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Research Article

Comparative Impact of Drying Techniques on Physicochemical, Proximate Compositions, and Antioxidant Properties of Roselle Calyces (*Hibiscus sabdariffa* L.) for Sustainable Agriculture

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ABSTRACT

This study evaluated the impact of different drying methods, freeze drying (-120℃), food dehydrator (65℃), and oven drying (65℃) on the quality of roselle calyces to extend their shelf life and availability, considering implications for sustainable agriculture. Key quality parameters assessed included physicochemical properties, proximate composition, and antioxidant activity. Freeze drying produced the highest quality results, closely resembling fresh roselle in several aspects. The colour analysis showed freeze-dried samples had the highest chroma values with L* (35.97), a* (31.86), and b* (17.3), indicating vibrant colour retention. The pH of freeze-dried roselle was the lowest at 2.74, indicating a desirable balance of acidity. In terms of proximate analysis, freeze-dried roselle had the lowest moisture content (7.55%) among dried samples, which is crucial for extended shelf life. It also had the highest ash content (8.06%), protein content (11.06%), and crude fat content (2.51%), highlighting its superior nutrient retention compared to other drying methods. Oven-dried roselle showed higher ash (8.69%) and fat (1.44%) contents but had a lower moisture content (6.36%) than dehydrator-dried roselle, which had the highest moisture content (8.30%) and the lowest ash (5.75%) and protein (5.86%) contents. Antioxidant analysis revealed that freeze drying preserved the highest levels of antioxidant activity (14.22% RSA), total phenolic content (12.3 mg GAE/mL), total flavonoid content (44.9 mg QE/mL), and ascorbic acid content (22.267 mg/100 g). These findings highlight the effectiveness of freeze-drying in maintaining the bioactive compounds and overall nutritional quality, which is crucial for sustainability agriculture practices aimed at preserving crop value and extending product availability.

Key words: Antioxidant, food-dehydrator drying, freeze-drying, oven-drying, physicochemical, proximate analysis, roselle

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INTRODUCTION

Roselle (*Hibiscus sabdariffa L.*), a member of the Malvaceae family, has been cultivated in West India since the 6th century and spread to Asia by the early 17th century, becoming popular worldwide. Traditionally used for medicinal purposes, it treats conditions like hypertension, wounds, ulcers, colds, and conjunctivitis, with emerging research indicating its potential as an anti-obesity agent (Nguyen & Chuyen, 2020). Rosella tea effectively manages metabolic syndrome by lowering blood pressure and fasting glucose levels, particularly in older diabetic women. Its natural compounds, including ascorbic acid and glycolic acid, contribute to diuretic and mild laxative effects, suitable for long-term use with minimal adverse effects compared to synthetic medications (Yusni & Meutia, 2020). Roselle calyces are versatile, used in beverages, syrups, jams, and jellies, while extracts are employed in beverages, supplements, yogurt, and confectionery.

Handling fresh roselle is challenging due to its high moisture content, which hastens deterioration, mechanical damage, colour degradation, microbial growth, and shortens shelf life. Extended exposure to room temperature can turn its red colour black due to high humidity. This short shelf life results in higher post-harvest losses, which is unsustainable as it reduces the overall yield that reaches consumers. Consequently, it could not fulfil the aim of sustainable agriculture to provide a consistent supply of agricultural products throughout the year. To ensure optimal quality for consumers, proper processing and storage post-harvest are essential to maintain freshness and preserve nutritional content. Improper drying, like using elevated temperatures, can diminish quality and affect the final product's sensory properties (Mahunu *et al.*, 2021). Drying is a processing technique that can control the moisture content of food and lengthen the number of days before spoilage (Djaeni *et al.*, 2018).

