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Evaluation of encapsulant type on product characteristics of encapsulation of bromelain enzyme crude extract from pineapple peel by spray drying

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Pineapple peel is an agricultural waste that could be a source of bromelain enzymes. This study aims to identify and obtain the type of encapsulant material that is efficient for product characteristics in the encapsulation of crude extract of bromelain enzyme from local pineapple peel (*Ananas comosus* [L.] Merr.) in Kediri Regency, East Java, Indonesia, using the spray dryer method. This study applied three encapsulant treatments, namely T0: 10% maltodextrin, T1: 8% maltodextrin and 2% chitosan, T2: 8% whey and 2% gum arabic. The parameters observed included yield, dry matter content, density, solubility, total protein content, enzyme activity, particle morphology (SEM), and identification of functional groups (FTIR). The results showed that the combination of 8% maltodextrin and 2% chitosan encapsulants (T1) produced the highest yield of 10.07%, with a total protein of 6.65% and enzyme activity of 8.79 IU/mL ($p<0.01$). T1 also produced smaller and more uniform particles (32.68 µm) and displayed characteristic bromelain functional groups in the FTIR spectrum. These findings suggest the use of maltodextrin-chitosan mixture as an efficient encapsulant material in maintaining the stability and activity of bromelain enzymes and have the potential to be developed as a supplement, feed additive, and functional food ingredient.

Keywords: Bromelain, Chitosan, Encapsulation, Maltodextrin, Pineapple peel, Spray drying

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Introduction

Pineapple peel accounts for 30-40% of total pineapple fruit processing, which is generally considered agricultural waste[1]. Pineapple peels hold great potential as a source of bromelain enzymes. Bromelain is a proteolytic enzyme extracted from pineapple plants, which is gaining much attention from the pharmaceutical and medical industries due to its medicinal and nutraceutical uses. Bromelain has a variety of functions, including antiplatelet, anti-inflammatory, anti-edematous, anti-thrombotic, and can support the immune system in the body by increasing the activation of immune system cells, and reducing oxidative stress[2]. Feeding bromelain to poultry reduces the population of *Escherichia coli* bacteria in the gut, can improve the morphology of the small intestine, and increase growth and performance[3].

Bromelain has autodigestion properties[4,5] and also, in liquid form, is unstable and quickly degraded, especially under acidic conditions and high temperatures, so this can lead to a decrease in enzyme activity over time[6]. This poses a challenge in the long-term utilization of bromelain. Encapsulation has become a widely used strategy in various industries due to its ability to defend the enzyme from degradation and extend its active life under less favourable conditions. In addition, encapsulation also provides control over the release of the encapsulated compound, allowing for targeted and controlled delivery[7].

The type of material used in bromelain encapsulation plays an essential role in determining stability, bioactivity, size, shape, and characteristics. Maltodextrin is often used as an encapsulant material because it can provide protection that minimizes enzyme denaturation during the drying process, increases solubility and dispersibility in aqueous environments, and has proven to effectively protect bioactive compounds and increase their stability[8]. The encapsulant combination of 8.5% maltodextrin and 1.5% chitosan showed a significant increase in antioxidant and antimicrobial activity and better

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stability during storage compared to the other formulations[9]. The combination of gum arabic and whey resulted in better true metabolizable energy (TME), nitrogen corrected true metabolizable energy (TMEn), and ileal protein digestibility values[10], as well as high encapsulant efficiency, smaller particle size, and more excellent retention of antioxidant activity compared to using a single encapsulant[11].

Encapsulation by spray drying is a commonly used encapsulation technique because the process is simple and uses equipment that is easy to find. The advantages of spray drying include fine spherical products (10-50 μm)[12], lower moisture content (9.83%)[13], longer shelf life (up to 6 months), increased stability[14], can maintain enzyme activity and protein content, and increase protein digestibility in the small intestine[15]. The results showed that the spray-drying encapsulation method could better protect the crude extract of bromelain than the freeze-drying method. With enzyme activity ranging from 23.70 to 100.83 U/g of human digestive simulation, the spray-drying encapsulation method is stable at low pH and can maintain its activity in the intestine[16].

This study aims to obtain an efficient encapsulant type for encapsulating crude bromelain extract from pineapple peel through spray drying. It will focus on identifying product characteristics and the enzymatic quality of bromelain.

