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SHORT COMMUNICATION

Preparation and quality evaluation of Ready-To-Serve beverage from cashew apple

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ABSTRACT

Cashew fruit, Anacardium occidentale, consists of two main parts; real fruit which is the nut and cashew apple which is the juicy swollen peduncle. Though cashew kernel has tremendous local and international market demand, cashew apples get wasted in bulk due to the lack of proper post-harvest management and its astringent taste despite its nutritious and medicinal value. Therefore, this study was aimed on developing cashew apple into a ready-to-serve (RTS) beverage and its quality analysis. Ready-to serve beverage from fresh cashew apple was prepared following Sri Lankan standards of preparation. The quality evaluation of the prepared beverage showed 3.44 ± 0.01 of pH, 0.38 ± 0.01% of titratable acidity, 20 ± 0.5^0 Brix of TSS, 1.02 g/mL of density, 4.48 mPa of viscosity, 10.91 ± 0.20 g /100 mL of total sugars and 25.13 ± 0.73 mg/100 mL of ascorbic acid. Total phenolic, flavonoid and tannin contents of the beverage were found to be 53.44 ± 1.84 mg gallic acid equivalent/g, 10.03 ± 0.57 mg quercetin equivalent/g and 31.37 ± 1.80 mg tannic acid equivalent/g respectively. Antioxidant properties of the beverage was evaluated using DPPH and FRAP assays and the IC_{50} in DPPH assay was 585.29 ppm whereas the FRAP value was 531.4 ± 8.7 μ mol Fe^{2+}/ g conveying promising antioxidant capacity of the final product. Sensory evaluation was conducted and it showed that RTS beverage followed stable trends in all organoleptic properties including color, appearance, taste, flavor, aroma and overall acceptability rendering the potential for commercialization that would allow consumers to relish the refreshing flavor of cashew apple throughout the year.

Keywords: Antioxidants**,** cashew apple, quality evaluation, Ready -To-Serve beverage.

INTRODUCTION

Anacardium occidentale (cashew) is a seasonal crop cultivated in tropical and sub-tropical region of the world with aim of obtaining cashew nuts (kernels). It is considered as a "gold mine" as high output is received with very low input applied in production. Cashew tree bears cashew nut, the true fruit, and cashew apple, the pseudo-fruit, accounting for 10 % and 90 % of the total weight respectively. Cashew apples are elongated, round or pear-shaped fibrous fruits and the flavor, aroma and sugar concentrations are very high whereas the acidity and astringency are low at the ripening stage. It has higher content of juice (80-90%) and bears higher nutritional value including vitamin C and antioxidants (Talasila *et al*., 2013). At the ripening stage, the apple with nut falls on the ground and the nut is easily detached from the apple for further processing but the apple get wasted without consuming, processing or used in generating income. It is estimated that about 30 million metric tons of cashew apples are produced yearly in the world (Talasila *et al*., 2013).

Despite the advanced nutritional profile, cashew apples encompass with some negative characteristics that have led it to become an underutilized fruit. The shelf life of cashew apple is very short (1-2 hours) and undergoes rapid microbial spoilage in fresh stage due to high content of moisture and sugar (Talasila *et al*., 2013). In addition, astringent taste of cashew apple which is resulted from high tannin content and oily substance present in the skin, have made it unfavorable to consumers (Das and Arora 2017).To overcome the issues related to limited shelf life, unfavorable taste and to make them available throughout the year, value addition is suggested. Several reports are available on value addition to cashew apple around the globe; processing cashew apple into fermented products such as wine, vinegar, bio-ethanol and

probiotic beverages using specific strains of microorganisms has been reported by Prommajak *et al*. (2014). The extraction of juice, pomace which is rich in dietary fiber and reducing sugars has been converted into value added products. However, the number of value added products available in the market is very limited and hence, the avenues are still open to formulate different products from cashew apple to satisfy the market demands and also to generate an additional income for cashew farmers. Unfermented cashew apple beverages have become a major category of value added products which preserves natural taste and quality. Nutritious and refreshing beverages like juice, juice concentrates, squash and syrup are prepared from the unfermented juice of cashew apple by adding varying concentrations of sugar, citric acid and preservatives as fresh or unfermented juice itself trends to alter its characteristics such as total soluble solids (TSS), total sugars, pH, ascorbic acid, alcohol content and tannin content very quickly (Gawankar *et al*., 2018, Talasila *et al*., 2013). Currently, the demand for Ready-to-Serve (RTS) beverages from cashew apple with high nutritional value and other health imparting attributes emerges in the local and global market (Hemalatha *et al*., 2018). Therefore, this study was carried out to develop nutritional Ready-To-Serve beverage from cashew apples in Sri Lanka, and assess the quality and sensory parameters.

