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Structural Analysis and Processing Characterization of Chitins Extracted from Different Sources.

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Abstract

The characteristics of three different types of chitin extracted from commonly used seafood organisms; namely crabs, shrimps, and squids were investigated. X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM) were employed to analyze these chitins' polymorphic structure and morphology. Bulk and tapped density measurements, along with Kawakita analysis, were used to assess the flowability and compression behaviour of the chitin powders. Chitin extracted from crabs and shrimp exhibited the α-polymorphic form, while chitin from squid showed the β-polymorphic form. SEM images of the two forms revealed distinct differences in chitin layer arrangement, with the α-form appearing more compact due to the anti-parallel alignment of its polymer chains. Both bulk and tapped density measurements and the calculated Hausner ratio (HR) and Carr Index (CI) indicated poor flowability for these chitins. However, Kawakita's analysis of compression and compaction properties demonstrated that both polymorphs are compressible, though they differ in their extent of compaction. Regardless of their source, chitin compacts exhibited high crushing strength. Based on the observed morphology, flowability, and compression characteristics, a blend of α- and β-polymorphic chitin could be an effective excipient for pharmaceutical solid dosage forms.

Paper type: Research paper

Keywords: Chitin; chitin sources, characterisation, compression analysis, chitin flowability.

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Introduction

Chitin is a polysaccharide made up of crystallized N-acetyl D-glucosamine monomers linked by 1–4 glycosidic bonds (Piekarska *et al.*, 2023). This structural polymer is widely found in nature, forming a key component of the shells of marine crustaceans, the cell walls of organisms like fungi and algae, and the exoskeletons of crustaceans, molluscs, and insects (Baharlouei and Rahman, 2022). Naturally, chitin can be found in three polymorphic forms: α, β, and γ (Rinaudo, 2006). These forms vary based on the crystalline structure, hydration levels, cell sizes, and polymerization degree (Kumirska *et al.*, 2010; Mucha *et al.*, 2003). The α form is the most stable and widespread, found in crustacean shells, insect skeletons, and mushrooms. The less common β form is found in organisms such as squids (Laval *et al.*, 2007). In α-chitin, molecules are arranged in an antiparallel manner, stabilized by strong hydrogen bonds. Conversely, the β form has parallel molecular packing with weaker hydrogen bonds, while γ-chitin features a pattern where one antiparallel molecule alternates with two parallel ones (Kumirska *et al.*, 2010). Variations in the crystal structure of chitin forms greatly influence their suitability for further processing. Its highly organized structure, combined with strong intra- and intermolecular interactions, limits the solubility of chitin, thereby restricting its use in industrial applications. Hajji et al. (Hajji *et al.*, 2014) examined the structural differences in chitin sourced from three marine organisms. They analyzed α-chitin from shrimp waste and crab shells, as well as β-chitin from cuttlefish bones, using ^{13}C NMR, FTIR, and XRD techniques. The ^{13}C NMR results revealed distinct structural differences between α-chitin and β-chitin. Additionally

