Exploring Morphological and Functional Changes in *Phaseolus vulgaris* L Subjected to Different Durations of Heat-Moisture Treatment

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ABSTRACT

Phaseolus vulgaris L, known as red bean, is a staple food in Indonesia. The temperature sensitivity of red bean limits the applications despite the high starch content. Therefore, this research aims to analyze the effects of heat-moisture treatment (HMT) on the properties of red bean starch, focusing on the functional and morphological characteristics. The effects of HMT (4, 5, and 6 hours at 110°C) were elevated on fresh and dried red bean starch using Completely Randomized Design. The results show that significant alterations were observed in starch properties due to HMT. Water absorption capacity (WAC) varied with HMT duration and fresh bean starch peaked at 4 hours (198.0 ± 0.1%) before declining at 6 hours (159.5 ± 0.7%). In contrast, dried bean starch showed a decrease from the native capacity of 223.5 ± 5.0% to 176.5 ± 5.0% after 6 hours of HMT. Oil absorption capacity (OAC) also changed markedly and fresh bean starch increased from 68.0 ± 2.8% to 85.5 ± 0.7%. Similarly, dried beans increased from 67.0 ± 1.4% to 83.5 ± 0.7% since the effectiveness of HMT enhanced starch interaction with water and oil molecules. In fresh and dry beans, swelling power decreased and increased with prolonged HMT, reaching a peak at 4 hours (8.57 ± 0.15 g/g). However, solubility index decreased in both types following an increase in HMT duration. The morphology of starch granules transitioned from round or elliptical shapes to more polyhedral and irregular forms, reflecting substantial structural changes. In conclusion, HMT effectively modified the functional properties of red bean starch to offer potential benefits for application in food industry.

Keywords: Functional properties; heat-moisture treatment; *Phaseolus vulgaris* L; starch morphology

INTRODUCTION

Phaseolus vulgaris L, commonly known as red bean or red kidney bean, is a staple legume in Indonesia, playing a significant role in agricultural output. Recent data from Badan Pusat Statistik in 2020 reported production of 66,210 tons, stating the prevalence and potential in Indonesian agricultural sector (Badan Pusat Statistik, 2023). Red bean is often underutilized but offer substantial nutritional value by providing rich sources of proteins, carbohydrates, minerals, and vitamins (Du et al., 2014; Mutmainah, 2019).

Globally, legumes such as red bean are valued for high protein and carbohydrate contents, playing an important role in the diets of many regions, including India, Africa, and America. The primary carbohydrate of red bean is starch, comprising 50–70% of the dry matter (Wani et al., 2010). Starch has been thoroughly analyzed for various applications in food and non-food sectors. In food industry, the product is used as a

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thickening agent (Koswara, 2009). The modification of red bean starch can enhance the functional properties, stability, and versatility.

Food industry has a long-standing practice of modifying the physical and chemical properties of starch to improve the functionality. Among various modification methods, heat-moisture treatment (HMT) is a significant physical method for enhancing the subpar functional qualities of native starch, specifically in the scope of food applications (Gani et al., 2016). As an important advantage, HMT does not require the addition of chemical substances in food processing. HMT includes controlled moisture, temperature, and heating time successfully applied to modify starches from diverse sources such as mung beans (Li et al., 2011), maize (Lestari et al., 2015), and cassava (Fetriyuna et al., 2016).

Gani et al. (2016) reported the benefits of HMT on the properties of resistant starch from various bean cultivars. There is a lack of research concerning the effects of HMT on the functional and morphological properties of red bean starch. Therefore, this research aims to address the gap by investigating the effects of varying durations of HMT on the functional and morphological aspects of red beans starch. The objectives are to enhance the understanding of HMT influence on water absorption capacity (WAC) and oil absorption capacity (OAC), swelling power, solubility index, and granule morphology of red bean starch. The value of HMT modification is determined as a basis for more practical application testing in food industry by understanding the effects.

