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Effect Of Fermentable Sugar Concentration In Pineapple Juice On Wine Quality

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Abstract

Pineapple juice at different concentrations (10, 20 and 25^o brix) was fermented with oenological yeast to produce pineapple wine and physico-chemical, microbiological and organoleptic parameters were analyzed after 10 and 20 days of fermentation, with the aim of selecting the concentration best suited to the production of quality pineapple wine. The physico-chemical analysis (pH, alcohol content, titratable acidity, fixed acidity and volatile acidity) revealed that on the tenth and twentieth post-fermentation days, the pH remained almost the same, fluctuating around 3.77 in the wine made with concentrated juice at 15^o brix. Wine made with 20^o Brix juice concentrate saw its pH drop from 3.76±0.015 on the tenth day after fermentation to 3.75±0.021 on the 20th day. Wine made with 25^o Brix juice had respective pH values of 3.80± 0.020, 3.78 ±2.300. Wine made with 20^o Brix juice had a lower pH than the others, but the difference was not significant at the 5% level.

The alcohol content of the wine increased with post-fermentation time for all the juice concentrations used, being the highest for the wine obtained with concentrated juice at 20^o Brix (14.5 ±0.021) and lowest for the wine prepared from juice at 15 ^{o} Brix (10.5 ±0.367). Statistical analysis shows a significant difference ($p<0.05$) at the 5% threshold.

Pineapple wine made with concentrated juice at 15 ^{o} Brix had titratable acidities of 4.5± 0.379) tartaric acid/l and 4.6± 0.30g tartaric acid/l pineapple wine respectively after 10 days and 20 days of fermentation.The wine made with 20 ^{o} Brix pineapple juice had titratable acidities of 4.9± 0.046g tartaric acid/l and 5.2 ±0.061g tartaric acid/l pineapple wine at the tenth and twentieth day after fermentation. On the tenth and twentieth post-fermentation days, pineapple wine made with concentrated juice at 25 ^{o} Brix had titratable activities of 5.2 ±0.061g tartaric acid/l pineapple wine and 5.4± 0.30g tartaric acid/l, respectively.

The wine made with the concentrated juice at 15 ^{o} Brix had a volatile acidity of 3.3 ±0.100 and 2.9 ±0.200g sulfuric acid /l respectively on the tenth and twentieth post-fermentation day. Wine made with juice at 20° Brix contained 3.3 ±0.010 and 3.0 ±0.200g sulfuric acid/l respectively, while wine made with concentrated juice at 25° Brix contained 1.8 ±0.10 and 1.4 ±0.20g sulfuric acid/l respectively.

After 20 days from the end of fermentation, the total aerobic mesophilic flora was 2.736 ±0.082, 2.248 ±0.141 and 2.446 ±0.182 for wines made with juices at 15, 20 and ^{25o} Brix respectively.

Yeasts found were 2.526 ±0.090, 2.202 ±0.073, 2.332± 0.101 respectively, while molds amounted to 1.840 ±0.081, 1.509 ±0.081 and 1.817 ±0.138 at day 20 after fermentation.

Salmonella, Shigella, Staphylococci and Clostridia, known to be pathogenic bacteria, were not detected at either the tenth or twentieth day of fermentation in any of the wines. Statistical analysis of sensory quality parameters revealed no significant differences between wines. The wine is light yellow in color, with a pleasant, less sweet taste, a good pineapple aroma and a pineapple aftertaste.

***Key words**: pineapple wine, sugar concentrations, oenological yeast, physico-chemical and microbiological parameters, organoleptic qualities.*

1. Introduction

Pineapple is a drought-resistant crop adapted to low and medium altitudes in tropical regions (South America, Brazil, northern Argentina and Paraguay) where it is grown for export. (Pyc., Lacoeuilhet jj., (1984), Sarah et al. 1997)).

In Burundi, pineapple is grown in certain natural regions, with the exception of the high plateaus of Mugamba, Bututsi and Buyenzi, where the altitude exceeds 2300m. There are two varieties of pineapple in Burundi: Queen and Cayenne: all are used for local consumption, for juice production and for the production of alcoholic beverages (Niyonkuru, 2014).