Various drying methods significantly impact the stability of bioactive compounds, antioxidant capacity, and the physical and sensory quality of dried products (Dadi *et al.*, 2018). Oven drying is known for its speed, uniform heat distribution, and energy efficiency, which makes it a practical choice for agricultural processing. This method uses penetrating heat to dry foods quickly while preserving quality. Similarly, food dehydrators, which are equipped with electric heating elements and efficient air circulation systems, provide consistent and high-quality drying conditions. They allow for temperature adjustments from 29℃ to 82℃, making them suitable for drying roselle and other foods (Lema *et al.*, 2022). Freeze-drying, on the other hand, involves the sublimation of ice from frozen products, preserving delicate items such as penicillin, hormones, and biological materials like fruits and vegetables. Although freeze-drying is energy-intensive, it operates in a low-oxygen, low-temperature environment that effectively retains bioactive compounds, making it highly favourable for maintaining food quality. However, this method requires more time and incurs higher costs than other drying techniques (Kumar *et al.*, 2024).

Studies have elucidated the influence of drying methods, particularly temperature, on roselle calyx dehydration. These investigations highlight temperature's role in affecting the retention of monomeric anthocyanins, phenolic compounds, polymeric colour, and antioxidant activity (Kumar *et al.*, 2023). Although higher drying temperature has the advantage of removing moisture rapidly, the heat-sensitive compounds will be decomposed (Dadi *et al.*, 2018).

In this study, various drying techniques such as oven drying, food dehydrator, and freeze drying were applied to fresh roselle under controlled moisture and low temperatures. Physicochemical characteristics, proximate compositions, ascorbic acid content, and antioxidant properties were analysed.

MATERIALS AND METHODS

Materials

Sodium Hydroxide (NaOH), Boric acid, hydrochloric acid (HCl), Sulphuric acid, Petroleum ether, Iodine solution, Gallic acid, 2,2-diphenyl-2-picrylhydrazyl (DPPH), and methanol were obtained from MERCK (Darmstadt, Germany).

Methods

Preparation of roselle sample

Fresh roselles (variety type UMKL) were obtained from a local supplier in Perlis. The fresh roselle was selected at the maturity stage of 6 months after budding. The freshly harvested roselles were washed under running tap water, the calyces plucked and sliced into strips of 1 cm width before the drying process. Fresh roselle was included as a control sample.

Drying of roselle calyx using conventional oven, food dehydrator, and freeze dryer

Preliminary drying tests on the roselle sample were conducted to achieve the required moisture content in the range of 6%-9% (Marak *et al.*, 2021) for a specific time for each drying method. After fresh roselle had been prepared, the roselle was divided into three groups (for conventional oven drying, food dehydrator, freeze dry) with 30 g roselle each. The conventional oven was set at 65℃ for 3.5 hr, the food dehydrator was set at 65℃ for 4.5 hr, whereas the freeze dryer was set at −120℃ for 24 hr.

Determination of colour and pH

The colour of dried roselle was determined using a CR-400 colorimeter (Konica Minolta, Japan) based on the CIE L*a*b* values. Simultaneously, the pH of the roselle solution was measured using a pH meter (Thermo Fisher Scientific, US). Approximately 1 g of dried roselle was added to 50 mL of distilled water and allowed to immerse. The pH probe was rinsed with distilled water between samples to prevent contamination. These analyses were conducted in triplicate to ensure the accuracy and consistency of the measurements.

Determination of proximate compositions

Proximate analysis was conducted following the AOAC standard (AOAC International, 2016). About 6 g of sample was used for triplicate sample readings. Moisture content was determined using a moisture analyser (A&D Weighing, Japan). Ash content was determined by heating the roselle sample overnight at 550℃ using a furnace (Cress, New Jersey). Protein analysis was done using a Kjeldahl machine (Gerhardt, Germany). The sample was digested using 12 mL concentrated sulfuric acid (H_2SO_4) at 420℃ for 2 hr, followed by distillation and titration process using 0.01 M hydrochloric acid (HCl). Fat content was determined using Soxhlet (FOSS, Denmark) using petroleum ether as solvent.

Determination of antioxidant capacity

The free radical scavenging activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A 4% DPPH solution was initially prepared. Then, 1 mL of methanol was mixed with 1 mL of the DPPH solution, and the mixture was incubated at room temperature in darkness for 30 min. Following incubation, the absorbance of the mixture was measured at 517 nm using a UV-vis spectrophotometer (Thermo Scientific, US).