Materials and Methods

Material

The pineapple peels used were from local pineapple fruits in Kediri that were almost ripe (15 months after planting). They were collected from the Ngancar sub-district, Kediri district, East Java, Indonesia. The pineapple plants used in this study were taxonomically identified as *Ananas comosus* [L.] Merr. The variety used is a local cultivar from Kediri, namely Queen.

Research method

This study used an experimental method with three treatments (T0, T1, T2) and six replications. The pineapple peel was manually separated using a knife, washed with clean water, dried, cut into small pieces, and juiced using a Hi-Cook slow juicer (SJ-LB No. 19A080, China). The juice was centrifuged at 4000 rpm for 20 minutes, and the supernatant was collected as a crude bromelain extract and stored at -4°C[17]. The extract was then encapsulated using a spray drying method with three formulations: T0 (10% maltodextrin), T1 (8% maltodextrin + 2% chitosan), and T2 (8% whey protein + 2% gum arabic). Each encapsulant mixture was homogenized with a magnetic stirrer (1000 rpm) and ultrasonic disruptor for 20 min before being dried in an SD-18A spray dryer at an inlet temperature of 165°C and an outlet temperature of 70°C.

The encapsulated powder was analyzed to calculate the yield, density[18], and solubility[19]. The analysis of total protein, dry matter, and enzyme activity was carried out in the nutrition laboratory of the Faculty of Public Health, Airlangga University, Surabaya, Indonesia. The calculation of total protein used the micro-Kjeldahl method (AOAC, 1995) with a nitrogen-to-protein conversion factor of 6.25, and for the enzyme activity test based on the amount of L-tyrosine released in one minute per mL of sample. The activity unit of bromelain is defined as 1 microgram of L-tyrosine released in 1 minute per mL of the sample when casein is hydrolyzed under standard conditions, namely at 37°C and pH 7.0 for 10 minutes. Morphological analysis was performed using SEM (Hitachi Flexsem1000) at 1,000x to 20,000x magnification, with surface area estimated using ImageJ software. Fourier-transform infrared (FTIR) analysis was performed using a Shimadzu IRSpirit spectrophotometer to identify functional groups. Data were statistically analyzed using one-way ANOVA and Duncan's multiple range test to determine significant differences between treatments.

Results and Discussion

The volume of the crude extract of pineapple peel

Pineapple peels as much as 500 g produced an average of 120 mL of crude extract, or 24%. Differences in oil extract yield are influenced by peeling techniques, pineapple varieties, extraction methods, and maturity levels[1,20]. Another study showed an increase in the volume of bromelain extraction from the peels of pineapple cultivars Nang Lae and Phu Lae when using extractants containing cysteine and ethylenediaminetetraacetic acid (EDTA), with a volume ranging from 152 to 162 mL per 100 g of fruit peels[21].

Effect of treatment on yield

The addition of 120 mL of bromelain enzyme crude extract in each treatment showed a significant difference in yield, and the highest value was found in treatment T1 (10.07%), followed by treatment T0 (8.86%) and T2 (4.71%) ($p < 0.01$) (Table 1). This

Table 1 — Characteristics of physicochemical properties and encapsulation quality of bromelain enzyme crude extract

Variable	Treatment			*p*-value
	T0	T1	T2	
Yield (%)	8.86±1.79[b]	10.07±0.87[b]	4.71±0.11[a]	0.001
Dry matter (%)	95.96±1.75[b]	97.83±1.80[b]	92.78±1.29[a]	0.001
Density (Kg/cm^3)	1.03±0.002[b]	1.03±0.003[b]	1.01±0.004[a]	0.042
Total Protein (%)	7.26±0.75	6.65±0.75	6.55±0.11	0.179
Enzyme Activity (UI/mL)	8.54±0.03[b]	8.79±0.13[b]	5.73±0.02[a]	0.001
Dissolved in:				
- n-hexane	Insoluble	Insoluble	Insoluble	
- Chloroform	Insoluble	Emulsified	Insoluble	
- 70% Ethanol	Insoluble	Insoluble	Emulsified	
- Methanol	Emulsified	Emulsified	Emulsified	
- Water	Soluble	Soluble	Soluble	

Data are presented as means±SD. Different superscript letters (a–b) within a row indicate significant differences.

shows that the combination of chitosan and maltodextrin encapsulant mixture, with good wrapping ability, resulted in a higher yield of encapsulated crude extract of bromelain enzyme.

