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Functional and Textural Characterisation of Flavoured Yogurt Supplemented with Millet Milk Extracted from *Paspalum scrobiculatum* (Kodo Millet)

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ABSTRACT

Background: The aim of the study was to develop a Sapodilla flavoured yogurt enriched with millet milk extracted from *Paspalum scrobiculatum* (Kodo millet). Millet milk was extracted by simple soaking and pressure cooking method.

Methods: Four variations and control sample of the yogurt were developed with incorporation of millet milk in the proportions; 10%, 20%, 30% and 40%, where 0% served as the control. Sensory evaluation was performed using the nine-point hedonic scale. Variation 2 of the yogurt (Y2-20%) having the highest overall acceptability (7.70±0.05) was finalized for further analyses.

Result: The physicochemical analysis including texture (hardness- 75.66±0.57), syneresis (4.50±0.10), pH (4.46±0.05), total titratable acidity (0.86±0.05) and total solids (23.2±0.20) were studied for the developed product. Antioxidant activity revealed 87.93% inhibition and total Phenolic content was found to be significant (P value <0.001) in comparison with the control. Development of flavored yogurt enriched with kodo millet milk is a novel technique. Hence, as indicated, great prospects exist in India for such value added ingredients made with traditional millets and milk byproducts along with advanced technologies for their processing.

Key words: Antioxidant activity, Flavored yogurt, Kodo millet, Millet milk, *Paspalum scrobiculatum*.

INTRODUCTION

Cereal grains were among early man's agricultural endeavors, which they may still enjoy depending on their environment. Millets are indigenous crops that are hard by texture and possess the ability to grow well in dry areas with minimal water and low fertility levels of soil (Stanley *et al.,* 2013). Besides having a shorter maturation time, millets are known to be that category of grains that are extremely nutrient dense possessing an array of therapeutic properties. Hence, the ease of growth, rich nutritional properties and importance in agro-industries make millets, the crops of greatest importance. Millet grains are a nutritious source for various food preparations in the food industry; however, they contain anti-nutritional factors which can be overcome by certain processing techniques (Bhuvaneshwari *et al.,* 2020). *Paspalum scrobiculatum* also known as Kodo millet or Koda millet is a millet crop that has originated in Africa but was domesticated in India around 3000 years ago. Kodo millet, a rich source of macronutrients like carbohydrates, fiber and protein and micronutrients like vitamin B12, calcium, iron, potassium and magnesium is utilized in formulations of various food products (Mitkal *et al.,* 2021).

Kodo millet of all the other millets is seen to have the highest antioxidant potential (Deshpande *et al.,* 2015). Millet milk is the byproduct of germinated millets that have attracted considerable attention in the food industry due to its versatility as a value added ingredient. With growing need for vegan and gluten free foods, millet milk can be used as an excellent replacement for dairy products having considered its high nutrient content and increased digestibility and bioavailability of micronutrients. Soaking of millets is a significant step in the extraction process which helps in the reduction of the antinutritional factors (Bhuvaneshwari *et al.,* 2020). Yogurt is considered as an excellent choice for those with gastrointestinal disorders, irritable bowel syndrome, inflammatory bowel disease and those looking for weight management due to its high digestibility and high bioavailability of nutrients. Microorganisms present in yogurt are known to improve colonic health and cure infantic diarrhea. It was also proven to be effective in preventing a relapse of ulcerative colitis. Yogurt is evidenced to be effective in cytokine production, T-cell function and natural killer-cell activity and thereby result an overall immunological enhancement (Weerathilake *et al.,* 2014). A recent meta-analysis showed a 14% lower risk of type 2 diabetes when

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yogurt consumption was 80-125 g/d compared with result of no yogurt consumption (Salvado *et al.,* 2017).

The objective of the present study was to develop a chikoo flavoured yogurt enriched with millet milk extracted from *Paspalum scrobiculatum* (Kodo millet) and to study the physicochemical properties, antioxidant potential and sensory qualities of the yogurt.

MATERIALS AND METHODS

Ingredients

Kodo millet grains, pasteurized toned milk fortified with vitamin D and vitamin A, commercial yogurt- Nestlé (starter culture), chikoo (flavouring agent), white sugar and agar obtained from plain china grass sheets (thickening agent) were procured from local retail stores (Bengaluru, India).

