International Journal of Scientific Multidisciplinary Research (IJSMR)
Vol.1, No.11, 2023: 1457-1470

Antioxidant Activity of DPPH Method on Stone Banana Flour with Blanching Drying and Temperature Treatment

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A R T I C L E I N F O

Keywords: Antioxidant, Ares, Stone Banana, Flour

Received: 17 October
Revised : 20 November
Accepted: 21 December

A B S T R A C T

Stone banana is a type of banana that has seeds in the fruit. Ares is a young banana stem. Banana stems called ares, located on the inside of banana stems. The objective of this research was to identify the antioxidant capabilities of stone banana fields as they are dried and heated with a UV-Vis spectrophotometer. The results showed that stone banana ares flour has very weak antioxidant activity. It's characterized by an IC50 value of over 500 ppm, which can be interpreted as having a low activity or potential as an antioxidant. The solar drying method and blanching temperature of 85 °C obtained the smallest IC50 average value of 42,429.90 ppm with a very weak category

DOI: https://doi.org/10.55927/ijsmr.v1i11.7065
ISSN-E: 2986-5042
https://journal.formosapublisher.org/index.php/ijsmr

INTRODUCTION

Indonesia is the largest banana producing country in Asia (Dhamayanti, Tiwow, & Nuryanti, 2018). Based on data from the Central Bureau of Statistics (2018), banana production in Indonesia reached 7,264,383 tons with East Java as the highest producing area. One type of banana that grows a lot in Indonesia is the klutuk banana or also known as the stone banana. Stone banana (Musa *balbisiana* Colla) is one type of banana that has seeds in the fruit, which is planted to be used for thick leaves for wrapping not for consumption like other bananas (Musita, 2012).

Young banana stems are used as one of the vegetables called "jukut ares" in the Bali area. Ares is another name for young banana stems. Jukut ares is a favorite vegetable that is always awaited at events in Bali. According to Margianti & Su'udi (2020), the stem of a banana plant called ares is located on the inside of a young banana stem. Ares has not been effectively utilized by the community. Ares bananas have high fiber, and according to nutritionists ares bananas contain serotonin, norepinephrine, tannins, hydroxytryptamine, dopamine, vitamin A, vtamine B, vitamin C, potassium and sugar (Margianti & Su'udi, 2020). Banana stems are known to contain flavonoids where most flavonoid compounds have antioxidant activity (Nurhaeni *et al*., 2019). Antioxidants can prevent oxidation of body cells by active oxygen such as hydrogen peroxide and hydroxyl radicals and other free radicals, so that the body can avoid degenerative diseases and premature aging (Afriani *et al*., 2014). Therefore, banana stems have good enough potential to be used as a source of antioxidants in food (Singapurwa *et al*., 2023).

Based on the above description, researchers are interested in using the DPPH (1,1-*diphenyl*-2-*trinitophenylhydrazine*) method to process banana young stem waste (Ares) into flour with different drying treatments and blanching temperatures to explore its Antioxidant activity. The sample used is stone banana ares, because usually stone banana ares are only discarded and not used.

LITERATURE REVIEW

Banana Stem (Ares)

Banana stems are potential waste from agricultural products that have not been widely used by the community (Nurrani, 2012). Young banana stems are made into a Balinese specialty, jukut ares (traditional Balinese vegetables), and banana hearts are made into banana heart stir-fry. The core of the banana plant stem, called Ares, is located inside the banana stem and is not yet fully utilized by the community. The liver part of banana stems tends to be discharged into waste that harms the environment. Ares bananas have high fiber, and according to nutritionists ares bananas contain serotonin, norepinephrine, tannins, hydroxytryptamine, dopamine, vitamin A, vtamine B, vitamin C, potassium and sugar (Margianti & Su'udi, 2020).

