

MORPHOLOGY AND SIZE DISTRIBUTION OF PALM OLEIN CRYSTALS DURING FRACTIONAL CRYSTALLIZATION WITH BEHENIC ACID

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Abstract. Fractional crystallization of palm olein is industrially practiced to produce superolein and soft palm mid fraction for food and non-food products. This process, however, is frequently complicated by prolonged nucleation induction times, sluggish crystal growth rates and inconsistent crystal sizes, often requiring seeding agents for process improvement. This study examined the morphology and size distribution of palm olein crystals during isothermal fractional crystallization at 15 °C with 0.25%, 0.5%, 0.75% and 1% behenic acid using optical microscopy and focused beam reflectance measurement (FBRM). Results showed that the addition of 1% behenic acid to palm olein significantly reduced the nucleation induction time to 30 min compared to 170 min in the control palm olein. Crystal size distribution analysis depicted that the highest crystal population was observed at 0.75% behenic acid concentration, with a mean crystal size of 31 µm. Above this concentration, the count rate decreased and crystal sizes exceeded 100 µm due to crystal growth and agglomeration. Furthermore, the addition of 1% behenic acid accelerated palm olein crystallization and increased the solid fat content of the crystallizing slurry to 22% from 15% in the control palm olein. Microscopic images revealed that behenic acid acted as heterogeneous nuclei, forming the cores of dense spherulites within the bulk oil. The findings from this study have demonstrated the potential role of behenic acid as a nucleation enhancer and crystallization rate promoter in facilitating palm olein fractional crystallization.

Keywords: Palm olein, fractional crystallization, behenic acid, crystal size distribution, focused beam reflectance measurement (FBRM)

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Introduction

Palm oil is a semi-solid oil originating from the *Elaeis guineensis* Jacq. fruit. It was the largest-produced vegetable oil in the world in 2021, at 76.39 million tonnes [1]. Fractional crystallization of palm oil produces two products, i.e., a liquid fraction termed palm olein (POo) and a solid fraction termed palm stearin, with POo forming approximately 75% to 85% of the initial palm oil feed. Malaysia's production of POo in 2021 was nearly 8.9 million tonnes, making this oil the second largest processed palm oil product after refined palm oil [2]. POo is a major ingredient in culinary oils and can be found in a multitude of food products. It is also frequently blended with other liquid oils for quality and nutritional improvement [3-4].

Despite POo being one of the most important palm oil-based fractions produced worldwide for the past half century, there remain processing and quality issues surrounding POo. The fractional crystallization of POo is frequently associated with extremely long crystallization times as a result of significantly slower nucleation induction [5]. This is attributable to the absence of higher melting triacylglycerols in POo which are removed during palm oil fractional crystallization [6]. In addition, the fractional crystallization process of POo often produces irregular crystal sizes, which further complicate the filtration stage and reduce separation efficiency due to increased entrainment of the liquid oil within the solid crystals [7].

It has been previously reported that improvements in nucleation induction and post-crystallization separation efficiency can be achieved by controlling the crystallization process of palm oil using seeding agents. This can be accomplished through either the blending of palm oil with small amounts of a higher melting fat such as palm oil and palm stearin, or by using food-grade additives such as monoacylglycerols (MAG), diacylglycerols (DAG), phytosterols, phospholipids, lecithin, essential oils, sugar, polyglycerol esters and talc [8]. These seeding agents have been found to assist in promoting or inhibiting crystallization at different stages and consequently influence the number, size and morphology of crystals produced, thus improving separation efficiency and product yields [9]. Incorporation of certain crystallization promoters or inhibitors not only aids in improving the overall fractional crystallization process but can also potentially enhance the quality of the finished product by offering better cold stability, increased oxidative stability for cooking and frying applications, as well as boosting the nutritional value of a product for a variety of food applications [10].