Extraction of millet milk

The process described by Punniyamoorthy *et al.* (2020) was used with slight modification for the extraction of the milk. Soaking was done for 8 hours to reduce the antinutritional factors after which it was pressure cooked with addition of 200 ml of water. The pressure cooked millet was cooled and blended with 100 ml of water and filtered using a muslin cloth. The milk yield obtained was 240 ml when 50 g of the millet was used. This millet milk was stored at a refrigerated temperature of 4°C for further analysis.

Preparation of millet milk yogurt

The formulation of the yogurt recipes was done by incorporating different proportions of millet milk in the standard recipe. Four variations were identified based on the acceptable levels and this was prepared by addition of 10%, 20%, 30% and 40% into samples Y1, Y2, Y3 and Y4. The control was labeled as YO which had 0% addition of the millet milk. Other ingredients (milk starter culture, sugar, chickoo fruit pulp and agar) were added in the same concentrations in both the control sample as well as the variations. The ingredients were weighed separately and kept aside. Sugar and agar powder was added to the milk and mixed thoroughly. The milk was pasteurized at 90°C for 15 min and cooled to 42°C. The pH was recorded. The milk was then inoculated with the commercial starter culture. To this, chikoo pulp and millet milk were added in the required amounts and altogether stirred to make it a homogenous mixture. The mixture was transferred to PPE (polyphenylene ether) containers and this was incubated at 42°C for 4 hours and cooled at 4°C. The control (without the addition of millet milk) was prepared following the same steps.

Sensory evaluation

For the sensory evaluation, 30 semi- trained panelists were chosen and it was carried out in a well ventilated and illuminated room. The panelists were invited for the evaluation based on their willingness and convenience to carry out the tasting. A 9 point hedonic scale was used to evaluate the sensory characteristics of the food products. The attributes taken into account for the evaluation of yogurt included appearance, taste, aroma, consistency, colour, mouthfeel and overall acceptability. In order to obtain accurate and unbiased results, all the four variations along with the control were coded with random numbers. The codes didn't follow any particular order and products were placed on the counter in a random sequence. This enabled panelists to rate the food product purely based on its acceptability without any prior knowledge about the food.

Texture analysis

Texture of the yogurt was analyzed using the equipment, Texture Analyzer by following the procedure described by Joon *et al.* (2017) with slight modifications. The textural characteristics analyzed were firmness, cohesiveness, consistency and index of viscosity.

Syneresis

Determination of syneresis was done by the centrifugal method as described by Mwizerwa *et al.* (2017). 30 g of the yogurt sample was transferred to a centrifuge tube (50 ml). The sampe was then centrifuged at 3000 rpm for 20 minutes in a Remi centrifuge. The supernatant (whey) was transferred into a measuring cylinder and volume was noted.

Total titratable acidity

Procedure for the measurement of the total titratable acidity of yogurt given by Zainoldin *et al.* (2009) was used. 1 ml of the yogurt sample was dissolved in 9ml of distilled water and this was titrated against 1 M NaOH with 0.1% of Phenolphthalein as the indicator. The volume rundown was equated in a formula to calculate the lactic acid percentage equivalent.

pH

The changes in the pH during fermentation of the yogurt were measured using a digital pH meter. The pH of the control and the variation sample was measured before fermentation and during the fermentation process until the pH reached 4.5.

Moisture content

The process employed for the determination of moisture was described by AOAC, (2016). 5 g of the sample was weighed and added to clean and dry Petri plates. The plates were weighed before and after addition of the sample. It was then placed in the hot air oven at 100°C for 3 hours and moisture % was calculated using an equation.

Total solids

Determination of total solids was done by following the procedure described by Matela *et al.* (2019). It involves the measurement of moisture and calculation to find out the percentage of total solids present in the sample.

$$\%\ \text{total solids} = (100 - \%\ \text{moisture})$$

Antioxidant activity (DPPH radical scavenging activity)

The procedure described by Rahman *et al.,* 2015 was followed where the analysis was based on electron-transfer that produced a violet color in methanol solution for which the absorbance was measured at 517 nm. An equation was then used to calculate the inhibition %.

