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RESEARCH ARTICLE

EVALUATION OF VIRGIN COCONUT OIL-IN-WATER EMULSION STABILITY: INSIGHTS FROM CREAMING INDEX AND POLARISED LIGHT MICROSCOPY ANALYSES

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Abstract. The stability of emulsions containing virgin coconut oil-in-water is crucial for their successful use in food, cosmetics, and medicinal products. The goal was to determine the most stable formulation by examining various ratios of VCO, deionised water, and mixed surfactants (methyl-a-D-glucopyranoside combined with either Span 20 or Span 80). This study examines the stability of these emulsions using two primary analytical techniques: the creaming index quantitatively measures the stability of an emulsion by evaluating the degree of phase separation over a period of time and the optical polarising microscope gives a qualitative evaluation by visualising the microstructure of the emulsion. The creaming index revealed that emulsions prepared with methyl- α -Dglucopyranoside and Span 20 exhibited superior stability compared to those prepared with Span 80. Samples with Span 20 showed low creaming indices, indicating minimal phase separation. Samples L1 and M1, including 15 % w/w of methyl- α -D-glucopyranoside and 15 % w/w of Span 20, were the most stable formulations; they did not phase separate at all throughout the storage time. These results were corroborated by the optical polarising microscope analysis, which showed that emulsions with Span 20 had more consistent, smaller droplet sizes at 9.44 µm and 10.00 µm, respectively, which added to their stability. On the other hand, emulsions stabilised with Span 80 exhibited decreased stability as indicated by bigger droplet sizes and greater creaming indices. In addition to providing essential information for long-lasting VCO-based emulsions for various industrial applications, the study emphasises the relevance of selecting the appropriate surfactants to improve the stability of emulsions. These discoveries enhance the progress of creating more enduring emulsions based on virgin coconut oil, which may be used in a wide range of applications such as cosmetics, food and drug deliveries.

Keywords: Virgin coconut oil, emulsion stability, creaming index, optical polarising microscope.

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1. INTRODUCTION

Emulsions are very relevant in several industries, such as pharmaceuticals, cosmetics, and food, as well as in practical applications like oil extraction and the creation of innovative nano-structured materials [1]. An emulsion is a mixture of two liquid phases that do not mix together, with one phase being dispersed into the other. An emulsion consists of two primary phases: a continuous phase, also called the external phase, where the droplets are spread, and a dispersed phase, also known as the internal or discontinuous phase [2]. An oil-in-water emulsion is a system that includes three components: a hydrophobic fat as the oil phase, an aqueous phase, and a surface-active compound interface that connects the two phases. The chemical makeup of an oil-in-water emulsion is distinct from that of a water-in-oil emulsion, and each is most effectively used in different products. Oil-in-water emulsions serve as the basis for water-based products [3]. In the pharmaceutical industry, they are commonly used in creams such as moisturisers and topical steroid treatments. Oil-in-water emulsions are a promising method for enhancing the skin penetration of lipophilic medicines due to their ease of production, strong thermodynamic stability, and potential to enhance the solubility of lipophilic drugs [4].

In order to facilitate the dispersion of all components in an emulsion, the surfactant must be able to reduce interfacial tension in the emulsion to almost zero throughout the preparation process [5]. Non-ionic surfactants are highly effective in emulsifying oils. Non-ionic surfactants provide exceptional emulsification properties and demonstrate resistance to the difficulties presented by hard water, rendering them essential in detergent and emulsifier compositions [6]. Although anionic surfactants are efficient, they have disadvantages, such as the capacity to induce skin irritation, especially in those with sensitive skin. Non-ionic surfactants, unlike other types, are milder in nature, which makes them appropriate for inclusion in personal care items [7]. Spans, also known as sorbitan esters, are non-ionic emulsifiers that are used in emulsions, lotions, and ointments. A class of commonly used, non-irritating, safe, and readily available non-ionic surfactants is the Span surfactant series. Common sorbitan monoseters include sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 80) [8].