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, XRD analysis indicated that α-chitins possess a higher degree of crystallinity compared to β-chitin. Azmi et al. (Azmi *et al.*, 2024) examined how the synthesis process affects the physical and chemical properties of the chitin extracted from Black Soldier Fly (BFS). The research indicated that chitin extracted using Natural Deep Eutectic Solvents (NADES) demonstrated greater efficacy in deacetylation and exhibited a superior conversion rate to chitosan compared to chitin obtained through organic solvents. Chitin extracted using organic solvents revealed a complex honeycomb-like structure, while the chitin obtained through NADES exhibited a more fibrous and irregular configuration. The results indicated that the chitin sourced from BFS was α-chitin. Moreover, the chitin isolated via organic solvents showed enhanced crystallinity and thermal stability relative to that derived from NADES. A comparative study was conducted by Haldar *et al.*, (2021) focusing on chitin sourced from crab shells, shrimp shells, and insect cuticles. Their findings revealed that crab shell chitin exhibited the highest degree of acetylation (95.8%) and crystallinity index (78.1%), followed by shrimp shell chitin (87.5% acetylation and 65.4% crystallinity), and insect cuticle chitin (72.4% acetylation and 52.8% crystallinity). Despite this, chitin from insect cuticles demonstrated a higher molecular weight (611 kDa) and greater thermal stability (240°C) than chitin from the other two sources. The study concluded that the properties of chitin vary significantly depending on the source, and the choice of chitin should be tailored to the specific application. Martínez-Rocha *et al*. (Martínez-Rocha *et al.*, 2019). Extracted chitin from two different types of algae (diatoms and kelp) and their properties were characterized. Algal chitin exhibited a reduced degree of acetylation and a lower molecular weight in comparison to alternative sources of chitin. Algal chitin presents promising opportunities for utilization within the agricultural sector, serving as a bio-stimulant and a soil conditioner. Hasan and Tan (2020) provided a comprehensive review concerning chitin derived from animal sources, specifically crustacean shells and fish scales. They noted that the characteristics of chitin and chitosan, such as their deacetylation and molecular weight, were influenced by the origin of the raw materials and the methods employed for extraction. The authors also explored the possible applications of these biopolymers in various sectors, including food, pharmaceuticals, and agriculture. Kumirska *et al*. (Kumirska *et al*. (2010) conducted a comprehensive review of the spectroscopic methods and techniques employed in chitin and chitosan characterization. They discussed the benefits and limitations of the used techniques. In their research, Joseph *et al*. (Joseph *et al.*, 2021) examined the characteristics and uses of chitin and chitosan, categorizing them according to their sources—marine, terrestrial, and fungal—as well as their chemical properties and the methods of extraction. Kaya *et al*. (Kaya *et al.*, 2014) conducted a physical and chemical characterization of the chitin structures in two common spider species. Their findings from XRD, FTIR, and TGA analyses confirmed that the chitin is in the α-form. Environmental scanning electron microscopy (ESEM) demonstrated unique surface morphologies for each species examined. In a review, Alimi *et al.* (Alimi *et al.*, 2023) summarized existing literature on the extraction and yield of chitin from various fruiting bodies of certain mushroom species. The authors examined techniques employed to measure the quantity of extracted chitin and detailed the physical and chemical characteristics of chitin and chitosan obtained from these mushrooms. Their review concluded by assessing the potential applications of chitosan derived from mushrooms in food packaging solutions. Understanding the characteristics of chitin from different sources is crucial for optimizing extraction and processing methods, as well as identifying its potential uses as a biomaterial in the pharmaceutical industry (Al-Hmoud *et al*., 2020). In their study, Piekarska *et al*. (Piekarska *et al*., 2023) provided a comprehensive overview of the methods for modifying chitin and chitosan, as well as conducting a life cycle assessment (LCA) that spans from the polymer's source, production, and its diverse applications.

From a processing perspective, the flow behaviour of chitin and chitosan powders is significantly compromised due to their low bulk density, which leads to inadequate flow. This phenomenon is likely a consequence of the fibrous composition of these natural polymers (Badwan *et al.*, 2015; El-hefian and Yahya, 2010). To alleviate this drawback, several approaches have been explored, including the addition of silicon dioxide to chitin and chitosan powders (Rashid *et al.*, 2008; Alonso and Belamie, 2010). This addition improves flowability, facilitating the smoother operation of tabletting machines. Moreover, combining chitin/chitosan with excipients like Avicel PH201, Starch 1500, calcium carbonate, or gelatin has been shown to significantly enhance the flow properties of composite powders (Rojas *et al.*, 2012; Chaheen *et al.*, 2018; Chaheen *et al.*, 2019). Additionally, Abu Fara et al. (2020) utilized roller compaction to improve the flow properties of chitin powder, making it compatible with industrial pharmaceutical machinery for compression and compaction. These studies collectively demonstrate that chitin is a promising excipient for pharmaceutical applications. As such, modifying chitin powder is essential for its commercialization as a novel excipient.

The current study focuses on characterizing chitin extracted from shrimp, crab, and squid, and examines the relationship between chitin's structure and its performance in the pharmaceutical tableting process.

1 Materials and Methods

1.1 Materials

Chitin powder extracted from shrimp was sourced from G.T.C. Bio Corporation (Qingdao, China). The powder was subjected to sieving and the fraction with a particle size smaller than 90 μm was selected for further analysis. In addition, chitin samples from crab and

squid were also acquired from the same corporation in the form of flakes and fibres, respectively. These samples were processed using a ring mill and subsequently sieved, with the portion having a particle size of less than 90 µm being designated for further examination.