The powder yield obtained after the spray drying is influenced by the type and amount of dressing material used. Some studies show that alginate-chitosan dressing has a higher encapsulation efficiency of 86% than alginate dressing alone at 77%[22]. Other studies have shown that using a combination of maltodextrin and gum arabic resulted in higher encapsulation efficiency, which supports the finding that a mixture of these ingredients can improve yields[23]. Other studies have shown that a combination of maltodextrin, gum arabic, and gelatin in a ratio of 77.5:20:2.5 at a density of 30% produced the highest powder yield[24].

The selection of the type and combination of encapsulant ingredients significantly affects the emulsion characteristics (viscosity, stability, droplet size) formed during the encapsulation process. These differences in emulsion characteristics will ultimately affect the drying efficiency and yield of the final product obtained[25].

Effect of treatment on dry matter

The results of dry matter showed significant differences, with the highest value found in treatment T1 (97.83%), followed by treatment T0 (95.96%) and T2 (92.78%) ($p < 0.05$) (Table 1).

The spray drying process produces dry materials with high dry matter content. In this process, liquid materials containing active substances or bioactive components are sprayed into a drying chamber at a high temperature, thereby evaporating the water and producing a dry powder. One of the key factors affecting dry matter content is the use of binders such as maltodextrin, which serves as a drying agent and protector of sensitive components against oxidation and degradation during the drying process[26].

The moisture content in encapsulated products can be influenced by several factors, including the ratio between the encapsulated material and the dressing material (wall), as well as the temperature of the incoming (inlet) and outgoing (outlet) air during the spray-dried process[27]. The spray-drying encapsulation method can produce products with a moisture content of around 3.70%[28].

Spray drying produces products with low moisture content that can increase the shelf life and stability of the encapsulant material[14]. However, too dry bromelain during spray drying can reduce enzyme activity, and it is recommended that bromelain enzyme products coming out of the spray drying machine have a minimum moisture content of 8 and a maximum of 10%[13].

Effect of Treatment on Density

The results showed significant differences in the density of the encapsulation with the highest value of T0 and T1 (1.03 g/cm³) and in the T2 treatment (1.01 g/cm³) ($p < 0.05$) (Table 1).

The inlet and outlet temperatures of spray drying affect the density; if the temperature is too low, it results in low evaporation, resulting in encapsulation with a high density and easy clumping, and vice versa. If the temperature is too high, it causes excessive evaporation, membrane cracks, degradation, or loss of the encapsulated core[12].

The difference in bromelain encapsulation density occurs because each coating type has a different density. The molecular weight of maltodextrin ranges from 500 to 3,600, depending on the Dextrose

equivalence (DE) value[29]. Like maltodextrin, the density of chitosan also varies, generally ranging from 10-500 kDa[30]. The density of whey protein hydrolysate ranges from 300 to 1400 Da[31]. Commercial gum arabic shows that the average molecular weight varies from 460,000 to 1,020,000[32]. Gum arabic has a relatively high molecular weight and complex molecular structure, and there is a large amount of starch in it, making it more hygroscopic and complex. As a result, the water in the material is more retained and difficult to evaporate.

Effect of treatment on total protein

The highest total protein results were found in treatment T0 (7.26%), followed by treatment T1 (6.65%) and T2 (6.55%). Although there were differences in values in the study's results, there were no significant differences in all treatments ($p > 0.05$) (Table 1).

Previous studies have shown that maltodextrin is often used in encapsulation due to its ability to increase the stability and bioavailability of active compounds in extracts. Maltodextrin acts as a protective barrier for proteins under dry conditions. Maltodextrin replaces the role of water in forming hydrogen bonds with proteins. In addition, maltodextrin also forms a rigid protective structure around the protein, making it difficult to deform and remain active[33]. In this context, using maltodextrin as a binder to encapsulate pineapple peel extract may explain why this treatment resulted in the highest total protein.