Methyl- α -D-glucopyranoside, a glucose derivative (one of the glycosides), is frequently employed in carbohydrate chemistry as a molecule that may mimic more complex carbohydrates. Although it may not work as a conventional surfactant on its own, its derivatives or related chemicals may display surfactant capabilities [9]. Glycosides, derived from sugars found in nature, are very desirable for use as surfactants due to their biocompatibility, which makes them well-suited for incorporation into cosmetics and pharmaceuticals. Glycoside surfactants are often gentler in comparison to specific synthetic counterparts, which makes them very suitable for use in personal care products [10]. It reduces the interfacial tension between the oil and water phases by positioning itself at the oilwater interface, which facilitates the formation of smaller, more stable droplets. Methyl-a-Dglucopyranoside forms a protective layer around these droplets, preventing them from merging (coalescing) over time. This protective film contributes to steric stabilisation, as the bulky glucopyranoside head group creates a physical barrier that keeps droplets apart, reducing their tendency to coalesce. Additionally, the hydrophilic portion of methyl- α -D-glucopyranoside can form hydrogen bonds with water, enhancing the hydration of the system. This leads to an increase in the viscosity of the aqueous phase, which slows down the movement of oil droplets, thereby improving the stability of the emulsion. Opting for a blend of diverse surfactants, rather than depending on a singular kind, is a strategic method in formulation for several persuasive justifications. The combination of several surfactants frequently results in a synergistic effect, which enhances overall performance by improving stability, emulsification, foaming, and wetting qualities. This technique provides flexibility, allowing for the customisation of goods for various applications by utilising the distinct advantages of each surfactant. The combination enhances stability in different settings and provides a cost-efficient option compared to utilising a solitary, possibly pricier surfactant [11]. Hence, the current study performed an optical polarising microscope and creaming index characterisation to assess the stability of virgin coconut oil-in-water emulsion.

2. MATERIALS AND METHODS

2.1 Emulsion Preparation

All samples were prepared at room temperature. Table 1 shows the preparation of the mixture with VCO involving mixed surfactants consisting of methyl- α -D-glucopyranoside and Span 20. The composition was prepared with deionised water using a mixture of VCO and mixed surfactants. The emulsion was prepared using the approved technique that was previously used to investigate the creation and description of emulsion based on virgin coconut oil (VCO) [12]. The composition was mixed using a vortex mixer.

Set 1 - Methyl-α- <i>D</i> -glucopyranoside & Span 20						
	VCO (g)	Deionised - water (g)	Surfactant	VCO: Deionised		
Samples			Methyl-α- <i>D</i> - glucopyranoside	Span 20	Water: Surfactant % (w/w)	
J1	2.0	6.0	1.0	1.0	20: 60: 20	
K1	2.0	4.0	2.0	2.0	20: 40: 40	
L1	3.0	4.0	1.5	1.5	30: 40: 30	
M1	1.0	6.0	1.5	1.5	10: 60: 30	

Table 2 illustrates the preparation of emulsion formulation using methyl- α -*D*-glucopyranoside and Span 80.

Set 2 - Methyl-α- <i>D</i> -glucopyranoside & Span 80						
	VCO (g)	Deionised - water (g)	Surfactant	VCO: Deionised		
Samples			Methyl-α- <i>D</i> - glucopyranoside	Span 80	Water: Surfactant % (w/w)	
J2	2.0	6.0	1.0	1.0	20: 60: 20	
K2	2.0	4.0	2.0	2.0	20: 40: 40	
L2	3.0	4.0	1.5	1.5	30: 40: 30	
M2	1.0	6.0	1.5	1.5	10: 60: 30	

Table 2: Ratios of different formulations using mixed surfactants

2.2 Creaming Index

All of the emulsion samples were placed in small vial tubes with tightly sealed caps and were stored for 32 days at 25 °C. During the storage period, observations were made every four days. Typically, the oil droplets, having a lower density than the surrounding aqueous phase, moved upwards during storage, leading to creaming [12]. The creaming index [5] was calculated as:

Creaming index % =
$$\frac{\text{Height of the droplet depleted lower layer (HD)}}{\text{Height of total emulsion (HE)}} \times 100\%$$
 (1)

2.3 Droplet Size & Distribution

Using a SOPTOP CX40M optical polarising microscope (OPM) connected to a DCLR Canon Fos 550D, the droplet size of the emulsion was determined. A drop of the sample was placed onto a glass microscope slide, which was then sealed with a glass cover slide. The sample was examined under 10x magnification through the objective glass. The droplet size was determined by calculating the diameter of each droplet using SOPTOP's image analysis software, which was provided after the images were captured with a camera.