1.2 Methods

1.2.1 X-ray Powder diffraction (XRPD), crystalline index (ICR)

The XRPD analysis was conducted utilizing an X-ray powder diffractometer (Bruker, Karlsruhe, Germany) within a 2-theta range of 2–40° in reflection mode. The X-ray system employed is a D2 Phaser, which features a copper tube that generates Kα X-rays at a power of 300 watts and a wavelength of 1.54184 Å. The crystalline index (I_{CR}) was determined from the normalized diffractograms. The peak intensities at the 110 lattice (I_{110}, at approximately 20° 2θ, which corresponds to the maximum intensity) and at around 16° 2θ (indicative of amorphous diffraction) were utilized to compute the I_{CR}, as given in Equation (1) (Al Sagheer et al. 2009).

$$I_{CR} = 100 \times \left(\frac{I_{110} - I_{am}}{I_{110}} \right) \quad (1)$$

1.2.2 Scanning Electron Microscopy (SEM)

The morphology of the samples was analyzed utilizing an Inspect F50 scanning electron microscope (SEM) from FEI Company (Eindhoven, The Netherlands) with an accelerating voltage ranging from 1 to 30kV. Approximately 0.5mg of each sample was affixed to graphite tape on an aluminium stub. Subsequently, the powder underwent sputter-coating with platinum (Emitech K550X device from Quorum Technology, based in Lewes, UK).

1.2.3 Bulk and tapped density measurement and flow determination

The bulk density of chitin powder samples (shrimp, crab, and squid) in g/mL was measured by pouring the powder into a 25mL volumetric cylinder. The bulk density of all samples was calculated as the ratio of the mass over the volume it occupies. Tapped density measurements were carried out by physical tapping of the cylinder for 100 mechanical taps then dividing the mass over the tapped volume. The cylinder was tapped again for 200 mechanical taps. If the decrease in volume (V100-V200) was less than 2 mL then the V200 was considered. If the difference is greater than 2mL, the increments are repeated, such as the 200 taps, until the difference between succeeding measurements is less than or equal to 2mL. The reduction in powder bulk volume due to tapping is considered to be an indication of powder flowability which was evaluated by the Hausner ratio (*HR*) and Carr Index (*CI*). As HR and *CI* increase in value, the flowability is reduced. HR is calculated using Equation (2), and *CI* is calculated using Equation (3) (The United States Pharmacopeia, 2020):

$$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \quad (2)$$

$$CI = 100 \times \left(\frac{\rho_{tapped} - \rho_{bulk}}{\rho_{bulk}} \right) \quad (3)$$

1.2.4 Tablet crushing strength

In order to assess the crushing strength, 415 mg of chitin powder was compressed using the Manesty single-punch tablet machine. A compression force of 35kN was exerted with a 10mm circular biconvex punch. Following this, ten tablets were tested for crushing force using the Pharma Test PTB 311E apparatus from Hainburg, Germany.

1.2.5 Compression analysis

Samples of chitin powder derived from shrimp, crab, and squid were subjected to compression into tablet form utilizing a benchtop tablet press (GTP-1, Gamlen Tablet Press Ltd, Nottingham, UK). The compression process was executed at five distinct loads: 100, 200, 300, 400, and 500kg. Each sample introduced into the die of the GTP maintained a consistent weight of 100±1mg, with the die having a diameter of 6 mm. The operation of the machine was controlled by software that generated the force-displacement (*F-D*) curve. The Kawakita model (Equation 4) was employed to analyze the compression characteristics of the powders (Nordstrom *et al.*, 2009). This analysis illustrates a linear correlation between the ratio P/C and P, where P denotes the pressure in MPa and C signifies the volume reduction. From the slope and intercept of this linear relationship, the constants ‘a’ and ‘b’ can be derived; ‘a’ indicates the

maximum volume reduction achievable by the powder, while '*1/b*' or '*PK*' represents the force necessary to decrease the powder bed volume to half of its initial value.

$$\frac{P}{C} = \frac{P}{a} + \frac{1}{ab} \quad (4)$$

Where volume reduction (C) is calculated using Equation (5)

$$C = 1 - \frac{\rho_b}{\rho_c} \quad (5)$$

where ρ_b and ρ_c are bulk and compact densities (kg/m^3), respectively.