METHODS

Materials

The selected material was red bean from a local market in Rawamangun, East Jakarta, for starch extraction and modification treatment. Edible mustard oil (R.R.Oomerbhoy PVT LTD, India) and distilled water were used for measuring OAC and WAC, respectively. These materials were selected for the specific qualities and relevance to the research objectives, ensuring the consistency and reliability of the experimental results.

The equipment used was carefully selected for precision and reliability. Key instruments included the JSM-6510 Series Scanning Electron Microscope (JEOL Ltd, Tokyo, Japan) and the ZEISS Axiocam ICc 1 Microscope Camera (Carl Zeiss Industrielle Messtechnik GmbH, Oberkochen, Germany). Additionally, the experiments adopted a universal lab oven UN55 (Memmert GmbH, Schwabach, Germany), a health centrifuge HC1180T, and IKA Shakers MS 3 digital (IKA Works Sdn Bhd, Selangor, Malaysia). Measurements were carried out using a Quattro electronic scale, and the heating of samples was performed on an Akebonno Inox Hot Plate MSP3201 (PT. Rama Makmur Sentosa, Jakarta, Indonesia).

Study Design

This research used a completely randomized design structured in a multi-stage experimental method, including starch extraction, HMT, and various functional and morphological properties analyses. The two groups of treatment used are fresh and dried red beans. Dried red bean is sun-dried in the open air for 3 days during the day with an average temperature of 32°C. The groups are subjected to the same extraction and HMT. WAC and OAC, swelling power and solubility index, and morphological properties were measured in the groups.

Starch Extraction

The extraction process for red bean was adapted from Huang et al. (2006) with certain alterations. This comprised initial mechanical skin removal through blending, followed by immersing the beans in cold water (4°C) for 24 hours at a 3:1 water-to-bean ratio. The soaked beans were blended at high speed, and the resulting mixture was strained through fabric sieves in three separate stages. The filtrate was periodically pH adjusted using 0.2% NaOH and 0.1 N HCl, allowed to sediment, dried at 50ºC for 24 hours, and sieved through an 80 mesh screen.

Heat-Moisture Treatment Procedure

Starch was subjected to HMT following the procedure of Collado et al. (1999) with modifications. The initial moisture content was determined, and a 100 g starch sample was adjusted to 20% and 25% w/w moisture levels. Starch was stored overnight in HDPE containers, followed by oven-induced HMT at 110ºC for durations of 4, 5, and 6 hours, with stirring every 60 minutes. After the treatment, starch was cooled for 1 hour and dried in an oven at 50°C for 4 hours. Subsequently, dried starch was ground, sieved, and reserved for further examination.

Water and Oil Absorption Capacity

Based on Ashwar et al. (2015) with some modifications, this measurement included mixing 0.2 g starch with 10 ml distilled water/mustard oil, followed by vortexing for 2 mins. The mixture was left to stand for 15 mins and centrifuged at 3000 rpm for 15 mins. After centrifugation, the supernatant was separated, and the remaining sample was weighed. The weight increase was expressed as WAC and OAC percentage.

Swelling Power and Solubility Index

The measurement of swelling power and solubility index was adapted from Ashwar et al. (2015) with some alterations. A 0.2 g starch sample was combined with 10 mL distilled water in a centrifuge tube and heated using a water bath at 90°C for 30 minutes, with brief vortexing at 5, 15, and 25 minutes. After cooling to room temperature, the mixture was centrifuged for 15 mins at 3000 rpm and the supernatant was separated from the sediment. Subsequently, the sediment was weighed for swelling power, and calculated as the weight compared against the dry weight of starch. The supernatant was dried at 105°C and weighed to calculate solubility.

Morphological Properties

The granule shape and size were observed under a light microscope at 100x magnification (Letviany et al., 2012). Starch granules were affixed to an aluminum stub with adhesive tape, coated with gold-palladium, and visualized using a JSM-6510 Series Scanning Electron Microscope at 20 kV and 1000x magnification (Gani et al., 2016).

Statistical Analysis

Each measurement was performed three times for each group and the data were presented as mean ± SD. IBM SPSS 20 was used for statistical analysis, applying a two-way Analysis of Variance (ANOVA) and Tukey's post hoc test for identifying significant differences among treatments.