3. RESULTS AND DISCUSSION

3.1 Creaming Index

More stability is indicated by a lower creaming index (CI), which gauges the degree of phase separation in emulsions [5]. The oil phase may climb to the top (creaming) or completely separate from the water phase, indicating more severe phase separation, as indicated by a higher CI. Because it jeopardises the emulsion's consistency and efficacy, this is undesirable. A lower creaming index indicates that the emulsion is well-stabilised when the dispersed phase (like oil droplets) is uniformly distributed throughout the continuous phase (like water). This uniform dispersion prevents the oil droplets from gathering and rising, a process known as creaming. A stable emulsion ensures consistent use and effectiveness throughout time by preserving the product's texture and appearance. For instance, lotions and creams with low CI will not separate, guaranteeing that the active components are distributed evenly for consumers.

Figure 1 displays the mixtures of methyl- α -*D*-glucopyranoside and Span 20 (Set 1) while Figure 2 shows the mixtures of methyl- α -*D*-glucopyranoside and Span 80 in Set 2. Set 1 exhibited initial stability in the emulsions on Day 1. However, with time, some fluctuations were apparent. Specifically, set 1 consisted of two samples (L1 and M1) that exhibited remarkable stability when stored. Set 1 consisted of a combination of Span 20 and methyl- α -*D*-glucopyranoside as surfactants. Consequently, the product's homogeneity was preserved and ensured to be consistent. Samples J1 and K1 also demonstrated high stability, as shown by creaming indices compared with samples J2 and K2 in Figure 2 with the same formulation. Methyl- α -*D*-glucopyranoside is a sugar derivative with increased hydrophilic characteristics, which enhances its compatibility with surfactants used to stabilise oil-in-water emulsions. The combination of methyl- α -*D*-glucopyranoside with Span 20 would exhibit synergistic effects due to their hydrophilic properties, which contribute to the improved stability of oil-in-water emulsions. One of the main causes of surfactants' synergistic behaviour is the energy contributions from the interactions of their head and tail groups [13]. A more stable interface may be created by combining surfactants with complementary head groups, which maximises advantageous interactions (such as hydrogen bonds) [14].



Figure 1: Creaming index for mixed surfactant using methyl-α-*D*-glucopyranoside and Span 20 from Day 1 to Day 30

The Span has a tiny, non-ionic, hydrophilic head that interacts well with the oil phase and a hydrophobic fatty acid tail. The head group of methyl- α -*D*-glucopyranoside's sugar-based structure is more hydrophilic and interacts favourably with the aqueous phase. This combination facilitates the reduction of interfacial tension between the oil and water phases, resulting in the formation of a durable film surrounding the oil droplets, which effectively prevents their coalescence. Span 20's hydrophilic properties enhance the interaction between methyl- α -*D*-glucopyranoside and the aqueous phase, resulting in improved stability and uniformity of the emulsion.

On the other hand, due to its higher affinity for lipids, Span 80 is more appropriate for waterin-oil emulsions and may have less effective interaction with methyl- α -D-glucopyranoside. The discrepancy in solubility preferences might result in less stable emulsions due to the surfactants' inability to build a cohesive interface around the dispersed droplets.

By comparing Figures 1 and 2, Set 2 did not reach the same level of stability as the formulations in Set 1. Samples K2 and L2 demonstrated initial stability on Day 1, however, variations persisted over time. Sample M2 exhibited the lowest level of stability compared to the other samples in Set 2, but Sample L2 had the highest level of stability among them. This discrepancy highlights the variations in the effectiveness of surfactant mixtures under different formulations and circumstances.

Analysis of Samples L1 and M1, which utilised a combination of surfactants, revealed an absence of creaming and separation, thereby demonstrating the emulsion's stability. This robust stability was attained through the employment of superior emulsifiers and the meticulous optimisation of droplet size, which together ensured consistent and enduring product quality. These findings highlight the critical role of strategic surfactant selection and precise droplet size management in sustaining emulsion stability.



