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Research Article

Physicochemical Properties and Stability of Microencapsulated Blue Colorant from *Clitoriaternatea L.*

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A B S T R A C T

Anthocyanins are water-soluble vacuolar pigments that provide wide range of colors depending upon the existing pH. Generally known for their free radical scavenging activity and numerous health-promoting benefits, these natural pigments appeared promising making them suitable to replace existing harmful synthetic ones. However, prolonged exposure to certain environmental conditions makes them susceptible to degradation. The purpose of this study is to microencapsulate anthocyanin pigments extracted from *Clitoriaternatea L.* at pH 5 and 7 through spray-drying technique by using 5% Maltodextrin DE 11.8 as the carrier agent. The resulting physicochemical properties, particle size distribution and surface morphology are intended for its future functional applications. Stability studies in relation to color characteristics, moisture and total monomeric anthocyanin content were also evaluated to monitor its quality throughout storage. No signs of inter-particle liquid bridges among powders and a minimal noticeable color difference ($\Delta E < 4$) were detected for each colorant after 12 weeks.

INTRODUCTION

The prevailing use of synthetic dyes normally obtained from petrochemical derivatives progresses its detrimental effects to every living organism due to the continuous emission of hazardous wastes from various industries. Studies have claimed how these wastes are chemically converted to a more toxic and carcinogenic substance after being exposed to certain environmental conditions (Ratna and Padhi 2012).This has advanced the preferences of most individuals to divert into the use of materials derived from natural alternatives deemed as safer, non-allergic and with proven health-promoting properties. Out of all the plant biological sources of pigments, anthocyanin has been regarded as predominantly substantial next to chlorophyll and is being considered the most significant among all the parent class of molecules called flavonoids (Delgado-Vargas 2000).Anthocyanins account for the wide array of colors ranging from red to blue in different parts of the plant from roots up to the stem, leaf, fruits and flowers and recognized abundant in wines, tea, nuts, fruits, cocoa, cereals, honey, olive oil, vegetables, blackcurrant, red cabbage, red radish, and black carrot (Grotewold 2006).

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The basic chromophore of anthocyanins is the 7-hydroxyflavylium ion, deemed as naturally occurring which normally obtains hydroxyl substituents at positions 3 and 5 with one or more hydroxyl or methoxy substituents in the 2-phenyl- or ß ring (Bueno *et al*. 2012). The diversity of color is then dependent on its interaction with other molecules in different conditions like in the case of altering the acidity and basicity of media as its structure is ionic in nature. Changing the pH allows reversible skeleton transformation that has a broad effect on color shades.

Several in vitro and in vivo epidemiological studies have already been conducted to demonstrate its diverse therapeutic benefits. Anthocyanins are well-known for their preventive effects from oxidative stress and protecting biomembranes from oxidation because of their high phenol content. It is believed that anthocyanin pigments have antioxidant capacity twice as much compared to standard ones namely catechin, vitamin E and even the most common synthetic antioxidants such as BHA and BHT (He and Guisti 2010; Wrolstad and Culver 2012;Martin *et al*. 2016;Eghbaliferiz and Iranshani 2016).Specific molecular mechanisms of anthocyanins were elucidated exhibiting their chemoprotective activities against many types of cancers (Kocic*et al*. 2011; Devi *et al*. 2011; Bishayee*et al*. 20120; Bunea*et al*. 2013) ameliorating inflammation-associated diseases (Sohn*et al*. 2014; Akiyama *et al*. 2012; Samuels *et al*. 2013) enhancing cognitive, memory

and motor performance due to its neuro-protective activity (Youdim*et al*. 2004; Strathearn*et al*. 2014),possessing anti-diabetic activity (Jurgonski*et al*. 2013; Wu *et al*. 2013) and reducing risks of cardiovascular diseases. Further studies with regards to the various cellular processes and biochemical metabolism of anthocyanins can offer more therapeutic targets and strategies making it more substantial in food, pharmaceutical and cosmetic applications.

