk in goatskin churn (Original, 2021)



Collection of J. phoenicea L leaves

J. phoenicea L. leaves were collected from the Mecheria region (Naâma province, west of Algeria) in November 2021. They were cleaned and dried in the dark and open air (**Fig. 2**). A mass of confined leaves in the form of a knot and covered with sterile gauze was added to the raw goat milk test sample and stored in a goatskin churn (Chekoua).



Figure 2: Cleaned and dried leaves of *Juniperus phoenicea* L. (Original, 2021)

Extraction process of aqueous extract

This process was carried out by maceration technique where a mass of 25g of dry juniper leaves was put in 250ml of distilled water. At 50°C and under continuous stirring conditions, this mixture was heated for 2 hours and then macerated under ambient conditions for 24 hours. After filtration, the filtrate was evaporated to dryness (dry extract). The extraction process and yield were done following the method described by Benyagoub *et al.* ^[9, 10, 11, 12].

Total phenolic content (TPC)

The phenolic content of the aqueous extract of *J. phoenicea* leaves was measured according to the Folin-Ciocalteu method using a spectrophotometer at 765nm, in which gallic acid (0–1mg/ml) was used as a positive control ^[13].

The aqueous extract was analyzed in three replication, and the result was expressed in gallic acid equivalents (GAE) per milligram of dry extract weight.

Monitoring the juniper leaves-supplemented goat milk Physicochemical analysis

The samples intended for fermentation (test and control samples) underwent physicochemical analysis according to the Algerian standards ^[14] by monitoring some parameters during natural fermentation, namely, pH, temperature (°C), ti-tratable acidity (TA in g/l), total dry matter (TDM in %) and lactose content (g/l) as follows:

- Temperature and pH of the goat milk were measured according to standard methods with a thermometer pH-metre (Adwa AD 1040, Romania)^[15].
- TA was established according to the titration method described by Nielsen ^[16] using sodium hydroxide (NaOH 0.1 N) (Sigma-Aldrich, Czech Republic), and phenolphthalein (1%) (Sigma-Aldrich, India) as a colour indicator (Volumetric method). The acidity was expressed in grams of lactic acid per litre (g/l) of milk^[17].
- TDM in (g/l) was determined after drying 10g of goat milk in an oven at 103±2°C (Drying method) according to the Algerian standard ^[18].
- Lactose content (%) was determined by UV-visible spectrophotometry (UV-1700). To 1ml of goat milk, we added 1ml of phenol water and 5ml of sulfuric acid. The assembly was mechanically homogenized using a vortex and then brought to the boil for five minutes. The absorbance was read at 490nm against the control sample prepared with distilled water. A calibration curve was plotted from a stock solution of 0.1% of lactose^[19].

Before pouring the milk into the goatskin churn 'Chekoua', a 500ml sample of raw goat milk was taken after a thorough mixing to analyze pH, acidity, lactose content, and total solids (TS) according to the methods mentioned above.

Fat content and milk density were analyzed as follows^[20]:

- The density of milk was measured by using a calibrated thermo-lactodensimeter^[19]
- The fat content was measured by the Gerber butyrometric method according to NF V 04-210^[21] which uses sulfuric acid to attack the milk and separation by centrifugation in the presence of isoamyl alcohol.

Bacteriological analysis

Peptone water was used as a diluent to prepare a series of sample dilutions to be analyzed up to the dilution factor of 10^{5} ^[22].

The analyzed bacteriological parameters were as follows:

- Detection and enumeration of the total aerobic mesophilic flora (TAMF) were carried out by the pour plate technique on PCA agar medium (Liofilchem Diagnostici, Italy). The Petri dishes were incubated in an inverted position at 30°C and 22°C for 72 hours^[23].
- Detection and enumeration of spores of sulfite-reducing clostridia at 46°C according to NF T 90-415^[24] by incorporation of meat liver sulfite iron agar (Fluka, India) in the deep tube.
- Detection and enumeration of thermotolerant coliforms were carried out by the pour plate technique on MacConkey agar medium (Liofilchem Diagnostici, Italy). The Petri dishes were incubated at 44°C for 24 to 48 hours^[25].
- Detection and enumeration of faecal streptococci (Enterococci) on azide dextrose broth and Litsky EVA broth (Lioflichem Diagnostici, Italy) by the multi-tube/most probable number technique (MPN). The tubes were incubated at 37°C for 24 to 48 hours^[26].
- Detection and enumeration of coagulasepositive staphylococci (CoPS) was carried out using the spread plate technique on Baird-Parker agar medium (Liofilchem Diagnostici, Italy)^[27].
- Detection of *Salmonella spp* was carried out according to JORA n.44 ^[28]. This included three consecutive steps: (i) pre-enrichment step on buffered peptone water (BPW); (ii) selective enrichment on Rappaport Vassiliadis broth (RVB) and selenite cystine broth (SCB) (Liofilchem Diagnostici, Italy) incubated at 41.5°C and 37°C, respectively; (iii) isolation on agar medium where drops of the selective enrichment broths (RVB)