For the roselle sample, approximately 10 mg of dried roselle was dissolved in 10 mL of distilled water to achieve a concentration of 1 mg/mL. The roselle mixture was then filtered using filter paper Whatman No 4 to remove the roselle impurities. Subsequently, 1 mL of roselle solution was mixed with 1 mL of the prepared DPPH solution. Similar to the standard DPPH assay, the mixture was kept at room temperature in the dark for 30 min. After incubation, the absorbance of the mixture was measured at 517 nm using a UV-vis spectrophotometer to determine the antioxidant activity of the roselle solution (Millinia *et al.*, 2024).

Determination of ascorbic acid content

A 125 mL conical flask was filled with 25 mL of oxalic acid. A 1% starch solution was then added in 10 drops. The burette was rinsed with a small amount of iodine solution and filled, and the initial volume was recorded. The titration proceeded until the endpoint was reached, indicated by a persistent blue colour after swirling for 20 sec. The final volume of iodine solution was noted, considering the starting volume for accurate measurement, ensuring results within 0.1 mL accuracy.

For the ascorbic acid content determination in fresh and dried roselle: Two 125 mL conical flasks were prepared, each filled with 25 mL of extract from fresh and dried roselle samples. The burette was rinsed with iodine solution and filled, and the initial volume was recorded. Titration was carried out until the endpoint, indicated by a stable blue colour lasting more than 20 sec. The titration was repeated at least three times for accuracy. The volume of titrant used in each flask was recorded, and the average was calculated to determine the amount of titrant required for standardization. The moles of reacting iodine were calculated, and from this, the quantity of ascorbic acid reacting was determined using the titration equation as follows:

$$\text{Ascorbic acid } (mg) = \frac{V_i \times 176.12}{V_s}$$

Equation 1

Where V_i is the volume of iodine (mL) used in the titration, 176.12 (g/moL) is the molecular weight of ascorbic acid, and V_s is the volume of sample (mL).

The concentration of ascorbic acid in the roselle juice solution was determined in moL L^{-1}, and the amount of ascorbic acid in the roselle sample was calculated in mg/100 mL based on the titration results (Zargarchi *et al.*, 2024).

Determination of total phenolic content

The method described by Anh-Dao *et al.* (2024) was slightly modified to determine the total phenolic content of dried roselle. Ethanolic roselle extract was prepared by mixing 10 g of roselle calyces with 70% ethanol in a 1:10 ratio. The mixture was left for 24 hr at room temperature, followed by a filtration procedure to remove solids. The ethanol was then evaporated to concentrate the extract. Extracts of fresh and dried roselle, each totalling 0.5 mL, were mixed separately with 2.5 mL of Folin-Ciocalteu reagent in test tubes and left at room temperature. After 5 min, 2 mL of 7.5% (w/v) Na_2CO_3 solution was added to each mixture, followed by incubation for 1 hr in a dark environment at room temperature. The absorbance of the reacted mixtures was measured at 750 nm using a spectrophotometer. The total phenolic content of the extracts was quantified as mg gallic acid equivalent (mg GAE) based on a standard curve prepared with gallic acid solutions.

Determination of total flavonoid content

Two 10 mL volumetric flasks were prepared by mixing 1 mL of fresh roselle extract with 4 mL of water, and 1 mL of dried roselle extract with 4 mL of water, respectively. After 5 min of incubation, 0.3 mL of 10% aluminium chloride and 0.3 mL of 5% sodium nitrite were sequentially added. The mixture was allowed to react for an additional 6 min at room temperature. Following this, 1 mL of 1 M sodium hydroxide was added, and the final volume was quickly adjusted to 10 mL with distilled water. Subsequently, the absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer, with a blank serving as the reference. To ensure precision, the entire experiment was repeated three times, and the results were reported as mg quercetin equivalent (mg QE) based on the quercetin standard.

RESULTS AND DISCUSSION

Colour and pH determination

Colour appearance of food will give the first impression of the food's quality that influences consumer acceptance. The L*, a*, and b* values of dried roselle were tabulated in Table 1. There was a significant difference between fresh roselle and dried roselle. The drying process caused a loss of moisture in large amounts; as water molecules disappeared, roselle became more concentrated. The freeze-dried roselle showed the lowest lightness (L*), highest redness (a*) (31.86), and highest yellowness (b*) (17.3) compared to the other two drying methods. The freeze-drying process involves the direct conversion of water from solid (ice) to vapor, bypassing the liquid state. Subsequently, water is desorbed from the 'dry' layer through sublimation. These processes preserve all nutritional components and colour pigments of the sample (Gat & Gawande, 2024). Therefore, water loss during the freeze-drying process not only prevents degradation of colour pigments but also enhances the intensity of redness.

