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Research paper

Effect of adding dried jambolan pulp (*Syzygium cumini*) to potentially symbiotic fermented milk

Efeito da adição de polpa de jambolão desidratada em leite fermentado potencialmente simbiótico

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ABSTRACT: The aim of this research was to determine the technological viability of adding dried jambolan pulp to potentially symbiotic fermented milk. Jambolan pulp samples were obtained from fruit from the city of Bambuí, MG, and four fermented milk formulations were obtained with different proportions of dried jambolan pulp (0, 1, 2 and 3%). Physicochemical and microbiological analyses were carried out in a 4x4 factorial scheme at different evaluation times (0, 10, 20 and 30 days). The results of the physicochemical analyses carried out on the dried pulp showed that this ingredient has moderate antioxidant activity (41.88% according to the DPPH method) and is rich in phenolic compounds (235 mg GAE 100 g^{-1}). The fermented milk's antioxidant activity and phenolic content became higher as the proportion of jambolan pulp increased. The acidity, protein and lipid values were within the standards established by the Regulation of Identity and Quality for fermented skim milk. The treatments' lactic acid bacteria counts showed values greater than 6,00 log CFU g^{-1} and presented the minimum count required to be classified as potentially probiotic fermented milk. The combination of *L. casei* and jambolan pulp resulted in a potentially functional product with feasible industrial application.
Keywords: Antioxidant. *Lactobacillus* spp. Dairy products. *Syzygium cumini*.

RESUMO: *O objetivo deste trabalho é determinar a viabilidade tecnológica da adição da polpa desidratada de jambolão em leite fermentado potencialmente simbiótico. Amostras de polpas de jambolão foram obtidas de frutos provenientes do município de Bambuí/ MG e quatro formulações de leite fermentado foram obtidas, com diferentes proporções da polpa desidratada de jambolão (0, 1, 2 e 3%). Foram realizadas análises físico-químicas e microbiológicas em esquema fatorial 4x4 em diferentes tempos de avaliação (0, 10, 20 e 30 dias). Os resultados das análises físico-químicas realizadas na polpa desidratada demonstraram que o ingrediente apresenta atividade antioxidante moderada (41,88% pelo método de DPPH) sendo rico em compostos fenólicos (235 mg AGE 100* g^{-1}*). A atividade antioxidante e teor de fenólicos dos leites fermentados foram superiores à medida que se aumentou a proporção de polpa de jambolão. Os valores de acidez, proteínas e lipídeos estavam dentro dos padrões previstos pelo Regulamento de Identidade e Qualidade para leites fermentados desnatados. As contagens de bactérias lácticas dos tratamentos apresentaram valores superiores a 6,000 log UFC* g^{-1} *e apresentaram a contagem mínima exigida para serem classificados como leite fermentado potencialmente probiótico. A junção de L. casei e polpa do jambolão resultou em um produto potencialmente funcional, com viabilidade de aplicação industrial.*
Palavras-chave*: Antioxidante., Lactobacillus spp. Produtos lácteos. Syzygium cumini.*

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INTRODUCTION

Despite their unique sensory characteristics and considerable nutritional values, many national fruit species have been vastly overlooked, and thus little explored (BIANCHINI et al., 2020). This richness in nutrients, however, is one of the main factors that has led to the growing interest in consuming these fruits and their products (RUFINO et al., 2010; FARIA et al., 2020). Among these fruits, jambolan (*Syzygium cumini*) stands out.

Jambolan is a plant from the Myrtaceae family that is native to tropical Asia, specifically India (SINGH et al. 2016). Found in several countries, it contains a complex composition of phytochemicals, especially total phenolic compounds, anthocyanins, tannins, and carotenoids (LESTARIO et al., 2017). Such compounds are considered responsible for antioxidant potential (VIZZOTO; PEREIRA, 2008; RUFINO et al., 2010), and several studies are being carried out with the aim of using jambolan powder as a food ingredient due to its bioactive potential (TAVARES et al., 2020).

Jambolan's therapeutic potential and the benefits that it can bring to the human diet encourage the exploration of its products and co-products as they contain residues rich in anthocyanins and other antioxidant substances. Therefore, preservation processes, such as drying, may be mandatory for later use (MUSSI et al., 2015).