and SCB) were spread on the surface of two selective media with a platinum inoculating loop (Hektoen enteric agar and SS agar (Liofilchem Diagnostici, Italy)). The inoculated Petri dishes were incubated at 37°C for 24 hours.

- Detection and enumeration of Listeria monocytogenes was carried out according to JORA n.3, ^[29] which involved three steps: (i) primary enrichment on half-Fraser broth (half the concentration of nalidixic acid and acriflavine) incubated at 30°C for 20 to 24 hours; (ii) secondary enrichment on Fraser broth complete; (iii) 0.1ml of Fraser broth complete that shows blackening was spread on the surface of PALCAM agar using a platinum inoculating loop. The agar was then incubated at 37°C for 26 ± 2 hours. Usually, 24 hours of incubation is sufficient for the development of a black colour resulting from esculin hydrolysis^[30].
- Detection of yeasts and moulds was carried out by spread plate technique on medium Oxytetracycline-Glucose-Yeast Extract Agar (OGA). The plates were incubated at 25°C for three to five days^{[31].}
- The assessment of the lactic acid bacteria load (LAB) was carried out using the pour plate technique ^[32] on MRS agar and M17 agar for lactobacilli and lactic streptococci species, respectively (Liofilchem Diagnostici, Italy). Before pouring the medium, cycloheximide at a concentration of 1% (v/v) was added to the agar plate. The Petri dishes were incubated at 30°C for 48 to 72 hours in microaerophilic conditions using an anaerobic gas jar pack system to reduce oxygen levels.

The horizontal method for the enumeration of bacterial loads given in the Algerian standard^[27] was followed. Monitoring of microbial loads expressed in Log₁₀ CFU/mL was done as a function of time.

Biochemical identification of bacterial isolates

Suspected pathogenic bacteria isolated on selective agar medium, namely *Staphylococcus aureus*, *Salmonella spp*, and *Listeria monocytogenes* were subjected to serial biochemical identification tests according to standard microbiological methods described by Tille^[33] and Benyagoub *et al.*^[34]

Interpretation of the analysis results

The microbiological analysis results were interpreted based on the contamination limit thresholds (m and M) given in the Official Journal JORA n.39^[35], while the physicochemical analysis results were interpreted according to Algerian standards^[36, 37].

The graphical presentations in the form of curves and histograms were plotted using the Origin LAB software (2018).

Results

Extraction yield and total phenolic content (TPC)

The yield extraction of aqueous extract showed a rate of $12.7\pm0.36\%$ (dry extract). TPC results were derived from a calibration curve (y= 3.7756x + 0.1182, R² = 0.9978) of gallic acid (Fig. 3).



Figure 3: Calibration curve for standard gallic acid

Based on the calibration curve shown in **Fig. 3**, the obtained results showed that the TPC of aqueous extract of *J. phoenicea* leaves was 322.8±38.33µg GAE/mg DW (dry weight).

Biochemical, physicochemical, and microbiological analyses of goat milk sample

Biochemical, physicochemical and microbiological analysis of the results of the collected goat milk used for fermentation are shown in **Figs. 4 and 5**.



Figure 4: Physicochemical and biochemical analysis results of collected goat milk - T. acidity: Titratable acidity

Upon receipt, the physicochemical results of the raw material showed that the milk was acidic, with an average pH of 6.56 and a TA of 2.3 g/l, exceeding the threshold set at 1.8g/l. It had a density value of 1.035, while the fat and lactose contents were 3.6% and 49g/l, respectively.