The content of banana stems consists of carbohydrates, fats, proteins, glycemic and fiber (Hasanah *et al*., 2022). Banana stems contain secondary metabolite compounds that act as antioxidants, antibiotics and antibacterials. Banana stems contain phytochemical compounds such as saponins, tannins, flavonoids, anthraquinones, quinones, and lectins (Raharjo & Andaka, 2020). Saponins, anthraquinones, and quinones function as antibacterial and

painkillers, flavonoids play a role in the growth of plant fiber roots, lectins function to stimulate skin cells (Nugroho *et al*., 2016), and tannins are aseptic (Raharjo & Andaka, 2020). Antioxidant compounds such as flavonoids, saponins, and tannins found in banana stems (Ananta, 2020). The antioxidant content of banana stems can be used as wound treatment, dental treatment, and bacterial infection treatment (Ananta, 2020)

(a) (b)

Figure 1. Stone Banana (*Musa balbisiana* Colla)
(Remarks: a. stone banana tree, b. stone banana ares)
Source: Personal Documentation (2023)

Ares Flour (Young Banana Stem Flour)

In the times, flour is often produced from tuber raw materials that have a high starch content, this is done to improve the economic value of the tubers themselves and the use of domestic products so that tuber-based flour processing is expected to be an alternative to the use of wheat flour whose raw materials still have to be obtained from abroad (Sudaryati & Nurmaini, 2023). The process of making flour from tubers can be done in various ways depending on the type of tubers used. Flour is made with a very low moisture content of about 2-10%. This shows that flour has a longer shelf life (Nurbaya & Estiasih, 2013). According to Sembiring (2017) banana kepok weevil flour contains 79.16% carbohydrates, 2.15% fat, 3.58% proteins, and according to Saragih (2013) states that kepok banana weevil flour contains 29.62% crude fiber, 0.99% water content, 1.83% ash content, the color of kepok banana weevil flour is brownish.

Ares flour is one type of flour obtained through drying and blaching. Processing banana ares into flour increases the added value of banana ares as they can be processed into different types of food products. The processing principle in making banana Ares flour is that it can be dried using sunlight or using a drying device such as a drum dryer, then ground using a crusher, then sifted using a 60-100 mesh sieving tool (Halisa, 2021). The following is the content contained in banana stems can be seen in Table 1.

Table 1. Content of Dried Banana Stems

No	Content	Up to (%)
1	Water content	8.98%
2	Ash content	25.84%
3	Fat	1.74%
4	Proteins	11.64%
5	Coarse fiber	41.88%
6	BETN (Nitrogenless Extract Ingredients)	18.90%
7	Carbohydrate	55,32%

Source: Dhamayanti *et al*. (2018); Handayani *et al*. (2023)

Blanching

Blanching is a way that can be done to inhibit the activity of an enzyme, both enzymes contained in food and enzymes (ecoenzymes) produced from putrefactive bacteria (Afrianto *et al*., 2014). According to Widyasanti *et al*. (2018), blanching aims to inactivate unwanted enzymes that may change the color, texture, flavor, and nutritional value of a foodstuff. Deactivated enzymes are enzymes that can help speed up the process of decay and discoloration in a product. Blanching will cause air in the tissue to come out and the movement of water is not hampered so that the drying process becomes faster (Khaerunnisya & Rahmawati, 2019). Chocolate in the drying process of food can be prevented by inactivating the enzyme system, inactivation of the enzyme system can be done by blanching food in boiling water or water vapor (Kusumadati *et al*., 2021). The blanching process is included in the thermal process which is generally carried out for 10 minutes using temperatures of 75-95 °C (Rukmana & Saidi, 2021).

Drying

Drying is one of the oldest and most widely used methods of food preservation, in which most of the water in food is evaporated using thermal energy (Hariyadi, 2018). The purpose of drying is to reduce the moisture content of food, thereby extending the shelf life of food, reducing the volume of food, and saving transportation, packaging and storage costs (Risdianti *et al*., 2016). According to Dharma *et al*. (2020) The drying method consists of drying with direct sunlight, drying with the oven, drying with the wind, and drying with the greenhouse.

Grinding using sunlight is the most economical and easiest drying process to do (Hamka & Geroda, 2017). Drying by drying in the sun has several disadvantages, namely depending on the weather, difficult to control, requires a large drying place, easily contaminated, and requires a long time. Drying using sunlight can also provide advantages in terms of production costs in a shorter time than the wind dry method (Widarta & Wiadnyani, 2019).