Oven drying and dehydrator-assisted drying, while both relying on hot air, differ in their impact on the quality of dried roselle. Oven drying typically involves a more direct and higher intensity heat application, which can cause more significant damage to heat-sensitive compounds such as ascorbic acid and pigments. This method often results in greater colour loss and browning due to the higher temperatures and prolonged exposure (Nainggolan *et al.*, 2024). In contrast, a food dehydrator usually operates at slightly lower and more controlled temperatures, often with better air circulation, which can result in less severe degradation of sensitive compounds. However, even with these advantages, dehydrator drying still causes some level of pigment loss and discoloration, though it may be less pronounced than with oven drying.

As for the pH values, this study shows that freeze-dried roselle had the lower pH value (2.74) compared to the other samples (2.86 – 2.98). The freeze-dried method preserves the natural organic acids, such as hibiscus acid, citric acid, and malic acid, which contribute to the acidic pH (Ozcelik, 2024). In contrast, other drying methods like sun drying or oven drying may cause some degradation or loss of these acids due to exposure to heat and prolonged drying times. The low pH value of roselle indicates good quality due to its high anthocyanin content, which thrives in acidic environments (Langyan *et al.*, 2022).

Proximate compositions

Fresh roselle calyx contained 88.7% moisture. After drying, the moisture content dramatically dropped to a range between 6% and 9%. A preliminary experiment was conducted to identify the suitable temperature and time for each drying method to ensure the moisture content remained below 10%, as suggested by Kolawole *et al.* (2024). This ensured that all the dried samples would likely last longer before being used or processed because their low moisture content could prevent microbial growth and extend shelf life (Vera Zambrano *et al.*, 2019).

The ash content of all dried roselle samples was higher (5-8%) than that of fresh roselle (0.49%) because the minerals and nutrients become more concentrated due to moisture loss. The low volatility of minerals ensures they remain unaffected by heating at around 100℃ (Lema *et al.*, 2022). Oven heating resulted in higher ash content (7.78%) in lemongrass (*Cymbopogon citratus*) compared to microwave and sun drying, as reported by Mabai *et al.* (2018).

For the protein content, fresh roselle showed higher (16.57%) protein content than dried roselle (5-11%). Besides, freeze-dried roselle exhibited the highest protein content (11.06%) compared to the other dried roselle from oven-dried and food dehydrator drying strategies. High temperature, such as 65℃, is a potential factor that can denature proteins, resulting in roselle having the lowest protein content. Low temperature can ensure better preservation of bioactive compounds in roselle calyx (Roslan *et al.*, 2020). Because of its high protein content, freeze-dried roselle can be used as an additive in the food industry and to produce bioactive peptides (Mahunu *et al.*, 2021). Freeze-drying induces fewer changes in chemical constituents compared to heat drying because thermochemical degradation slows at low temperatures, whereas the Maillard reaction, forming complexes between proteins and carbohydrates, occurs at high temperatures (Amoasah *et al.*, 2018).

Table 1. L*, a*, and b* values of the dried roselle from different drying methods.

Sample	L*	a*	b*	pH
Oven-dried roselle	35.92 ± 0.00^{c}	29.24 ± 0.07^{b}	15.92 ± 0.01^{b}	2.86 ± 0.005^{a}
Dehydrator-dried roselle	35.69 ± 0.03^{d}	23.36 ± 0.01^{c}	8.78 ± 0.005^{c}	2.98 ± 0.005^{a}
Freeze-dried roselle	35.97 ± 0.01^{c}	31.86 ± 0.01^{a}	17.3 ± 0.015^{a}	2.74 ± 0.024^{b}
Fresh roselle	57.26 ± 0.26^{a}	0.96 ± 0.01^{e}	0.95 ± 0.17^{d}	2.96 ± 0.001^{a}

Different superscript letters within the same column indicate significant differences ($P \leq 0.05$).