MATERIALS AND METHODS

Chemicals: All the chemicals and reagents used in the experiments were of analytical grade, purchased from Sigma Aldrich.

Sample Collection: The fully ripened yellow cashew apples were collected in fresh form from Giradurukotte, Uva Province, Sri Lanka. Ripened yellow cashew apples were washed using running tap water followed by distilled water, and quickly used for the experiments.

Preparation of the RTS beverage: Ready-to serve (RTS) beverage from cashew apple was prepared by following Sri Lankan standards for beverage (SLS 729:1985) (Saranyah and Mahendran, 2015). Cashew apples were cut into small pieces and were pulped using a blender. About 150 g of the pulp was weighed and the juice was extracted from the pulp by pressing and straining through a muslin cloth. An amount of 2.5 g of potassium metabisulfite (Gawankar *et al*., 2018) and 2.0 g of citric acid (Hemalatha *et al*., 2018) were added to fruit pulp and the mixture was stirred well. Gelatin solution which contains 3.0 g of gelatin in 10 mL of water was added to the above mixture. After mixing well, it was allowed to stand for 24 hours in a cool dark place for clarification to occur. The upper clarified juice layer was separated and mixed with a solution which was prepared by mixing 85 g of sugar and 0.5 g of citric acid in 100 mL water under boiling conditions (Hemalatha et al., 2018), it was filtered using a muslin cloth. Finally prepared beverage was filled into a pre-sterilized glass bottle, immediately sealed and stored under refrigeration.

Quality Evaluation of the Cashew Apple RTS Beverage

All the measurements were done in triplicate.

Measurement of pH: An aliquot of 25.0 mL of the RTS beverage was used and the pH of the sample was measured using a calibrated pH meter (ORION STAR A211).

Total Soluble Solids: Total Soluble Solid content was measured using a portable hand refractometer (QUANBU) and the average value was considered as the Total Soluble Solid (TSS) content of the RTS beverage (Ranganna 1999).

Density: Density of the RTS beverage was calculated using the weight measurements taken from the pycnometer, water as the reference.

Viscosity: Viscosity was calculated using the given equation, according to the time measurements taken using the Ostwald viscometer, using water as the reference (Maheshwar, 2018).

Titratable Acidity: AOAC Methods (2002) was used to measure titratable acidity. An aliquot of 25.00 mL of the RTS beverage was titrated with

$$\text{Viscosity}_{\text{sample}} = \frac{\text{Viscosity}_{\text{water}} \times \text{Density}_{\text{sample}} \times \text{Time}_{\text{sample}}}{\text{Density}_{\text{water}} \times \text{Time}_{\text{water}}}$$

0.1 N NaOH using phenolphthalein as the indicator. The mean value was taken to calculate the titratable acidity using the following equation. Titratable acidity was expressed as g citric acid/100 g of juice and was calculated using the formula:

$$\% \text{ Titratable Acidity} = \frac{\text{Normality}_{\text{titrant}} \times V_{\text{titrant}} \times \text{Eqv. weight}_{\text{acid}}}{V_{\text{sample}} \times 1000} \times 100$$

Ascorbic acid content: Indophenol method was used to determine ascorbic acid content (AOAC Methods, 2002). Standardization of the 2,6-dichloroindophenol dye was carried out using the mixture of metaphosphoric-acetic acid solution and standard ascorbic acid and distilled water was used as the blank. A solution containing 5.00 mL of metaphosphoric-acetic acid and 2.00 mL of the RTS beverage was titrated against the indophenol dye until a distinct rose pink color that persisted for more than 5 seconds appeared. Ascorbic acid content was calculated using the following equations.

$$\text{Titre} = \frac{\text{mg of Ascorbic Acid in volume of standard solution titrated}}{[\text{Mean volume of dye for standard}] - [\text{Mean volume of dye for blank}]}$$

$$\text{mg of Ascorbic Acid}/\text{mL} = (V_{sample} - V_{blank}) \times \frac{\text{Titre}}{V_{assayed}}$$

Total Sugars: Total sugar content of the RTS beverage was determined using phenol-sulfuric colorimetric method as explained below.