An oven is one of the devices used to dry food using heat in an enclosed space. The purpose of drying in the oven is to reduce the moisture content of food. The advantage of using the oven method for drying is that the

temperature and speed of the drying process can be adjusted at will, and hygiene and sanitation can be controlled regardless of the weather (Saleh & Yusnaini, 2022). Drying using the oven method has the disadvantage of requiring more expensive costs.

Drying by aerating is one of the drying processes carried out traditionally. The principle of the wind dry method is to utilize the air flow rate to reduce the moisture content in a sample without the influence of temperature increase. The purpose of the wind dry method itself is to maintain the content of secondary metabolite compounds so as not to experience damage due to temperature increases or decreases. The disadvantage of the wind dry method is that it takes days or even up to several weeks in the drying process depending on the type of material being dried.

Antioxidant

Antioxidants are substances that can prevent or inhibit cell damage caused by free radical oxidation (Antarti & Lisnasari, 2018). Antioxidants work by capturing free radicals so that they can inhibit oxidative reactions in the body (Adawiyah *et al*., 2015). Antioxidant compounds act as free radical scavengers, thereby inhibiting the formation of free radicals (Dungir *et al*., 2012). The formation of free radicals through three stages of reaction, namely initiation, propagation, and termination.

Depending on the source, antioxidants are of two types namely natural antioxidants and synthetic (artificial) antioxidants. Natural antioxidants are antioxidants extracted from natural ingredients. Natural antioxidants inhibit the onset of disease, protect the body from damage caused by reactive oxygen compounds, and inhibit lipid peroxidation. Examples include vitamin A, vitamin E, carotenoids, vitamin C, vitamin B2, zinc (Zn), selenium, wheat gliadin, copper, ovalbumin, phenols (thyroid alcohol, vanillin, hydroxytyrosol), Tannins (gallic acid, ellagic acid), and polyphenols (flavonoids, flavones, flavonols, bisflavonoids). Synthetic (artificial) antioxidants are antioxidants formed during the synthesis of chemical reactions. There are concerns that excessive amounts of synthetic antioxidants can lead to cancer-causing diseases. Examples include tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). (Sayuti & Yenrina, 2015).

According to the function and mechanism of preventing free radicals, antioxidants are divided into three types, primary, secondary and tertiary antioxidants. Primary antioxidants are antioxidants that act as chain-breaking antioxidants and prevent the formation of new free radical compounds. The working principle is to break the chain of radical reactions and donate hydrogen atoms quickly to radical lipids. The resulting product will be more stable. Examples include superoxide dismutase (SOD), catalase, metal-binding proteins, glutathione peroxidase (GPx), ascorbic acid, tocopherols, and antioxidants. Supplementary antioxidants are antioxidants that act as oxygen scavengers, singlet oxygen reactivators, ultraviolet radiation absorbers, metal ion binders, and decomposers of hydroperoxides into non-radical compounds. The working principle is to chelate metals that are pro-oxidant, capture radicals,

and inhibit chain reactions that produce new radicals. Examples include bilirubin, transferrin, isoflavones, beta-carotene, albumin, vitamin C, and vitamin E. The role of tertiary antioxidants is to inhibit the accumulation of ecological enzymes and biomolecules and repair biomolecule damage caused by free radicals. Examples are proteins oxidized by proteolytic enzymes, and DNA repair is carried out by methionine reductase enzymes (Sayuti & Yenrina, 2015; Wulansari, 2018)

Antioxidant Activity

Antioxidant activity is the ability of a substance to inhibit oxidative reactions caused by free radicals. The increase in antioxidant activity is expressed as IC50. IC50 is defined as the concentration of an antioxidant compound that results in a 50% loss of DPPH activity. There are four methods that can be used to measure antioxidant activity, namely the free radical soaking method 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), 2,2-azobis(3-ethylbenzene) thiazoline-6-sulfonate) (ABTS), iron reducing antioxidant capacity (FRAP), copper ion reducing antioxidant capacity (CUPRAC), and oxygen radical absorption (ORAC) (Kurniawati and Sutayo, 2021).

1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) method The DPPH method is based on a substance that can reduce free radicals: 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) to test antioxidant activity. The test sample was reacted with the DPPH solution and its absorption at the maximum wavelength was measured by UV-visible spectroscopy. DPPH is dark purple in color and has an absorption maximum at a wavelength of 517 nm. This method works on the principle that when a DPPH solution reacts with an antioxidant, the antioxidant compound donates its hydrogen atoms to DPPH.