Using dried jambolan pulp as an ingredient in fermented milk is an interesting alternative for obtaining a potentially symbiotic product. Symbiotic products are those that contain a synergistic combination of probiotic microorganisms and prebiotic substances (OLIVEIRA; GONZÁLEZ-MOLERO, 2016). Such combinations may have technological and physiological advantages as they enable better probiotic culture viability in the product and stimulate the growth of these cultures in the consumer's intestinal tract (GALLINA et al., 2012).

The aim of this study was to analyze the technological viability of adding dried jambolan pulp to potentially probiotic fermented milk.

METHODS

Approximately 5 kg of jambolan fruits (*Syzygium cumini)* were manually harvested from the fruit cultivation sector at the Federal Institute of Minas Gerais (IFMG) Campus in Bambuí, MG. Only fruits at the mature stage were selected, considering the visual observation of the intense purple skin color and the sensory characteristics of maturation (AYYANAR; SUBASH-BABU, 2012; ARAÚJO, 2014).

After collection, the fruits were packed in plastic containers to avoid mechanical damage and transported to the vegetable pilot plant at the IFTM Uberaba Campus. The fruits were selected, washed in running water, sanitized by immersion in a solution with chlorinated water (200 mg L^{-1} de cloro) for 15 minutes, rinsed and dried at room temperature. The pulping of the fruits was carried out in an electric pulper (Itametal). The pulp was packed in polyethylene plastic bags wrapped in aluminum foil and the packaging was closed with a metal seal. The packed pulp was stored in a cold room at 18±1°C until the drying process.

Fermented milk processing

Drying was performed by placing the pulp in aluminum molds and then in a tray dryer with forced-air circulation (Pardal) at 60±1°C for 24 hours. After drying, the material was

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ground in a knife mill with a thermostatized rotor (Tecnal) and sieved (48 mesh) to standardize the particles and obtain a powder (dried jambolan pulp). The material was stored in closed plastic bottles in a freezer (-18°C) and protected from light. This storage did not exceed seven days.

Four fermented milk formulations were processed with different proportions of dried jambolan pulp (0, 1, 2 and 3%), resulting in four treatments. The base ingredients used to make the fermented milk were: Itambé brand skim milk powder (15%), União brand refined sugar (8%), Newnutrition brand fructooligosaccharides (FOS) (2%) and Chr. Hansen brand lactic culture *Lactobacillus casei* (5%). The ingredient proportions were defined based on a volume of 100 ml of drinking water. The ingredients (milk powder, sugar and FOS), after weighing, were dissolved in water, and the mixture was subjected to pasteurization at 80ºC for 25 minutes in a water bath (Biomatic), followed by cooling to 40ºC in an ice bath. The dried jambolan pulp and the lactic culture *L. casei* were added to the treatments according to the proposed formulations. The samples were again placed in a water bath for incubation at 40ºC until the pH range reached 4.8-5.0, followed by cooling in an ice bath. The fermented milk formulations were stored in 250 ml polyethylene plastic bottles with polypropylene caps under refrigeration conditions at 5±1ºC in a refrigerator. Processing was performed with two replications.

Physicochemical and microbiological analyses

The physicochemical and microbiological analyses were performed in triplicate and with two replications, respectively, at the Bromatology and Microbiology Laboratories at the IFTM Campus in Uberaba, MG.

For the dried jambolan pulp samples, phenolic, antioxidant activity, moisture, acidity, water activity and pH analyses were carried out, and for the fermented milk, lipid and protein analyses. Antioxidant activity, phenolic content, color, pH and total titratable acidity were quantified in the fermented milk formulations after 0, 10, 20 and 30 days of storage.

Phenolic compounds and antioxidant activity (DPPH method) were determined, respectively, using the methodologies of Singleton; Rossi (1965) and Brand-Williams; Cuvelier; Berset (1995).