The analyzed microbial parameters, namely TAMF and faecal coliforms (FC) followed the thresholds set by national regulations, in that the sample was free from *Salmonella spp*, *Listeria monocytogenes*, and *Staphylococcus aureus* (CoPS) as pathogenic bacteria. However, the analysis recorded a CoNS (coagulase-negative staphylococci) load of 3.6 Log₁₀ CFU/ml which exceeds the set threshold 'Maximum (M) contamination limit' given for CoPS in the Official Journal.

Monitoring the fermentation of juniper leaves-supplemented goat milk

Biochemical and physicochemical parameters Fig. 6 shows the change in pH, temperature, TA, lactose content and TDM of the goat milk (test and control samples) during fermentation.



Figure 6: Evolution of the physicochemical parameters of goat milk during fermentation (test and control samples) **TA**: Titratable acidity; **LC**: Lactose content (g/l); **T°C**: Temperature in degrees Celsius; **TDM**: Total dry matter (%); **pH**: Potential of hydrogen; **C**: Control sample; **T**: Test sample.

Fig. 6 shows an increase in TA and consequent decrease in pH during natural fermentation with relatively high values for the test sample compared to the control sample (without juniper leaves). Thus, for lactose content and TDM, a decrease was revealed as a function of time at the room temperature ranging from 19°C to 22°C.

Bacterial parameters

Total count of aerobic and LAB load

Fig. 7 shows the evolution of the TAMF, fungal flora (FF), and LAB load of goat milk (test and control samples) during fermentation over time.





Lb: Lactobacilli; Str: lactic Streptococci; C: Control sample; T: Test sample.

Fig. 7 shows an increase in the bacterial load of the TAMF incubated at 22°C and 30°C ranging from 6.42 to 6.84 Log_{10} CFU/ml, which increased over time for the control sample, contrary to test one.

For the analyzed samples, a load ranging from 4.08 to 4.46 Log_{10} CFU/ml and from 4.08 to 4.83 Log_{10} CFU/ml was recorded for *Lactobacillus* and *Streptococcus spp* lactic strains, respectively.

Pathogenic and bacterial contaminants

Bacterial contaminants

Fig. 8 shows the evolution of coliform organisms (total coliforms) and thermotolerant coliforms (faecal coliforms) of goat milk (test and control samples) during fermentation.



Figure 8: Evolution of thermotolerant coliforms in goat milk (test and control samples) during fermentation.
TC: Total coliforms; FC: Faecal coliforms (Thermotolerant coliforms); F Str: Faecal Streptococci; SRC: Spores of Sulfite-reducing clostridia; C: Control sample; T: Test sample. The test sample showed a decrease in the microbial load from 4.34 and 3.94 Log_{10} CFU/ml to 3.94 and 3.3 Log_{10} CFU/ml for TC and FC, respectively. However, the control sample showed an increase in total and faecal coliform loads as a function of time, increasing from 4.83 and 4.6 Log_{10} CFU/ml to 5.04 and 4.91 Log_{10} CFU/ml, respectively. Faecal streptococci results showed a decrease as a function of time for the test sample, in contrast to the control sample. Both samples (test and control) were sulfite-reducing clostridia spore-free.

Pathogenic bacteria

Fig. 9 shows the evolution of contaminating and pathogenic bacteria, namely coagulase-negative staphylococci, *Salmonella spp*, and *Listeria monocytogenes* in goat milk (test and control samples) during fermentation.



Figure 9: Evolution of CoNS and pathogenic bacterial strains of goat milk (test and control samples) during fermentation. **CoNS:** Coagulase-negative Stpathylococci; **Listeria:** *Listeria monocytogenes;* **Sal:** *Salmonella spp;* **C:** Control sample; **T:** Test sample.

National regulations ^[35] set a load of m=2.95 Log₁₀ CFU/ml and M= 3.95 Log₁₀ CFU/ml for CoPS of fermented milk. The collected sample tested negative. The control sample showed an increase in the staphylococcal load up to a maximum value of 4.48 Log₁₀ CFU/ml, which exceeds the range given by national regulations. The test sample revealed a maximum load of 3.95 Log₁₀ CFU/ml, followed by a decrease in the bacterial load to a minimum load of 2.3 Log₁₀ CFU/ml after five days of storage. The analyzed samples were *Salmonella spp* and *Listeria monocytogenes*-free.

The identification results of the strains isolated during the search for pathogenic bacteria are given in **Table 1** and **Fig. 10** below.

