Stability and microstructure of powdered pulp of the Palmer mango obtained by the process of lyophilisation

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ABSTRACT - The aim of this work was to study the stability of powdered mango pulp obtained through the process of lyophilisation, as well as its structural characteristics using electron microscopy analysis. In response to an earlier experimental design using maltodextrin concentrations of 0%, 10%, 20% and 30% and periods of 24 and 30 hours, the condition of 20% maltodextrin and 24 hours drying was chosen as the best for drying mango in a freeze dryer. The stability study was therefore carried out using powdered mango pulp obtained under the above best-drying conditions, stored for 90 days and monitored every two weeks by analysis of the moisture, ascorbic acid content, b* colorimetric parameter and hygroscopicity. Storage was at room temperature (27 °C) in vacuum and non-vacuum laminated packaging, and in non-vacuum plastic packaging. The vacuum laminated packaging maintained the initial characteristics of the stored product for longer, especially the ascorbic acid content, hygroscopicity and b* colour parameter. The microstructure of the sample was then studied under different concentrations of maltodextrin (0%, 10%, 20% and 30%) so as to understand the effect of the drying agent on the final product. Close-up images of the powder revealed the porous surface formation and the effect of the agent on particle size, where higher concentrations of maltodextrin resulted in more porous powders with smaller particles.

Key words: Drying. Maltodextrin. Hygroscopicity. Microstructure.

RESUMO - O objetivo deste trabalho foi o estudo de estabilidade da polpa de manga em pó obtida pelo processo de liofilização, assim como das suas características estruturais através de análise de microscopia eletrônica. Em resposta ao delineamento experimental realizado precedentemente utilizando concentrações de maltodextrina de 0%; 10%; 20% e 30% e tempos de 24 e 30 horas, foi escolhida a condição de 20% de maltodextrina e 24 horas de secagem como a melhor para secagem de manga em liofilizador. Portanto, o estudo de estabilidade foi realizado com a polpa de manga em pó obtida a partir desta melhor condição de secagem armazenada durante 90 dias, com acompanhamento quinzenal através de realização das análises de umidade, teor de ácido ascórbico, parâmetro colorimétrico b* e higroscopicidade. O armazenamento foi feito a temperatura ambiente (27 °C) em embalagem laminada com e sem vácuo, e plástica sem vácuo. A embalagem laminada a vácuo apresentou maior manutenção das características iniciais do produto armazenado, principalmente para o teor de ácido ascórbico, higroscopicidade e parâmetro de cor b*. Posteriormente foi realizado o estudo da microestrutura da amostra em diferentes concentrações de maltodextrina (0%; 10%; 20% e 30%) com finalidade de compreender a interferência do adjuvante no produto final. As imagens aproximadas do pó revelaram a formação de superfície porosa e a influência do adjuvante nas dimensões da partícula, na qual maiores concentrações de maltodextrina resultaram em pó de menores e mais porosos.

Palavras-chave: Secagem. Maltodextrina. Higroscopicidade. Microestrutura.
INTRODUCTION

Mango is one of the main species of tropical fruit grown in Brazil, with Brazilian production reaching 1,132,449 tons of fruit in 2014 (BRAZILIAN INSTITUTE OF GEOGRAPHY AND STATISTICS - IBGE, 2017). The pulp is used in the preparation of sweets, purees, slices in syrup or canned, jellies, juices and nectars, and can be added to ice creams, juice mixtures, liquors and other products. The fruit is of nutritional importance, being especially rich in phenolic compounds, carotenoids, ascorbic acid and dietary fibres (CAPARINO et al., 2012; JAHURUL et al., 2015).

The Palmer variety of mango is characterised by fruit with a yellowish, firm flesh, good flavour (21.6º Brix) and little or no fibre. It is well accepted for direct consumption in the domestic market, and is used by the canning industry for processing, which has resulted in a marked increase in cultivated area (BRAZILIAN AGRICULTURAL RESEARCH CORPORATION - EMBRAPA, 2004; MONACO, 2015).

However, the mango is highly perishable due to its high moisture content (>80%), and deteriorates over a short period of time if not handled properly, resulting in a loss of quality and in total deterioration. The combined effect of a lack of post-harvest facilities and of technical knowledge by the farmers concerning proper handling and storage, contributes to these losses (CAPARINO et al., 2012).

