Changes in colour, ascorbic acid and 5-hydrohymethylfurfural concentration in grapefruit and carrot juices during storage

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Summary
Effect of time and temperature on ascorbic acid (AA), 5-hydroxymethylfurfural (HMF) and colour is described in grapefruit and carrot juices with pulp that were stored at (2 ± 1) °C, (7 ± 1) °C, and (20 ± 2) °C for 168 days. Degradation of AA followed the first-order kinetic model and showed some inverse correlations with HMF formation ($r \approx 0.6665$–0.7953), together with subsequent changes in colour characteristics L^*, a^*, b^*, browning index and total colour difference. The reaction rate of AA decomposition increased with an increase in temperature and the half-time increased with temperature decrease. Changes in AA, HMF and colour were significantly lower at (2 ± 1) °C and (7 ± 1) °C comparing to (20 ± 2) °C in both juices ($p < 0.05$). The kinetic model of AA decomposition may be a useful diagnostic tool for control of juice quality and for prediction of the shelf life.

Keywords
ascorbic acid; colour; 5-hydroxymethylfurfural; juice; storage

Thermal treatment is the widest applied technology for the extension of shelf life and for preservation of juices [1]. Pasteurization and sterilization steps usually inactivate or destroy enzymes and microorganisms, but they could also cause changes in colour, flavour and nutritive value [2]. Besides thermal processing, also storage conditions significantly affect the quality of juices. Juice protected from external impacts and filled in proper packaging material is quite stable. However, combination of the intrinsic Maillard reaction (non-enzymatic browning) and the extrinsic increase in storage temperature can cause serious deterioration of juices that occurs slowly at ambient temperatures from 20 °C to 25 °C, but rapidly above 30 °C [3]. High processing temperatures are closely related to generation of 5-hydroxymethylfurfural (HMF), which is not naturally present in fresh and untreated fruits. Its formation is associated not only with thermal degradation of sugars, in particular hexoses, but also with the Maillard reaction and decomposition of ascorbic acid (AA) [4]. In general, HMF is regarded as a chemical indicator of heat processing and storage [5].

An important attribute of juice quality is colour, which is derived from natural pigments, such as chlorophylls, carotenoids, anthocyanins and betalains [6], and which is closely related with ripening of fruits, technological processing, storage conditions, actvity of microorganisms, etc. [7]. Changes in colour during processing and storage of juices can be due to AA degradation and Maillard reaction producing compounds that can participate in juice browning [4, 6].

Decomposition of AA is reported to be the major deteriorative reaction occurring during the storage of orange juice [8]. AA is the most sensitive vitamin among the other vitamins, which is destroyed under air oxygen, light and heat exposure [9]. AA degradation in orange juice can take place by aerobic and anaerobic mechanisms [10] that occur simultaneously [11]. Decline in AA is also actively supported by the low pH, residual hydrogen peroxide and trace metal ions present in juice

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[12]. As AA degrades more quickly than other nutrients, its critical decrease can indicate the end of the shelf life. Understanding of AA degradation process is essential to predict its loss during storage as well as the shelf life. Some data were reported on changes of AA during storage of citrus (orange, lemon, lime, grapefruit), mango, pawpaw and carrot juices [13, 14]. Also, several kinetic studies were conducted on the degradation of AA in some fruit and vegetable juices such as orange juice [15] and citrus juice concentrates (orange, lemon, grapefruit, tangerine) [16]. In these studies, decomposition of AA was investigated in association with various storage conditions and duration ranging from few days to several months.

In contrast to fruits, vegetables are generally low-acid foods (pH > 6.0), with the exception of tomatoes [2]. Since AA is unstable in neutral-alkaline environment [14], carrot contains less natural AA in comparison with fruits. Only limited information is available on changes in phytonutrients during processing and storage of carrots [17]. Few studies can be found on kinetics of water-soluble antioxidants during thermal processing of carrot juice [18], on prolongation of the shelf life of fresh juices by modification of atmosphere or acidification [19], and on Maillard reaction kinetics in carrot juice concentrate [20].

The aim of this study was to examine the effect of long-term storage (during 168 days, including 56 days after the declared expiration date) on stability of AA and colour of grapefruit juice (GJ) and carrot juice (CJ) at different storage conditions. Also, the formation of HMF was followed during the time period. Further, some kinetic indices were calculated for AA decomposition on the basis of the obtained experimental data.