Table 1: Identification of presumed pathogenic bacteria iso-

lated from fermented goat milk (test and control samples)



B. parameter: Bacterial parameter, **Samp.:** Goat milk samples, **CoPS:** Coagulase-positive staphylococci, **CoNS:** Coagulase-negative staphylococci, *L. monocytogenes: Listeria monocytogenes;* **-ve:** A negative culture for a presumed isolate.

Figure 10: Isolation of microbial species from fermented goat milk flavoured with leaves of Phoenician juniper, and stored in goatskin churn (Chekoua) (Original, 2022).

(a): FC on MacConkey agar (test sample); (b): TAMF on PCA at 30°C (control sample); (c): Presumed Listeria strains on PALCAM *Listeria* agar; (d): Presumed *Salmonella* strains on Hektoen enteric agar; (e): Presumed *Salmonella* strains on SS agar; (f): Search for Sulfite-reducing clostridia on meat liver sulfite iron agar (control sample); (g): Multi-tube/MPN technique for faecal streptococci on azide dextrose broth (test sample); (h): Confirmatory test for faecal streptococci on EVA broth (control sample); (i): *Lactobacillus* lactic strain on MRS agar; (j): Total coliforms on MacConkey agar (control sample); (k): Staphylococci strains on Baird-Parker agar (control sample).

Discussion

The physicochemical and microbiological analysis of the collected test sample of goat milk, which is used for fermentation through traditional storage in goatskin churn (Chekoua) at room temperature, showed the good quality of the goat milk. Analyzed parameters were within the national regulations threshold established for the 'raw milk' category.

The pH and TA of raw milk are related to hygienic conditions during milking, the total microbial flora load and its metabolic activity as well as to milk handling ^[38, 39]. The variation of physicochemical parameters of raw goat milk, namely fat content, and TDM may be a consequence of variations in the negative effect of heat stress on feeding intake, poor nutrition that is typical of desert habitat conditions, variations in the quality and quantity of feed available for such regions, and genetic factors ^[40, 41]. Thus, density variability depends on the dry matter content, fat content, increase in temperature, and food availability ^[39, 42, 43].

The microbiological analysis carried out in this study included several parameters, exceeding those recommended by the national regulations (five microbiological criteria, namely TAMF, FC, CoPS, Salmonella spp, and L. monocytogenes). Milk quality can be assessed based on the presence or absence of certain types of pathogenic bacteria, TBC (where a high load reflects manual milking carried out in unhygienic conditions, e.g. absence of systematic teat washing, rarely renewed litter, etc.) and the absence of refrigeration^[44]. Moreover, coliforms and the enterococci group represent a good indicator of faecal contamination, especially in the case of water pollution [45], inappropriate hygienic conditions and practices during milking, processing, storage, and transport of dairy products between manufacture and sale points^[44].

The achieved quality confirms compliance with good hygiene practices recommended by FAO/FIL^[46], where the raw milk quality depends on various factors such as processing methods, the health of the animal, the environment, acceptability levels, etc^[6]. According to Matallah *et al.*^[39], the absence of *Staphylococcus aureus*, sulfite-reducing clostridia, and *Salmonella spp* could be explained by the absence of udder infection.

Analysis of bacteriological parameters during milk storage showed a gradual decrease in microbial parameters, namely TAMF, TC, FC, and CoNS for the test sample. This was in contrast to the control sample, which experienced an increase in the load of these microbial parameters. Many studies have shown that traditionally produced dairy products are the most frequently contaminated by a high load of FMAT, FC, and *S. aureus*^[47].

The FF load and that of LAB increased during fermentation, where both samples (test and control) were *Salmonella* and *L. monocy-togenes*-free.

Yeasts and moulds are common food contaminants. They can be transported by the environment and be found in milk and dairy products. Although yeasts do not cause food poisoning, they can cause organoleptic alteration of the food. Unhampered by acidity and with an abundant source of energy with the residual sucrose and lactose, yeasts can develop in acidified dairy products and cause alterations. The FF generally comes from poor hygiene conditions during handling, and storage, but above all from the ambient air (spores) ^[38].