There are several preservation techniques that can be applied to extend shelf life and add value to the fruit. According to Shofian et al. (2011), freeze drying is the method that achieves the highest standard of quality in the dried product in relation to colour, taste, texture and nutritional value; it is suitable for drying foods which contain considerable amounts of heat-sensitive antioxidants, such as ascorbic acid, carotenoids and phenolic compounds. The method prevents the proliferation of microorganisms and reduces the rate of lipid oxidation, thereby increasing shelf life. Studies are carried out under varying operating conditions to establish the ideal concentration and drying agent for each process and each type of raw material (SAGAR; KUMAR, 2014).

Knowledge of the storage conditions of the powdered product is of great importance for maintaining quality; for example, determining the best environmental conditions and packaging to protect the product from external influences (JAYA; DAS, 2009; SAGAR; KUMAR, 2014).

Scanning electron microscopy can rapidly provide information on the morphology, particle-size distribution and identification of chemical elements in a solid sample. It is a versatile technique that allows the observation and analysis of the microstructural characteristics of solid objects through three-dimensional images of the sample. By means of this tool it is possible to analyse the porous and amorphous structure of the surface of the powders obtained through lyophilisation (EINFALT; PLANINŠEK; HROVAT, 2013).

Based on the above, the aim of this work was to study the stability of powdered mango pulp over 90 days, in order to predict the behaviour of the material during its shelf life, in addition to a comparative study of the effect of adding a drying agent on the structural characteristics of the powder, using scanning electron microscopy.

MATERIAL AND METHODS

The mature, in natura Palmer mangoes used in this research were obtained from Embrapa Semiárido, in Petrolina in the State of Pernambuco. The mangoes were first selected and washed in running water to remove any dirt and other physical contaminants that might be present. After processing, the mango pulp was kept in a freezer at -20 ºC for analysis.

The experimental design of the research project used mango pulp formulated in four concentrations of maltodextrin DE20, 0% (control), 10%, 20% and 30%, and 2 drying times, 24 and 30 hours. On the day of the drying process, the pulp was thawed at room temperature and separated through 16-mesh (1 mm aperture) stainless steel sieves to remove pieces of skin and pit (from the pulping). The drying agent (maltodextrin DE20) was then added to the pulp, and the mixtures were homogenised in a Tecnal Turratec model TE-102 stirrer for 5 minutes at a speed of approximately 15,000 rpm.

The analyses of moisture, hygroscopicity, ascorbic acid content, colorimetry and yield were used as response variables. At the end of the trial, the drying condition of 20% maltodextrin and 24 hours was chosen as the best for lyophilisation of the pulp of the Palmer mango based on the analysed parameters. Thus, by affording the best responses in relation to the raw material in question, this drying condition was used to obtain the powdered mango for the stability study.

For the process of lyophilisation, the pulp had been previously arranged in stainless steel trays (18 cm in diameter) and placed in a Coldlab model CL 120-86V ultra-low freezer at -40 ºC to form a frozen sheet of the product. After 24 hours, the trays were placed in a Liopol L101 freeze dryer, where the samples remained for 24 hours in a vacuum at -50 ºC, and where under these conditions drying takes place by sublimation of the water.
At the end of the lyophilisation process, the samples were removed from the trays and ground (Marconi model 048 mill, 20-mesh screen) to obtain the powdered product. The drying stages to obtain the powdered mango pulp used in the analysis were carefully developed under similar conditions, so as to avoid changes due to unwanted interference when obtaining the powders.

For the stability study, the powdered mango pulp was packed into three different types of packaging immediately after obtaining the powder: vacuum laminated packaging, non-vacuum laminated packaging and non-vacuum plastic packaging, and stored at room temperature. The study was carried out for 90 days, with analysis every two weeks. Each analysis was carried out in triplicate. The results were analysed by analysis of variance (ANOVA) to determine whether there were differences between the samples, and by Tukey’s test at a confidence level of 95% to determine any difference between the mean values.

The moisture content was measured on a series ID-V1.8 model ID50 infrared balance at 105 ºC and a significance level of 0.05%. The ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol indicator, as described by the Association of Official Analytical Chemists (1997). The colour parameters were established using a Konica Minolta model CR-410 spectrophotometer, with the results expressed in the CIELAB colour system. Hygroscopicity was determined as per the methodology described by Goula and Adamopoulos (2010), in hygroscopic cells at a relative humidity of 75.0%.