MATERIALS AND METHODS

Materials

Samples of CJ and GJ were obtained directly from the producer (McCarter, Dunajská Streda, Slovakia). Fresh juices with added pulp had been pasteurized at 95 °C for 20 s and filled into polyethylene terephthalate bottles. For the storage experiment, one batch of respective juice was produced. Juices were stored during 168 days at defined conditions, i.e. (2 ± 1) °C, (7 ± 1) °C and (20 ± 2) °C. Bottles were kept in refrigerated/tempered boxes in order to mimic the storage conditions in sales sections equipped with refrigerating boxes at 2–10 °C. CJ and GJ samples analysed immediately after bottling were taken as reference samples. All analyses were performed in duplicate, i.e. from two bottles ($n = 2$).

Methods

Concentrations of AA and HMF were determined by HPLC, and CIE *L*a*b** colour coordinates were evaluated as described by Tobolková et al. [21].

The validation characteristics for AA determination by HPLC were as follows: limit of detection (*LOD*) 1.4 mg·l^{-1}; limit of quantification (*LOQ*) 1.8 mg·l^{-1}; mean recovery of AA was (88 ± 3) %. The relative standard deviation (*RSD*) of intra-day repeatability ($n = 8$) was 2.7 % and 36.0 % using control standard solutions with AA (Fluka Chemie, Buchs, Switzerland) concentration of 60.0 mg·l^{-1} and 3.0 mg·l^{-1}, respectively.

In HMF analysis, *LOD* of the procedure was 0.26 mg·l^{-1} and *LOQ* was 0.87 mg·l^{-1}. The uncertainty of the measurement was ± 0.09 mg·l^{-1} at the concentration level 1.41 mg·l^{-1}, and ± 0.3 mg·l^{-1} at the level > 4.0 mg·l^{-1}.

Browning index (*BI*) and total colour differences (ΔE) of stored juices were calculated from evaluated CIE *L*a*b** colour coordinates according to Maskan [22].

Data analysis

Statistical evaluation was performed using the software package Unistat 6.0 (Unistat, London, United Kingdom). Multiple comparisons were carried out by ANOVA Tukey's HSD test at the level of significance of $p < 0.05$. Correlation analysis between all monitored parameters at 95% significance level was performed.

The MicroMath Scientist (MicroMath, St. Louis, Missouri, USA) was utilized to fit the experimentally obtained dependencies of AA decomposition to the first-order kinetic model in the integral shape:

$$c_t = c_{t=0}\, e^{(-kt)} \quad (1)$$

in which $c_{t=0}$ and c_t represent an original concentration of AA ($t = 0$) and the concentration at particular time t of storage, respectively; k is the first-order rate constant of AA degradation expressed as reciprocal day.

The kinetic stability of AA throughout the entire storage period was evaluated by a half-time period $t_{1/2}$ (in days), i.e. as the time required to achieve a half of the original concentration of AA:

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

in which k represents the calculated first-order rate constant.

RESULTS AND DISCUSSION

The initial parameters of the reference juice samples were as follows: AA concentration (276 ± 10) mg·l^{-1} in GJ and (616 ± 21) mg·l^{-1} in CJ; HMF concentration at *LOD* level: 0.26 mg·l^{-1} in CJ and 0.92 mg·l^{-1} in GJ; the CIE $L^*a^*b^*$ colour coordinates: $L^* = 99.42 \pm 0.83$, $a^* = 0.004 \pm 0.000$, $b^* = 0.834 \pm 0.002$, $BI = 0.829 \pm 0.002$ in GJ and $L^* = 99.76 \pm 0.91$, $a^* = -0.033 \pm 0.000$, $b^* = 0.374 \pm 0.002$, $BI = 0.347 \pm 0.002$ in CJ.