RESULT AND DISCUSSION

The functional characteristics of red bean starch were systematically evaluated after HMT for varying durations. The results showed significant differences in WAC, OAC, swelling power, and solubility index across different treatment times and bean conditions.

WAC of red bean starch was affected by both the condition of beans and the duration of HMT. In fresh bean starch, WAC initially showed no significant difference from native starch at 4 hours of HMT, registering 198.0 ± 0.1%. According to Gani et al. (2016), HMT enhanced WAC of native bean starch considerably, a trend in line with the observation of higher water absorption in dried beans (Gani et al., 2016). This increment is attributed to starch gelatinization, which is supported by the significant increases reported in autoclaved, HMT, and annealed starches (Ashwar et al., 2015).

As the duration of HMT increased beyond 4 hours, a decrease in WAC was observed, reporting 187.5 ± 3.5% and 159.5 ± 0.7% at 5 and 6 hours of treatment, respectively. In line with Ariyantoro et al. (2022), WAC serves as an indicator of starch ability to integrate into aqueous food formulations, with modifications such as HMT (Ariyantoro et al., 2022). However, WAC begins to decrease beyond an optimal HMT duration, suggesting an equilibrium state. The observation is consistent with Abraham et al. (1993), which observed a decrease in water-binding capacity of modified starches. This is attributed to the formation of a hard surface layer on the granules affecting water penetration (Abraham et al. , 1993). The present results suggest that while HMT can initially disrupt molecular structures to increase water absorption, excessive treatment may lead to the development of a restrictive layer, reducing starch capacity to absorb water. However, the peak or plateau of WAC cannot be determined due to less than 4 hours or more than 6 hours of HMT treatment. The reduction in WAC influences red bean starch properties in absorbing water, diminishing effectiveness as a thickener in culinary products.

Table 1. Functional properties of the fresh and dried red bean starch after various durations of HMT

	Duration of HMT treatment	Water absorption (%)	Oil absorption (%)	Swelling power (g/g)	Solubility index (g/g)
Fresh bean starch	Native bean	174.5 ± 5.0^{a}	68.0 ± 2.8^{a}	6.74 ± 0.12^{a}	0.027 ± 0.001^{a}
	4 h	198.0 ± 0.1^{a}	73.0 ± 1.4^{a}	6.03 ± 0.01^{b}	0.020 ± 0.001^{b}
	5 h	187.5 ± 3.5^{b}	79.5 ± 0.7^{b}	6.13 ± 0.01^{b}	0.021 ± 0.001^{b}
	6 h	159.5 ± 0.7^{c}	85.5 ± 0.7^{b}	6.20 ± 0.11^{a}	0.020 ± 0.001^{b}
Dry bean starch	Native bean	223.5 ± 5.0^{d}	67.0 ± 1.4^{a}	6.83 ± 0.04^{c}	0.025 ± 0.001^{c}
	4 h	208.5 ± 2.1^{d}	69.0 ± 1.4^{a}	8.57 ± 0.15^{d}	0.022 ± 0.001^{d}
	5 h	191.5 ± 2.1^{e}	86.5 ± 2.1^{b}	8.10 ± 0.07^{d}	0.022 ± 0.001^{d}
	6 h	176.5 ± 5.0^{f}	83.5 ± 0.7^{b}	7.19 ± 0.09^{c}	0.022 ± 0.001^{d}

*Values in the column for a specific parameter with different superscripts are significantly different at ($p < 0.05$)

Olayinka et al. (2008), reinforced the premise that HMT could drastically increase the hydrophilic nature of starches. The hydrophilic properties increased with the level of HMT, showing a modification in the granular structure. Moreover, the quadratic relationship between HMT duration and WAC is evident as reported by Fetriyuna et al. (2016). HMT process enhances the hydrophilic properties of starch due to slight expansions in the amorphous regions, disrupting some hydrogen bonds to form with water molecules (Fetriyuna et al., 2016).