2 Results and Discussion

2.1 X-ray Powder diffraction (XRPD) analysis

The XRPD monographs of chitins derived from various sources are illustrated in **Figure 1**. The XRPD profiles for shrimp and crab chitins exhibit four distinct crystalline reflections, which are characteristic of α-chitin, at 2θ values of 9.3, 19.1, 20.6, and 23.2°. In contrast, the XRPD profile for squid chitin reveals two wide peaks at 8.7 and 19.8°, suggesting that squid chitin is classified as β-chitin. The size of crystals in the case of shrimp and crab chitins is nearly identical, whereas squid chitin displays a smaller crystal size, as evidenced by the significantly broader peaks at 9° and 19° compared to those of crab and shrimp chitins. The crystalline index (I_{CR}) for the different chitins is presented in **Table 1**. These findings imply that α-chitin possesses a more ordered crystalline structure due to its inter-sheet and intra-sheet arrangements (Al Sagheer *et al.*, 2009; Jang *et al.*, 2004).

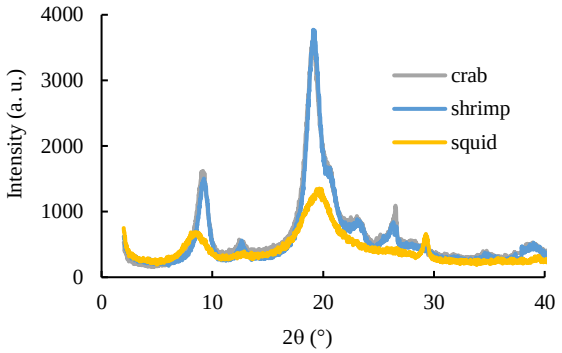

Fig. 1 X-ray powder diffraction (XRPD) patterns of crab, shrimp, and squid chitins.

2.2 Scanning electron microscope (SEM)

Table 1 Crystalline index (I_{CR}) for the different chitins.

Chitin Type	Crab	Shrimp	Squid
I_{CR} (%)	83.3±0.1	86.2±0.2	73.1±0.2

Figures 2 and **3** illustrate scanning electron microscopy (SEM) images of chitin derived from shrimp, crab, and squid at 16,000x and 30,000x magnifications, respectively. At the magnification of 16,000x (Fig. 2), it is evident that the chitin fibers in the case of squid exhibit a more organized structure compared to those from shrimp and crab. At the higher magnification of 30,000x (Fig. 3), shrimp chitin reveals two distinct fiber types: one that forms the layers and another that connects these layers. In contrast, squid chitin does not display any connecting fibers between its layers. The SEM images further highlight the parallel arrangement of β-chitin sheets, which exhibit clear spacing, suggesting a greater capacity for hydration and gelling compared to α-chitin (Jang *et al.*, 2004; Kaya *et al.*, 2015).

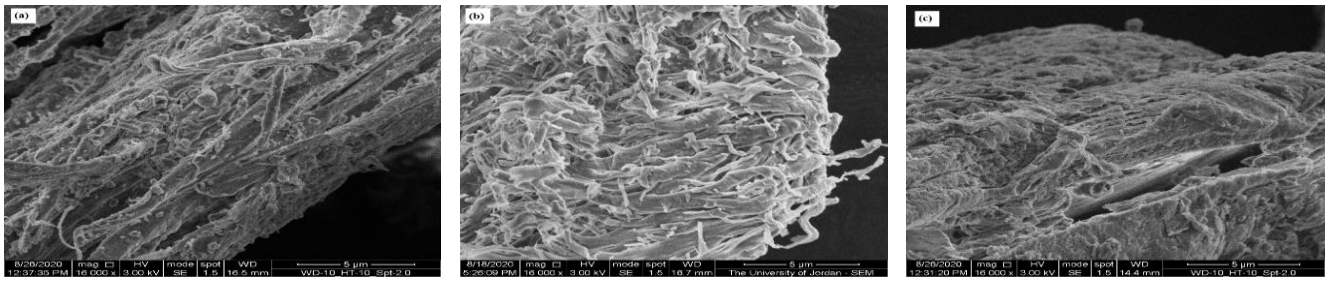

Fig. 2 SEM image of (**a**) crab chitin, (**b**) shrimp chitin, and (**c**) squid chitin at 16,000×magnification.