Figure 2: Creaming index for mixed surfactant using methyl-α-*D*-glucopyranoside and Span 80 from Day 1 to Day 30

When deciding whether Span 20 or Span 80 is better to blend with methyl- α -D-glucopyranoside for stabilising an emulsion, it is crucial to consider the properties of each surfactant and how they interact with the glucopyranoside. Span 20 (sorbitan monolaurate) has an HLB (hydrophilic-lipophilic balance) value of around 8.6, making it more hydrophilic and suitable for oil-in-water (O/W) emulsions in which water is the continuous phase. Span 80 (sorbitan monoleate), on the other hand, has an HLB value of around 4.3, making it more lipophilic and suitable for water-in-oil (W/O) emulsions in which oil is the continuous phase [15].

When comparing surfactant combinations, Set 1, which employed both Span 20 and methyl- α -*D*-glucopyranoside, achieved excellent stability, with samples exhibiting minimal creaming. This is attributed to the synergistic effect of combining Span 20's hydrophilic properties with the surfactant-like characteristics of methyl- α -*D*-glucopyranoside, enhancing the stability of O/W emulsions. On the other hand, Set 2, which combined Span 80 with methyl- α -*D*-glucopyranoside, did not match the stability observed with Span 20, reflecting the less optimal interaction between the lipophilic Span 80 and the hydrophilic glucopyranoside.

In contrast, Span 80's more lipophilic nature means it is better suited for W/O emulsions and may not interact as effectively with methyl- α -D-glucopyranoside. This mismatch in solubility preferences can lead to less stable emulsions because the surfactants may not form a cohesive interface around the dispersed droplets.

3.2 Droplet Size Distribution

The analysis of droplet size in emulsions using an optical polarising microscope reveals several factors influencing the variation in droplet size. The concentration and type of surfactant are critical determinants. Surfactants reduce the interfacial tension between the oil and water phases, facilitating the formation of smaller droplets. Higher surfactant concentrations generally lead to smaller droplet sizes due to better stabilisation of the oil droplets in the continuous water phase [16]. Additionally, the proportion of oil to water in the emulsion significantly influences droplet size. A higher oil content can result in larger droplets because there is more oil to be dispersed within the same amount of water, while a higher water content tends to produce smaller droplets due to the higher dilution of the oil phase.

Different surfactants have varying efficiencies in stabilising emulsions. For instance, the use of methyl- α -*D*-glucopyranoside, Span 20, and Span 80 in the provided samples affects the droplet size differently due to their distinct molecular structures and hydrophilic-lipophilic balance (HLB) values. Because of its hydrophilic nature with an HLB value of 8.6, which enables it to interact with the water phase and stabilise oil droplets more effectively, Span 20 is generally more effective at producing finer dispersions in O/W emulsions. In contrast, Span 80 which has an HLB value of 4.3 is better suited for W/O emulsions or situations where larger droplet sizes are acceptable [17]. It is possible to optimise the formulation for particular applications, such as in food goods, medicines, or cosmetics, by having a better understanding of the HLB value and molecular properties of each surfactant. The method and intensity of mixing during emulsion preparation also play a crucial role. High-shear mixing or ultrasonication typically results in smaller droplet sizes due to the increased energy input that breaks down the oil phase more effectively.

Notable synergistic effects were seen when methyl- α -*D*-glucopyranoside was combined with either Span 20 or Span 80 in mixed surfactant compositions. In the experiment described in Table 3, when methyl- α -*D*-glucopyranoside and Span 20 were combined (Set 1), Sample K1 had an average droplet size of 9.44 \pm 0.405 µm. This droplet size was much lower than the droplet sizes seen in formulations with just one surfactant. Combining different surfactants may significantly decrease the size of droplets and improve the stability of emulsions by reducing the tension between interfaces in a synergistic manner [18].

methyl-a-D-glucopyranoside and Span 20 (Set 1)				
Sample	Mean (µm)	Confidence interval (µm)		
J1	20.30	± 1.121		
K1	9.44	± 0.405		
L1	NA	NA		
M1	10.00	± 0.754		

Table 3: Particle Size Analysis of Oil-in-Water Emulsion using methyl-α-D-glucopyranoside and Span 20 (Set 1)