Other research results show that using chitosan coating can protect the total protein of the bromelain enzyme by up to 84%[34]. Using chitosan coating and cross-linking with glutaraldehyde resulted in an average encapsulation efficiency of 96.29% with a bromelain protein content of around 89.40 µg/mL[35].

Effect of treatment on enzyme activity

The results showed significant differences in enzyme activity, and the highest was found in treatment T1 (8.79 UI/mL), followed by treatment T0 (8.54 UI/mL) and T2 (5.73 UI/mL) ($p < 0.01$) (Table 1).

Encapsulation of bromelain using maltodextrin as a dressing in the spray dryer process can increase the stability and ease of use of the encapsulated powder in the food industry and protect the activity of protease enzymes in the digestive process in humans[16]. Low molecular weight chitosan as a coating can maintain bromelain enzyme activity up to 79%[4]. Chitosan as a dressing material can protect enzyme activity in gastric fluid conditions, and bromelain encapsulants can be released in the intestinal environment with a dressing success rate of 96.29%. These results indicate that maltodextrin and chitosan can protect bromelain enzyme activity during encapsulation[35].

Effect of encapsulant material on solubility

The results showed that the encapsulation of bromelain enzyme crude extract in treatments T0, T1, and T2 tended to be soluble in polar solvents; more specifically, water solvents showed perfectly soluble results. Treatment using non-polar solvents showed that the results were insoluble (Table 1).

Bromelain has properties that are soluble in water but insoluble in organic solvents. The structure of bromelain has many polar functional groups, such as hydroxyl (-OH), amino (-NH2), and carboxyl (-COOH) groups[36]. These polar groups can form hydrogen bonds with water molecules, which causes bromelain to dissolve easily in water.

Maltodextrin microcapsules showed high solubility in water solvents[37]. Microencapsulation of stevia extract with maltodextrin increased its solubility by about 35% compared to without encapsulation[38]. Encapsulation of pineapple juice with a spray dryer using 20% maltodextrin and 1% alginic acid had a solubility in water of up to 99.76%[16]. The solubility of bromelain plays a vital role in determining its absorption rate in the digestive tract, especially in the intestine, which ultimately affects its effectiveness as a health supplement.

FTIR Analysis

The FTIR test results showed similar spectrum patterns between treatments T0, T1, and T2 because they showed identical absorption bands despite using different encapsulants. Further analysis revealed the presence of broad hydroxyl O-H bands at wave numbers 3270.02 (T0), 3275.77 (T1), and 3303.77 (T2), indicating the presence of alcohol or phenol. The absorption bands at 1589.06 (T0), 1595.52 (T1), and 1594.8 (T2) indicate the presence of NH2 amine groups. The absorption bands at 2681.92 (T0), 2922.48 (T1), and 2928.95 (T2) indicate the presence of carboxylic acids, characterized by the presence of C-H stretching vibrations. The presence of alkanes was also detected, supported by C-H absorption bands at 2921.76 (T0), 2922.48 (T1), and 2928.95 (T2). The absorption bands at 1027.54 (T0), 1028.25 (T1), and 1036.15 (T2) may be the result of C-O vibrations on