The microstructure was studied using the powdered mango pulp obtained by freeze drying for 24 h with the addition of maltodextrin at concentrations of 10%, 20% and 30%. The aim of the methodology was to verify the effect of the drying agent on the morphology and surface of the resultant powder.

The morphology and surface of the powders were evaluated by a model Inspect 50 scanning electron microscope (SEM). A gold support was used as the target for the electron beam, to which the powders were adhered in a QUORUM model 150T ES coater using double-sided adhesive tape.

**RESULTS AND DISCUSSION**

**Stability study**

The samples were kept in powdered form, showing a little agglomeration during the last 30 days of storage, especially for the samples stored in a vacuum, which did not prevent analysis of the stability study of the powder (shown below), each quality attribute being evaluated over 90 days at intervals of two weeks. Table 1 shows the results for the moisture of the powdered mango pulp during the storage period of the stability study.

Starting from the 45th day of the stability study, there was no significant difference (p<0.05) between the vacuum and non-vacuum laminated packaging. But it can be seen that there was a significant difference throughout the stability period between the laminated and plastic packaging. It can therefore be inferred that the presence or absence of oxygen in the storage medium is not a determinant factor of changes in the moisture content of the powdered mango pulp. On the contrary, the superior barrier properties of the laminated material compared to the plastic material have a positive impact on maintaining the initial moisture of the powder due to its chemical composition and multilayers.

### Table 1 - Mean values and standard deviation for moisture of the powdered mango pulp in vacuum and non-vacuum laminated packaging and in non-vacuum plastic packaging during the stability period

| Time (days) | VL<sup>1</sup> | NL<sup>2</sup> | NP<sup>3</sup> |
|-------------|---------------|---------------|---------------|
| 0           | 4.37 ± 0.06 bc,A | 4.37 ± 0.06 bc,A | 4.37 ± 0.06 b,A |
| 15          | 3.52 ± 0.31 a,c | 4.73 ± 0.32 c,B | 5.76 ± 0.03 e,A |
| 30          | 4.26 ± 0.09 c,c | 3.69 ± 0.19 a,c | 5.77 ± 0.11 e,A |
| 45          | 4.41 ± 0.26 b,c | 4.82 ± 0.15 c,B | 6.34 ± 0.11 d,A |
| 60          | 4.18 ± 0.10 c,B | 4.18 ± 0.21 ab,B | 6.53 ± 0.10 cd,A |
| 75          | 4.85 ± 0.11 b,B | 4.73 ± 0.10 c,B | 6.75 ± 0.14 c,A |
| 90          | 4.09 ± 0.23 c,B | 4.37 ± 0.19 bc,B | 7.58 ± 0.15 a,A |

Significant at a confidence level of 95% (p<0.05). Mean values followed by the same lowercase letter in the same column do not differ statistically. Mean values followed by the same uppercase letter on the same line do not differ statistically. <sup>1</sup>VL - Vacuum laminated. <sup>2</sup>NL - Non-vacuum laminated. <sup>3</sup>NP - Non-vacuum plastic
In the vacuum laminated packaging, there was no significant difference at a confidence level of 95% between the initial value and the periods of 30, 45, 60 and 90 days. The greatest moisture content seen in relation to the VL packaging was for the period of 75 days, with a gain of ±11% relative to the initial moisture content. There was no significant difference between the moisture content at the start and the end of the 90 days for the non-vacuum laminated packaging. Similar results were obtained by Moura (2010) for acerola pulp dehydrated in a spray dryer and also stored in laminated packaging, in a stability study over a period of 360 days. The use of non-vacuum plastic packaging together with the atmosphere resulted in the greatest increase in moisture, with a gain of ±73% during the stability period, showing a significant difference between almost all the stability times, with results between 4.37 ± 0.06% and 7.58 ± 0.15% moisture.