Results for fat content showed no significant difference between fresh roselle and dried roselle as well as among the different dried roselle. However, a high value of fat content was observed in freeze-dried roselle. The prolonged exposure of the samples to heat (65℃) and light inside the dehydrator and oven dryer may be the cause of the low-fat content of roselle. Factors such as heat, light, and radiation contribute to lipid oxidation, which can reduce the fat content (Wang *et al.*, 2023). In general, drying roselle calyces in a freeze dryer might be preferable because flavour and fat-soluble vitamins as well as crude fat content are preserved.

Antioxidant capacity and Ascorbic acid content

The free radical scavenging activity of roselle was analysed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Results in Table 3 revealed that freeze-dried calyx has the highest percentage of Relative Scavenging Activity (14.22%), followed by oven drying (9.77%) and dehydrator (0.57%). The low operating temperature (-50℃) during the moisture removal process ensures better preservation of bioactive compounds such as anthocyanins, carotenoids, ascorbic acid, and phenolic compounds (Samoticha *et al.*, 2016). The use of an oven and dehydrator for thermal treatment can lead to nutritional and organoleptic decline, alteration of carotenoid, ascorbic acid, and phenol levels, resulting in reduced antioxidant capacity (Banwo *et al.*, 2022).

Ascorbic acid is a well-known antioxidant that can neutralize free radicals, reducing oxidative stress in the body. It donates electrons to reactive oxygen species (ROS), preventing cellular damage. The highest ascorbic acid content was in the freeze-dried sample (22.27 mg/100 g) with no significant difference from the fresh roselle (21.13 mg/100 g) (Table 3). The other thermally dried roselle had lower ascorbic acid content (10.85-15.14 mg/100 g). The possible reason for the highest amount of ascorbic acid in freeze-dried roselle is using a low temperature, where the low temperature can ensure better preservation of bioactive compounds in roselle calyx (Roslan *et al.*, 2020). Thermal processing, highly sensitive to elevated temperatures, can decrease levels of ascorbic acid, thereby reducing antioxidant capacity and other bioactive compounds, and leading to nutritional and organoleptic deterioration (Ibrahim & Mazuki, 2013). To preserve ascorbic acid, it is essential to employ short drying times and low temperatures. Freeze drying allows for food rehydration while maintaining quality similar to that of fresh products (Gat & Gawande, 2024).

Phenolic content and total flavonoid content

This study showed the highest total phenolic content (TPC) was obtained in freeze-dried roselle (12.3 mg GAE/mL), while the lowest phenolic content was observed in dehydrator-dried roselle (4.7 mg GAE/mL) (Table 4). The breakdown of cellular components induced by the freeze-drying process (through ice crystal formation) can hasten the release of phenolic compounds from the food matrix (Arslan & Özcan, 2012). Fresh roselle contains enzymes that are prone to degrading phenolic compounds and are susceptible to oxidation, even without the introduction of heat. The dried sample led to enzyme inactivation due to limited water availability, as evidenced by the low concentration of phenolic compounds observed in the dehydrator-dried sample (Lema *et al.*, 2022). Oven drying uses higher temperatures for extended periods, which can accelerate the degradation of heat-sensitive compounds. Phenolic compounds, particularly, are prone to thermal breakdown when exposed to high temperatures, leading to a loss in bioactivity. Although oven drying retained more phenolic content than the dehydrator drying, the elevated temperatures likely caused a reduction in flavonoid levels, as seen in the significantly lower flavonoid content (14.32 mg QE/mL). This could be due to the thermal sensitivity of flavonoids, which can break down or undergo chemical transformations when subjected to heat (Lema *et al.*, 2022).