OAC is a crucial functional property in food processing, contributing to the retention of flavor, improved palatability, and extended storage life, specifically in baked goods and meat products (Gani et al., 2016). In this research, both fresh and dried bean starches showed an increase in OAC with the duration of HMT, with no significant differences. After 5 hours of HMT, OAC reached a threshold where further treatment did not show a significant increase. This plateau suggests that the structural modifications induced by HMT may have reached an optimum for the interaction with oil molecules.

Gani et al. (2016) and Ashwar et al. (2015) reported that HMT enhanced OAC of starch more significantly than citric acid treatment (CT). Meanwhile, treated starch reported a higher amount compared to the native counterpart. The elevated OAC in treated starch is due to the creation of amylose-lipid complexes and the exposure of more hydrophobic moieties under high temperatures. Comparable results were observed by Olayinka et al. (2008), where an increase in OAC was observed in sorghum starch (Olayinka et al., 2008).

According to Mathobo et al. (2021) and Abraham et al. (1993), the annealing of starch such as red sorghum augments is believed to result from slight expansions in the amorphous areas and the breaking of hydrogen bonds to reinforce the lipophilic properties of the exterior. These studies also documented an increase in the oil-binding capacity of modified cassava starch, with a significant portion of the granules subjected to gelatinization during HMT. Enhancing OAC can improve the application in food products by increasing the ability to retain and stabilize oils. This is essential for creating richer textures, improving flavor retention, and enhancing the mouthfeel of products such as sauces, dressings, and baked goods.

Swelling power and solubility index are indicative of the interactions between starch chains in crystalline and amorphous structures, crucial for application in food industry as thickeners, gelling agents, stabilizers, and fat substitutes (Singla et al., 2020). Swelling power showed a decrease in fresh bean starch but an increase in dried bean starch with prolonged HMT, a behavior also observed by Gani et al. (2016). The reduction in swelling power and solubility of HMT bean starches, compared to the native counterparts, is attributed to increased granular stability and the formation of amylose-lipid complexes (Gani et al., 2016). Additionally, swelling power showed complex behavior in response to HMT. For dried bean starch, swelling power reached the peak at 4 hours of HMT (8.57 ± 0.15 g/g). This peak suggests a disruption in the molecular order in starch granules of dried beans, which enhances water retention capacity relative to native bean starch. Swelling power between fresh and dried red bean starches is significantly different. Dried red bean starch has a higher value because the product is initially shrunk before the treatment.

The crystallinity of starch is altered after heating in excess water, leading to gelatinization, which can affect swelling power and solubility (Singla et al., 2020). Ashwar et al. (2015) discovered that swelling and solubility index are functions of temperature, increasing significantly due to the weakening of binding forces between the granules. This research shows significant differences between swelling power and solubility index of native bean starch after HMT treatment. The effect of HMT treatment on gelatinization needs further research to strengthen the result.

In contrast to the expected trend, solubility index reported a decrease following HMT. Despite an increase in swelling power, starch solubility decreased, with fresh bean starch showing higher results. Ashwar et al. (2015) observed a comparable reduction in solubility index after autoclaving-retrogradation treatment due to increased crystallite perfection and additional interactions between starch chains. This decrease might be associated with the extent of granule disruption, which may have led to a more compact structure or a reduction in leachable amylose to decrease water-dissolving ability (Ashwar et al., 2015). The reduced index for dried bean starch treated for 4 hours (0.022 ± 0.001 g/g) shows that HMT enhances swelling but does not uniformly improve starch solubility. Swelling power and solubility index respond differently to HMT. These changes are important for starch usage in baked goods and other food items (Fonseca et al., 2021).

Letviany et al. (2012) reported that high amylose content led to lower swelling power. In this research, prolonged HMT increases amylose-amylopectin interaction and decreases swelling (Letviany et al., 2012). This result is significant for food industry, where controlled swelling power is desirable, such as in noodle or pasta production (Pranoto et al., 2014).