Total phenolic content

The phenolic compounds in yogurt samples were assessed through the procedure described by Sahu *et al.,* (2009). Gallic acid was used as the standard and the total phenolics were expressed as gallic acid equivalents (GAE). For the samples, distilled water, different concentrations of the sample and were mixed together in different amounts. Sodium carbonate was then added and after incubation for 45 mins in the dark, the readings were taken using the spectrophotometer at 765 nm. A graph was plotted of the concentration and the absorbance to find out the phenolic content in the sample.

Shelf life evaluation

Assessing the microbial content of milk is a very important factor that determines the quality of the product. (Frew *et al.,* 2020)**.** A milk product which has high nutritional content allows the growth of both harmful as well as beneficial bacteria. Growth of harmful bacteria can be minimized if the shelf life of the product is detected (Tomar *et al.,* 2024).

The freshly prepared samples of yogurt were stored at 4°C for 8 days and microbial testing was done on different days (Day 0, Day 2, Day 4 and Day 8). The shelf life study was conducted using the standard spread plate technique. Nutrient agar was prepared and used in order to check the bacterial growth. Around 1ml of the sample of dilution factors 10-7 and 10-8 were taken for nutrient agar and bacterial Petri plates were prepared after which it was incubated at 37°C for 24 hours. Similarly, 1 ml of the sample of dilution factors 10-4 and 10-5 were taken for potato dextrose agar and fungal Petri plates were prepared and incubated for 48 hours.

Statistical Analysis

Mean, standard deviation and p value were obtained for the results of the sensory evaluation and physicochemical properties. Results were analysed by applying one way ANOVA test and Pearson's correlation test to find the significance of acceptability of the developed yogurt.

RESULTS AND DISCUSSION

Standardization of yogurt

Kodo millet grains, pasteurized toned milk fortified with vitamin D and vitamin A, commercial yogurt- Nestlé (starter culture), chikoo, white sugar and agar were used in the formulation of the yogurt. (Table 1).

Physicochemical properties of yogurt

From the p value, a significant difference can be observed between Y0 and Y2 (Table 2). The syneresis % obtained for Y0 is 7.30±0.10 whereas for Y2 it is 4.50±0.10. The higher value of syneresis in Y0 is probably due to decrease in the water holding capacity which ultimately leads to more release

Table 1: Standardization of yogurt.

Ingredient	Y0	Y1	Y2	Y3	Y4
Milk	70	60	50	40	30
Millet milk	0	10	20	30	40
Starter culture	15	15	15	15	15
Chikoo pulp	10	10	10	10	10
Sugar	5	5	5	5	5
Total	100	100	100	100	100

Key: YO= Control, Y1= 10% MM incorporated, Y2= 20% MM incorporated, Y3= 30% MM incorporated, Y4= 40% MM incorporated.

Table 2: Physicochemical properties of yogurt.

Parameter	Products		P- value
	Y0	Y2	
Syneresis %	7.30±0.10	4.50±0.10	<0.001*
pH	4.60±0.10	4.46±0.05	0.116NS
Total titratable acidity (LA %)	1.07±0.01	0.86±0.05	0.004*
Moisture %	78.66±1.00	75.00±1.00	0.07NS
Total solids %	22.3±0.05	23.2±0.20	0.07NS
Hardness/ Firmness %	61.33±0.57	75.66±0.57	<0.001*
Hardness work cycle (g/cm)	58.30±0.10	70.50±0.10	<0.001*
Adhesiveness (g/cm)	9.37±0.07	10.44±0.03	<0.001*
Adhesive force (g)	14.20±0.02	15.36±0.05	<0.001*
Correlation between pH and total titratable acidity			
pH	4.60±0.10	4.46 ± 0.05	R value-
Total titratable acidity (LA %)	1.07±0.01	0.86± 0.05	1.000
Correlation between syneresis and total titratable acidity			
Syneresis %	7.30±0.10	4.50±0.10	R value-
Total Titratable acidity (LA %)	1.07±0.01	0.86±0.05	+0.999

All values are means of triplicate determinants ± standard deviation (S.D). *P- value is <0.05 by ANOVA; NS- not significant. Y0- Control; Y2- 20% millet milk.

