

Differences of Quercetin Content in Fresh and Extracts Local Apples using High Performance Liquid Chromatography Method

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Abstract

Background: Oxidative stress is an imbalance between pro-oxidants and endogenous antioxidants resulting in cell damage and degenerative diseases. As horticultural commodities, apples have superior varieties of Manalagi and Romebeauty. Apples contain exogenous antioxidants, namely quercetin that effectively contributes to the management of oxidative stress.

Purpose: The study used laboratory experimental methods to determine differences in quercetin content in fresh apples and apple extracts of Manalagi and Romebeauty.

Research methodology: Determination of quercetin content of fresh apples and apple extract used the High Performance Liquid Chromatograph (HPLC) method. The samples used were fresh apples and apple viscous extract of Manalagi and Romebeauty obtained respectively from UPT Medika Material Laboratory of Batu City and Food Engineering Laboratory of Soegijapranata Catholic University Semarang using maceration process with ethanol solvent.

Findings: The results showed that the average levels of quercetin in fresh apples of Manalagi and Romebeauty varieties were 13,685 ppm and 15,544 ppm respectively. Extraction of 70% ethanol from 100 grams of Manalagi and Romebeauty varieties resulted in 38.123 grams and 52.699 grams of thick extract. The average levels of quercetin in Manalagi and Romebeauty extracts were 422.235 ppm and 243.454 ppm. It can be concluded that the extract can optimize the quercetin content.

Research limitations: The quercetin content in local apple extracts of Manalagi and Romebeauty can be used as an alternative to antioxidant therapy due to oxidative stress.

Originality/value: Analyzing differences in quercetin content in fresh apples and apple extracts.

Keywords: *Quercetin, Romebeauty apples, Manalagi apples, Extraction, HPLC*



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INTRODUCTION

Apples are fruits from *Malus domestica* species of the Rosaceae family. Apple plants grow and develop well in sub-tropical areas. These fruits become a horticultural commodity and increasingly develop in Indonesia. Apples started to spread widely in 1930 and began to be planted in Indonesia in 1960, namely in Malang and Batu which have altitudes of more than 900 asl (Muhamad and Istis, 2017). The trend of Indonesian local fruit production including apples is positive with an average increase of 10.12% in the last 4 years so that their abundance availability does not rely on the season (Muhamad and Istis, 2017). The increase in local fruit production since the COVID-19 pandemic is associated with increased awareness of the benefits of fruit for body immunity and health (Perekonomian, 2020; Isaskar and Perwitasari, 2021). This is in line with a survey by Statistics Indonesia that there was an increase in local apple production of around 35 thousand tons from 2019 to 2020 (Indonesia, 2020). The most widely local apple variety cultivated in Indonesia

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is the Romebeuty and Manalagi. Romebeauty varieties have the characteristic of reddish-green color with a round shape, a sour taste, and crunchy texture, while Manalagi varieties have yellowish-green color, round shape, sweet taste, and crunchy texture (Putri et al., 2017).

Apples are known for their high content of vitamin C and antioxidants such as flavonoids to prevent free radicals (Ventianingsih et al., 2016; Xiang et al., 2020). Oxidative stress occurs when the body produces more pro-oxidants than antioxidants (Muliando, 2020; Nowotny et al., 2015). Free radicals are molecules with an imbalance number of electrons that can be very reactive causing the body experiences large chain chemical reactions (Sinaga, 2016; Oyenihini et al., 2015). Long-term oxidative stress can contribute to the development of various chronic conditions such as diabetes, heart disease, and cancer disease (Midah et al., 2021; Reis et al., 2016). Handling degenerative diseases with pharmacological therapy are one of the curative steps, but its side effects are common (Kemenkes RI, 2017). To treat degenerative diseases, an appropriate alternative with low side effects is needed such as utilizing local fruits containing high antioxidants. The most abundant source of flavonoid is vegetables and fruits. Human daily intake of flavonoids is estimated around 20-50 mg/day consisting of an estimated 13.82 mg/day in the form of quercetin (Siswarni MZ et al., 2017).