Moisture content is an important variable in powdered mango, as it is related to the drying efficiency and stability of the product. The moisture content of a dehydrated product plays an important role in determining its fluidity, viscosity, quality and storage stability due to its effect on glass transition and crystallisation behaviour (SANTHALAKSHMY et al., 2015). The moisture content of the powdered mango pulp stored in laminated packaging ranged from 3.52 ± 0.31% to 4.85 ± 0.11%, which is sufficient to make the powdered food microbiologically safe.

Ascorbic acid is a natural antioxidant sensitive to heat and to degradation in the presence of light and oxygen due to adverse conditions of handling, processing and storage. The oxidation of ascorbic acid, besides reducing or eliminating vitamin activity, results in undesirable flavours in the product (OLIVEIRA et al., 2013). The packaging evaluated in the study showed degradation of the ascorbic acid content to a greater or lesser extent depending on the barrier properties of the material, as can be seen in Table 2.

The initial ascorbic acid content at the end of the stability period showed a significant difference (p<0.05) for each type of storage. Vacuum laminated packaging proved to be the best barrier to the degradation of the ascorbic acid in the powder. There was a significant difference (p<0.05) between the initial content of 86.51 ± 0.01 mg/100g and that at the end of the stability period of 70.45 ± 2.79 mg/100g, with losses of 18.56%; however this value can be considered low compared to losses under the other storage conditions. The use of non-vacuum laminated packaging doubles the losses of ascorbic acid, with a 48.29% reduction in the initial value. The absence of a vacuum increases losses to 57.46% when using plastic packaging, with a value for ascorbic acid at the end of the stability period of 36.80 ± 2.79 mg/100g.

Sousa et al. (2016) presented similar results in research with powdered cashew pulp when comparing storage in laminated and plastic materials, where the plastic packaging presented a marked reduction in ascorbic acid after 45 days of storage until the end of the stability period, with losses of ±22%, while the vacuum laminated

| Time (days) | Ascorbic Acid (mg/100g) |
|------------|------------------------|
|            | VL¹ | NL² | NP³ |
| 0          | 86.51 ± 0.01 ab,A    | 86.51 ± 0.01 b,A | 86.51 ± 0.01 a,A |
| 15         | 68.47 ± 0.02 c,A    | 65.17 ± 2.82 d,A | 68.43 ± 4.90 c,d,A |
| 30         | 84.61 ± 2.71 a,A    | 65.17 ± 2.84 d,b | 74.86 ± 2.81 d,B |
| 45         | 87.95 ± 0.10 a,A    | 59.23 ± 2.76 d,B | 64.04 ± 2.79 c,B |
| 60         | 81.45 ± 2.80 b,A    | 79.80 ± 2.86 b,c,A | 76.48 ± 2.76 d,B |
| 75         | 70.05 ± 2.80 c,A    | 75.04 ± 2.83 c,A | 71.77 ± 2.84 c,d,A |
| 90         | 70.45 ± 2.79 c,A    | 44.73 ± 2.79 a,B | 36.80 ± 2.78 b,C |

Significant at a confidence level of 95% (p<0.05). Mean values followed by the same lowercase letter in the same column do not differ statistically. Mean values followed by the same uppercase letter on the same line do not differ statistically. ºVL - Vacuum laminated. ºNL - Non-vacuum laminated. ºNP - Non-vacuum plastic
packaging showed losses of ±12%. The authors explain that the laminated packaging has good barrier properties to light, gas and water vapour, which are responsible for the degradation of ascorbic acid.

The results relative to the mean value of the b* colour parameter during the 90 days of storage are described in Table 3, and reveal the behaviour of the powders for the different types of packaging.

The b* colour parameter is associated with yellow (+b*) and blue (-b*), relating the decrease in values for this parameter during the stability period to colour degradation in the yellow band for the loss of colour characteristic of mango pulp. The storage showed a significant difference (p<0.05) between the vacuum and non-vacuum laminated packaging throughout almost the whole stability period. However, non-vacuum storage proved to be independent of the material used, showing no significant difference (p<0.05).

There was a decrease of only 6.12% between the initial value of b* (14.05 ± 0.15) and the value at the end of the 90-day study period (9.15 ± 0.19). However, the absence of a vacuum in the laminated packaging caused a fall of 17.86% between the initial value and the final value of 11.54 ± 0.14 for the 90-day study period. The non-vacuum plastic packaging showed a significant difference for storage time, with a reduction of 16.01% in the initial value of b* during the stability period.