of whey (Zainoldin *et al.,* 2009). This stipulates that millet milk possesses greater water holding capacity due to which syneresis % in Y2 is much lesser than Y0. From the table it can be seen that both Y0 and Y2 have a similar pH to 4.7 as reported by Anand and Kapoor (2011). This acidic pH is seen due to the action on lactose which produces lactic acid having an acidic pH. Total Titratable acidity is the measure of the amount of alkali required to change the pH of milk from its original value (6.8) to a more acidic pH. The acceptable range for fermented products is between 0.7% and 1.2% (Choi *et al.,* 2016)**.** The values obtained for Y0 and Y2 are in good agreement of the standard suggesting that the acidity is in the desirable range. The moisture % obtained for Y0 is 78.66±1.00 and for Y2 is 75.00±1.00 which is similar to the previously reported values of 76.44% and 79.64% (Matela *et al.,* 2019). The amount of moisture content present in the yogurt affects the texture and the mouthfeel. Total soluble solids include the organic acids such as citric, malic, tartaric acids, sugars which can be monosaccharides, disaccharides, or oligosaccharides and soluble amino acids (Al-syed *et al.,* 2002). The TSS in Y0 was found to be 22.3±0.05 and in Y2 it was 23.2±0.20. The slight increase in the total solids of Y2 could be because of the increased total solids % in millet milk which ideally varies from 18.94% to 38% (Jha *et al.,* 2013). A value greater than 20% inhibits the growth of *Lactobacillus bulgaricus* and a value lesser than 20% would most likely result in the malfunction of the starter culture or inoculums. (Matela *et al.,* 2019).

The mean score obtained for hardness of Y0 is 61.33 ± 0.57 which is slightly lower than the score obtained for Y2 which is 75.66±0.57. This difference is due to the incorporation of millet milk into Y2 which is known to enhance the firmness property of the product (Mwizerwa *et al.,* 2017). From the values, it can be seen that Y2 has a greater adhesiveness and adhesive force as compared to Y0. The reason behind this could be due to the fact that millet milk has greater viscosity and greater ability to promote gel formation hence, increasing the chance of cross-linking with polymerization whey protein. In addition to this, the addition of a thickener such as xanthan gum, agar, *etc* may further solidify the yogurt and enhance its cohesiveness (Song *et al.,* 2020). Fig 1 illustrates the graph obtained as a result of the texture analysis of Y0 and Y2. The higher the peak greater is the hardness/ firmness. Hardness is one of the most important parameter with which the texture of yogurt can be understood. It is referred to as the force required to achieve a certain deformation and is also regarded as a measure of firmness of the yogurt (Mudgil *et al.,* 2021). Since hardness is directly related to better texture, it can be deduced from the figure that the peak of Y2 is higher than Y0 indicating that Y2 possesses greater firmness and hence, has better texture than Y0.

It can be observed from the table that the two parameters pH and Titratable acidity are inversely related. As per literature, when pH reduces the Titratable acidity of the yogurt increases. This is due to the action of lactic acid released during the fermentation process (Zainoldin *et al.,* 2009). With respect to the relationship between syneresis and total Titratable acidity, it can be observed that the R value obtained is positive (+ 0.999) suggesting that the two parameters share a direct relationship. According to Rani *et al.,* 2012, one of the significant parameters responsible for this is titratable acidity. It is agreed that higher acidity of yogurt results in greater separation of whey and total solids hence, more syneresis.

Antioxidant Activity of Yogurt

Results obtained for the DPPH inhibition assay and total Phenolic compounds is presented in Table 3. From the table, it is evident that as the concentration increases, the % inhibition also subsequently increases. There was a 87.9% inhibition at 100 mcg/ml seen for Y2. The percentage inhibition values obtained for both control and variation at 100 mcg/ml concentration is close to the % inhibition of ascorbic acid indicating that both the products have high antioxidant potential. However, the percentage inhibition for the variation at 100 mcg/ml is higher than the values obtained for control. This is because of the incorporation of millet milk in the variation which is known to have high antioxidant

Table 3: Antioxidant capacities of yogurt.

Antioxidant analyses	Percentage of inhibition	
	Y0	Y2
Antioxidant activity (% inhibition of DPPH assay)	79.08	87.92
Total phenolics (µg GAE/mL)	12.55	34.36

Y0- Control; Y2- 20% millet milk.

Table 4: Sensory scores of Yogurt.