The oven method with forced-air circulation at 105°C for 24 hours was used for the moisture content. Protein analysis was performed using the Kjeldhal method and a conversion factor of 6.25 for the jambolan pulp and of 6.38 for the fermented milk. To determine the total lipids in the jambolan pulp, the "Soxhlet" method was used based on the amount of material solubilized by the solvent (Instituto Adolfo Lutz - IAL, 2008). For the determination of lipids in the fermented milk, the Rose-Gottleib methodology was used (IAL, 2008).

For color, the Minolta CR-400 colorimeter was used to determine the coordinates L* (brightness), a* (chromaticity on the green (-) to red (+) axis and b* (chromaticity on the blue (-) to yellow (+) axis. Water activity was determined by Aqualab equipment.

For the pH analyses, a digital potentiometer (Gehaka) was used. The total titratable acidity was determined by potentiometric titration with a 0.01 N NaOH solution titrated to a pH of 8.3, with the results expressed as a percentage of lactic acid (BRASIL, 2006) for the fermented milk and of citric acid for the dried jambolan pulp (IAL, 2008).

The viability analysis of probiotic lactic acid bacteria was performed on the treatments and at 0, 10, 20 and 30 days of refrigerated storage. To count *Lactobacillus* ssp., De Man,

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Rogosa and Sharpe Agar (MRS) was used according to the Lopez, Medina and Jordano methodology (1998).

The study consisted of four fermented milk treatments, respectively, T0 (control), T1 (addition of 1% dried jambolan pulp), T2 (addition of 2% dried jambolan pulp) and T3 (addition of 3% dried jambolan pulp) and 4 evaluation times (0, 10, 20 and 30 days), in a 4x4 factorial scheme. A completely randomized design (CRD) was adopted with two replications for each analysis. The effects of the treatments were compared by analysis of variance, and when there was significance, the Scott-Knott or Tukey Test at 5% ($p<0.05$) and regression analysis were used, performed according to the SISVAR software's usual techniques (FERREIRA, 2019).

RESULTS AND DISCUSSIONS

The average values obtained from the chemical composition of the dried jambolan pulp from the municipality of Bambuí,MG were: phenolic content = 235 mg GAE 100 g^{-1}; antioxidant activity: 41.88%; Humidity: 9.27%; Acidity: 2.42%; water activity: 0.33 (25.25ºC) and pH = 3.6. It was observed that the dried pulp showed acidic characteristics, low moisture, low water activity, a high phenolic content and moderate antioxidant activity.

Araújo (2014) evaluated the physicochemical characteristics of dried jambolan pulp (dried in a spouted bed and freeze-dried) and found the products' water activity values to be below 0.6, as in the present study, and to have higher phenolic contents, which ranged from 418.0 to 515.1 mg GAE 100 g^{-1}. Differences in the phenolic contents are expected because the drying processes and the composition of the raw material interfere in the results.

The moisture content and pH results of the dried jambolan pulp were close to those of Freitas et al. (2021), who obtained freeze-dried jambolan pulp with 9.48% moisture and a pH value of 3.64. However, the acidity values were much lower (0.23%) than in this study, which may be due to the different fruit maturity characteristics. The phenolic compounds (512.9 mg GAE 100 g^{-1}) and antioxidant activity (77.55%) of freeze-dried jambolan pulp were higher than in the present study, and this may be due to differences in the adopted drying methods. Freeze-drying is a method that preserves the jambolan pulp's bioactive compounds. The conventional process, even when using a not so high process temperature (60ºC), can lead to phenolic compound loss and, consequently, reduced antioxidant activity.

The acidity values of the fermented milk formulations are within the standards established by the Regulation of Identity and Quality of Fermented Milk (BRASIL, 2007), ranging from 0.6 to 2.0 g of lactic acid 100 g^{-1} (**Table 1**).

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Table 1. Results of the legal physicochemical requirements of fermented milk with different proportions of dried jambolan Pulp

Treatments	Acidity (g of lactic acid 100 g^{-1})	Protein (%)	Lipids (%)
T0	1.50^{a}	5.17^{a}	0.24^{a}
T1	1.45^{a}	5.04^{a}	0.23^{a}
T2	1.51^{a}	4.45^{b}	0.25^{a}
T3	1.51^{a}	4.43^{b}	0.20^{a}

T0 = fermented milk with 0% dried jambolan pulp; T1 = fermented milk with 1% dried jambolan pulp; T2 = fermented milk with 2% dried jambolan pulp; T3 = fermented milk with 3% dried jambolan pulp. Means followed by the same letter on the same line do not differ according to the Scott-Knott Test ($p \geq 0.05$).