Quercetin is a pigment of flavonoid compounds found in many vegetables or fruits such as red grapes, apples, or tomatoes. Shallot has the highest content of flavonoids, namely 347 mg/kg, while apples and red grapes have 36 mg/kg and 11 mg/kg respectively (Masyhur et al., 2011). Quercetin can act as an antioxidant to prevent lipid peroxidation and suppress oxidation processes which can protect the body from several degenerative diseases such as diabetes mellitus, coronary heart disease, kidney failure and stroke. Apples contain high quercetin levels with varying levels due to differences in varieties, nutritional growth, processing, and storage (Ventianingsih et al., 2016; Snyder et al., 2016).

An extraction process is a method of separating molecules by withdrawing the chemical components contained in natural products with the help of appropriate solvents to maximize the withdrawal of quercetin compounds (Mukhriani, 2014; Sari et al., 2022). In line with the previous study, the ethanol solvent is effective in extracting quercetin compounds from Dutch eggplant (Siswarni MZ et al., 2017). Other studies reveal that the use of 70% ethanol solvent in Bangle rhizomes contains flavonoids, saponins, alkaloids, tannins, essential oils and glycosides (Mutton, 1998). Based on the description above, this study tries to identify the differences in the quercetin content in fresh local apples and apple extracts of Manalagi and Romebeauty varieties.

LITERATURE REVIEW

Oxidative Stress

Reactive Oxygen Species (ROS) are compounds with one or more unpaired electrons so that they are very active in finding partners by attacking or freely binding the surrounding electron molecules and even causing cell damage (Muliando, 2020; Forrester et al., 2018). Increased lipid peroxidation occurs due to the presence of free radicals in the body as characterized by increased levels of malondialdehyde (MDA), a dialdehyde compound as a marker of oxidative stress (Mashinchian et al., 2014). Experimental research by giving oxygenated water, namely pentagonal and hexagonal to rats showed no significant difference in MDA levels which means that there is no

cell damage due to oxidative stress (Zaetun et al., 2019). This is different from studies of acne vulgaris patients with mild-moderate, and severe levels showing MDA levels of 58.371 ng/ml; 99.121 ng/ml, and 171.779 ng/ml respectively. Thus, it is proven that oxidative stress is directly proportional to MDA levels (Anggraeni et al., 2017).

The occurrence of oxidative stress in a long term can trigger the occurrence of non-communicable diseases such as diabetes mellitus, heart disease, and cancer (Midah et al., 2021). The diabetes mellitus rat model experienced an increase in MDA levels with an average of 8.3 μ M. Thus, it can be concluded that degenerative diseases such as diabetes mellitus affect the body's oxidative stress events (Muhajirin and Marjan, 2019). Degenerative diseases such as diabetes mellitus due to uncontrolled blood glucose can cause oxidative stress which finally damages cell membranes and cause various disorders of a body function to organ damage (Diyah, 2014; Byun et al., 2017). Uncontrolled blood glucose levels are proven to be at risk for kidney disease in Type 2 Diabetes Mellitus patients at Bangli Hospital. This is associated with uncontrolled blood glucose levels which can cause oxidative stress which has an impact on the complications as evidenced by cases of diabetes mellitus with kidney disease with a total of 84.8% (Sumarya et al., 2020; Zhu et al., 2016).

Quercetin

Quercetin has the chemical formula of $C_{15}H_{10}O_7$ included in flavonoid compounds and can be found in vegetables and fruits (Masyhur et al., 2011; Chen et al., 2016). The antioxidant properties of quercetin are due to its ability to bind to free radicals associated with quercetin compounds which have catechol groups on ring B and 3-OH groups on rings A and C so that they can prevent oxidation processes and protect the body from degenerative diseases such as diabetes mellitus, coronary heart disease, kidney failure, and stroke (Ventianingsih et al., 2016; Salehi et al., 2020). Administration of 20 mg/kg of quercetin and 5 mg/kg of glibenclamide has been proven effective in reducing blood glucose levels. This is in line with the function of quercetin which can slow down glucose absorption supported by the function of glibenclamide which is able to increase insulin secretion (Eka Fitriani et al., 2014). Other studies reveal that higher quercetin levels are proven to increase the percentage of decreased fasting blood glucose levels (Malini et al., 2019). The combination of 20 mg/kg and glibenclamide can improve conditions due to dyslipidemia. Besides the function of glibenclamide as a therapy for diabetes mellitus, quercetin plays a role in reducing the expression of sterol regulatory element-binding protein 1c (SREBP-1c) and increasing the expression of peroxisome proliferator-activated receptor-1c. γ (PPAR- γ) so that it can reduce levels of triglycerides and plasma cholesterol in the body (Monika and Lestariyana, 2014).