Studies by Costa et al. (2013) with atomised powdered passion-fruit pulp showed similar results to the present study when using VL packaging. Lisboa et al. (2012) also reported the same behaviour, with no change in the b* parameter during an 80-day storage period in laminated packaging of Indian figs subjected to drying on a foam layer.

According to Harnkarnsujarit and Charoenrein (2011), mango is rich in β-carotene, a carotenoid that provides several health benefits, being pro-vitamin A and displaying antioxidant activity. However, β-carotene is unstable and susceptible to oxidative reactions due to the large number of unsaturated double bonds. Its degradation depends on many factors, such as oxygen level, heat, acid, light, enzymes and storage time. The loss of β-carotene leads to lower nutritional quality and colour loss in the product.

In relation to the hygroscopicity of the powdered mango pulp, it can be seen in Table 4 that the type of storage presented practically no significant difference (p<0.05) during the stability period. Each type of packaging used caused a reduction in the initial hygroscopic content of the powder. The use of laminated packaging, with or without a vacuum, caused no significant difference at a significance level of 5%, for almost all study times, with similar results at the end of the stability period.

Oliveira et al. (2013) found similar behaviour in studies with lyophilised macaúba powder (Acrocomia aculeata) explaining the low values for hygroscopicity as due to larger dust particles, which would entail the exposure of a smaller contact surface for bonding with the water.

The non-vacuum plastic packaging had the lowest values for hygroscopicity, with a statistical difference between the initial and final values. This behaviour is associated with the greater moisture of the sample stored in NP packaging during the stability period, since with the greater moisture, fewer sites would be available to bind to water molecules in the environment, there being a lower water concentration gradient between the environment and the product. The greater moisture with the plastic packaging is due to the inefficient barrier of the material

| Time (days) | Colour (b*) |
|------------|-------------|
|            | VL*         | VL*         | VL*         |
| 0          | 14.05 ± 0.15 a,A | 14.05 ± 0.15 a,A | 14.05 ± 0.15 a,A |
| 15         | 12.72 ± 0.04 b,A | 12.76 ± 0.04 b,A | 12.61 ± 0.09 c,A |
| 30         | 12.90 ± 0.03 b,A | 11.76 ± 0.14 e,F | 12.16 ± 0.13 d,B |
| 45         | 13.27 ± 0.18 b,A | 12.04 ± 0.05 d,e,B | 11.78 ± 0.02 e,B |
| 60         | 13.11 ± 0.11 b,A | 12.27 ± 0.07 d,B | 12.35 ± 0.12 c,d,B |
| 75         | 12.76 ± 0.49 b,A | 11.08 ± 0.08 c,B | 11.43 ± 0.02 b,B |
| 90         | 13.19 ± 0.19 b,A | 11.54 ± 0.14 f,B | 11.80 ± 0.03 e,B |

Significant at a confidence level of 95% (p<0.05). Mean values followed by the same lowercase letter in the same column do not differ statistically. Mean values followed by the same uppercase letter on the same line do not differ statistically. *VL – Vacuum laminated. **NL – Non-vacuum laminated. *NP – Non-vacuum plastic
to water, which was easily bound to the molecules of low molecular weight (fructose, glucose and sucrose) present in the powdered pulp (RIBEIRO, 2014, SOUSA et al., 2016).

Studies by Molina et al. (2014) with lyophilised pitaya pulp displayed different results to the present study, with a considerable increase in the amount of water absorbed by the powder, especially in the plastic packaging. There was an increase in hygroscopicity in the powders for both types of packaging in relation to storage, with a significant difference between the plastic and laminated packaging.

According to Sousa et al. (2016), packaging plays an important role in maintaining quality when storing powdered pulp, as it protects the product from external environmental factors such as temperature, relative humidity, light and physical damage. Therefore, selecting the best condition is relevant to minimise undesirable changes and prolong the shelf-life of the product.

**Scanning electron microscopy**

The samples used to study the microstructure of the powdered mango pulp were dried for 24 h in a freeze dryer and formulated with 10%, 20% and 30% concentrations of maltodextrin, so as to verify the effect of the drying agent on the morphology and surface of the resultant powder. The results generated by the SEM showed the powder obtained through the process of lyophilisation as being entirely amorphous, without the long-range transactional orientation symmetry that characterises a crystal (Figure 1).