The fermented milk showed reduced lipid values without statistical differences ($p<0.05$), classifying them as fermented skim milk according to criteria established by legislation (maximum of 0.5 g 100 g^{-1}) (BRASIL, 2007). It was found that the protein content was lower in treatments with a higher proportion of jambolan pulp (T2 and T3), with differences in relation to T0 and T1 (**Table 1**).

Despite the differences in the protein values of the fermented milk treatments, all treatments are within the standards set forth by the Regulation of Identity and Quality of Fermented Milk (BRASIL, 2007), which establishes minimum milk proteins at 2.9%.

According to the analysis of variance of the physicochemical parameters of the fermented milk treatments with different proportions of jambolan (0%, 1%, 2% and 3%), there was no significant difference in the effects analyzed for acidity, pH, color (L*, a* and b*), phenols and antioxidants during the refrigerated storage times (0, 10, 20 and 30 days).

The results of the analyses related to a* color, phenols and antioxidants (**Table 2**) showed statistical differences in relation to treatments ($p<0.05$).

Table 2. Means of physicochemical parameters of fermented milk with different proportions of dried jambolan pulp (0%, 1%, 2% and 3%) during refrigerated storage time (0, 10, 20 and 30 days)

Treatment	pH	L*	a*	b*	Phenols (mg GAE 100 g^{-1})	Antioxidants (%)
T0	3.91^{a}	77.86^{a}	-5.85^{b}	8.73^{a}	49.16^{b}	15.27^{b}
T1	3.96^{a}	67.48^{a}	-0.23^{a}	5.50^{a}	55.58^{b}	25.11^{ab}
T2	3.88^{a}	66.43^{a}	2.10^{a}	5.28^{a}	58.93^{b}	36.42^{a}
T3	3.86^{a}	64.48^{a}	2.97^{a}	5.23^{a}	82.34^{a}	40.83^{a}

T0 = fermented milk with 0% dried jambolan pulp; T1 = fermented milk with 1% dried jambolan pulp; T2 = fermented milk with 2% dried jambolan pulp; T3 = fermented milk with 3% dried jambolan pulp; GAE = gallic acid equivalent in milligrams. Means followed by the same letters in the same column do not represent a statistical difference according to the Scott-Knott test ($p \geq 0.05$).

Sample T0 showed an average a* color value lower than the average values for the samples to which Jambolan pulp was added. This was expected since the pulp's natural pigments, such as anthocyanins, increase the intensity of the product's red color (**Table 2**).

The antioxidant activity in the treatments was higher as the proportion of jambolan pulp increased, confirming the pulp's considerable antioxidant activity. Sample T3 obtained

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antioxidant activity of approximately 2.5 times greater than T0. The same trend was observed for the phenolic content as sample T3 had a phenolic content of approximately 1.5 times greater than T0 (**Table 2**). It is assumed that samples with a higher phenolic percentage present greater oxidizing activity, as attested in this research.

It was observed that, at the 5% level for pH, there was no significance for interaction, main effect or treatment. There was, however, a significant difference for the times. The fermented milk showed a reduction in pH during storage, indicating the production of acidic compounds (**Figure 1**). The reduction in pH may have been caused by the fermentation activity of *L. casei*, which continues its activity even under refrigeration.

The fermented milk treatments also showed a reduction of phenolic contents during refrigerated storage, with statistical differences in relation to storage times ($p<0.05$) (**Figure 2**).

Figure 1. Variation in the pH of fermented milk with different proportions of dried jambolan pulp (0%, 1%, 2% and 3%) during refrigerated storage (0, 10, 20 and 30 days).