The Effectiveness of Apples

Apples have been cultivated and developed in Indonesia since 1960 (Muhamad and Istis, 2017). Apple varieties that can grow well in Indonesia are the Romrbeauty and Manalagi (Putri et al., 2017). Increased consumption of apples is associated with their high content of antioxidants, vitamins, and minerals (Perekonomian, 2020). Antioxidants contained in apples such as quercetin is included in the group of flavonoid compounds and have been widely used as a therapy for some diseases. Differences in quercetin content in apples depend on the varieties, harvesting age, plant nutrition, apple processing and storage (Ventianingsih et al., 2016). Apple varieties can affect

differences in the quercetin content of 27.16 mg/mL and 17.4 mg/mL for Romebeauty and Fuji varieties, while the quercetin content of Manalagi apples that have been stored in cold temperatures for one month increased to 51.79 mg/mL. Meanwhile, Fuji apples that had been stored in cold temperatures for 1 month decreased the quercetin content to 15.33 mg/mL (Ventiyaningsih et al., 2016). Processed apples such as juices and smoothies show a decrease in quercetin content compared to fresh apples (Anggun et al., 2014).

Extraction

Extraction is a separation of a substance or compound according to its solubility in a particular solvent. Maceration is extraction by immersing the sample using organic solvents so that there is a difference in pressure inside and outside the cell and the soaked sample experiences a breakdown of the cell wall and cell membrane causing compounds or metabolites contained in the cytoplasm to dissolve in organic solvents so that the compound content or molecules contained in material samples are optimally absorbed through the extraction (Suhendra et al., 2019; Ćujić et al., 2016). Common solvents used in maceration such as methanol and ethanol are adjusted to the polarity of each material. Compounds that have the same polarity as the solvent, make them easy to dissolve. Determination of the amount of solvent refers to the amount of simplisia used. The amount of solvent is directly proportional to the amount of simplisia where the higher the simplisia used, the greater the amount of solvent used. The effectiveness of dissolving the active substance in the sample is influenced by the fineness level of the simplisia, the finer the simplisia, the greater the surface area with the solvent (Effendi et al., 2015). A study reveals that the maceration method is less effective in producing phenolic levels in corncob extract compared to the reflux method with the results of phenolic levels of 312.420 mg/kg and 396.768 mg/kg in samples using the maceration and reflux methods respectively (Susanty and Bachmid, 2016). Another study reports different results that the maceration method in white dragon fruit extract shows that the flavonoid test is more effective than the digestion method with the results of 74.167 mgEK/g and 8.87 mgEK/g respectively (Nudiasari et al., 2019). Thus, the maceration method was selected as the extraction treatment in Manalagi and Romebeauty varieties.

High Performance Liquid Chromatography

Chromatography is a method of separating molecules referring to the differences in movement patterns between the stationary and mobile phases which has the function of separating the molecular components contained in the solution (Seal, 2016). Examination of the quercetin content in fresh apples and apple extracts used the High-Performance Liquid Chromatography (HPLC) method. This method refers to a chromatographic technique in which the stationary phase is in the form of a liquid or solid and the mobile phase is in the form of a liquid. The HPLC method showed better results used for antioxidants in white galangal extract compared to the spectrophotometer method with the results of IC50 levels using the spectrophotometer and HPLC methods of 462.89 and 62.17 ppm. In other words, the lower the IC50 value, the better the results. The difference in IC50 levels from white galangal extract and it can be concluded that the HPLC method is more effective than the spectrophotometer method (Cahyono et al., 2021). The HPLC method can be applied in testing the quercetin content which has been validated method with a value of 0.0086% and can be applied in determining the quercetin content in the ethanol extract of Leunca fruit

(Husnia and Budiarti, 2021).