Clitoriaternatea L. is a local plant in tropical countries with acknowledged medicinal properties. Lines of *C. ternatea* located in its petals comprise of acylatedanthocyanins called ternatins with extensive anti-inflammatory effects (Kazuma*et al*. 2004). The extraction and microencapsulation of its bioactive compounds would provide a more adequate delivery of its functions as anthocyanins are regarded as unstable and are very susceptible to degradation rising from a lot of environmental factors including temperature, pH, presence of enzymes, and oxygen. Spray-drying technique has been commonly used widely in this manner because of its several advantagesnot to mention its simpleness, speed and low cost. Reports have also claimed how anthocyanin-derived pigments in acidic conditions are more stable. This is due to the fact that at low pH (pH < 2), additional pyran ring in the pigment structure is formed from the cycloaddition of nucleophiles at C4 and at the –OH attached to C5. The presence of the fourth ring and therefore the absence of the availability to nucleophilic attacks makes it more stable and resistant to oxidative degradation as compared to other anthocyanin precursors (Freitas and Mateus 2010; Turturica*et al*. 2010). This study then aims to microencapsulate blue anthocyanin-derived colorants of *Clitoriaternatea L*. at low acidic conditions (pH 5 and 7) through spray-drying technology in order to identify its physicochemical properties and further assess its stability.

MATERIALS AND METHODS

Materials

The plant materials were obtained from Naga City, Camarines Sur, Philippines and were kept in an ultra-low freezer to ensure preservation of its components prior to extraction. 50% (w/v) citric acid and 0.1M NaOH were used to adjust the pH, Maltodextrin DE 11.8 as the microencapsulating carrier and buffers which are 0.04M CH_3COONa (pH 4.5) and 0.025M KCl (pH 1.0). All of which are of food grade and purchased from chemical suppliers.

Extraction of Anthocyanin Pigments

Samples were subjected to liquid-solid extraction using deionized water (1:5), macerated and let stand for 24h in a cool environment (4°C). The solution was filtered using a muslin cloth followed by the adjustment of pH of the resulting extract. To achieve a vibrant blue color, the extract was adjusted to pH 5.0 and 7.0 consequently**.** Two trials per corresponding pH were to test the reproducibility.

Microencapsulation Process

A total of 5% of the microencapsulating carrier, Maltodextrin DE 11.8 was added to each solution in order to protect the bioactive compounds present. Spray-drying technology was utilized in producing the desired microcapsules. The spray-dryer was pre-heated up to 160°C with 20 mL/min feed flow rate and the airflow pressure was set to 2kg/cm^3 with a pump of 115V.

Encapsulation Yield

The encapsulation yield was calculated according to Itaciara*et al*. 2007.

% yield = MSA/MSB

MSA – total mass of solids after encapsulation
MSB – total mass of solids before encapsulation

Physicochemical properties of colorant powder

Color Characteristics

The color analysis was examined using an L100 LovibondSpectrocolorimeter. The L* a* b* values were determined followed by the conversion to their respective RGB values. Significant changes on the color characteristics were detected by computing for *ΔE*.

Moisture Content

A moisture balance (MOC-120H) from Shimadzu Corporation was used in determining the moisture content, where 1g of powder was weighed and exposed in a temperature of 105°C. A total of three replicates were performed.

Hygroscopicity

The test for hygroscopicity was based from Cai and Corke 2000 method. Approximately 1g of each sample was placed inside a desiccator with saturated NaCl solution. The samples were weighed after one week where the hygroscopicity was expressed as a gram of adsorbed moisture (g/100g)

Total Monomeric Anthocyanin Content

The determination of the total monomeric anthocyanin content was described in AOAC 2005 (Lee *et al*. 2005) using the pH differential method. 5% (w/v) of each powder in separate volumetric flasks was added with deionized water and diluted with buffers. The 3-cyanidin-glucoside equivalents were read at 520 and 700 nm in a L7 Double Beam UV Vis Spectrophotometer after 20-50 minutes.