Effect of storage time

Due to the limited stability of AA, tested juices varied in degree of AA retention during storage. Independently of other storage conditions, concentration of AA started to decrease immediately after juice production. For both CJ and GJ samples, the decrease in AA had comparable progress during the following storage time (Tab. 1). In the period to 70th day of storage, decrease in AA was very rapid especially in GJ, although AA degradation was not progressive during the entire period of storage. This phenomenon was also observed by Abbasi and Niakousari [23] when a rapid degradation of AA in non-pasteurized lemon juice during two weeks changed to a slower but gradual decrease during subsequent 10 weeks of storage. An unpasteurized sour orange juice showed an analogical trend, in which major loss of AA occurred during the first days of storage [24]. Citrus fruit juices were found to follow a similar pattern of AA degradation, which differed from other fruits species [14]. This supported the former suggestion that factors influencing the stability of AA in oranges and lemons are also highly applicable to grapefruit [25].

Since decomposition of AA is an important factor for HMF formation, consequential changes were also recorded in this parameter. According to results in Tab. 2 it can be presumed that pasteurization process had been carefully adjusted by the producer, as the concentration of HMF was very low, around *LOD* and *LOQ* in both juices. Any prolongation of the storage time was reflected in a higher concentration of HMF. The increase of HMF concentration was, on average, from 0.92 mg·l^{-1} to 1.93 mg·l^{-1} and from 0.92 mg·l^{-1} to 9.22 mg·l^{-1} in CJ and GJ, respectively. Significant difference in HMF concentration between the two tested juices ($p < 0.05$) could be also due to the diverse levels of oxalic acid [26], which usually acts as a catalyst in HMF formation. The increase in HMF concentration correlated highly with the level of browning in lemon juice and conduced to the formation of brown pigments [27]. Wang et al. [20] found a good correlation between HMF formation and the degree of browning during storage of CJ at 25 °C and 37 °C ($p < 0.01$). Evolution of both parameters followed a first-order reaction, as a function of storage and the soluble solid content in CJ. In this case, the formation of HMF in CJ took place by the Maillard reaction between amino acids and reducing sugars, in absence of AA.

As to our results, respecting individual colour characteristics, minor changes were observed in lightness (L^*) of both juices. Only trifling increase in redness (a^*) was noticed in GJ, while a^* values of CJ were shifted to more negative values

Tab. 1. Changes in ascorbic acid concentration during storage at various temperatures.

Juice	Time of storage [d]	(2 ± 1) °C		(7 ± 1) °C		(20 ± 2) °C	
		Ascorbic acid					
		Concentration [mg·l^{-1}]	Loss [%]	Concentration [mg·l^{-1}]	Loss [%]	Concentration [mg·l^{-1}]	Loss [%]
Carrot	28	573 ± 21	8	564 ± 23	9	528 ± 43	13
	70	418 ± 36	20	449 ± 17	20	386 ± 36	34
	84	479 ± 46	23	451 ± 32	24	345 ± 9	39
	98	471 ± 20	26	441 ± 11	27	333 ± 17	44
	168	360 ± 30	41	381 ± 40	42	265 ± 27	63
Grapefruit	42	236 ± 21	27	192 ± 20	33	146 ± 14	44
	70	116 ± 12	42	110 ± 11	51	90 ± 9	62
	98	121 ± 5	54	103 ± 8	61	82 ± 9	75
	112	109 ± 12	59	102 ± 9	67	50 ± 5	79
	168	90 ± 9	74	63 ± 6	81	44 ± 5	90

The loss of ascorbic acid in percent for each temperature was calculated following the first-order kinetic equation (Eq. 1).

Tab. 2. Concentration of 5-hydroxymethylfurfural during storage at various temperatures.

Juice	Time of storage [d]	(2 ± 1) °C	(7 ± 1) °C	(20 ± 2) °C
		5-Hydroxymethylfurfural [mg·l^{-1}]		
Carrot	28	*LOD*	*LOD*	*LOD*
	70	0.92 ± 0.09	0.92 ± 0.09	0.99 ± 0.09
	84	1.98 ± 0.09	1.27 ± 0.09	1.35 ± 0.09
	98	1.85 ± 0.09	1.70 ± 0.09	1.63 ± 0.09
	168	1.70 ± 0.09	1.56 ± 0.09	2.27 ± 0.09
Grapefruit	42	0.92 ± 0.09	0.92 ± 0.09	0.92 ± 0.09
	70	1.21 ± 0.09	1.21 ± 0.09	1.78 ± 0.09
	98	1.56 ± 0.09	1.56 ± 0.09	2.20 ± 0.09
	112	3.20 ± 0.09	3.20 ± 0.09	8.81 ± 0.30
	168	8.24 ± 0.30	8.20 ± 0.30	11.58 ± 0.30

LOD – limit of detection (0.26 mg·l^{-1}).