Attributes	Y0	Y1	Y2	Y3	Y4	P- value
Appearance	7.76±0.05	6.36±0.05	7.46±0.05	5.53±0.05	5.76±0.05	0.001*
Taste	7.66±0.05	6.56±0.05	7.66±0.05	5.66±0.05	6.03±0.05	0.001*
Aroma	7.86±0.05	6.56±0.05	7.66± 0.05	5.66±0.05	6.03±0.05	0.001*
Colour	7.77±0.05	6.46±0.05	7.76±0.05	5.36±0.05	6.13±0.05	0.001*
Consistency	7.66±0.05	6.46±0.05	7.76±0.05	5.66±0.05	6.26±0.05	0.001*
Overall acceptability	7.77±0.05	6.50±0.05	7.70±0.05	5.70±0.05	6.3±0.05	0.001*

All values are means of triplicate determinants ± standard deviation (S.D). *P- value is <0.05 by ANOVA; NS- not significant. Y0- Control; Y1- 10% millet milk; Y2- 20% millet milk; Y3- 30% millet milk;Y4- 40% millet milk.

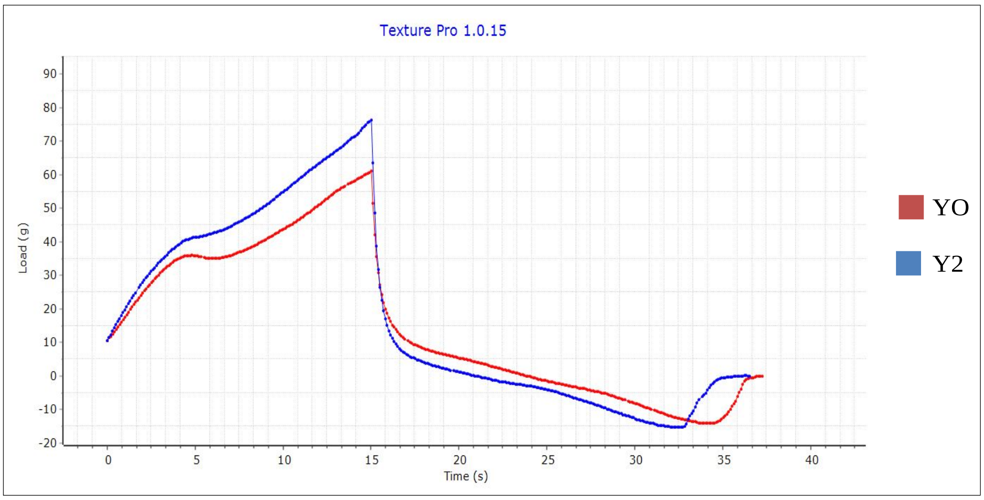

Fig 1: Texture properties of yogurt.

activity when subjected to the process of fermentation. Similar results were obtained by Peerkhan *et al.* (2022) where kodo and little millet milk had exhibited the highest antioxidant potential in comparison to proso and barnyard millet.

The total phenolic content of Y0 is 12.55±0.01 which is slightly lower than 34.36±0.05 obtained for Y2. The results of the present study are similar to 20.34 mg GAE/g reported by Nguyen *et al.* (2016).

Sensory evaluation

From the p- values in Table 4 it can be seen that there is a significant difference for all the sensory attributes (P-value <0.05). The mean scores of overall acceptability obtained for all the variations and control lie between the range 5.7 to 7.8 indicating that the panellists either showed neutral behaviour towards the food or liked the food product to a certain degree. The lowest score was seen for variation 3 (Y3) consisting of 30% millet milk followed by variation 4 (Y4) consisting of 40% millet milk. This could possibly be due to the bland taste of the millet milk hence, reducing the taste and flavor of the product. An overall acceptability score of 7.70 ± 0.05 was given to variation 2 (Y2) which was seen to be the highest in comparison to the other variations hence, this formulation was finalized as the most accepted variation. (Fig 2)

Fermentation profile

The change in pH is one of the most significant indicators of the fermentation process. The drop in the pH during the process of fermentation is explained in Fig 3**.**

Shelf- life evaluation

From table 5, it can be observed that there was slight bacterial growth on the 0th day and this growth gradually increased by the end of 8 days. Considering the fact that