To date, there are a limited number of studies which have looked into the effect of additives or seeding agents on the microstructural behaviour and crystal size distribution during POo fractional crystallization. Polyglycerol esters additives were previously reported to have a dual role, which could either increase or decrease the induction time and crystallization rate of POo, depending on the type and concentration [11-12]. Limonene addition to POo has been shown to impart a crystal retardation effect through the reduction in solid fat content, producing POo with enhanced cold stability [13]. In addition, sucrose esters were also found to influence the crystallization kinetics of POo, depending on the structure and concentration of the emulsifier [14]. In this present study, the effect of adding behenic acid as a potential seeding agent on the fractional crystallization of POo was examined in relation to the morphological aspects and size distribution of crystals using optical microscopy and FBRM.

Materials and Methods

Materials

Refined, bleached and deodorised POo was purchased from MOI Foods Malaysia Sdn. Bhd. (Pulau Indah, Malaysia). Behenic acid (99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Fractional Crystallization Procedure

Fractional crystallization experiments on POo with behenic acid at concentrations of 0.25%, 0.5%, 0.75% and 1% were performed using a LabMax Automatic Lab Reactor (Mettler Toledo, Redmond, USA). Crystallization of pure POo without the addition of behenic acid served as a control. Approximately 700 mL of the sample was subjected to isothermal fractional crystallization at 15 °C for 6 h in a 1 L glass jacketed vessel. Continuous monitoring of the crystal behaviour within the slurry was performed using a Lasentec FBRM Model D600L probe (Mettler Toledo, Redmond, USA) installed through the top of the vessel (Figure 1). The agitation speed was maintained at 50 rpm throughout the experiments. The FBRM data was analysed using the Lasentec FBRM Data Review software (Mettler Toledo, Redmond, USA). FBRM provided real time data on crystal characteristics such as crystal population and crystal size distribution within the slurry.



Figure 1: Fractional crystallization set-up with the FBRM probe

Solid Fat Content Measurement

At the end of each fractional crystallization experiment, the crystallizing slurry was vacuum filtered into liquid and solid fractions. The solid fat content (SFC) of the crystallizing slurry was measured immediately prior to filtration by a Bruker mq20 pulsed nuclear magnetic resonance (Bruker Optics Inc., Ontario, Canada) by direct SFC measurement [15].

Morphological Observation

The morphology of crystals within the slurries was observed using optical microscopy. Crystals were visually examined using a Leica DMLP polarizing light microscope equipped with a temperature-controlled stage (Linkam TP94 and LNP). Images were captured at 20 x magnification and processed using Leica Qwin software (version 3.0, Leica Microsystems Digital Imaging, Cambridge, UK).

Statistical Analysis

One-way analysis of variance (ANOVA) was performed to evaluate significant differences between results using Tukey's test at 95% confidence level ($p < 0.05$). All statistical analyses were conducted using the Minitab 16 software (Minitab Inc., State College, USA).

Results and Discussion

Crystal Morphology

In this study, the effect of adding 0.25% to 1% behenic acid on the morphology of POo crystals during isothermal fractional crystallization was investigated using a PLM. Micrographs showing POo crystal microstructures with increasing behenic addition are illustrated in Figure 2. Morphological observation of the crystallizing slurry showed that crystals in the control POo were small in number and possessed irregular shapes (Figure 2(A)). When 0.25% behenic acid was added to POo, it can be seen that behenic acid acted as heterogenous nuclei within the bulk oil and crystallized as tiny, radially-oriented needles separately from the POo crystals (Figure 2(B)). Above 0.5% behenic acid addition, there was a significant increase in the count rate where crystals appeared tightly packed and the earlier observed behenic acid nuclei were not visible microscopically (Figure 2(C)). Crystal sizes appeared to be more consistent (below 100 μm) at 0.75% behenic acid concentration, with behenic acid nuclei visible at the central core of POo crystals (Figure 2(D)).