The production of pineapple wine is difficult and less well known in certain regions of the country, and it is one of the products that is inaccessible to Burundi's low-income population due to its high selling price and the lesser-known production techniques used (Nzigamasabo and Nimpagaritse, 2009).

Pineapple wine is a beverage obtained by fermenting pineapple juice using the oenological yeast “saccharomyces cerevisiae Bayanus”. This yeast was chosen because of its higher fermentative capacity than baker's yeast, which has an impact on the organoleptic quality of wine.

It tolerates high sugar concentrations and has regular kinetics, resulting in high fermentation yields and complete exhaustion of fermentable sugars. It produces low levels of acetic acid, SO_2, volatile sulfur compounds and urea excretion (Hencke, S., (2000).

It has a good flocculation capacity, which facilitates wine clarification. It has the power to convert all sugars into alcohol, organic acid and other compounds capable of preserving wine quality. Saccharomyces cerevisiae Bayanus is used to improve wine's unique taste, odor or aroma, and to reduce the astringency of certain fruit wines. (Renouf, V, 2006) in contrast to the baker's yeast formerly used to ferment pineapple wine, which is responsible for the wine's poor organoleptic quality (Ndayarinze, 2022).

The aim of this study was to evaluate the performance of the oenological yeast genus Saccharomyces cerevisiae Bayanus on the fermentation of pineapple wine from pineapple juice at different Brix concentrations (15, 20 and 25), to assess the physico-chemical,

microbiological and organoleptic quality parameters of the wine at different storage times, i.e. 10 and 20 days after fermentation and to choose the best concentration suitabe for a best pineapple wine.

2. Materials and methods

2.1. Pineapple collection and processing

A variety of cayenne pineapples produced in Cibitoke province were purchased on the local market from Sion, transported to the technology hall and laboratory of the Centre National de Technologie Alimentaire (CNTA) for processing and analysis. Artisanal processing techniques as described by Nimpagaritse and Nzigamasabo (2009) were used. In brief, ripe pineapples were selected and subjected to pre-treatments to ensure juice uniformity. The pineapples were stemmed, topped, washed, peeled, cut into pieces and ground to a purée. The pulp was then pressed with an artisanal press until all the pulp was depleted of juice. The juice was filtered through nylon sieves and pasteurized to reduce the microbial load that could interfere with the fermentation process (Wimalsiri et al. 1971).

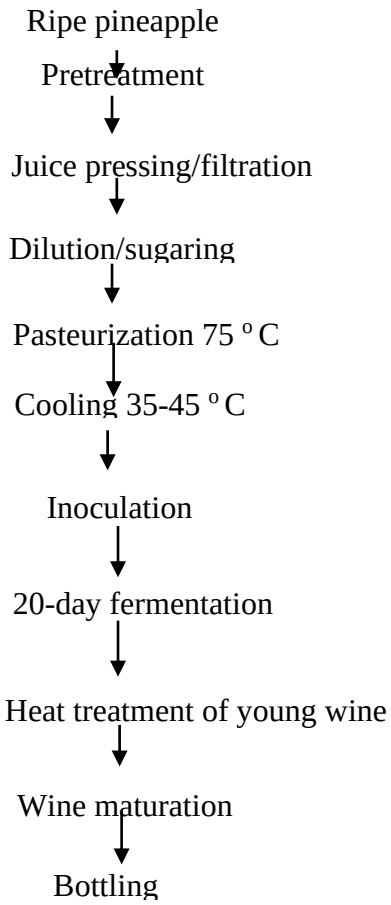

Fig1. Pineapple wine production diagram

2.2. Determination of physico-chemical characteristics

After fermentation, pH, titratable acidity, fixed acidity and alcoholic strength were determined using the official AOC analysis methods (2012).

2.3. Microbiological analysis

2.3.1. Material sterilization

All materials used for microbial content analysis were washed with potable water, dried and sterilized in autoclaves at 121ºC for 15 minutes for petri dishes, and pipettes were sterilized with 70% ethanol. All work tables were cleaned and disinfected with 80% ethanol to avoid cross-contamination during pretreatment operations.