Particle Size Distribution

The samples were suspended in an HPLC grade water prior to analysis. The equipment used was a Malvern ZetasizerNanoseries Nano-ZS90 where the particle size analysis was conducted through dynamic light scattering technique. Average particle size was determined using three measurements at 90° angle. (UPLB Nanoscience and Technology Facility Analytical and Instrumentation Service Laboratory, Laguna, Philippines)

Morphological Characterization

Micrographs of each colorant were obtained using Phenom Pro Scanning Electron Microscopy (SEM) to evaluate its surface morphology after spray-drying. The samples were placed on separate pin stubs where a thin layer of gold was applied through sputter coating technique with the use of a Jeol JFC-1200 Fine Coater. Afterwards, post-processing of images by ImageJ was conducted to retrieve analysis of 100 particles of different sizes. (PHENOM-DLSU, Malate, Philippines)

RESULTS AND DISCUSSION

At a fixed feed flow rate (20 mL/min) and temperature (160°C), the physicochemical properties of spray-dried *Clitoriaternatea* colorant at pH 5 and 7 were shown in **Table 2** with percent yields varying from 11.99% to 20.07% as presented in **Table 1**. Anthocyanins are tagged as weak diacids. The phenolic OH groups of the flavylium ion at C4', C5' and C7' are regarded as moderately acidic brought about by their conjugation with the electron-withdrawing pyrylium ring (Pina *et al*. 2012; Pina 2014). When subjected to alkaline conditions, the C4'-OH undertakes a secondary proton loss (A−) and produces an anionic base with maximized electron delocalization over the three rings. Due to this phenomenon, the wavelength of maximal visible absorption following the deprotonation sequence would normally shift from 20-30 nm (AH+ → A), then by 50–60 nm (A → A−) turning the red acidic color into purple-blue shade (Nave *et al*. 2010). The resulting L, a, b values verified this theory where the spray-dried colorant at pH 7 produced deeper blue shade than that of pH 5. The corresponding L* values indicate light (+) versus dark (-), a* corresponds to red (+) versus green (-) while b* dictates yellow (+) versus blue (-) according to Hunter's Lab color scale (Hunter and Harold 1987).

Table 1. **Yield of *Clitoriaternatea* colorant after spray-drying**

COLORANT	Powder yield (g)	Percent yield (%)
BT15-T1	150.5	20.07
BT15-T2	149.5	19.93
BT16-T1	89.9	11.99
BT16-T2	112.2	14.96

Table 2. Physicochemical properties of *C. ternatea* colorant

COLORANT	pH	Moisture content (%)	Hygroscopicity	Color Analysis L	a	b	RGB	TMA (mg/L)
BT15-T1	5.0	7.11 ± 0.69	4.96 ± 0.08	52.0	2.5	-22.3		22.38 ± 0.03
BT15-T2	5.0	7.29 ± 0.12	4.70 ± 0.04	51.9	1.7	-23.5		36.25 ± 0.18
BT16-T1	7.0	10.07 ± 0.12	3.58 ± 0.14	46.9	-1.6	-21.8		23.15 ± 0.09
BT16-T2	7.0	9.07 ± 0.15	4.51 ± 0.13	46.3	-0.9	-22.1		24.17 ± 0.02

Table 3. Color Stability of *C. ternatea* colorant for 12 weeks

COLORANT	1st week	Day 10	Day 11	Day 12	Day 13	2nd week	3rd week	4th week	12th week
BT15-T1									
BT15-T2									
BT16-T1									
BT16-T2									

Table 3.1. Color difference (ΔE) values for each colorant after 12 weeks

COLORANT	Storage duration	L	a	b	RGB	
BT15-T1	After 12 weeks	53.8	2.2	-20.3		ΔE : 2.70
BT15-T2	After 12 weeks	53.4	3.5	-20.9		ΔE : 3.50
BT16-T1	After 12 weeks	47.5	0.8	-20.8		ΔE : 2.67
BT16-T2	After 12 weeks	46.8	0.3	-20.0		ΔE : 2.47

Fig 1 ***C.ternatea***colorant at pH 5 (left) and pH 7 (right)

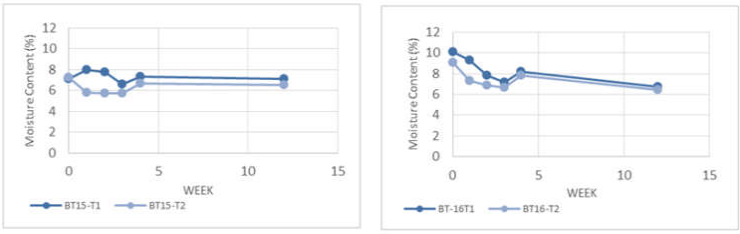


Fig 2 Moisture Analysis of *C. ternatea* colorant pH 5 (a) and pH 7 (b)