($p < 0.05$). In general, decrease in a^* values is connected with degradation of carotenoids and anthocyanins [28]. Significant increase ($p < 0.05$) in b^* values (yellowness) was identified for both types of juices, while Koca et al. [29] reported significant decrease of L^* and b^* values in GJ as a function of storage time and temperature. Along with the increase in b^* values, also an increase in *BI* and ΔE were determined (Fig. 1). The values of *BI* increased by approximately 30 % on average in both juices. Based on the observed ΔE values, the storage conditions caused not noticeable (ΔE up to 0.5) to slightly noticeable (ΔE up to 1.5) changes, a little higher for CJ. In opposite, Dede et al. [30] reported ΔE values up to 10 in pasteurized CJ. The statistical analysis confirmed that *BI* and ΔE values were mostly related to changes in b^* parameter.

Lee and Nagy [31] found a high correlative relationship between the loss of AA and increase of browning in stored grapefruit juices. During the initial stage of browning, possible reactions happen between amino compounds and some degradation products of AA, followed by condensation of present products with each other [32]. In absence of oxygen, oxidation of AA to dehydroascorbic acid (DHAA) is suppressed and only little browning occurs in juices. However, browning can proceed further under anaerobic conditions, if DHAA has been already formed in juices [4].

Effect of temperature

Although the rates of AA degradation in the two tested juices were different, they showed similar trends of decrease at various temperatures. ANOVA analysis revealed significant differences ($p < 0.05$) in AA degradation between all storage temperatures, except for (2 ± 1) °C vs (7 ± 1) °C ($p > 0.05$) for both types of juices. The changes in AA concentration in juices stored for 168 days at (20 ± 2) °C were the most critical (Tab. 1), resulting in 90% and 63% loss in GJ and CJ, respectively. Correspondingly, Lee and Nagy [31] detected more than 98% decrease of AA in GJ during 6 weeks, but at an elevated temperature of 50 °C.

Based on the results of a previous study on orange juice with vitamin E addition [33], we could suppose that an increased retention of AA in CJ might be attributed to the presence of vitamin E, which could stabilize AA by slowing down its oxidation to DHAA. Carrot contains approximately five-fold more vitamin E in comparison to grapefruit. Likewise, any higher initial concentration of AA in CJ may contribute to better stability of AA and DHAA, as indicated by the study dealing with behaviour of both forms of vitamin C in pure solutions of different concentrations [34].

In accord with AA changes, ANOVA analysis showed also significant differences in all colour characteristics as a function of temperature. Colour differences between (2 ± 1) °C and (7 ± 1) °C were negligible, but both GJ and CJ stored at (20 ± 2) °C exhibited significantly lower a^* values and higher b^*, *BI* and ΔE values in comparison to samples stored at (2 ± 1) °C and (7 ± 1) °C. The results confirmed that an enhanced storage temperature can boost the colour losses in both juices. Data on certain negative effects of higher storage temperatures were previously published [29, 30]. Koca et al. [29] also reported that the development of browning in orange and tangerine juices was temperature-dependent, whereas browning in lemon and grapefruit juices might be

attributed to their higher acidity. AA as the main agent in browning of citrus juices and concentrates [35] can decompose easily in strongly acidic media, thus accelerating the colour changes.

The changes in concentration of HMF showed some opposite trends towards AA behaviour (Tab. 2). Accumulation of HMF was in connection with a concurrent degradation of AA, which might relate with the fact that the Maillard reaction, AA decomposition and caramelization are reactions mostly responsible for HMF generation [32]. After 168 days, the highest level of HMF was recorded in both juices stored at (20 ± 2) °C, representing the mean increase from 0.26 mg·l^{-1} to 2.27 mg·l^{-1} in CJ, and from 0.92 mg·l^{-1} to 11.58 mg·l^{-1} in GJ. At this temperature, a strong inverse correlation of AA and HMF was determined (r = –0.7953 in CJ and r = –0.7584 in GJ), whereas a moderate inverse correlation was found for both juices at (7 ± 1) °C (r = –0.6665). Our results are similar to those of Ordóñez-Santos et al. [36], who observed an increase in HMF content from 3.95 mg·kg^{-1} to 9.94 mg·kg^{-1} in tomato purée after 180 days of storage at 20 °C. The growth of HMF correlated well with a decrease in organic acids concentration, e. g. ascorbic, citric and malic acid. The referred study stated some dependency of HMF production on temperature, pH value, fruit variety and juice extract concentration.