In general, the addition of behenic acid from 0.25% to 0.75% increased the overall crystal population compared to the control POo. However, a substantial decrease in the number of crystals and a significant increase in crystal sizes to above 100 μm were noticeable at 1% behenic acid concentration. This could be due to the occurrence of crystal growth and agglomeration within the crystallizing slurry, as observed in Figure 2(E). Large crystal agglomerates consisting of 2 or 3 individual spherulites merged together were clearly visible, and these crystals also appeared denser, signifying increased transformation of liquid oil into the crystalline solid phase. The darker cores of the crystals showed behenic acid surrounded by POo needles, forming large and dense spherulitic agglomerates within the bulk oil. This observation appears to be similar to earlier studies that showed polyglycerol ester molecules

acting as initiators for heterogenous nucleation and cluster formation in palm oil crystallization [16-17].

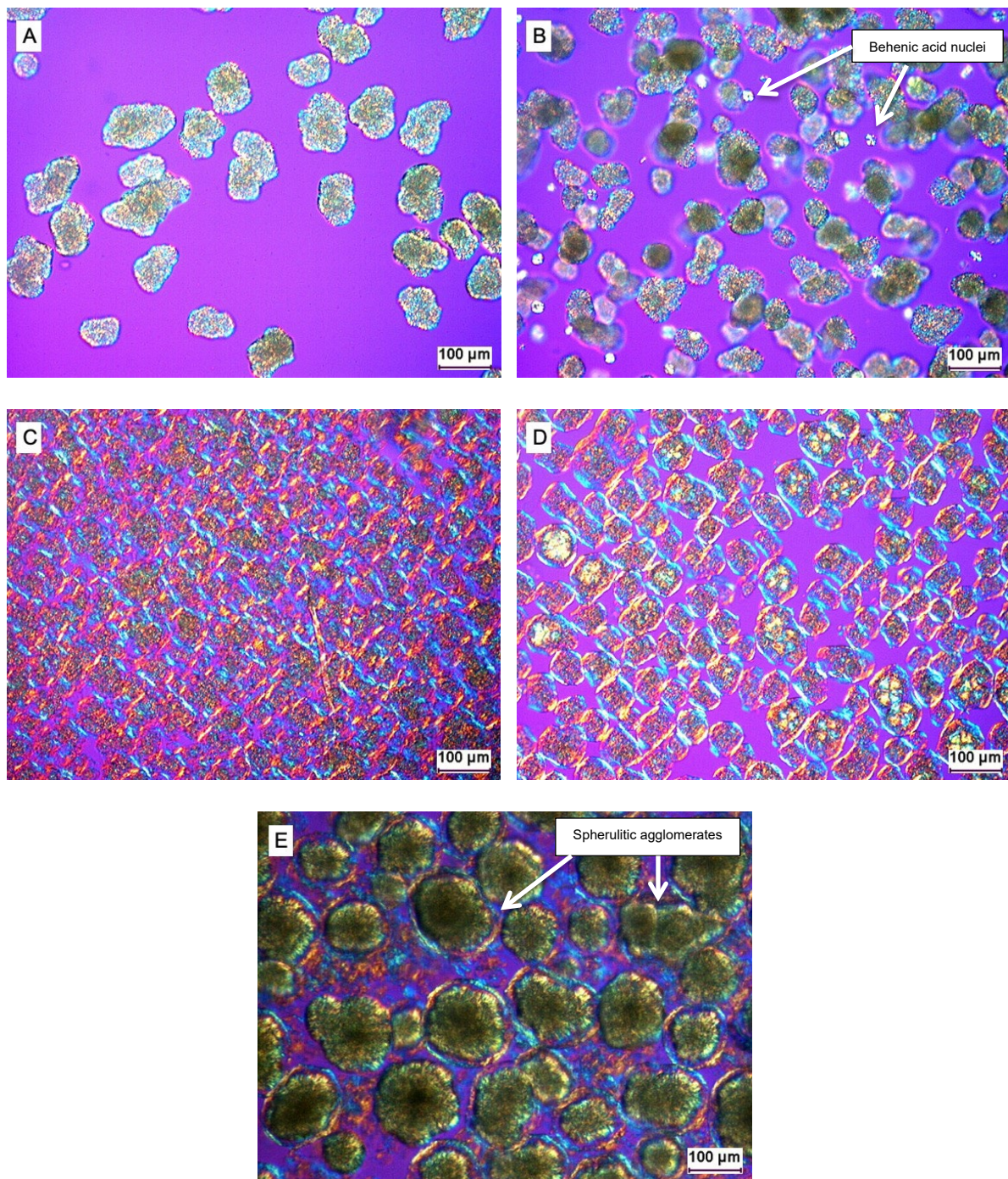


Figure 2: PLM images of POo fractional crystallization with behenic acid at 15 °C.
 (A) Control POo, (B) POo + 0.25% behenic acid, (C) POo + 0.5% behenic acid,
 (D) POo + 0.75% behenic acid and (E) POo + 1% behenic acid (Magnification: 20 x)

Crystal Population