2.3.2. Culture medium preparation

1. PCA was used to determine the total aerobic mesophilic flora.

22.3g were suspended in 100 ml of water, heated to complete dissolution and autoclaved at 121^{O}C for 15 minutes, cooled to 45-50 ^{O}C and carefully poured into petri dishes (Bunani et al, 2020, Kavishe and Matenu,2015)

2. Mac conkey agar was used for the isolation and differentiation of total and fecal coliforms, staphylococci, salmonella and shigella.

50g were suspended in 1000ml of water, heated to complete dissolution and autoclaved at 121 ^{O}C for 15 minutes, cooled to 45-50 ^{O}C and carefully poured into petri dishes (Bunani et al, 2020, Kavishe and Matenu,2015).

3. CSA (Clostridium selective Agar used for clostridia isolation,

44g were suspended in 100 ml water, heated to complete dissolution and autoclaved at 121 ^{O}C for 15 minutes, cooled to 45-50 ^{O}C and carefully poured into petri dishes (Bunani et al, 2020, Kavishe and Matenu,2015).

4. PDA was used for the isolation and differentiation of molds and yeasts.

39g were suspended in 1000 ml water, heated to complete dissolution and autoclaved at 121 ^{O}C for 15 min, cooled to 45-50 ^{O}C and carefully poured into petri dishes. Chloramphenicol was used to suppress bacterial growth (Bunani et al, 2020, Kavishe and Matenu,2015).

2.3.3. Serial dilution

Dilutions were very important to obtain a countable number of colonies. Seven test tubes were filled with 1ml of sterilized, homemade pineapple wine as shown in fig.1. Using a micropipette, 1ml of the original sample was taken and placed in 9ml of peptone water (dilution 10^{-1}).

After mixing, 1ml was removed from the tube and added to the next tube (dilution 10^{-2}).The same process was repeated until tube number seven was reached.

2.3.4. Inoculation

Inoculation was performed by placing 1000 microliters of diluted suspension samples in petri dishes using a micropipette. The samples were then spread onto the agar medium using a spreader.

2.3.5. Incubation

Petri dishes were placed in an incubator for 24 hours at 37 ^{O}C to allow aerobic mesophilic flora to develop on PCA, clostridia on SCA, faecal coliforms, E. coli, staphylococci, salmonella and shigella on MAC. Petri dishes of PDA were kept for 72 hours at 25-30 ^{O}C to allow yeasts and molds to grow (Bunani et al, 2020, Kavishe and Matenu,2015).

2.3.6. Description of colonies

In order to differentiate microorganisms, it is necessary to describe the colonies. For our study, colonies were differentiated by the following characteristics

Size: diameter in millimeters, shape: punctiform, circular, filamentous, irregular, rhizoidal Elevation: flat, convex in relief, pulvinate, umbonate, umbiliate, margin: whole, wavy, lobed, color: white, yellow, buff black, orange, pink, density: opaque, translucent, transparent, consistency: viscous, membranous, brittle, butyrins.

2.3.7. Enumeration

The microbial load was counted on the surface, after the appropriate incubation period for each microorganism. This was done using an electric colony counter with a magnifying glass and separating colonies on Petri dishes with a marker pen.

2.5. Sensory analysis

Sensory evaluation was carried out to know the acceptability of the wine by carrying out In-house consumer acceptability test using in-house panelists, according to the method described by Nwobodo (2013). Sensory evaluation was carried out by 10 untrained panelists who were selected based on their availability, objectivity and being conversant with wine tasting. The sensory attributes evaluated were color, taste/ mouth feel, smell and clarity on a 5-point hedonic scale (where 1 represents dislike very much and 5 represents like very much). The wine samples were served in clean plastic cups to individual panelist in a booth in a well-lit Environment Where There Was No Interference For Bias Expression.

2.4. Statistical analysis

The experiments were conducted in triplicate and the results were expressed as mean with standard deviation. Statistical analysis of the data was performed using SPSS Package Program. Statistical significance was taken at 95% confidence interval when $p<0.05$. When

Analysis of Variance (ANOVA) revealed a significant effect ($p<0.05$), the data means were compared by the least significant difference (Duncan's Multiple Range test) test.

3. **Results and discussion**

3.1. Physico-chemical parameters

The physico-chemical analysis of pineapple wine processed at different sugar concentrations (Brix degree) covered pH, alcoholic strength, volatile acidity, fixed acidity and total titratable acidity. The results are shown in Table 1.