Kinetics derived from ascorbic acid degradation

Tab. 3 summarizes the data on kinetics of AA degradation at various temperature conditions. With respect to non-linear evolution of AA degradation, the kinetic data suggested the model of the first-order kinetic reaction for describing the observed trends (Fig. 2). The first-order rate constant k and the half-time $t_{1/2}$ were calculated from the experimental data on AA degradation, according to Eq. 1 and Eq. 2, respectively.

As follows from Tab. 3, GJ exhibited faster decrease of AA than CJ in the temperature range of 2–20 °C, as a consequence of much higher k values obtained. Burdurlu et al. [16] reported

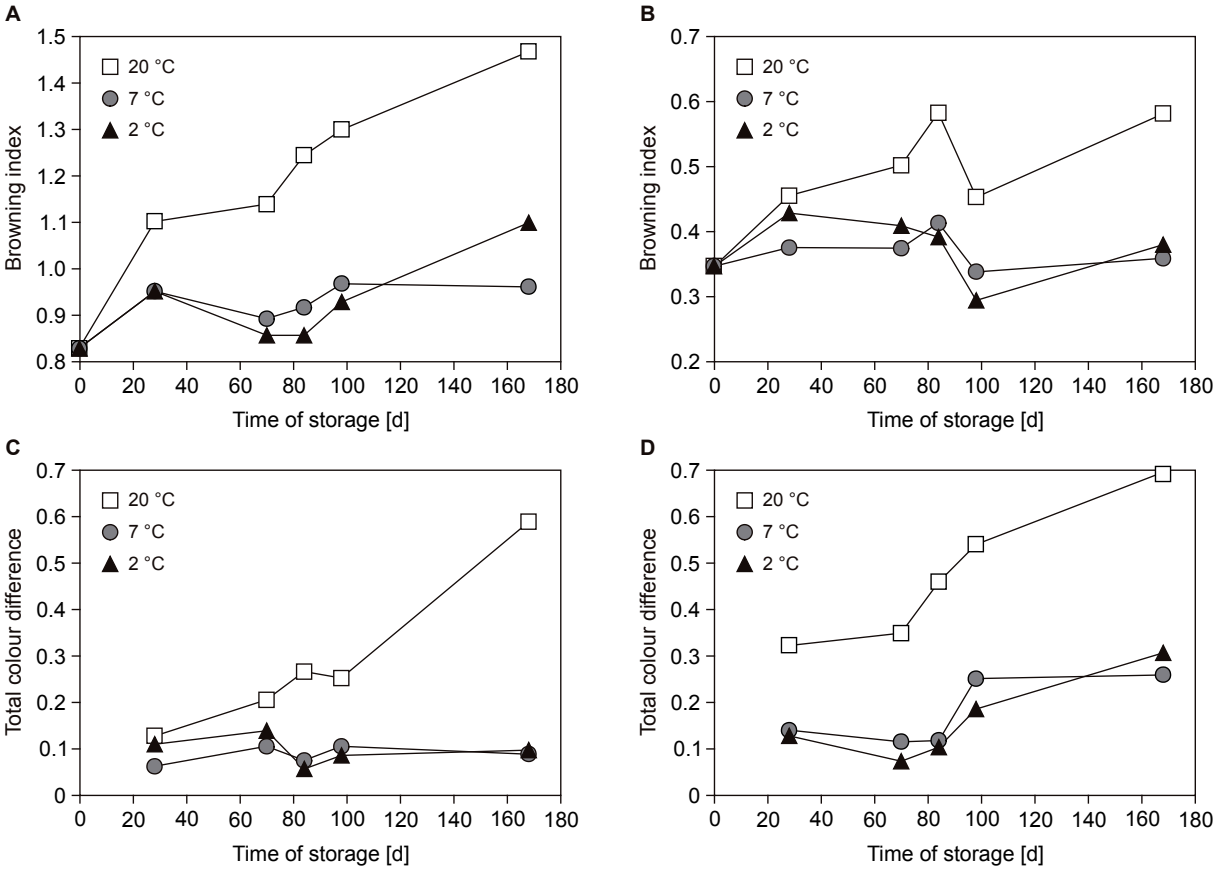

Fig. 1. Changes in browning index and total colour differences of grapefruit and carrot juices stored at various temperatures.