The effect of behenic acid addition on the crystal population during POo fractional crystallization was determined using FBRM. The addition of behenic acid to POo was found to shorten nucleation induction from 170 min in the control POo to 30 min with 1% behenic acid addition, as shown in Figure 3. The faster nucleation rate observed when 1% behenic acid was added to POo can be attributed to the higher melting temperature of behenic acid at 80 °C, as compared to the melting temperature of POo at 22 °C, which allowed behenic acid to crystallize out at a higher temperature and act as a template for heterogeneous nucleation. Heterogeneous nucleation enhancement has been previously reported to occur by molecules acting as foreign materials and crystallizing out earlier compared to the TAG species within the crystallizing slurry [8,18]. The absence of trisaturated triacylglycerols in POo, which could form the first nuclei, has also been reported to cause delays in POo fractional crystallization [6]. This would result in longer induction times as the system relies on spontaneous heterogeneous nucleation on impurities or the crystallizer wall [19].

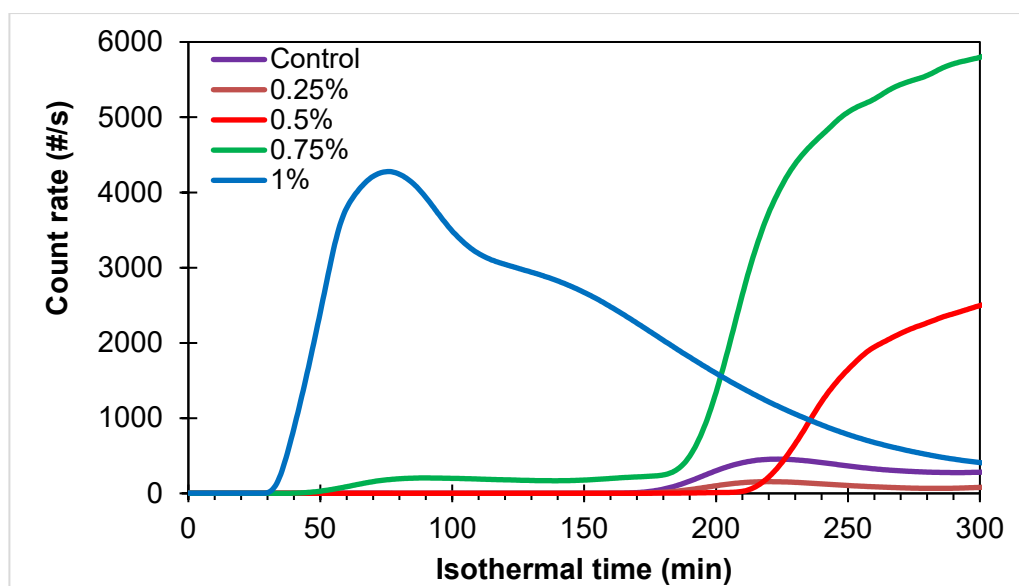


Figure 3: Effect of different behenic acid concentrations on the evolution of count rate in POo isothermally crystallized at 15 °C