The pH of the wine was determined because it has a major influence on the wine's properties, as well as on its biological and chemical stability. Acidity gives the wine greater microbiological and physico-chemical stability by limiting the development of microorganisms and increasing the antiseptic fraction of sulfur dioxide, and is also a pillar of the taste balance of pineapple wine.

The results show a decrease in pH on the tenth and twentieth day after fermentation for wine concentrated at 20o Brix and at 25o Brix, with pH falling from 3.76± 0.02 to 3.73± 0.021 and 3.80± 0.020 to 3.78 ± 0.300 respectively. It is 3.77± 0.010, 3.77± 0.021 for wine concentrated at 15oBrix respectively 10 and 20 days after fermentation. Wine concentrated at 20oBrix had a pH of 3.76± 0.015, 3.75 ±0.021 respectively 10 and 20 days after fermentation. While that concentrated at 25^{o} Brix had a pH of 3.80± 0.020, 3.78 ±2.300 respectively after 10 and 20 days of fermentation. We also found that pH gradually decreased over the 20-day fermentation period. The same trend was observed by Montney and Gould (1988).

The results also show that the pH of pineapple wine concentrated at 20^{o} Brix is appreciated and has a lower pH than that of other wines of different wine concentrations after 20days post fermentation. the results of the statistical analysis did not reveal any significant difference at the 5% threshold. The pH of pineapple wine produced at different sugar concentrations (15, 20 and 25 ^{o}Brix) remains within the recommended wine norms, i.e. a pH varying between 3 and 5.

The results of the pH values in the experiment show a gradual decrease in pH value as a function of the 20-day fermenting time. These results show that the wine should be slightly acidic as the maturation time progresses. The drop in pH after fermentation records the utilization of the sugar present in the must by the yeast. The results of this study suggest that the acidic pH may combat yeast spoilage of the wine (Akubor et al., 2003), and that these yeasts were not completely inactive during the post-fermentation process.

Table 1. Physico-chemical parameters of pineapple wine treated at different Brix concentrations.

	10 days after fermentation			20 days after fermentation		
Parameters	15 ^{0}Brix	20 ^{0}Brix	25 ^{0}Brix	15 ^{0}Brix	20 ^{0}Brix	25 ^{0}Brix
pH	3.77^a±0.01	3.76^a±0.015	3.80^a ±0.02	3.77^a±0.021	3.73^a±0.021	3.78^a±2.30
Alcohol content (%)	10.71^a±0.367	14.51^c±0.042	14.05^b±0.021	10.71^a±0.367	14.51^c±0.042	14.05^b±0.021
Titratable acidity (g /l)	4.5^a±0.137	4.9^b±0.041	5.2^c±0,061	4.6^b±0.3	5.2^c±0.061	5.4^c±0.30
Fixed acidity (g /l)	3.3^c±0.10	3.3^c±0.10	1.8^a±0.10	2.9^b±0.20	3.0^b±0.2	1.4^a±0.20
Volatile acidity (g /l	1.4^a ± 0,096	2.1^b±0.20	3.6^c±0.20	1.8^d±0.20	2.5^b±0.10	3.8^c±0.20

Values followed by different letters in the ranges are significantly different ($p<0.05$). Mean ±SD (n=3).

The alcohol content of a wine is the result of the total or partial transformation of the sugar contained in the must under the action of yeast. Alcohol content influences pH, wine quality, shelf life and market value. Its content depends on the initial sugar concentration of the must before fermentation, and on fermentation conditions, which can lead to slight variations in yield during conversion (Otgbayo, Akwatanimola, 2020, Randrianantoandro and Aandriamamisa,2018).

Alcohol content increased with fermentation time for all concentrations, being the highest for wine produced with a 20^o brix juice concentration (14.5 ±0.021) and the lowest for wine concentrated at 15 ^{o} Brix (10.5 ±0.367). A significant difference ($p<0.05$) was observed between these wines made at different must sugar concentrations, and this was observed in the 15 ^{o} Brix concentrated wine, which had the lowest alcohol content compared with the other wines. Despite the small difference in significance observed between the samples, we note that the concentrated wine at 20 ^{o}brix is the best in comparison with the other wines.