Preparation of the standard: A 10-fold dilution of the stock prepared by dissolving 0.1000 g glucose in 100.00 mL water was used as the working standard.

Preparation of the sample: A volume of 7.63 mL of the RTS beverage was hydrolyzed using 5.0 mL of 2.5 N HCl for 3 hours. After neutralizing with Na_2CO_3, it was centrifuged, and the supernatant was diluted up to 100.00 mL using distilled water. Sample solution of 0.20 mL was made up to 1.00 mL using distilled water (triplicate).

Measuring absorbance: Phenol (1.00 mL) and conc.H_2SO_4 (5.00 mL) were added into standard series /sample prepared above and, allowed to stand at room temperature for 10 minutes followed by at 30^0C for 20 minutes. Absorbance was measured at 490 nm using the UV spectrophotometer (SHIMABZUN UV-1601) (Nielsen, 2017).

Total phenolic content (TPC): The TPC content of RTS beverage was determined using the Folin-Ciocalteu (FC) reagent (Bulugahapitiya *et al*., 2020). A volume of 1.00 mL of the RTS beverage was diluted up to 50.00 mL using MeOH. Standard gallic acid solution (200 mg/L) was prepared and the standard series was prepared as 20, 40, 60, 80 and 100 mg/L solutions in MeOH. Test solution was prepared using 0.50 mL of standard/ sample with 2.50 mL of FC reagent and was allowed to stand at room temperature for 5 minutes and 2.00 mL of 7.5% Na_2CO_3 was added. MeOH was used as the blank. All the test solutions were placed in dark at room temperature for 1 hour, and absorbance was measured at 740 nm using the UV spectrophotometer (SHIMABZUN UV-1601). TPC content of fruits was expressed in gallic acid equivalents (GAE).

Total Flavonoid Content (TFC): Aluminium chloride colorimetric method was used to determine the TFC (Kamtekar *et al*., 2014). Quercetin stock solution (10000 µg/mL) and the standard series were prepared by diluting the stock solution with methanol. Test solution was prepared by mixing 1.00 mL of RTS beverage/ stock solution with 4 mL distilled water and 0.30 mL 5% $NaNO_3$. After standing for 5 minutes, 0.30 mL of 10% $AlCl_3$, 2.00 mL of 1M NaOH and 2.4 mL of distilled water were added. Absorbance was measured at 510 nm using UV-Vis spectrophotometer (SHIMABZUN UV-1601). TFC of RTS beverage was determined using

a standard curve prepared for quercetin (0.01 - 0.1 mg/mL) and was expressed as mg quercetin equivalents (QE) per 100 g of beverage.

Total tannin content: A colorimetric method was used to determine the total tannin content in RTS beverage (Bulugahapitiya *et al.,* 2020).Tannic acid stock solution (15000 µg/mL) and the standard series were prepared (1000 µg/mL to 15000 µg/mL). Test solution was prepared by mixing 1.00 mL of the standard/sample with 1.00 mL of 7.5% Na_2CO_3, 0.5 mL FC reagent and 7.50 mL of distilled water and it was allowed to stand at room temperature for 15 minutes. Absorbance was measured at 725 nm using the UV spectrophotometer (SHIMABZUN UV-1601).

Determination of anti-oxidant capacity

DPPH free radical scavenging assay: DPPH assay was employed to investigate the radical scavenging potential of the cashew apple RTS beverage. A working series of 5, 10, 20, 25, 50, 75, 100, 250, 500, 750 and 1000 ppm was prepared using the 1000 ppm stock solution of RTS beverage.