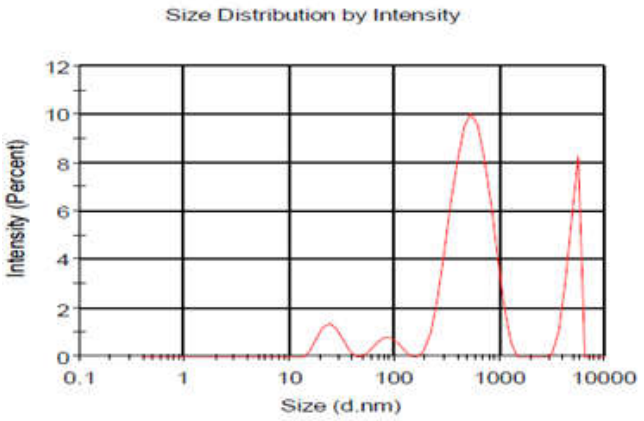


Fig 3 Size Distribution Report of *C. ternatea* colorant

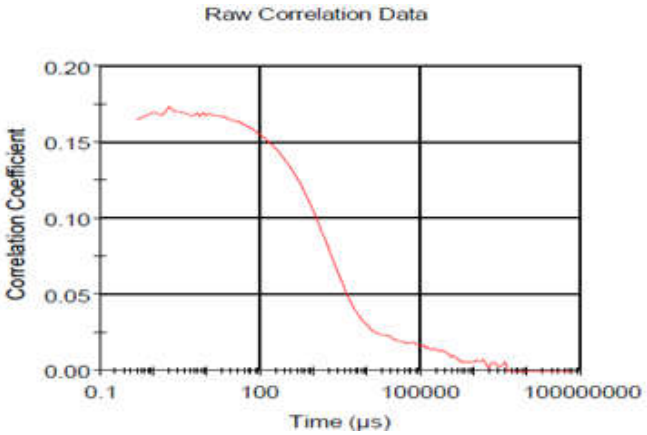


Fig 4 Size Quality Report of *C. ternatea* colorant

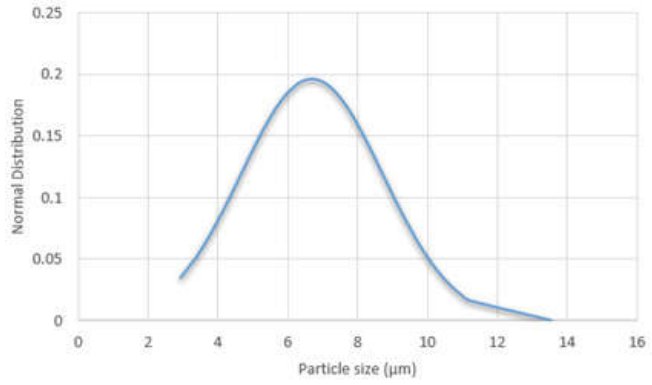
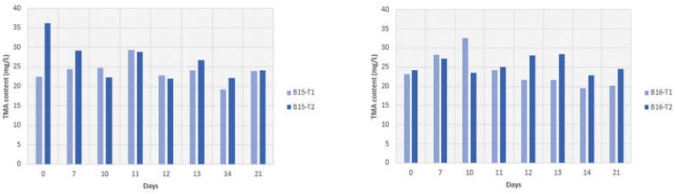


Fig 5 Distribution Curve of particle size of *C. ternatea* colorant



a b

Fig 6. Total Monomeric Anthocyanin content of *C. ternatea* colorant pH 5 (a) and pH 7 (b)