*NA: Not Available

The use of a combination of surfactants improves the organisation of molecules at the interface between oil and water, resulting in a more effective reduction of the force between the two phases. This leads to the creation of smaller droplets and increased stability of the emulsion [19]. The mean droplet size in Sample J1 was $20.30 \pm 1.121 \mu m$. Table 4 shows particle size analysis of oil-in-water emulsion using methyl- α -D-glucopyranoside. Figure 3 shows polarised optical microscopy images of emulsions using methyl- α -D-glucopyranoside as a surfactant for Sample A1, Sample B1, Sample C1 and Sample D1. According to Table 4 (control samples) and Figure 3 in which methyl- α -D-glucopyranoside was solely used as a surfactant, the findings were compared which indicated that the combination of surfactants resulted in smaller droplets compared to formulations with just one surfactant. Figure 4 depicts polarised optical microscope images of emulsions formed by the combination of surfactants methyl- α -D-glucopyranoside and Span 20.

Additionally, Sample M1 exhibited a mean droplet size of $10.00 \pm 0.754 \mu m$. The lower oil content combined with mixed surfactants effectively maintains smaller droplet sizes, indicating a stable emulsion. This aligns with the studies which stated that formulations with lower oil content and mixed surfactants provide a more stable and homogenous emulsion due to the enhanced synergistic effect of the surfactants [20]. Overall, the data suggest that mixed surfactant formulations, particularly those combining methyl- α -D-glucopyranoside with Span 20, significantly improve the emulsion properties by reducing droplet size and increasing stability.

Table 4: Particle Size Analysis of Oil-in-Water Emulsion using methyl-α-D-glucopyranoside

Sample (Control Samples)	Mean (µm)	Confidence interval (µm)
A1	31.11	± 1.694
B1	21.00	± 1.850
C1	31.00	± 3.438
D1	27.00	± 6.796



Figure 3: Polarised optical microscopy images of emulsions using methyl-α-D-glucopyranoside as surfactant (a) Sample A1 (b) Sample B1 (c) Sample C1 and (d) Sample D1

The inability to measure the particle size distribution for Sample L1 can be attributed to several factors, despite the molecules being visibly identifiable under microscopy. One of the primary reasons is the high polydispersity index (PDI) of the emulsion, indicating a broad range of droplet sizes. This diversity often results in droplets with irregular, non-spherical shapes, which pose significant challenges for most particle size measurement techniques [21]. Emulsions with non-spherical droplets can be difficult to analyze accurately because standard measurement techniques typically assume spherical geometry, leading to potential size underestimation or overestimation depending on the droplet orientation.



Figure 4: Optical polarising microscopy images of emulsions using methyl-α-D-glucopyranoside and Span 20 as surfactants (e) Sample J1 (f) Sample K1 (g) Sample L1 and (h) Sample M1

Furthermore, manual measurement under microscopy may be required for non-spherical droplets; however, defining their boundaries precisely is challenging, which can affect accuracy. Another contributing factor could be the agglomeration of droplets within the emulsion, where partial coalescence results in clusters that are visually detectable but do not represent individual particles suitable for size analysis. These clusters form due to droplets partially merging, creating larger, irregularly shaped aggregates that distort particle size readings. Additionally, the limitations of the measurement instruments used may play a significant role. Particle size analyzers have specific detection ranges, and if the droplet sizes in Sample L1 fall outside these ranges—whether due to being too small, too large, or due to the sample's viscosity or dilution—it can compromise the sensitivity and accuracy of the measurements. These factors collectively highlight the complexities involved in obtaining precise particle size data for emulsions with heterogeneous characteristics.

Table 5 shows that the average size of droplets in Sample J2 is $30.90 \pm 1.090 \ \mu\text{m}$. This somewhat larger droplets suggest that the lower hydrophilic-lipophilic balance (HLB) value of Span 80 has an impact on the size of the droplets, in comparison to the mixture containing Span 20. Surfactants with lower HLB values typically result in larger droplet sizes in oil-in-water emulsions. The average droplet size of Sample K2 was $10.80 \pm 0.739 \ \mu\text{m}$. The combination of methyl- α -D-glucopyranoside with Span 80 results in a notable reduction in droplet size compared to using Span 80 alone in formulations. The mean droplet size in Sample L2 was $21.20 \pm 0.812 \ \mu\text{m}$. The benefits of mixed

surfactant systems are seen in the relatively small reduction in droplet size compared to single surfactant systems. Figure 5 indicates the size of droplets in O/W emulsion images using methyl- α -D-glucopyranoside and Span 80 surfactants.