These results corroborate previous findings reported by Benyagoub ^[48] where a decrease in microbial load was the origin of a developed acidity that was greater in the test sample than the control sample, as well as the antibacterial and/or acidifying effect of juniper leaves added to goat milk. The *J. phoenicea* L. *aqueous* leaves macerate had a pH value of 5.29^[48], which can provide real protection against deterioration caused by unwanted microorganisms^[49].

Spontaneous acidification of different kinds of milk is linked to the relative proportions of flora of technological interest and milk spoilage flora, which are proteolytic and lipolytic ^[45].

The storage temperature of milk influences its physicochemical and microbiological quality; high ambient temperatures can lead to a proliferation of any bacterial contaminants already present in the product during production, transportation, and storage, which increases TA^[43, 50]. TA is linked to the technology used to produce fermented products^[32]. However, the ambient temperature makes the conditions favourable for an increase in acidity, which results in a high rate of LAB^[45]. Moreover, good progress in the fermentation process was confirmed by the evolution of both physicochemical and microbial parameters of the milk over time^[48].

The absence of pathogens in both samples, as well as the decrease in the coliform load for the test sample, can be justified by the action of the fermented goat milk acidity stored in the goatskin churn, where the protective effect of LAB against pathogenic microorganisms could be a major safety factor ^[38], and to the effect of secondary metabolites ^[48], particularly the high TPC of *J. phoenicea* L leaves on the growth of microbial contaminants. TPC results were higher than the findings reported by Telaidji ^[51] and Draoui *et al.* ^[52] for methanolic and aqueous extracts which were 124.88 µg GAE/mg and 2.42 mg GAE/g DW. Thus, the number of hydroxyls (OH) functional groups in the phenol group is toxic to microorganisms, disruptive to bacterial cell walls, and precipitates proteins of bacterial cells ^[53]. According to the inhabitants of the study area, the twigs, leaves, and fruits of Phoenician juniper are used in traditional medicine; their phytochemical compounds are incorporated into pharmaceutical preparations, particularly for antiseptic use. These qualities are attributed to the presence of essential oils in the leaves of the plant, which are usually used as a herbal decoction against diabetes, diarrhoea, and rheumatism ^[48, 54, 55].

Several studies have highlighted interest in the consumption of fermented goat milk [6, 56] and its benefits for infants and adults. Fermented goat milk has many medicinal properties such as anti-thrombotic, anti-oxidative and anti-atherogenic effects that are beneficial for cardiovascular diseases. It is also useful for the treatment of gastrointestinal diseases, cancer, allergies, and immunological properties. Also, goat milk acts as a probiotic and prebiotic ^[6, 56]. Thus, the series of previous studies have shown that fermented goat milk, flavoured with J. phoenicea L. leaves allows the development of lactic strains which contribute to the preservation of fermented milk by improving its hygienic and organoleptic quality, where compounds associated with herbaceous and fruity aromas added during milk processing are considered key odorants in wide varieties of dairy products produced in the study area [48, 57].

In rural areas known for animal husbandry, traditional products are the most appreciated, made using conventional methods to ensure a healthy product that is fit for consumption. This explains their higher consumption and processing rates, despite the diversity of industrial products on the market^[58].

Conclusion

Milk consumption patterns and the diversification of milk products in Algerian traditions remain insufficiently known and unstudied.

Through this study, we contributed to the physicochemical and microbiological characterization of fermented goat milk flavoured with juniper leaves and stored in a traditional goatskin churn 'Chekoua' at room temperature. The obtained results showed an evolution of the physicochemical parameters during five days of conservation by increasing the TA, which proved to be significant for the test sample supplemented with J. phoenicea leaves compared to the control sample. The microbiological quality was characterized by the absence of pathogenic species, and a reduction of bacterial contaminants (probably due both to the effect of milk fermentation and the addition of juniper leaves as an aromatic plant endowed with antimicrobial properties).

The current study results may contribute to the overall knowledge on fermented goat milk with juniper leaves where it constitutes a starting point to an approach with the objective of determining the value of goat milk and its derivatives as well as the traditional practices of the region's population. More research should be conducted to better understand this combination, which is not limited by the addition of juniper leaves, but also other medicinal and aromatic plants. In this way, the specific characteristics of this product can be protected.

Production and consumption of fermented or raw goat milk should be promoted due to its high nutritional, therapeutic, nutraceutical, and physiological values. The author also hopes that the results of this study can offer useful information for the improvement of this product.

Conflict of interests

None.

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