Scavenging free radicals results in electron pairing and a decrease in the amount of DPPH. The absorption value of DPPH decreases and the color initially changes from dark purple to light yellow. When DPPH radicals acquire hydrogen radicals from the sample, a color change occurs due to reduction of the conjugated DPPH double bonds. The DPPH radical will undergo reduction to 1,1-diphenyl-2-pyrryhydrazil (DPPH-H). The solution will be measured for absorbance with a spectrophotometer. This method has the advantage of being a fast, inexpensive and simple method for measuring antioxidants. The disadvantage of the DPPH method is that radicals can only dissolve in organic solvents (Shalaby & Shanab, 2013; Liaudanskas *et al*., 2014). The relationship between IC50 values and the strength of antioxidant activity is presented in Table 2.

Table 2. Antioxidant Power Level

IC50 value (mg/L)	Antioxidant Power
<50	Very powerful
50-100	Strong
100-150	Keep
150-200	Weak
>200	Very weak

Source: Jami'ah *et al*. (2018)

METHODOLOGY

Place and Time of Research

This study was conducted at the Food Processing and Analysis Laboratory, Faculty of Agriculture, Valadewa University, Denpasar, Bali Province. This study was conducted from July to September 2023.

Material

The materials used in this study were 6-8 month old stone banana Ares with banana trees 165-175 cm tall from Asahduren Village, Pekutatan District, Jembrana Regency, Bali Province. Ares flour as sample. The materials used for analysis were methanol and 1,1-*diphenyl*-2-*picrylhydrazyl* (DPPH).

Tool

The tools used in the study were analytical balances (Ohaus), test tubes, beakers, ovens (Memmert), test tube racks, Micropipettes (Joanlab), Spectrophotometers (Biochrom), gloves, masks, stirring rods, blue tips, aluminum foil, measuring flasks, spatulas, filter paper, and funnels.

Research Design

This study adopted a factorial design of completely randomized design (RAL), consisting of two factors and three replications, namely: Factor 1 is that drying consists of 3 treatments: P1 = Drying using oven temperature 48±2 °C, P2 = Drying using sunlight temperature 30±3 °C, P3 = Drying using wind temperature 25±5 °C. Factor 2 is that the temperature used for the blanching process consists of 3 treatments: T1 = Dry blanching 65 °C, T2 = Dry blanching 75 °C, T3 = Dry blanching 85 °C. Overall in this study the number of observation combination units was 3 x 3 = 9 treatment combinations as follows P1T1, P1T2, P1T3, P2T1, P2T2, P2T3, P3T1, P3T2, P3T3 were repeated as many as 3 so that 27 treatment units were obtained.

Research Procedure

The research procedure for processing ares flour is the first to start from taking stone banana ares from the tree. Stages of processing with the ingredients used ares stone bananas, salt, and water. The process begins with the weighing of stone banana ares, after which they are cut into pieces with a thickness of 2.74-4.57 mm. Sliced ares are soaked using 2.5% salt concentration for 10 minutes with a ratio of 1: 3, ie 1 kg of ares soaked with 3 liters of 2.5% salt solution. The purpose of soaking using saline solution is to facilitate cleaning and remove sap. Ares bananas that have been cleaned of sap are then washed with running water so that the sap and salt taste attached to the banana stems disappear. Banana stems are blanched using temperature treatment of 65 °C, 75 °C, and 85 °C for 10 minutes, the blanching method used is by steaming. The blanched banana stems are then soaked in water for 2 minutes. This is so that the heat from the blanching process does not cause over blanching. Next, the soaked banana stems are drained, then dried. The drying process is carried out using three treatments, namely oven, sunlight, and wind dry. Drying using an oven was carried out for 2 days (48 hours) with a temperature of 48±2 °C with a humidity of the first day of 84±5 RH%, and the second day of 22±12 RH%, drying with sunlight 9 days (72 hours) with a temperature of 30±3 °C with a humidity of 50±24 RH%, and drying with wind dry for 14 days (336 hours) with a temperature of 25±5 °C with a

humidity of 78±8 RH%. Coat the dried banana stems evenly with a blender and pass through a 100-mesh sieve. The signs of dry banana peels are brown color, shrinkage in volume, and crispy feeling when kneaded.