A standard ascorbic acid stock (1000 ppm) and working series were prepared. Test solutions were prepared by mixing 1.00 mL of the sample/standard with 1.00 mL of DPPH solution. After standing for 30 minutes at room temperature, absorbance was measured at 517 nm using the UV-Vis spectrophotometer (SHIMABZUN UV-1601). Radical scavenging activity was measured as the percentage inhibition of DPPH as described below where A_0 and A_S are the absorbance of the control and the sample respectively. The IC_{50} values were obtained by plotting the percentage inhibition vs. concentration (Bulugahapitiya, *et al*., 2020)

$$\% \ Inhibition = \frac{A_o - A_s}{A_o} \times 100$$

Ferric reducing anti-oxidant power (FRAP) assay: A working series of 5, 10, 20, 25, 50, 75, 100, 250, 500, 750 and 1000 ppm of the RTS beverage, a standard series of 100, 200, 400, 600, 800, 1000, 1200 ppm of $FeSO_4.7H_2O$ and working FRAP reagent (50.00 mL of acetate buffer with 5.00 mL of TPTZ and 5.00 mL $FeCl_3$) were prepared. Test solution was prepared by mixing 0.20 mL of sample/standard with 3.00 mL of working FRAP reagent. All the test solutions were incubated at 37^0C for 30 minutes under dark conditions. Absorbance was measured at 593 nm using the UV-Vis spectrophotometer (SHIMABZUN UV-1601) (Bulugahapitiya *et al.*, 2020).

Sensory evaluation: Sensory analysis was carried out for RTS beverage by a trained panel (n=10), using a 9-point hedonic scale for color, taste, flavor, aroma, appearance and overall acceptability. The beverage was randomly served in white paper cups just after the preparation, after 7, 14 and 21 days and the scores given by the panel were recorded to calculate the mean for each sensory attribute. Sensory profiles were constructed according to the calculated scores (Ranganna, 1999).

RESULTS AND DISCUSSION

A ready-to serve beverage from cashew apple was successfully prepared by following Sri Lankan standards for beverage (SLS 729: 1985), according to that a beverage should follow the following conditions; 15% juice, 0.3% acidity and 15% TSS (Saranyah and Mahendran, 1985). All the parameters were in the range of the above with very minor deviation for TSS (Table-1).

Value of pH: The pH of the RTS beverage was recorded as 3.44 ± 0.01 which is resulted from the presence of acids in the formulation. Cashew apple is rich in inherent organic acids such as malic, tartaric acids, and in addition citric acid was added externally in the development process of the beverage, which supposes to act as the acidulant and the antimicrobial agent, flavor enhancer and improving the shelf life (Joye, 2019). Inhibition of microbial growth and retention of nutrient quality are important to prolong the shelf life of cashew apple RTS beverage.

Total Soluble Solids: The TSS of RTS beverage formulations was adjusted at the time of preparation by changing added sugars, citric acid and water and the values obtained for this formulation was 20 ± 0.5^0Brix. Total soluble solids include simple sugars such as monosaccharides (glucose, fructose), oligosaccharides like sucrose, organic acids, such as citric, malic, tartaric acids etc. and soluble amino acids. Other miscellaneous compounds, such as soluble fat, minerals, alcohol, flavonoids (Vitamin

C and Vitamin A) can also be included. As noted above TSS content of RTS beverage has slight deviation from the recommended guidelines nevertheless it is in the favorable condition.

Density: Density of RTS beverage was recorded as 1.02 g mL^{-1} at the temperature (25^0C).Weight measurements of the pycnometer was carried out at 25^0C for the determination of density.

Viscosity: The viscosity is an important parameter for RTS beverage. Viscosity of RTS beverage was calculated as 4.48 mPa as given in the Table 1. Beverages are generally considered as non-Newtonian fluids. Time taken for the RTS beverage to move within the capillary arm of the Ostwald viscometer was incorporated in calculating the viscosity at 25^0C. The prepared RTS beverage was a semi-transparent, light cream color.

Titratable acidity: As given in the table 1, titratable acidity of RTS beverage is 0.38 ± 0.01 % which is in the acceptable range of SLS 729:1985. Acidity of a food product is generally expressed as percentage anhydrous citric acid and considered as an important attribute of fruit beverages. Citric acid is the most preferred acidulant due to its ability of balancing acid to sugar ratio while acting as an anti-microbial agent. Inherent organic acids like citric acid, malic acid and the added citric acid contributes to the total titratable acidity of the cashew apple RTS beverage prepared in this study.