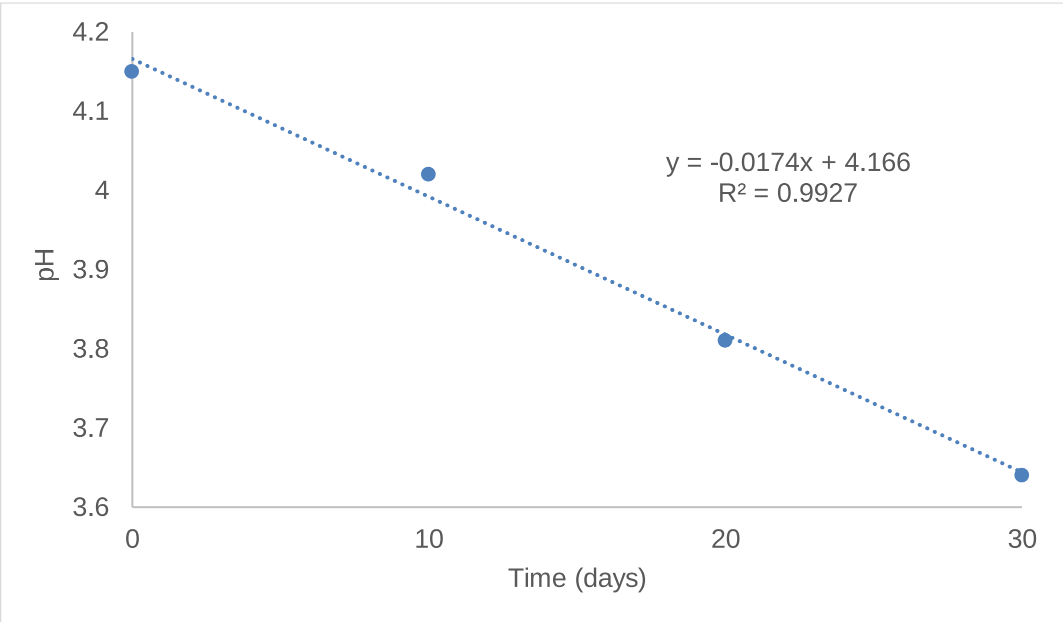

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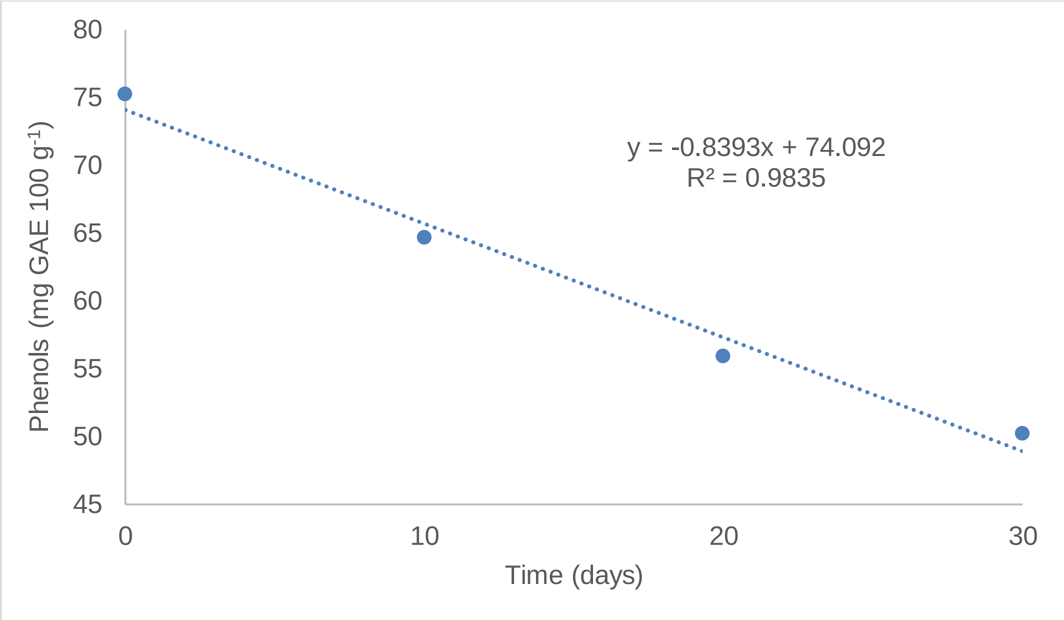
Figure 2. Variations in the phenolic content of fermented milk with different proportions of dried jambolan pulp (0%, 1%, 2% and 3%) during refrigerated storage (0, 10, 20 and 30 days).