RESEARCH METHOD

Research Design and Method

This research used an analytical experiment covering producing simplisia, extract preparation and testing for the content of quercetin in fresh apples and apple extracts of the Romebeauty and Manalagi varieties. This research was conducted in a different laboratory, namely at the UPT Laboratory of Materia Medika Batu City for producing local apple simplisia which required 3 days and the Food Engineering Laboratory of Soegijapranata Catholic University Semarang for producing the apple extracts and analysis of the quercetin content.

Tools and Materials

This research used some tools such as winnowing, plastic, scales, basins, oven, cutting boards, analytical scales, mesh, Siler Press, knife, blender, beaker glass, rotary evaporator, filter paper, Erlenmeyer tube, viscous extract container glass, thermohygrometer, silica gel, HPLC syringe and refrigerator. Then, the materials needed were Romebeauty apples, Manalagi apples, methanol, and 70% ethanol.

Procedure for producing local apple simplisia

The research procedure was started by making 13 kg of local apple simplisia, namely 7 kg of Manalagi and 6 kg of Romebeauty apples. They were washed with running water which aims to produce clean simplisia that is free from dirt or pathogens in order to have attractive physical appearances (Handoyo and Pranoto, 2020). The apples were thinly cut to speed up the drying process and make it easier to grind. Drying was carried out in an oven temperature of 50°C for 2 days. The grinding process aimed to widen the surface area in order to increase the effectiveness of extraction (Handoyo and Pranoto, 2020).

Procedure for producing local apple extracts

The simplisia produced was extracted with 70% ethanol for 3 days in stages and then the solvent was evaporated using a rotary evaporator at 40°C with a speed of 100 rpm to obtain a thick extract from the maceration of the material. The levels of quercetin in fresh apples and apple extracts were measured directly after processing. Then, they were extracted with methanol and sonicated and filtered using ordinary filter paper and a 0.2 µm HPLC syringe filter in stages. Before injection into the HPLC, the sample was stored at a cold temperature. The injection into the HPLC was for measuring the absorbance of quercetin with a wavelength of 254 nm and a column of C18. The results of quercetin levels are determined in ppm units (D'Mello et al., 2011).

Statistical Testing

Data were analyzed descriptively and the analysis of quercetin in fresh apples and apple extracts used statistical tests of SPSS version 20, namely the Analysis of Variance (ANOVA) to prove the relationship between quercetin in fresh and extracts of Manalagi and Romebeauty apples with significant values of ($p < 0.05$). It was then followed by the Duncan test to find out the differences

between all treatment groups.

FINDINGS AND DISCUSSION

Identification of fresh apples and apple simplisia

Fresh apples and apple simplisia have been determined by the UPT Herbal Laboratory of Materia Medika Batu, namely Romebeauty and Manalagi varieties. The results of macroscopic observations covered shape, weight, color, aroma and characteristics of fresh fruit and simplisia of Romebeauty and Manalagi varieties as presented in the following table.

Table 1. Results of macroscopic observations of fresh apples and apple simplisia

No	Observation	Manalagi	Romebeauty
1.	Shape	Round	Round
2.	Weight	122 gram	147 gram
3.	Color	Yellowish green	Reddish green
4.	Aroma	Fresh apples	Fresh apples
5.	Simplisia	Smooth and yellowish brown	Smooth and yellowish brown

The results of macroscopic observations showed some differences such as the weight and color of the apple skin. The difference is associated with physical characteristics such as yellowish-green skin color for Manalagi and reddish green for Romebeauty. The level of thickness of the skin color of each local apple is associated with the maturity level of the apples, while the differences in weight namely 122 grams and 147 gram for Manalagi and Romebeauty is associated with the maturity level of the fruit. The difference in weight is in line with the results of a study which stated that the maturity level of Manalagi and Romebeauty apples is between 10 to 18 weeks. Besides, the influence of the level of maturity based on the age of harvesting of the apple is proven to affect the development of the diameter and height of apples (Kristianto, 2019).