Ascorbic acid content: RTS beverage showed 25.13 ± 0.73 mg of ascorbic acid per 100 mL of beverage. Ascorbic acid is considered as a powerful antioxidant and a major nutrient in maintaining redox homeostasis and also there is a known correlation between ascorbic acid and the reduction of risk of non-communicable diseases (Chambial *et al.*, 2013).Therefore the presence of ascorbic acid is an added advantage to a food product. The quantity of ascorbic acid in fresh juice was reported as 181.4 mg/100 mL in the initial work of this study (Abeysuriya *et al.*, 2020). The value is less in RTS beverage, this reduction must be due to the partial degradation of ascorbic acid while heating at the preparation stage.

Total sugar content: RTS beverage contains the quantity of 10.91 ± 0.20 g of sugar per 100 mL and it was calculated using the calibration curve of standard glucose. While formulating the beverage, 8.5 g of table sugar was added per 100 mL to improve the taste, texture and the quality of the final product. Cashew apple itself contains 2.32 ± 0.31 g sugar per 100 mL (unpublished data of part of this study). Many of the sugars in regular diet come from "added sugars" (sugars that are added to food prior to consumption or during preparation or processing). Added sugars contribute to increase the shelf-life of food but the quantity should be in the permitted level. The amount of sugar preset in the prepared RTS beverage is in the permitted level and is considered as a medium sugar beverage according to Sri Lanka standards (FOOD ACT, No. 26 OF 1980).

TFC and TPC in beverage: Total Flavonoids and total phenols of cashew apple RTS beverage were found to be 10.03 ± 0.57 mg QE/g and 53.44 ± 1.84 mg GAE/g respectively. Flavonoids, and total phenols are very important phytochemicals found in cashew apple fresh fruit (Abeysuriaya *et al*., 2020). Those are remaining to some extent in the RTS beverage which is still higher that can contribute to maintain its nutritional and medicinal benefits(Nayak *et al*., 2015). Flavonoids are a class of plant secondary metabolites in the category of polyphenols that possesses a wide spectrum of pharmacological activity including anti-oxidants, anti-cancer and anti-inflammatory activities etc.

Total tannins content (TTC): Presence of a large amount of tannins is a major negative attribute of cashew apple in fresh state since tannins lead to astringency and an unpleasant sensory profile. Juice clarification was carried out using gelatin prior to product preparation in order to reduce the tannin content (Talasila *et al.*, 2013). The TTC of fresh cashew apple juice was found to be 143.1 ± 1.2 mg TAE / g (unpublished data of part of this study). After the gelatin clarification for 24 hours, the TTC has been reduced to 31.37 ± 1.80 mg TAE / g implying the successful clarification of juice by the action of gelatin.

Antioxidant Capacity: Antioxidant capacity of the prepared RTS beverage was investigated in terms of radical scavenging ability and Ferric reducing anti-oxidant power (FRAP) assay (Bulugahapitiya *et al.*, 2020). The radical scavenging ability was expressed as the concentration of the beverage required to inhibit

50% of DPPH radicals and the recorded IC_{50} was 585.29 ppm (Table 1) indicating significant anti-oxidant activity. In the results of FRAP assay was expressed in FRAP value and the RTS beverage recorded as 531.4 ± 8.7µ mol Fe^{2+} /g further supporting the anti-oxidant capacity of prepared beverage. Fruits are significant dietary sources of natural antioxidants which give many positive healthcare effects. Consumption of fruits has a strong correlation to reduce the rate of many NCDs like cardiac problems, aging, and cancers of respiratory tract, alimentary canal etc. Different antioxidants in fruits may act individually or synergistically by various mechanisms to reduce or eliminate oxidative stress which is known to be associated many NCDs (Almeida *et al*., 2011).

Sensory Evaluation: Maintenance of a favorable sensory quality throughout the shelf life is a major concern when preparing a fruit beverage. The organoleptic properties were assessed according to the 9-point Hedonic scale in which 1 represents the lowest score and 9 represents the highest score. The sensory profile of cashew apple RTS beverage just after preparation, after 7 days, 14 days and 21 days are given in figure 1. The sensory profile showed acceptable trends in color, appearance, taste, flavor, aroma and overall acceptability enhancing the preferable organoleptic quality of the cashew apple RTS beverage.