Table 5: Particle size analysis of oil-in-water emulsion using methyl-α-D-glucopyranoside
and Span 80

Sample	Mean (µm)	Confidence interval	
		(μm)	
J2	30.90	± 1.090	
K2	10.80	± 0.739	
L2	21.20	± 0.812	
M2	31.40	± 1.562	



Figure 5: Optical polarising microscopy images of emulsions using methyl-α-D-glucopyranoside and Span 80 as surfactants (h) Sample J2 (i) Sample K2 (j) Sample L2 and (k) Sample M2

The significance of surfactant concentration and selection in producing stable emulsions is shown by the examination of droplet sizes and confidence intervals across different formulations. Because surfactants improve stabilisation by efficiently lowering interfacial tension between the water and oil phases, increasing their concentration often results in smaller droplet sizes [22]. As an example, Span 20, which is well-known for its moderate hydrophilicity and efficient lowering of interfacial tension, usually results in emulsions with smaller and more uniform droplets, particularly at higher concentrations. On the other hand, Span 80 is less successful in lowering interfacial tension in oil-inwater (O/W) systems because of its larger molecular structure and lower hydrophilic-lipophilic balance (HLB) value. Because Span 80 has a lesser capacity to stabilise the interface in comparison to Span 20, increasing its concentration often results in emulsions with bigger and less uniform droplet sizes.

While certain formulations (J1, K1, L1, and M1) showed no evidence of phase separation, suggesting stability, others (J2, K2, L2, and M2) showed physical changes that indicated instability as shown in Table 6. These behaviours are explained by variations in droplet size and distribution, two

important elements affecting the general characteristics of the emulsion. Because of their higher surface area, which enables surfactants to effectively inhibit droplet aggregation, smaller droplets contribute to improved stability during storage. On the other hand, the phase separation seen in samples J2, K2, L2, and M2 indicates that the presence of bigger or more polydisperse droplets may have jeopardised their stability. Over time, creaming or sedimentation may result from larger droplets being more vulnerable to gravitational forces. To achieve the appropriate droplet size distribution, the kind of surfactant and its associated HLB value must be chosen [23]. Higher HLB surfactants, such as methyl-a-Dglucopyranoside, have strong hydrophilic properties and are thus better at creating smaller droplets in O/W emulsions. Methyl-α-D-glucopyranoside and Span 20 together produced notably more stable emulsions with smaller droplets, indicating a synergistic interaction between the two surfactants. Increased emulsion stability and better droplet dispersion result from this synergistic interaction that improves the stabilisation process. These results highlight how crucial it is to maximise the kind and makeup of surfactants in order to get the required characteristics in emulsion systems, whether for use in food, medicine, or cosmetics. Formulators in a variety of sectors must take into account the capacity to manage droplet sizes via careful surfactant selection since it affects the emulsion's texture and appearance and prolongs its shelf life.

Sample	Phase separation
J1	/
K1	/
L1	/
M1	/
J2	Х
K2	Х
L2	Х
M2	Х

Table 6:	Observation	of	virgin	coconut	oil-in-water	emulsion	phase
			se	paration			

/ = stable or no physical changes

X= not stable and physical changes occur

4. CONCLUSIONS

The study performed on the stability of emulsions prepared with virgin coconut oil and water, using a combination of methyl- α -*D*-glucopyranoside and Span surfactants, indicates that the selection and combination of surfactants have a substantial impact on the stability of the emulsion. When Span 20 was combined with methyl- α -*D*-glucopyranoside, it showed better stability, as shown by lower creaming indices and smaller, more consistent droplet sizes. On the other hand, Span 80 demonstrated lower effectiveness in stabilising the emulsions, emphasising the significance of surfactant characteristics and their interactions. These results indicate that this study will be valuable for the rational design of virgin coconut oil-based formulations in food, beverage, cosmetic, and pharmaceutical products, optimising stability and performance across these applications. Thus, the knowledge gathered from this research contributes to our comprehension of emulsion stabilisation and serves as a basis for creating better formulations in these fields.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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