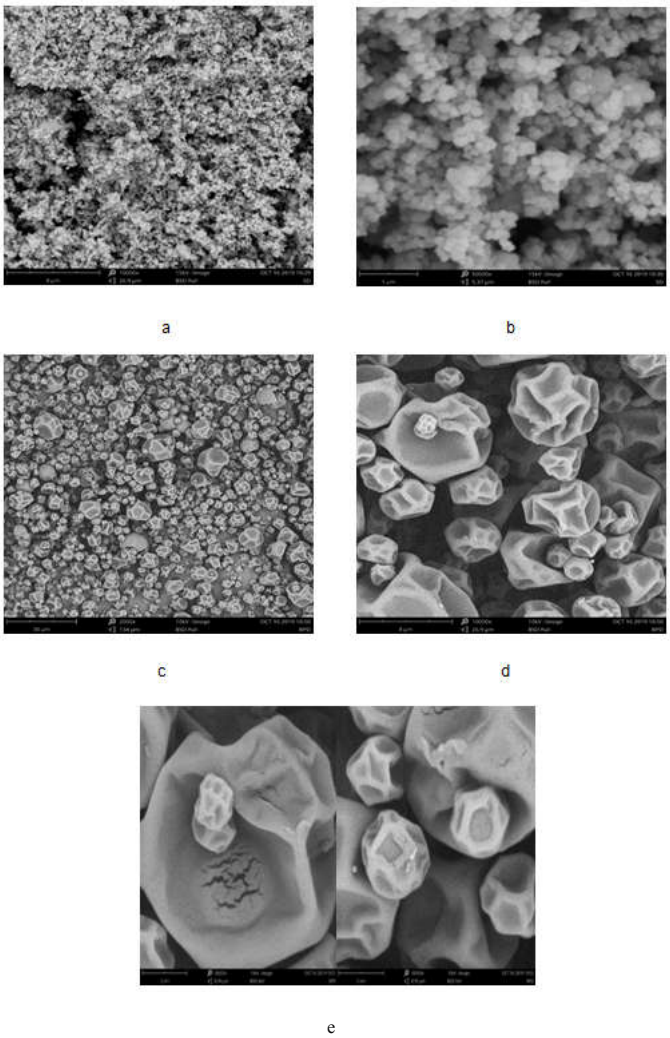


Fig 7. Scanning Electron Microscopy (SEM) micrographs of synthetic colorant and microencapsulated *C. ternatea* colorant through spray-drying at 160°C with different magnifications: 2000x, 10000x, 30000x, 50000x (a) & (b) synthetic colorant (c), (d), (e) *C. ternatea* colorant

In addition to that, it was observed that the initial moisture content of the spray-dried *C. ternatea* colorant at pH 7 was significantly higher compared to that of pH 5. Moisture affects the physical and chemical aspects of a material relative to its stability for storage and potential acquisition of microbial growth. This property should be kept evaluated throughout storage relative to the hygroscopicity. The hygroscopicity values of *C .ternatea* colorant ranged from 3.58% to 4.96%. This represents the capacity to bond with water molecules from the atmospheric air at room temperature. Furthermore, the initial total monomeric anthocyanin content for each powder were found to be 22.38mg/L (BT15-T1), 36.25mg/L (BT15-T2), 23.15mg/L (BT16-T1) and 24.17mg/L (BT16-T2).

Anthocyanins are known to have high sensitivity with heat and are the most thermally unstable among classes of flavonoids. The corresponding aglycones, are said to be degraded at alkaline and physiological conditions in less than an hour (pH = 7.4, 37 ◦C) (Fleschhut*et al*. 2006; Cabrita*et al*. 2014). Results presented in **Table 3 and Table 3.1** elaborated the changes in color characteristics for each *C. ternatea*colorant. A ΔE value less than 2 but greater than 1 denotes a noticeable color difference only by an experienced observer while ΔE at a range of 2-3 signifies a possible noticeable color difference by an unexperienced observer (Mokrzycki and Tatol, 2011). The least significant change in color was observed in B16-T2 at pH 7. The small noticeable differences in color after 12 weeks may suggest the accomplished objective of maltodextrin to reduce the reactivity of bioactive components with certain environmental conditions.

The interaction of powder with environmentally acquired moisture significantly affects the appearance and behavior. Undesirable “caking” or agglomeration can take place when too much surface moisture is adsorbed forming liquid bridges between particles that might interrupt flowability (Armstrong *et al*. 2014). Each colorant was packed with 1g moisture adsorbent after spray-drying. From the data shown in **Fig. 2**, the moisture content for each *C. ternatea* colorant decreased after the first week except for BT15-T1. A continuous decrease in moisture content was then observed during the 2nd and 3rd week for all four colorants. After a month, the values started to rise but were still lower than that of the initial readings (day 0). Unexpected further decrease in moisture content resulted after 12 weeks, the final moisture contents for each powder were 7.14% (B15-T1) 6.56% (B15-T2), 6.76% (B16-T1) and 6.41% (B16-T2). Qualitatively, following thestate of physical appearance and texture, there was no observed increased cohesiveness from the powders, denoting possible good flow properties without caking for enhanced final product performance.