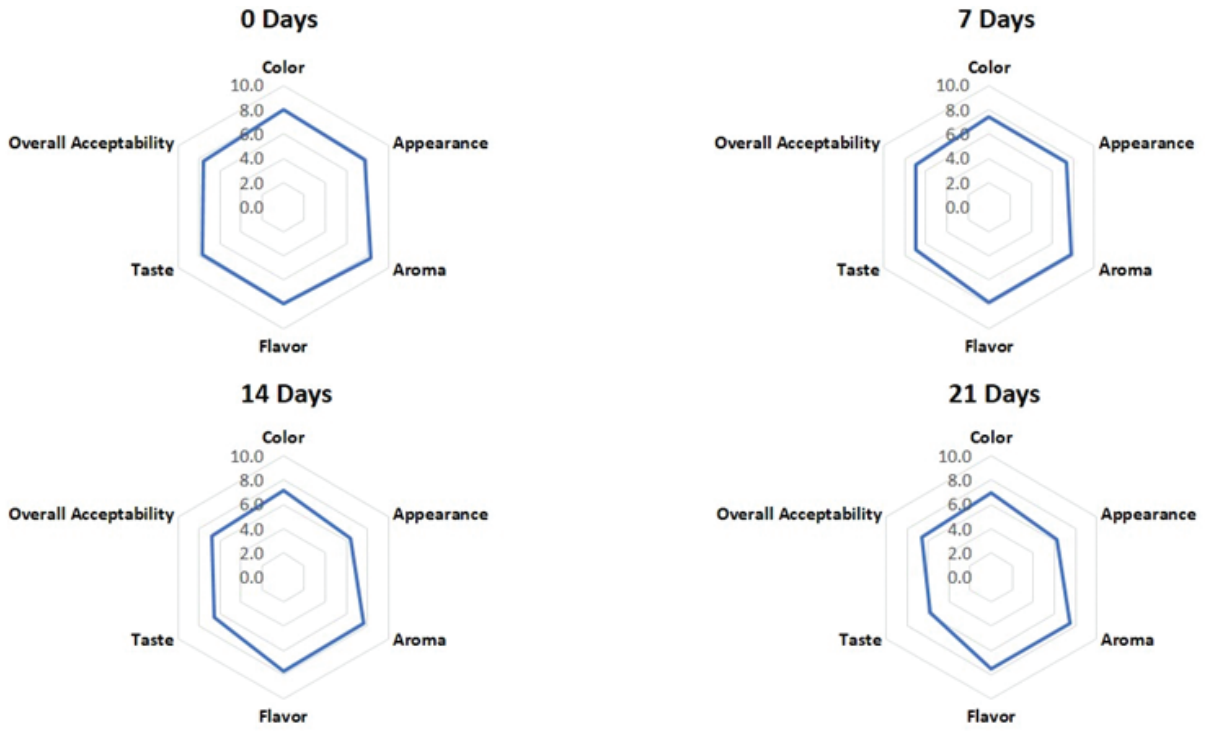

Fig. 1: Sensory profile of the cashew apple RTS beverage

CONCLUSION

Ready-To-Serve beverage using cashew apple was successfully prepared in this study and the values of all important physicochemical parameters confirm the acceptable level of quality and the nutritional value of the beverage. The sensory evaluation proves that the RTS beverage is in consumer satisfactory condition. Therefore, as an outcome of this study, cashew farmers are encouraged to convert cashew apple into functional beverage in order to generate additional income in Cashew nut industry and to provide healthier drink to the society.

Table 1: Results of the quality evaluation of beverage

Parameter	Value
pH	3.44 ± 0.01
TSS	(20 ± 0.5) ^{0}Brix
Density	1.02g mL^{-1}
Viscosity	4.48 mPa s
Titratable acidity	(0.38 ± 0.01) %
Ascorbic acid	(25.13 ± 0.73) mg/100 mL
Total sugar	(10.91 ± 0.20) g/100 mL
Total phenolic content	(53.44 ± 1.84) mg GAE/g
Total flavonoid content	(10.03 ± 0.57) mg QE/g
Total tannin content	(31.37 ± 1.80) mg TAE/g
IC_{50} in DPPH assay	585.29 ppm
FRAP value	(531.4 ± 8.7) μ mol Fe^{2+} / g

(Value ± indicates the standard deviation calculated using the results of replicate experiments)

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