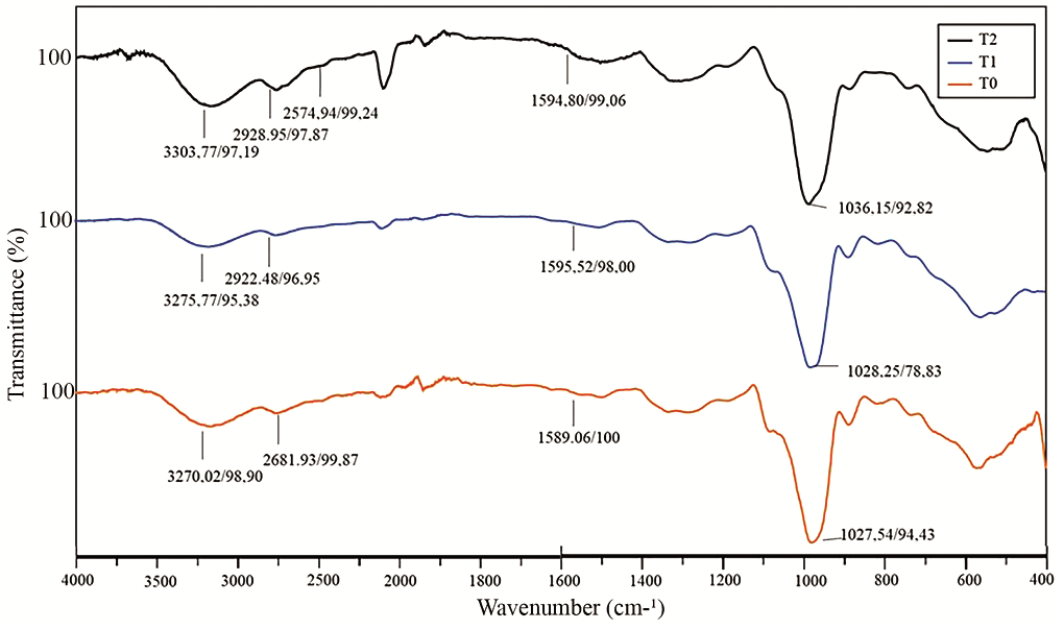

Fig. 1 — FTIR of TO, T1, and T2 treatments.

the aromatic ring of tyrosine, C-O vibrations of hydroxyl groups, or C-N vibrations on amides. These functional groups are shared characteristics of the amino acids' side chains and leading chains comprising bromelain (Fig. 1 and Table 2).

Encapsulation of bromelain showed the presence of hydroxyl (OH) bands at wavelengths of 3400, 1600, and 1400, which are related to symmetric and asymmetric carboxylate ion vibrations[39]. General characteristics of bromelain protein with the presence of nitrogen-hydrogen bonds in the spectrum between 3338-3380, 1517-1587, 1255-1290, and 1179-1149, which indicate the presence of carbon-nitrogen bonds, and at 1640-1700 indicates the presence of carbon-oxygen double bonds (C = O)[40].

Morphology of bromelain enzyme crude extract encapsulation

The encapsulation morphology of the bromelain enzyme crude extract, with a magnification of 1,000, 5,000, and 10,000 times showed differences in the treatments T0, T1, and T2.

SEM results at 10,000 times magnification showed that the T0 and T1 treatments had relatively similar morphology and particle distribution. The particles were round in shape and tended to clump together. The particle surface looks smooth, but the particle size in the T1 treatment is more uniform than T0. In the T2 treatment, the particle shape becomes oval and irregular, and the size is larger than in the T0 and T1 treatments (Fig. 2).

Table 2 — Typical bands of bromelain functional groups matched with IR research results

Typical absorption band of bromelain	Wave number (cm^{-1})		
	T0	T1	T2
O-H Stretching	3270.02	3275.77	3303.77
C-O Stretching	1027.54	1028.25	1036.15
NH_2 Bonding	1589.06	1595.52	1594.8
O-H Stretching	2681.93	2922.48	2928.95
CH_3, CH_2 and CH	2921.76	2922.48	2928.95

Some studies show that spray drying using maltodextrin dressing materials produces spherical particles, smooth surfaces, and little clumping[38]. Chitosan-bromelain nanoparticles produce smaller particles and a more uniform size distribution[4]. Powders obtained by spray drying, using maltodextrin as a wall material, can agglomerate due to the high water absorption properties of maltodextrin, which causes particles to stick together and cause agglomeration[37].

Based on the diagram in Fig. 3 at 1000 magnification, each treatment shows a different particle size sample distribution. The T0 treatment's average particle distribution is 45.89 µm in diameter. In treatment T1, the average particle distribution was 32.68 µm in diameter; in treatment T2, the average particle distribution was 131.43 µm in diameter.