Increases in the amount of drying agent (Figure 2) caused greater fragmentation of the dried product, with particles of smaller diameter and more regular size. The lyophilisation process caused the formation of obvious pores on the surface of the powder, especially in Figures (c) and (d) with 20% and 30% maltodextrin.

Caparino et al. (2012), researching the drying of mango pulp, found greater porosity in powders obtained by lyophilisation compared to other drying methods. This happens because the solid state of the water in the material helps to prevent shrinkage and the collapse of the structure and shape, resulting in an insignificant change in volume.

Unlike the present study, research with lyophilised guava pulp carried out by Conceição, Fernandes and Resende (2016) demonstrated that at higher concentrations of sucrose, lyophilised powder presents lower surface porosity, thereby reducing hygroscopicity during storage; a behaviour explained by the high hygroscopicity of sugars, especially non-reducing sugars such as fructose, being more hygroscopic than sucrose.

Obtaining amorphous solid material occurs either through the abrupt freezing of the material, rapid precipitation and/or direct solid conversion. Fast freezing prior to lyophilisation avoids crystallisation of the solutes. Solutes that do not crystallise are converted into amorphous solids at the end of the lyophilisation process, when the temperature drops below the glass transition temperature (Tg) of the concentrated solute, mostly due to sublimation of the solvent. The Tg is determined by components of the formulation and by the presence of residual water, which may act as a plasticiser, reducing the glass transition temperature. The grinding process used on the dry material to obtain the powder is responsible for the direct conversion of a crystalline form to the amorphous form by mechanical action (EINFALT; PLANINŠEK; HROVAT, 2013).

### Table 4 - Mean values and standard deviation for the hygroscopicity of the powdered mango pulp in vacuum and non-vacuum laminated packaging and in non-vacuum plastic packaging during the stability period

| Time (days) | VL\(^{1/}\) | NL\(^{2/}\) | NP\(^{3/}\) |
|------------|------------|------------|------------|
| 0          | 7.06 ± 0.31 a,A | 7.06 ± 0.31 b,A | 7.06 ± 0.31 a,A |
| 15         | 4.56 ± 0.63 b,B | 5.83 ± 0.28 bc,A | 4.64 ± 0.35 bc,B |
| 30         | 6.55 ± 0.57 ab,A | 6.00 ± 0.98 bc,A | 5.32 ± 0.74 b,A |
| 45         | 4.76 ± 0.49 b,A | 4.79 ± 0.21 ac,A | 3.78 ± 0.62 c,A |
| 60         | 6.16 ± 1.18 ab,A | 3.51 ± 0.90 a,A | 4.05 ± 0.41 c,A |
| 75         | 5.33 ± 1.11 ab,A | 5.96 ± 0.44 bc,A | 4.48 ± 0.25 bc,A |
| 90         | 5.20 ± 0.19 ab,B | 5.21 ± 0.72 ac,B | 3.81 ± 0.06 c,A |

Significant at a confidence level of 95% (p<0.05). Mean values followed by the same lowercase letter in the same column do not differ statistically. Mean values followed by the same uppercase letter on the same line do not differ statistically. \(^{1/}\)VL - Vacuum laminated. \(^{2/}\)NL - Non-vacuum laminated. \(^{3/}\)NP - Non-vacuum plastic.

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Figure 1 - Scanning Electron Microscopy (SEM) of the powdered mango pulp obtained by drying in a freeze dryer, without the addition of maltodextrin (a), with the addition of 10% maltodextrin (b), with the addition of 20% maltodextrin (c) and with the addition of 30% maltodextrin (d). 500x magnification in all images

CONCLUSION

The process of obtaining powdered mango pulp with added maltodextrin by lyophilisation results in material of excellent quality in terms of physical, chemical, rheological and nutritional characteristics, and of shelf life. The procedure guarantees physical and physicochemical stability during 90 days of storage for the types of packaging under study. Analysis by scanning electron microscopy (SEM) identifies characteristics of amorphous powder in the powdered mango pulp, demonstrating that increases in maltodextrin concentration cause the formation of smaller particles. Pores are formed on the surface of the powder, especially in samples with 20% and 30% maltodextrin.

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