The particle size can significantly affect the efficiency and appropriateness of the powder in terms of transport, storage, bulk density, flowability, handling, rehydration capacity, solubility and dispersibility (Akhavan*et al*. 2016; Paim*et al*. 2016). Particles were assessed through dynamic light scattering technique as shown in**Fig. 3 & 4**.

The size quality report claimed the presence of large particles exhibiting a high degree of polydispersity resulting to a poor sample quality. This has caused the slower decay of correlation function and intensity of scattered light due to the slowing down of movement of particles in a suspension (Brownian motion) altering the accurate interpretation of sizes. In

comparison, the average particle size was also examined through Scanning Electron Microscopy (SEM) using ImageJ with an observed peak between 6µm - 8µm as presented in **Fig. 5**.

Moreover, the chemical degradation which depicts the gradual loss of total anthocyanin brought about by irreversible degradation is due from the cleavage of the chromophore forming benzoic acid and an aldehyde derivative (Trouillas*et al*. 2016). Studies have claimed how heat exposure, both during process and storage, can influence rapid anthocyanin degradation (Cisse*et al*. 2012; Ma *et al*. 2012; Sadilova*et al*. 2007). That is, the anthocyanin stability decreased as temperature increased. In this manner, **Fig.6** showed the inconsistent peaks of the total monomeric anthocyanin concentration for each *C. ternatea* colorant after examining it per day (10^{th}-14^{th} day) and per week up to 3 weeks kept only at room temperature. The total monomeric anthocyanin content of microencapsulated powders at pH 5 and pH 7 were observed to obtain 20-30mg/L of the said pigment after subjecting to spray-drying process and being stored at constant temperature (25°C).

To support this data, a morphological characterization through Scanning Electron Microscopy (SEM) was performed. The surface morphology of powders spray-dried at 160°C presented irregularly shaped microcapsules **Fig. 7c & 7d** contrary to the morphological characteristics of synthetic colorant which possessed smooth, spherical shaped surface. **Fig. 7a & 7b**

Evident cracks and fissures on the outer surface were observed in some particles of the colorants **Fig.7e** that might be related to the slow movement of water diffusion allowing more time for the particles to deform, wrinkle and collapse. This phenomenon generally happens when heat is not high enough (Beirao-da-Costa *et al*. 2013). Higher drying inlet temperatures tend to produce particles with a smoother surface and with a low degree of teeth and concavities and this fact may be attributed to faster water evaporation and higher pressure inside the particles during microencapsulation disallowing shrinking (Loksuwan 2007). This might also be one of the main reasons behind the inconsistencies with regards to anthocyanin stability. The susceptible external physical structure of some microparticles with slight damage allows gas permeability that might lead to unwanted oxidation of bioactive compounds and undesired release (Jyothi*et al*. 2010) whilst others with higher retention of core materials remained efficiently protected by the carrier agent.

CONCLUSION

Factors significantly affecting the quality of natural colorants shall be monitored and are equally important during process and throughout storage. Protecting the core material from environmental conditions with the use of maltodextrin gave rise to reduced effects of oxidation and interaction of particles with moisture after 12 weeks. The resulting physicochemical properties and morphological characteristics of each colorant will direct to its specific applications to food, pharmaceutical, cosmetic and other industrial purposes.

Abbreviations

DE – dextrose equivalent

Declarations

Author's Contribution

Torres, R.C. devised the project, the main conceptual ideas and experimental framework, Jose, C.F., Walde, R.L., Yumang, R.G. and Canillo, D.P. contributed to the implementation of the research, to the analysis of the results and to the writing of the manuscript.

Competing Interests

The authors' declare that they have no competing interests

Availability of data and materials

The data supporting the conclusions of this article are included within the article. Any queries regarding these data may be directed to the corresponding author

Consent for publication

Authors have agreed to submit it in its current form for consideration for publication in the Journal

Ethics approval and consent to participate

Not applicable. No tests, measurements or experiments were performed on humans as part of this work

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