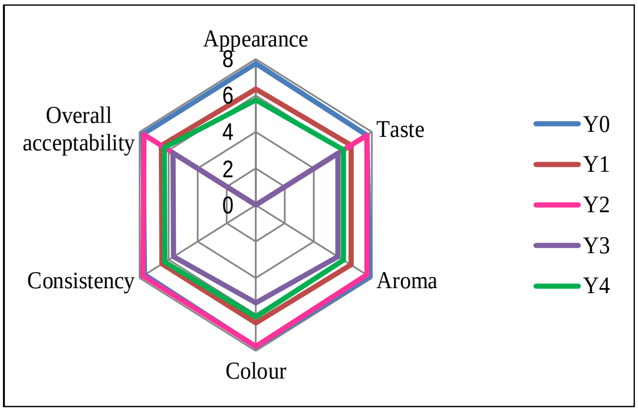

Fig 2: Sensory evaluation of yogurt.

the food product is yogurt which generally consists of live bacteria, it can be said that the growth is of the beneficial bacterial strains responsible for the fermentation process. The colony forming units (CFU) for Y0 on the 8th day of analysis was found to be higher than that of the variation. As per literature, a CFU value for yogurt bacteria must lie between the range of 2 x10-7 and 8.65 x 10-7. A value which is greater than 8.65×10^{-7} indicates presence of spoilage organisms in curd. On the other hand, a value below this standard is ineffective in providing the desired therapeutic effect (Ertem *et al.,* 2018). Hence, it can be said that the Y0 and Y2 prepared, consisted of bacterial growth in the acceptable range suggesting that the product has optimal probiotic potential due to the presence of beneficial strains.

With respect to fungi, no colony forming units (CFU) were seen on 0th and the 2nd day for Y0 as well as the Y2. However, by the 8th day, there was slight growth of fungi of both the control and variation samples. Since the microbial

Table 5: Shelf life evaluation.

Sample	0^{th} day	2^{nd} day	4^{th} day	8^{th} day
Total plate count for dilution 10^{-7} (Bacterial)				
Y0	0.24×10^{-7}	2.0×10^{-7}	4.88×10^{-7}	6.0×10^{-7}
Y2	0.16×10^{-7}	0.33×10^{-7}	1.20×10^{-7}	2.1×10^{-7}
Total Plate Count for dilution 10^{-4} (Fungal)				
Y0	0	0	0.41×10^{-4}	2.16×10^{-4}
Y2	0	0	0.21×10^{-4}	1.88×10^{-4}

Y0- Control; Y2- 20% millet milk.

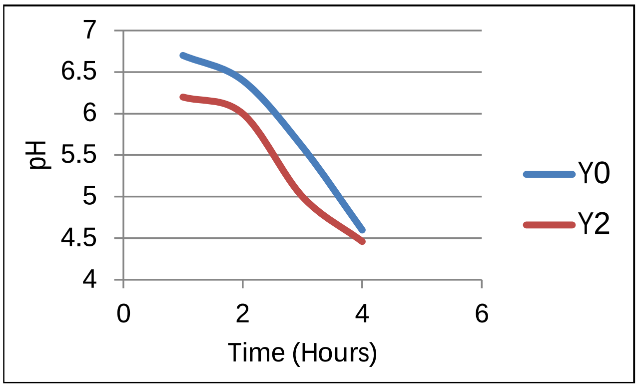

Fig 3: Drop in pH.

load was higher on the 10^{th} day and the bacteria CFU exceeded the acceptable range, the shelf life for both the products was concluded to be 8 days provided; it is stored at 4°C.

CONCLUSION

In conclusion it can be said that, with growing awareness about health aspects of everyday food, the importance of traditional foods is also increasing and since millets have been an integral part of traditional recipes from years before, popularity for these and their byproducts has seen a tremendous rise. With this view, millet milk was chosen as the key ingredient for the study to throw light on the lesser known and studied facts about the versatility and benefits of making use of this as an ingredient in the yogurt formulation. The product developed was sensorially acceptable and had proven to possess various physicochemical attributes which improved its eating quality. However, there is a need to perform clinical trials in order to check the therapeutic effect of the developed product in humans.

Conflict of Interest: The authors declare that they have no conflict of interest.

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