It can be seen that chitins from shrimp and crab exhibit bulk and tapped densities higher than squid chitin. Regarding the powder flowability of the different chitins, the Carr index (*CI*) and Hausner ratio (*HR*) were calculated (Equations 2 and 3). The results of

flowability, as presented in **Table 2**, demonstrate that chitin derived from various sources shows inadequate flowability. The criteria for interpreting flow, based on Hausner Ratio (*HR*) and Carr Index (*CI*), are detailed in the literature (Daraghmeh *et al.*, 2015).

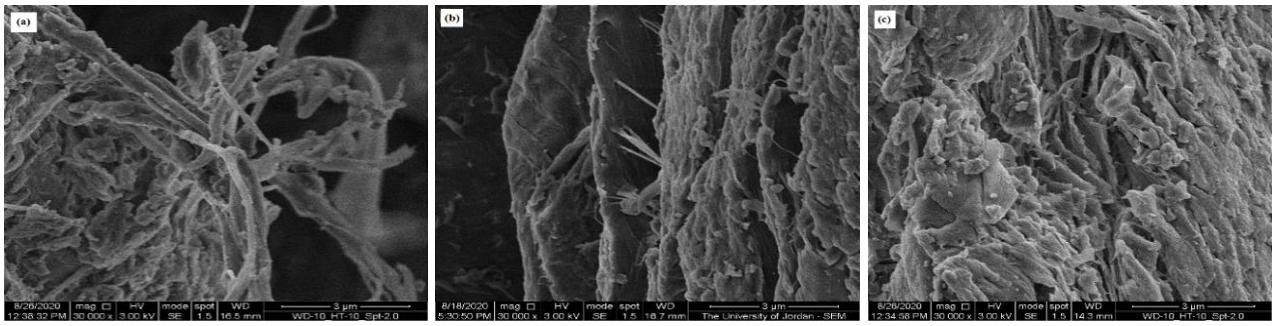

Fig. 3 SEM image of (**a**) crab chitin, (**b**) shrimp chitin, and (**c**) squid chitin at 30,000×magnification**.**

2.3 Density and flow characteristics

Table 2 presents the measured and calculated powder characteristics for chitins from different sources. The bulk densities of the three types of chitin (Table 2) differ even though shrimp and crab chitin share the same polymorphic form (α-chitin). The variation in bulk density between shrimp and crab chitin could be attributed to differences in the natural arrangement of the material in these organisms, likely influenced by their specific functional roles. Similar findings have been reported in previous studies, such as Kaya *et al.*, (2015), where chitins extracted from male and female grasshoppers showed structural differences. Therefore, it is reasonable to expect variations in chitin extracted even from the same species. The higher bulk densities of crab and shrimp chitin suggest that these powders contain more densely aggregated particles. This aggregation may result from particle-particle adherence, likely caused by the highly fibrous structure of crab chitin and shrimp chitin, while squid chitin has a much less fibrous structure (Figures 2 and 3). The smooth surface of squid chitin lacking fibrous extensions, prevents the formation of an interlocking structural network, unlike the aggregation seen in crab and shrimp chitins. As anticipated, none of the chitin varieties demonstrated satisfactory flowability, as evidenced by their elevated Hausner Ratio (*HR*) and Carr Index (*CI*) values (Table 1), attributable to their low bulk densities. This finding has prompted researchers to recommend pre-treatment of these powders via roller compaction before their application as excipients (Abu Fara *et al.*, 2020). Of the three types examined, shrimp chitin is deemed the most appropriate for tablet compression, given its lowest HR and CI values, which render it more advantageous than crab and squid chitins.

Table 2 Powder characteristics of chitins from various sources.