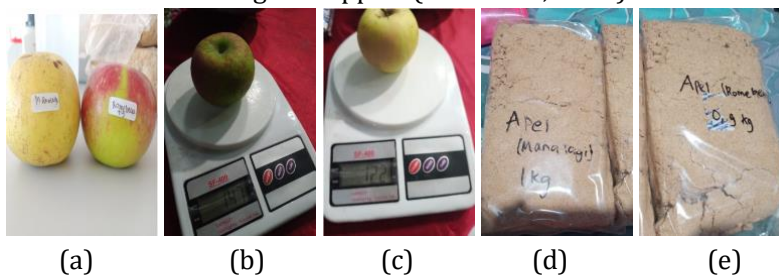


Figure 1: (a) macroscopic color test of Manalagi and Romebeauty apples; (b) weight of Romebeauty apples; (c) weight of Manalagi apples; (d) macroscopic test of Manalagi apple simplisia; (e) macroscopic test of Romebeauty apple simplisia.

Yields of Local Apple Simplisia

Extraction was started by making simplisia from Romebeauty and Manalagi apples that had been sorted and washed using clean running water in order to remove any dirt attached to the surface of the fruit. The sample used 13 kg of local apples consisting of 7 kg of Manalagi apples and 6 kg of Romebeauty apples. They were cut thinly to speed up the drying process. The drying was performed at oven temperature to effectively reduce the moisture content in order to increase in the shelf life of simplisia (Azzahra, 2022). The drying used an oven at a temperature of 50°C which was

monitored through a thermohygrometer for 2 days. Then, simplisia weights from the local varieties of Romebeauty and Manalagi apples were obtained. The results of observing the yield of local apple simplisia of Romebeauty and Manalagi affected by the drying method are presented in table 2 below.

Table 2. Yields of fresh local apples and apple simplisia

Varieties	Weight of fresh apples (Kg)	Weight of extracts (Kg)	Yields (%)
Manalagi	7	1,3	18,6
Romebeauty	6	0,93	15,5

Drying with an oven temperature of 50°C was monitored through a thermohygrometer for 2 days producing 1.3 kg of mashed simplisia from 7 kg of fresh Manalagi apples and fo 0.93 from 6 kg of fresh Romebeauty apples. The yield of the simplisia is calculated in percent by comparing the final weight of the simplisia with the fresh weight of each variety. The yields were 18.6% and 15.5% for Manalagi and Romebeauty simplisia respectively. The difference in yield produced is associated with the water content of each apple variety namely 81.18% and 86.11% for Romebauty and Manalagi respectively (Putri et al., 2017). Thus, it can be concluded that besides the speed of the drying process, the water content is directly proportional to the weight of the simplisia. The lower the water content, the greater the weight of the dried simplisia produced.

Yields of local apple extracts

Simplisia extraction of Manalagi and Romebeauty varieties was performed by maceration method with 70% ethanol solvent due to its polar properties and effective use as a solvent for phenolic compounds like quercetin (Effendi et al., 2015). Ethanol-type solvents have been proven to maximally absorb bioactive components as evidenced by the results of examining the most effective antioxidant activity (Suhendra et al., 2019). Soaking local apple simplisia with 70% ethanol solvent broke the cell walls and membranes of the simmpisia powder resulting in the breakdown of cytoplasm. The maceration process causes the solvent to pass through the cell wall to bind the quercetin metabolite compound contained in the cell. The stirring process in the simplisia during immersion aimed to bind all the polar components contained in the simplisia of each local apple variety and evenly distribute the heat. Besides, it affected the amount of extract yield produced. The extraction process was carried out for 4 consecutive days and every 24 hours was filtered with filter paper. The resulting filtrate was evaporated using a rotary evaporator, resulting a thick extract. The yield of romebeauty and manalagi apple variety extracts are presented in Table 3.

Table 3. Yields of local apple extracts

Extract	Yield (%)
Manalagi	38.123
Romebeauty	52.699

The result obtained was 38.123 grams of condensed extract from 100 grams of Manalagi apple simplisia and 52.699 grams from 100 grams of Romebeauty apple simplisia. The yield of the extract was determined from the ratio of the percentage of the weight of the condensed extract produced to the initial weight of the simplisia (Suhendra et al., 2019). Differences in yield results may be influenced by some factors such as the type of solvent, the stirring process during maceration, the quality of the simplisia, the powder size of the simplisia, and the temperature and

time used during the extraction process (Tamrin, 2022).