Wine titratable acidity is one of the essential constituents for quantifying organoleptic properties as well as storability. It is therefore important to monitor it periodically throughout the transformation or vinification process, particularly during the alcoholic and malolactic fermentation period but also up to bottling (Randrianantoandro and Aandriamamisa,2018). Total acidity is linked to all the acids present in the wine and reflects the wine's taste characteristics.

Pineapple wine concentrated to 15 ^{o} Brix had respectively a titratable acidity of 4.5± 0, 379 tartaric acid/l pineapple wine after 10 days of fermentation and 4.6± 0.30g tartaric acid /l pineapple wine after 20 days of fermentation. The wine prepared from concentrated juice at 20 ^{o} Brix has a titratable acidity of 4.9± 0.046g tartaric acid/l pineapple wine and 5.2 ±0.061g tartaric acid/l pineapple wine after 10 and 20 days respectively. Whereas wine prepared with concentrated juice at 25 ^{o} Brix contains 5.2 ±0.061g tartaric acid/l pineapple wine and 5.4± 0.30g tartaric acid/l pineapple wine after 10 and 20 days respectively. There is a progressive increase in titratable acidity as fermentation time progresses.

Alcohol production is due to the activity of yeasts and other bacteria. These results show that alcohol production does not totally inactivate the micro-organisms, as the oenological yeasts continue to degrade the residual substrates, with some production of alcohol and acids.Despite the simple difference in significance observed between samples, we note that wine prepared with concentrated juice at 20 ^{o} Brix is the best compared to other wines.

Volatile acidity is an important wine quality parameter (Delanoë D et al., 2007). It gives wine its characteristic aroma and odor. Volatile acids are formed naturally in very small quantities during alcoholic and malolactic fermentation. They can also be formed accidentally as a result of bacterial growth. In our study, we found that the quantity of acids produced became high, causing the wine produced to become slightly cloudy.

Determining a wine's volatile acidity also provides information on its sanitary condition. Wine made from concentrated juice at 15 ^{o} Brix has a volatile acidity of 3.3 ±0.100 and 2.9 ±0.200g sulfuric acid/l after 10 and 20 days respectively. Wine made from juice concentrated at 20 ^{o} Brix contains 3.3 ±0.010 and 3.0 ±0.200g sulfuric acid/l, while wine made from juice concentrated at 25 ^{o} Brix contains 1.8 ±0.10 and 1.4 ±0.20g sulfuric acid/l respectively.

As wines with low volatile acidity are recommended, it is the wine made with concentrated juice at 20 ^{o} Brix that is of better quality than the others, as the low volatile acidity does not deteriorate the wine on contact with air.

3.2. Microbiological analysis results

The purpose of microbiological analysis of wines is to monitor alcoholic and/or malolactic fermentations, and to detect the risk of microbial alterations that could adversely affect wine quality. This then makes it possible to detect any anomalies, not only in the finished product but also during the various phases of its manufacture. (OIV, 2015).

The results of microbiological analyses of pineapple wine obtained from juice at different concentrations are illustrated in Table 2 and concern total aerobic mesophilic flora, fecal coliforms including E. coli, salmonella and shigella, clostridia, staphylococci, yeasts and molds.

Table 2. Microbiological parameters of pineapple wine

MICROORGANISM	Storage period					
	10 days after fermentation			20 days after fermentation		
	15 ^{0}Brix	20 ^{0}Brix	25 ^{0}Brix	15 ^{0}Brix	20 ^{0}Brix	25 ^{0}Brix
Total Aerobic Mesophilic Flora	**3.452^{b}**±0.098	**3.370^{b}**±0.091	**2.692^{c}**±0.061	**2.736^{c}**±0.082	**2.248^{d}** ±0.141	**2.446^{c}**±0.182
Yeasts	**3.083^{b}**±0.052	**3.181^{b}**±0.054	**2.450^{c}**±0.088	**2.526^{c}**±0.090	**2.302^{c}**±0.073	**2.332^{d}**±0.101

Moulds	**2.627**^{b}±0.625	**1.932**^{a}±0.576	**2.571**^{b}±0.648	**1.840**^{a}±0.149	**1.509**^{a}±0.081	**1.817**^{c}±0.138
Faecal coliforms: E.COLI	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}
Salmonella and shigella	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}
Staphylococcus	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}
Clostridia	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}	**00**^{b}

Values followed by different letters in the rows are significantly different ($p<0.05$). Mean ± SD (n=3).