Chitin Type	Bulk Density (kg/m^3)	Tapped Density (kg/m^3)	*HR*	*CI*
Crab	333±9	532±8	1.60±0.04	60±4
Shrimp	280±6	401±6	1.43±0.02	43±2
Squid	190±4	338±4	1.78±0.03	78±3

3.1 Tablet crushing strength

The tablet-crushing strength of chitin derived from various sources is given in Table 3. The crushing strength of tablets from shrimp and crab chitins is approximately 50% of that of squid tablets. This disparity in results may be linked to the variations in the microstructure of the three types of chitin, as previously illustrated in the SEM images (Figs.2 and 3).

Table 3 Crushing strength of tablets.

Chitin type	Crap	Shrimp	Squid
Crushing Strength (*N*)	54±2	115±3	310±5

3.2 Compression analysis

The compression characteristics of the three varieties of chitin were examined using the Kawakita analysis method (Nordstrom *et al.*, 2009). **Figure 4** illustrates the Kawakita plots for shrimp, crab, and squid chitins. Additionally, **Table 4** provides the Kawakita parameters values for the various chitin types.

The Kawakita parameter *(a)*, which denotes the maximum achievable volume reduction, indicates that crab chitin exhibits the lowest value, while squid chitin demonstrates the highest volume reduction during the compression process. The parameter P_K, which indicates the pressure required to decrease the powder volume to half its original value, is the most critical Kawakita parameter to evaluate. This significance arises from its correlation with the hardness of chitin granules, thereby influencing their suitability for direct compression applications. As presented in Table 4, the P_K value for shrimp chitin is the highest among the three chitin types. Additionally, the Kawakita parameter *(ab)* reflects the extent of rearrangement of powder particles. According to Table 4, squid chitin exhibits the greatest degree of particle rearrangement during compression, while shrimp chitin shows the lowest value of particle rearrangement. The variations in powder compression behaviour, as indicated by the Kawakita parameters (*a*, P_K, *ab*), can be linked to variations in the particle morphology and crystallography of the different chitin types, as demonstrated by XRPD and SEM analyses.

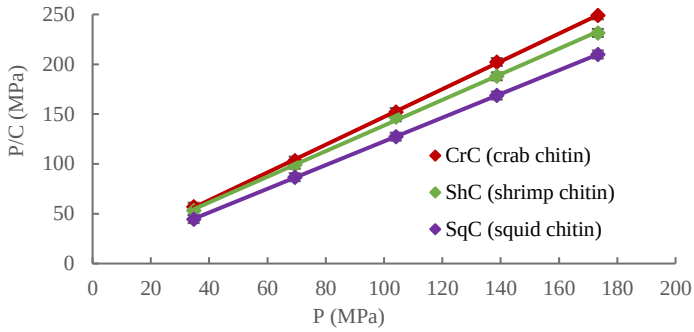

Fig. 4 Kawakita plots of crab, shrimp, and squid chitins**.**

Table 4 Kawakita parameters for crab, shrimp, and squid chitins.

Chitin Type	Slope	Intercept (MPa)	*a*=1/Slope	*b*=Slope/Intercept (1/MPa)	*ab* (1/MPa)	P_K=1/*b*(MPa)
Crab	1.392±0.065	7.83±0.35	0.718	0.177	0.126	5.65
Shrimp	1.285±0.062	9.93±0.45	0.778	0.128	0.102	7.74
Squid	1.191±0.055	3.64±0.18	0.841	0.325	0.275	3.07

Conclusions

The results confirmed that chitins extracted from various sources could differ in crystallinity and morphology, in addition to bulk and tapped densities. Consequently, such chitins would behave differently in the compression process as an excipient in the pharmaceutical industry. From a compression perspective, it can be concluded that α-chitin (shrimp and crab chitins) gives a fairly good flowability while β-chitin (squid chitin) produces tablets having good crushing strength. Consequently, for an excipient comprising chitin to have good compression behaviour and tablets, α- and β-chitin types would be mixed.

Nomenclature

C	=Powder volume reduction	[fraction]
CI	=Carr Index	[-]
HR	=Hausner Ratio	[-]
I_{CR}	=Crystalline Index	[-]
SEM	=Scanning Electron Microscopy	[-]
XRPD	=X-ray powder diffraction	[-]
ρ_b	=Bulk density	[kg/m^3]
ρ_c	=Compact density	[kg/m^3]

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