A – changes in browning index in grapefruit juice, B – changes in browning index in carrot juice, C – total colour difference in grapefruit juice, B – total colour difference in carrot juice.

Tab. 3. Kinetic stability of ascorbic acid.

Juice	Storage temperature [°C]	k [d^{-1}]	R^2	$t_{1/2}$ [d]
Carrot	20 ± 2	0.0059 ± 0.0006	0.9689	117.5
	7 ± 1	0.0032 ± 0.0004	0.9399	216.6
	2 ± 1	0.0032 ± 0.0006	0.8693	216.6
Grapefruit	20 ± 2	0.0156 ± 0.0019	0.9595	44.4
	7 ± 1	0.0098 ± 0.0010	0.9638	70.7
	2 ± 1	0.0092 ± 0.0014	0.9216	75.3

k – first-order rate constant, R^2 – correlation coefficient, $t_{1/2}$ – half-time of degradation.
The half-life period $t_{1/2}$ refers to the whole period of storage, e.g. 168 days.

the rate constant of k ~ (0.0470 ± 0.0067) in reciprocal week (R^2 = 0.9326), which corresponds with the value of (0.0069 ± 0.0007) d^{-1} for AA decomposition in grapefruit juice concentrate stored at elevated temperature of 28 °C during 8 weeks. This value appears to be much lower when compared with our result acquired for GJ (k ~ (0.0156 ± 0.0019) d^{-1}; R^2 = 0.9595) related to GJ stored for 168 days at (20 ± 2) °C (Tab. 3).

The half-time of AA degradation $t_{1/2}$ represents the kinetic index derived from the rate constant k. It was affirmed in our experiments that the higher the rate constant, the shorter the half-time, which is in agreement with some previous kinetic investigations [16, 37]. Regarding the temperature effect, $t_{1/2}$ of AA degradation was indirectly proportional to the storage temperature. The half-life of AA degradation was evidently longer in CJ than that in GJ at (7 ± 1) °C and (20 ± 2) °C. This result may indicate a potential to re-adjust the shelf life of CJ. According to data in Tab. 3, the loss of 50 % of the initial AA concentration could be achieved after 216.6 days and 70.7 days in CJ and GJ, respectively, at storage conditions that comply with the juice producer's recommendation (between 2 °C and 10 °C for single-strength juices).

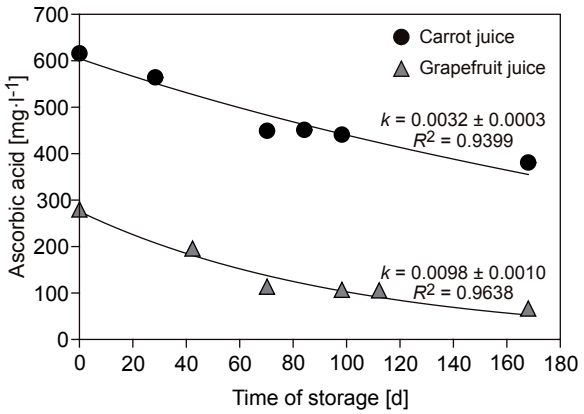

Fig. 2. The first-order reaction model of ascorbic acid degradation in carrot and grapefruit juices stored at (7 ± 1) °C.

CONCLUSION

In this study, juices of different origin were investigated at setting that simulated the storing conditions in common sales areas, usually equipped with refrigerating boxes thermostated at 2–10 °C. Based on the examined loss of AA, formation of HMF as well as colour changes, it was found that long-term storage conditions, in particular time and temperature, can markedly affect the quality and shelf life of juices. Results of this study may be useful in a practical sense, as a tool for prediction or re-adjustment of the shelf life of juices, to commercialize them during the entire interval when their quality is acceptable.

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