Before fermentation, the must must be pasteurized to reduce the microbial load without altering the pineapple's fruity aroma. This is understandable, as these microorganisms come from the manufacturer, the equipment, the environment and the raw materials used.

As fermentation and storage time progressed, we observed a gradual decrease in these microorganisms, and this was true for all the wines prepared, as the alcohol content and low pH prevented them from multiplying.

After 20 days of fermentation, the total aerobic mesophilic flora was 2.736 ±0.082, 2.248 ±0.141 and 2.446 ±0.182 for wines prepared with concentrated juice at 15, 20 and 25 degrees Brix. These values are lower than those found by Sanni et al, in 1999 when analyzing wines purchased from local market.

Yeasts found were 2.526 ±0.090, 2.202 ±0.073, 2.332± 0.101 respectively, while molds amounted to 1.840 ±0.081 1.509 ±0.081 and 1.817 ±0.138. However, these values are lower than those found by sanni et al, in 1999 who found mean values between 2.6 and 5.6. According to Ogbule et al (2007), yeasts are the predominant flora during the first hours of fermentation.

After 20 days of fermentation, the wine concentrated at 20 degrees Brix contained fewer microorganisms than the other wines (Table 2). As mentioned by Bunani et al (2020), Bourgeois, Mescle and Zuccay (1996), the high alcohol content and low pH would be responsible for the reduction in these microorganisms.

Salmonella, shigella, staphylococci and clostridia, known to be pathogenic bacteria, were absent after the entire fermentation period, as they are destroyed during must pasteurization. They were not detected on either day 10 or 20 of fermentation. These results differ from those obtained by Ogbulie et al (2007), who also isolated indicators of fecal contamination

(E. coli) in palm wine. Their presence reveals poor hygienic conditions during must extraction or packaging of these wines.

Microbiological analysis allows us to conclude that the final product is perfectly safe for human consumption, as the values obtained for various parameters are below the acceptable standards in force in Burundi.

3.4. Sensory analysis results

Sensory analysis shows that panelists greatly appreciated the color, taste and smell of the pineapple wine, and no significant differences ($p<0.05$) were detected between wines obtained at different concentrations (15 ,20 and 25^o Brix), although it should be noted that panelists preferred the wine made with juice concentrated to 20 degrees Brix.

The color is light yellow, the pleasant taste is less sweet with a pineapple aftertaste, and the characteristic pineapple odor is less pungent.

Table 3. Analyzed sensory parameters

Parameters	15 ^{0}Brix	20 ^{0}Brix	25 ^{0}Brix
Color and clarity	Light yellow 4,4^a±0,1	Light yellow 4,5^a±0,2	Light yellow 4,4^a±0,2
Taste (flavor)	Pleasant, slightly sweet taste 4,5^a±0,2	Pleasant, slightly sweet taste. 4,7^a±0,1	Pleasant, slightly sweet taste 4,5^a±0,2
Smell and aroma	Slight pleasant aroma , less pungent aroma with a pineapple aftertaste. 4,5^a±0,2	Slight pleasant aroma , less pungent aroma with a pineapple aftertaste 4,6^a±0,1	Slight pleasant aroma , less pungent aroma with a pineapple aftertaste 4,4^a±0,2

Values followed by different letters in the rows are significantly different ($P<0.05$). Mean ± SC (n=3).5=strongly accepted, 4=accepted, 3=moderately accepted, 2=disliked and 1=strongly disliked.Values= mean ± SD (n=3).

Conclusion

The production of pineapple wine with different concentrations of pineapple juice (10, 20 and 25^o Brix), followed by qualitative analysis after the tenth and twentieth day after the end of fermentation, reveals that the wine prepared with the juice concentrated at 20^{o}Brix is the best from a physico-chemical, microbiological and sensory point of view. It can then be recommended to manufacturers.

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