Test Parameters

Antioxidant Activity Test was conducted using DPPH method (Burda & Oleszek, 2001). Samples of banana stem extract (ares) weighed 0.1 g using analytical balances. Dissolve the extract in methanol to mega mark using a 10 mL volumetric flask. Prepare solutions at concentrations of 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1,000 ppm, then pipette 1 ml of each concentration into a test tube. Additionally, add up to 1 mL of 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), then vortex and incubate in a dark room for 45 min. Test samples were absorbed at wavelength 517 using UV-visible spectrophotometry. Calculate the percent inhibition value expressed as IC50 using the following formula:

$$inhibition = \frac{absorbantion\ blanco - absorbantion\ sample}{absorbantion\ blanco}\ x\ 100\%$$

Data Analysis

The data obtained from the study results are analyzed using the fingerprint analysis method or (Test F) to obtain objective data on whether the treatment achieves a real or very real effect, and then continues to maintain a realistic level of 5% using the Minimum True Difference Test (BNT) (BNT 0.05%).

RESULTS AND DISCUSSION

The results show that the IC50 values obtained by treatment, drying method and blanching temperature, as well as the interaction between the two treatments, have a very real impact on the IC50 value of stone banana powder ($p<0.01$). The analysis results of IC50 value in stone banana ares flour are shown in Table 3.

Antioxidant activity was analyzed using the DPPH (1,1-diphenyl-2-trinitrophenylhydrazine) method (Bentel et al., 2023). The DPPH method works on the presence of an antioxidant compound that donates H+ to DPPH, converting the purple DPPH free radical into the light yellow or color-lost non-radical compound DPP-hydrazine (diphenyltrihydrazide) (Ani et al., 2021). The lighter the color of DPPH formed after reacting with antioxidants, the stronger the antioxidant capacity in the sample (Suma & Urooj, 2012). Additionally, measurements were performed using a UV-Vis spectrophotometer at a wavelength of 517 nm. If the IC50 value is below 50 ppm, the compound can be described as a very strong antioxidant. If the IC50 value is between 50 and 100 ppm, it can be described as strong; if the IC50 value is between 150 and 200 ppm, if the IC50 value is between 100 and 150, it can be described as weak. If the IC50 value of a substance is greater than 500 ppm, it can be interpreted that the substance is less or very weakly active but still has potential as an antioxidant (Pratiwi et al., 2023).

Table 3. Effect of Drying Method and Blanching Temperature on IC50 "*Stone Banana Ares Flour*"

Influence of Interaction			
Drying Method	Dry Blanching		
	65°C	75°C	85°C
Oven	117,165.66 b (a)	506,616.57 a (a)	56,400.47 c (a)
Sun	103.887,04 b (ab)	145.443,74 a (b)	42.429,90 c (a)
Wind Dry	84.086,98 a (b)	53.318,86 b (c)	49.346,93 b (a)
BNT (0.05)	288.442,93		

Information: The average value followed by different letters in the same row or column shows a very noticeable difference in the 5% BNT test

Absorption values were measured by UV-Visible spectrophotometer and then calculated to determine the percentage value of inhibitory/antioxidative activity. Free Radical Inhibition Percent inhibition is the ability of a material to inhibit free radicals depending on the concentration of the material tested, while IC50 is a parameter often used to indicate DPPH test results (Muktisari & Hartati, 2018). The parameter used to indicate antioxidant activity is the inhibitor concentration (IC50). The IC50 value is defined as the antioxidant concentration required for a test compound to reduce free radicals by 50%. The smaller the IC50 value, the higher the free radical reduction activity, indicating that the test sample contains stronger antioxidants.