Table 4 also demonstrates the level of flavonoid content in dried roselle. A similar trend was observed for flavonoid content compared to phenolic content. The highest flavonoid content was found in freeze-dried roselle (44.9 mg QE/100 g), followed by fresh roselle 22.02, oven-dried roselle 14.32, and lastly food dehydrator-dried roselle (6.4 mg QE/100 g). Water in leaves may evaporate during drying, which could result in a decrease in the activity of an enzyme that breaks down flavonoids (Dadi *et al.*, 2018). The significantly high ($P < 0.05$) flavonoid content in freeze-dried roselle can be attributed to the formation of numerous flavonoid compounds due to moisture loss during drying (Rabeta & Vithyia, 2013). In comparison to oven- and dehydrator-dried roselle, freeze-dried roselle generally displayed higher total flavonoid and phenolic content. Flavonoids effectively neutralize a variety of oxidizing molecules, singlet oxygen, and other free radicals associated with several diseases, making it crucial to carefully select an appropriate drying method to preserve and maximize the total flavonoid content (TFC) in plant extracts and optimize the development of nutraceutical products.

Table 2. Proximate compositions of dried roselle.

Sample	Ash (%)	Moisture (%)	Protein (%)	Fat (%)
Oven-dried roselle	$8.69^{\#} \pm 0.013^{a}$	$6.36^{\#} \pm 0.80^{c}$	5.42 ± 0.23^{c}	$1.44^{\#} \pm 0.01^{a}$
Dehydrator-dried roselle	$5.75^{\#} \pm 0.002^{a}$	$8.30^{\#} \pm 0.50^{c}$	5.02 ± 1.58^{b}	0.45 ± 0.03^{a}
Freeze-dried roselle	$8.06^{\#} \pm 0.018^{a}$	$7.55^{\#} \pm 0.36^{c}$	11.06 ± 0.35^{a}	$2.51^{\#} \pm 0.05^{a}$
Fresh roselle	$0.49^{\#} \pm 0.001^{b}$	$88.7^{\#} \pm 3.24^{a}$	16.57 ± 0.4^{a}	0.52 ± 0.03^{a}

Different superscript letters within the same column indicate significant differences ($P \leq 0.05$)

Table 3. DPPH scavenging activity and ascorbic acid content of dried roselle from different drying methods.

Sample	DPPH (%RSA)	Ascorbic acid content (mg/100 g)
Oven-dried roselle	9.77 ± 0.002^{b}	15.14 ± 3.24^{b}
Dehydrator-dried roselle	0.57 ± 0.014^{c}	10.85 ± 0.49^{c}
Freeze-dried roselle	14.22 ± 0.005^{a}	22.27 ± 0.85^{a}
Fresh roselle	14.24 ± 0.00^{a}	21.13 ± 1.30^{a}

Different superscript letters within the same column indicate significant differences ($P \leq 0.05$).

Table 4. Phenolic content and flavonoid content of dried roselle from different drying methods.

Sample	Total Phenolic Content (mg GAE/mL)	Total Flavonoid Content (mg QE/mL)
Freeze-dried roselle	12.3 ± 0.00[a]	44.9 ± 0.06[a]
Oven-dried roselle	11.7 ± 0.33[b]	14.32 ± 0.08[c]
Dehydrator-dried roselle	4.7 ± 0.00[c]	6.4 ± 0.02[d]
Control-Fresh roselle	11.3 ± 0.34[b]	22.02 ± 0.005[b]

Different superscript letters within the same column indicate significant differences ($P \leq 0.05$).

CONCLUSION

Fresh roselle's high moisture content makes it prone to deterioration, necessitating drying to extend shelf life and increase availability, which is crucial for sustainable agriculture. Freeze-dried roselle was found to be the highest quality of dried roselle with the highest colour values (L: 35.97, a: 31.86, b: 17.3), lowest moisture (7.55%) among dried samples, highest ash (8.06%), protein (11.06%), crude fat (2.51%), and superior antioxidant properties (14.22% RSA). Freeze drying preserved the highest total phenolic (12.3 mg GAE/mL) and flavonoid contents (44.9 mg QE/mL). These findings support using freeze drying for producing high-quality dried roselle calyces, enhancing the sustainability of agricultural practices by reducing post-harvest losses, increasing market reach, and providing a consistent year-round supply. Future research should explore different parameters such as thermal properties, shelf-life tests, and the application of roselle-derived edible films. Adding antimicrobial agents could further enhance the properties of dried roselle. Hence, freeze-drying is recommended for commercializing dried roselle calyces due to its superior preservation of quality, contributing to more sustainable agricultural systems.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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