The reduction of phenols may be due to the refrigeration conditions of the samples, which result in the production of reactive oxygen species, in turn resulting in a higher phenolic compound consumption (JUNMATONG et al., 2015).

Bacterial counts showed variations over time but remained with counts equal to or greater than 7,000 log CFU g^{-1} at 30 days, considered fermented milk's usual shelf-life limit. The smallest variation in count over time, comparing 0 and 30 days, occurred in treatment T3 with only 0.30 logarithmic cycles. In the other treatments, the variations were greater: 0.70 for T0; 0.84 for T1 and 1.08 for T2. There was greater bacteria viability in T0 and T3 over time, which may indicate that the dried jambolan pulp did not influence the formulations, i.e., the pulp's presence had no influence on the viability of lactic acid bacteria during 30 days in refrigerated storage (**Table 3**). The presence of FOS (proportion of 2% m/m) in all formulations may have been sufficient for the viability of lactic acid bacteria.

Table 3 shows the counts of viable lactic acid bacteria in fermented milk at 0, 10, 20 and 30 days and expressed in log colony forming units per gram (log CFU g^{-1}). There were significant differences in the means for the treatments, time and interaction ($p < 0.05$).

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Table 3. Results of lactic acid bacteria counts in log CFU g^{-1} performed in fermented milk with different proportions of dried jambolan pulp (0%, 1%, 2% and 3%) during refrigerated storage times (0, 10, 20 and 30 days)

	Time (days)			
Treatments	0	10	20	30
T0	8.320 ^{aA}	7.670 ^{aB}	7.600 ^{aB}	7.625 ^{abB}
T1	8.175 ^{aA}	6.900 ^{bC}	7.260 ^{bB}	7.335 ^{cbB}
T2	8.075 ^{aA}	7.040 ^{bB}	6.950 ^{bB}	7.000 ^{cB}
T3	8.150 ^{aA}	7.885 ^{aA}	7.000 ^{bB}	7.850 ^{aA}

T0 = fermented milk with 0% dried jambolan pulp; T1 = fermented milk with 1% dried jambolan pulp; T2 = fermented milk with 2% dried jambolan pulp; T3 = fermented milk with 3% dried jambolan pulp. CFU g^{-1} = Colony Forming Units per gram of sample. Means followed by the same lower-case letter in the columns and the same uppercase letter in the rows do not differ according to Tukey's test ($p \geq 0.05$). CV = 1.55%

Lactobacillus casei was used as the sole yeast, which classifies milk as fermented or cultured. Although counts decreased over time, the number of viable cells remained above the recommended minimum limit of 6,000 log CFU g^{-1} (1.0 x 10^6 CFU g^{-1}) (BRASIL, 2007). Several authors propose that the minimum daily dose of the probiotic culture considered therapeutic is 8,000 to 9,000 log CFU (GALLINA et al., 2011), corresponding to the consumption of 100 g of product containing 6,000 to 7,000 log CFU g^{-1}. As all formulations reached these minimum counts, the fermented milk can be considered potentially symbiotic, with the presence of FOS and/or dried jambolan pulp in the formulations.

Thus, the results obtained in this research confirmed that jambolan pulp is a valuable source of bioactive compounds and in powdered form can be incorporated into the development of new food products. Therefore, the drying method and other factors that negatively affect the product quality must be selected and adapted to guarantee its stability (MUSSI et al., 2015).

CONCLUSIONS

The feasibility of adding dried jambolan pulp to fermented milk was verified, with an increase in the intensity of the red color, phenolic content and antioxidant activity as the proportion of pulp increased. There were some differences in the viability of lactic acid bacteria across formulations, which were not significantly influenced by the presence of dried jambolan pulp. However, all products had minimum counts of lactic acid bacteria.

The combination of *L. casei* and jambolan pulp resulted in potentially functional fermented milk formulations.

Further studies on the processing of jambolan pulp under more favorable conditions are suggested to preserve its active compounds and its application in probiotic dairy products.

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