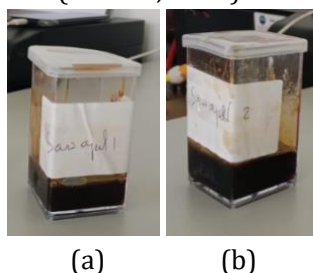


Figure 2: (a) Romebeauty apple extract; (b) Manalagi apple extract

Examination of quercetin levels

Examination of quercetin levels in fresh apples and apple extracts was started by determining the wavelength in obtaining the maximum light absorption for the compound with a maximum wavelength of 254 nm (Sukmawati et al., 2019). Each sample was extracted with methanol and sonicated and filtered using ordinary filter paper and 0.2 um HPLC syringe filter in stages. Prior to injection into the HPLC apparatus, the sample was stored at a cold temperature. Then, it was continued by injection into the HPLC apparatus for measuring the absorbance of quercetin with a wavelength of 254 nm and column C18. The results of quercetin levels were determined in ppm units. The quercetin examination of each sample was repeated 4 times. The average quercetin levels of each sample can be seen in Table 4.

Table 4. Results of statistical test for the quercetin level

Component	Quercetin levels			
	Manalagi apples	Romebeauty apples	Manalagi apple extracts	Romebeauty apple extracts
quercetin	13.685 ± 3.905 ^a	16.544 ± 1.101 ^a	422.235 ± 50.518 ^b	243.454 ± 23.237 ^c

Notes a, b, c = letter notation indicates that there is no significant difference between the Manalagi and Romebeauty apples based on the Ducan test.

Quercetin levels were measured three times in each sample with an average quercetin content of 13.685 ppm and 16.544 ppm for fresh Manalagi and Romebeauty apples respectively, and 422.235 ppm 243.454 ppm for Manalagi and Romebeauty apples extracts. The results of ANOVA statistical test showed a significance value of 0.00 and a P value of <0.05. This means that H0 is rejected so there are significant differences in the quercetin content in Manalagi and Romebeauty apples and extracts. This was continued with the Ducan test to find out the significant group. The results of the Ducan test showed that the quercetin content in Manalagi apples was not significantly different from the quercetin levels in Romebeauty apples, but the quercetin levels in Manalagi apples were significantly different from those in Manalagi and Romebeauty apple extract. The quercetin content in Romebeauty apples was not significantly different from Manalagi apples, but the quercetin levels in Manalagi apples were significantly different from those in Manalagi and Romebeauty apple extracts. Quercetin levels in Manalagi apple extracts showed significant difference from Romebeauty apple extracts. Quercetin levels in Romebeauty apple extract showed significant difference with Manalagi apple extract.

Fresh apples taken from Malang were tested for quercetin content in the Soegijapranata Catholic University laboratory. It was found that fresh apples have a lower quercetin content than

apple extracts with an average quercetin content of 19.11 mg/mL in imported apples and 21.05 mg/mL in local apples because imported apples experience a long distribution channel which affects the decrease in the quercetin levels (Ventianingsih et al., 2016). Room temperature storage can also reduce antioxidant activity such as quercetin as evidenced in previous studies that at room temperature storage, rosella flower extract decreased antioxidant activity (Khotimah et al., 2018).

CONCLUSIONS

The statistical test used one-way ANOVA with a significance value of 0.00 and a P value of <0.05. This means that H₀ is rejected so there is a significant difference in the quercetin content in Manalagi apples. Then, it was continued Duncan's test. It reveals that there is no significant difference between Manalagi and Romebeauty fresh apples, but there is a significant difference between Manalagi and Romebeauty apple extracts. The most effective quercetin levels were showed by Manalagi apple extracts.

Based on the results of this preliminary study, the quercetin content in local apple extract is higher than that of fresh local apples due to the ability of the extraction method to separate the molecules or compounds contained with the help of an appropriate solvent. Manalagi apple extract is the most effective with an average quercetin content of 422.325 ppm, followed by Romebeauty apple extract with an average quercetin content of 243.454 ppm. This means that the maceration method using 70% ethanol solvent is proven effective in removing the quercetin content of Romebeauty and Manalagi apples so it is expected to be used as an alternative therapy for degenerative diseases.

LIMITATION & FURTHER RESEARCH

In vivo tests have not been done to prove the effectiveness of local apple extracts containing high quercetin levels on certain diseases. It is expected that it can be developed as a fortification in processed food or beverages to be utilized by the majority of the public in overcoming degenerative diseases due to the body's oxidative stress.

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