The addition of 0.5% and 0.75% behenic acid to POo also caused a substantial rise in the count rate from 3000 #/s to 7000 #/s. On the other hand, behenic acid addition at 1% significantly increased nucleation rate and count rate to 4500 #/s during the initial stage until 80 min, before a gradual decrease in count rate to 250 #/s occurred towards the end of the experiment. This can be ascribed to the agglomeration process of individual single crystals into larger crystal clusters, as depicted earlier in the microscopic observation in Figure 2(E), further corroborating the data obtained by both FBRM and microscopic imaging techniques. A similar observation was reported by [5] in their study on palm olein crystallization at 9 °C, whereby after reaching a maximum count rate mid-experiment, a sudden decrease was detected along with a simultaneous increase in the crystal sizes. They attributed this phenomenon to the rapid agglomeration of crystals due to increased contact and crystal attachment between a large number of nuclei present within the system. Several other studies [12,20] have also reported a decrease in crystal numbers with an increase in crystal size when polyglycerol esters of fatty

acids (PGEs) were added during the crystallization of palm olein and a diacylglycerol-rich oil, respectively. These studies suggested that the addition of PGEs caused an overall reduction in the nucleation rate within the crystallizing system, which may also explain the decrease in count rate with the addition of 1% behenic acid observed in this present study.

Crystal Size Distribution

The final crystal size distribution of POo crystals measured by FBRM showed a peak size of 31 μm when 0.75% behenic acid was added, with the highest count rate as depicted by the area under the size distribution curve, as illustrated in Figure 4. A further increase in behenic acid concentration up to 1% shifted the distribution towards larger crystal sizes exceeding 100 μm with a significantly smaller curve area. It can be inferred from these results that the increase in crystal sizes and concomitant decrease in count rate at 1% behenic acid addition were a result of the agglomeration of individual spherulites, as depicted by the microscopic images in Figure 2, which further support the FBRM results. This observation also suggests 0.75% as the maximum amount of behenic acid for inducing nucleation and increasing the count rate in POo crystallization. These findings are in line with a previous study [16] which reported that accelerated nuclei formation and smaller crystal sizes were only observed when 0.7% of polyglycerol esters were added during palm oil crystallization.

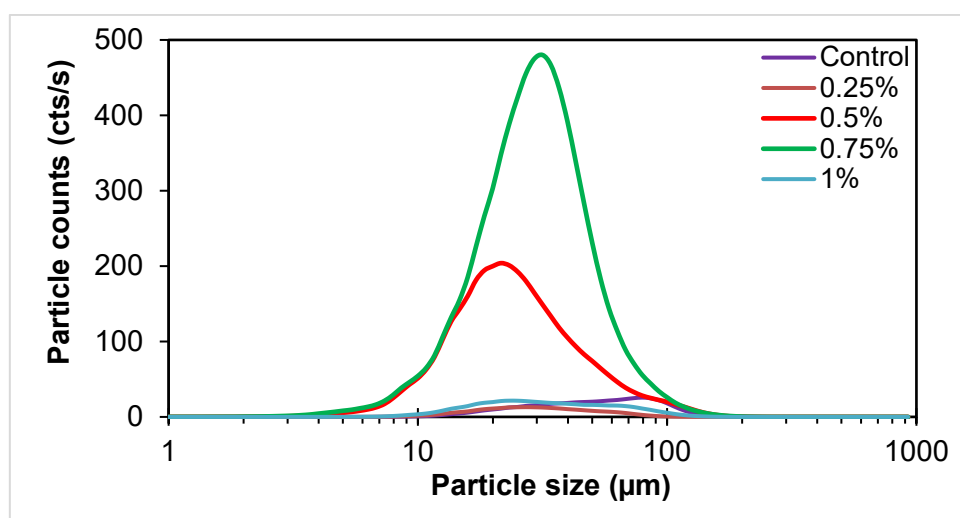


Figure 4: Effect of different behenic acid concentrations on the final crystal size distribution of POo isothermally crystallized at 15 °C

Solid Fat Content

Increasing the behenic acid concentration in POo from 0.25% to 1% caused a significant ($p < 0.05$) increase in the SFC of the slurry, from 15% in the control POo to 22% in the POo containing 1% behenic acid (Figure 5). Despite the lower number of crystals observed at 1% behenic acid addition, as shown in the microscopy analysis in Figure 2(E) and FBRM results in Figure 3, crystal growth was apparent in the individual crystals, with the formation of denser and more granular crystal structures. This clearly indicated increased liquid phase

conversion into the crystalline solid phase as POo molecules continuously attached themselves to the existing growing crystals, thus resulting in the higher SFC obtained.