The DPPH method was chosen because it is a simple, easy, and sensitive method that requires less sample and relatively short processing time (Muktisari & Hartati, 2018). DPPH is a free radical that is stable at room temperature and easily oxidized by light and air. Compounds with antioxidant activity react with DPPH and the color changes from violet to yellow as the antioxidant donates hydrogen atoms to DPPH. The degree of antioxidant activity is expressed by the IC50 value, which represents the amount of sample solution required to inhibit 50% of DPPH free radicals. The IC50 value is the antioxidant concentration required to scavenge DPPH free radicals by up to 50%. The IC50 value can be determined from a linear equation that gives the relationship between solution concentration (x) and percent inhibition (y). Calculate the concentration of the sample.

The fingerprint analysis results show that the lowest IC50 value is obtained when the sun drying method and blanching temperature are 85°C. The average IC50 value is 42,429.90 ppm with a very weak category. While the largest IC50 value is found in the treatment of the oven drying method and blanching temperature of 75°C with a value of 506,616.57 ppm with a very weak category. IC50 parameters based on the results of this study show that the interaction between the two has a very real effect ($p < 0.01$) on the drying method and

blanching temperature. Harnowo (2008) stated that the process of making food products can affect antioxidant activity. This is because food products undergoing processing processes such as frying / roasting in the oven / boiling can damage antioxidants and reduce their activity by 20%, but in processing processes such as drying can remove about 40% of antioxidant levels and reduce the level of reactivity by 50% (Hermayudha, Izzati, & Saptiningsih, 2013).

The temperature of the drying process greatly affects antioxidant activity, this causes a decrease in antioxidant activity in stone banana Ares flour products. The drying process in making stone banana Ares flour affects the content of antioxidant activity in stone banana Ares flour, this is because in the drying process there is evaporation caused by heat. According to Bentelu *et al*., (2023), the decrease in antioxidant activity in samples was influenced by heating treatment which caused damage to bioactive compound components. Temperature treatment has a damaging effect on phenolic compounds and antioxidant activity (Bentelu *et al*., 2023). These damaging effects depend on several factors such as heat treatment, air exposure, light exposure, washing process, antioxidant bioactive structure, cutting process, cooking method, bioavailability, and heat stability (Laga *et al*., 2021). The following is a graph of the interaction of drying method and blanching temperature against IC50 stone banana Ares flour seen in Figure 2.

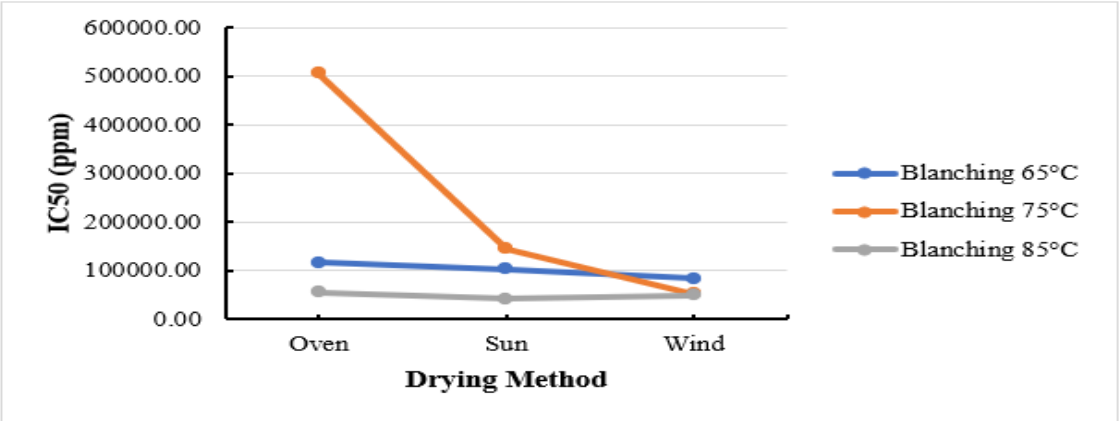

Figure 2. Graph of the Effect of Drying Method Interaction and Blanching Temperature on IC50 Stone Banana Ares Flour

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the studies conducted it can be concluded that the antioxidant activity of stone banana Ares flour after drying and blanching temperatures is very weak. It is characterized by an IC50 value of over 500 ppm, which indicates that the substance is less or very weakly active but still has potential as an antioxidant. Using solar drying and a blanching temperature of 85°C, the lowest average IC50 value for the very weak category was 42,429.90 ppm.

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