These results indicate that treatment T1 using maltodextrin and chitosan by spray drying produces finer particles. Encapsulation produces particles of different sizes and shapes through several techniques[41]. The combination of maltodextrin and chitosan

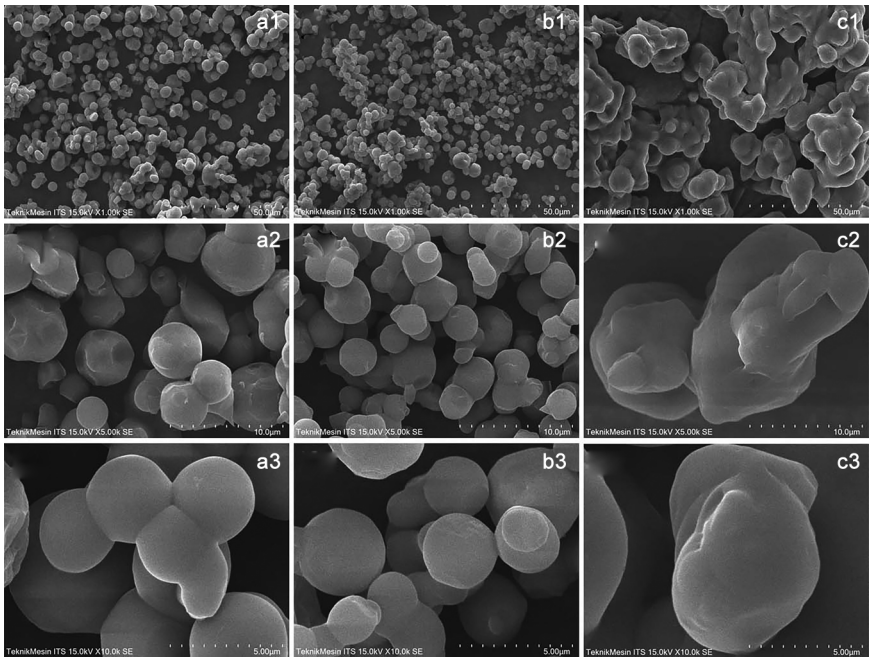

Fig. 2 — Magnification 1,000, 5,000 and 10,000 times. Treatment T0: code a, T1: code b, and T2: code c.

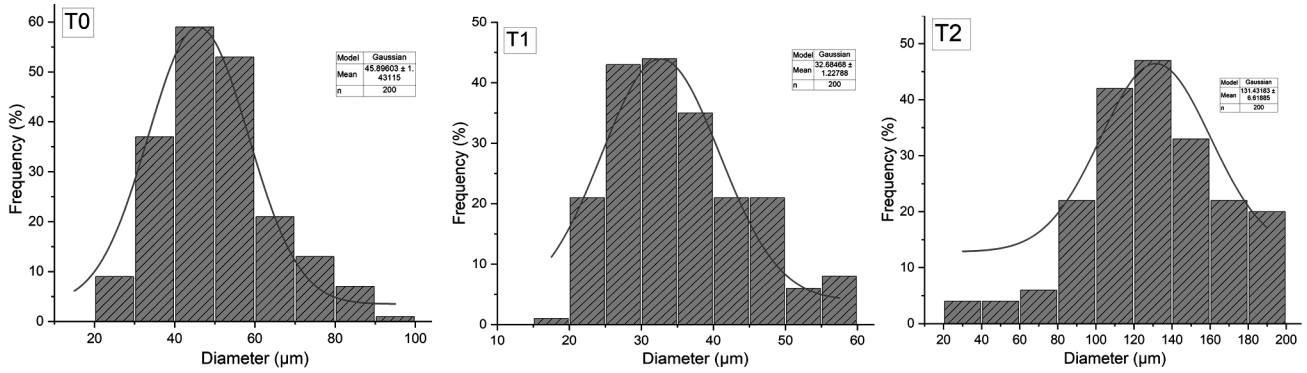

Fig. 3 — Particle size distribution diagram T0, T1, and T2.

produced nanocapsules with the smallest particle size (13.43 nm) compared to other treatments[9]. Spray-drying encapsulation technology produces suitable particles if the size is less than 40 μm[42].

Conclusion

Encapsulation of crude bromelain enzyme extract from pineapple peel by spray drying using a combination of maltodextrin and chitosan (T1) resulted in the highest yield (10.07%), maintained the highest enzyme activity (8.79 UI/mL) and produced smaller and more uniform particles with an average surface area of about 32.68 μm compared to the treatments of maltodextrin (T0) and gum arabic-whey (T2). These results demonstrate the efficiency of using maltodextrin-chitosan mixture as a promising encapsulant to improve the yield, enzymatic functionality, and microstructural characteristics of bromelain for various industrial applications.

Conflict of interest

The authors declare no conflict of interest.

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