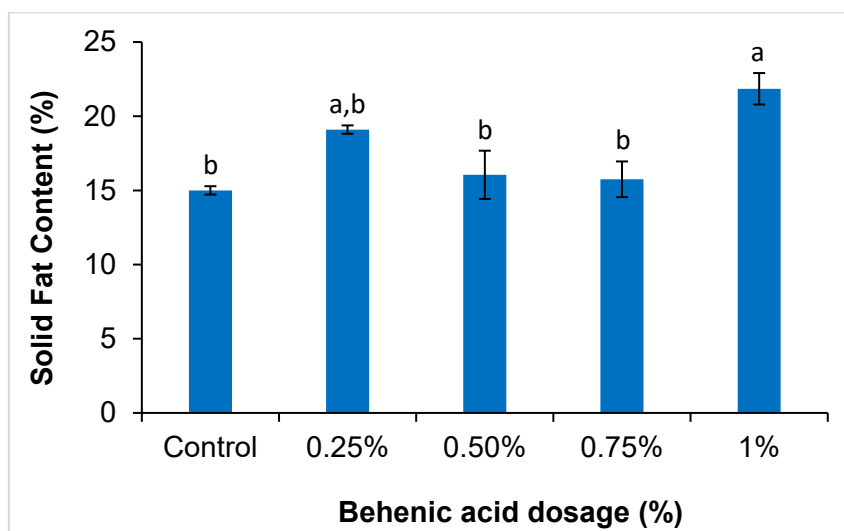


Figure 5: Effect of different behenic acid concentrations on the final solid fat content of POo isothermally crystallized at 15 °C. Different letters indicate significant differences at $p < 0.05$ according to Tukey's test

These findings were in agreement with previous studies [21-22] which showed that emulsifier additives exerted the greatest effect on the solid fat content of fats when the fat contained a higher amount of liquid phase. Thus, this may explain the higher SFC at 0.25% behenic acid addition, where the POo still contained a high amount of liquid phase and adding even a small amount of behenic acid resulted in a significant increase in SFC. At 0.5% and 0.75% behenic acid dosages, the similarity in the SFC at both concentrations indicates that a slight increase in behenic acid had a nucleation promoting effect, as illustrated earlier in Figure 3. However, as shown in Figures 2(C) and (D), these crystals were less dense and possessed more of a vitreous, gel-like structure similar to that reported by [5], and this may have directly affected the SFC measurement as these crystals were not entirely solid structure-wise. It is postulated that the sudden surge in the SFC observed at 1% behenic acid addition was due to the combined nucleation and crystal growth promoting effects through agglomeration, which led to the production of dense and large solid crystal entities (Figure 2(E)), and significantly increased the SFC measurements [22].

Conclusions

This article presents findings from an investigation on the effect of behenic acid addition on the morphology and size distribution of palm olein crystals during fractional crystallization. Behenic acid was found to significantly decrease nucleation induction time and accelerate crystal formation in palm olein. Microstructural observation revealed behenic acid had acted as heterogeneous nuclei forming the core of dense spherulitic crystals. Moreover, adding behenic acid to palm olein increased the solid fat content of the bulk oil, thereby indicating increased transformation of the liquid phase into solid crystals. This study has demonstrated that behenic acid may act as a potential nucleation enhancer and crystallization rate promoter in improving the fractional crystallization process of palm olein.

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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 declare no conflict of interest in the publication of this work.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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