Repeated heat-moisture treatment (RHMT) has been found to modify starch properties, allowing a

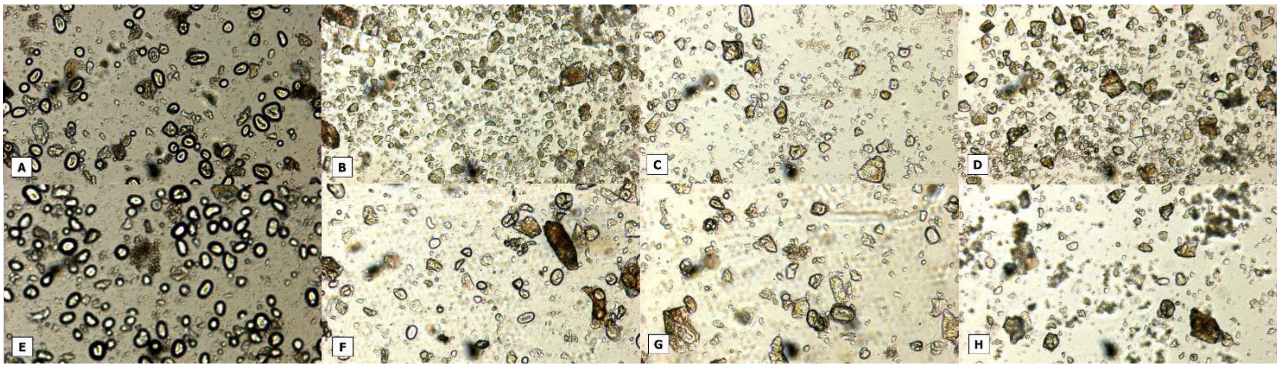

Figure 1. Light micrograph (magnification 100x) of fresh (A-D) and dried (E-H) red bean starch in the following order: native starch, 4, 5, and 6 hours duration of HMT

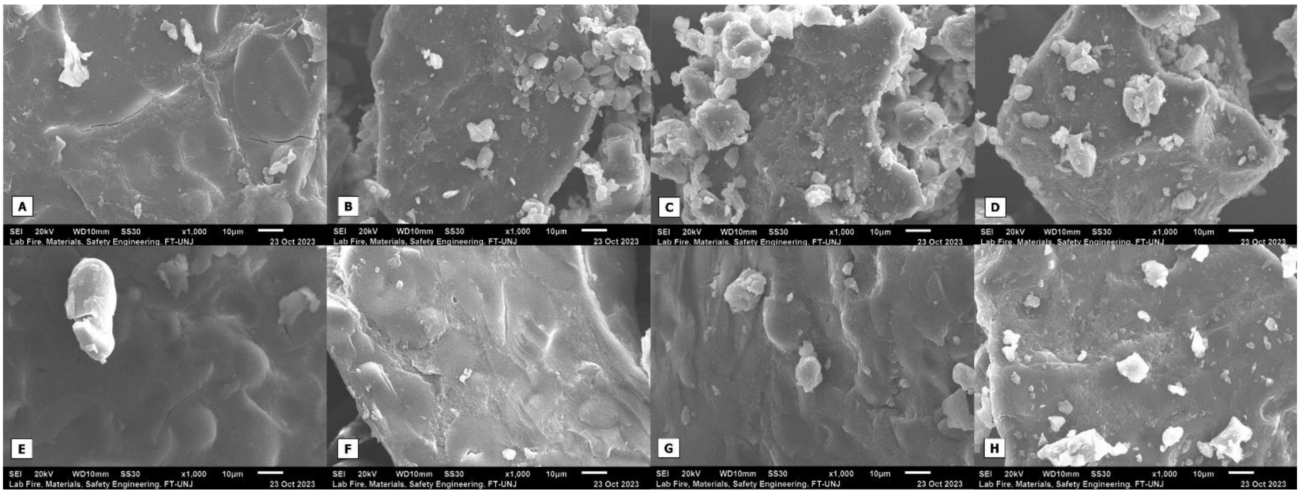

Figure 2. Scanning electron micrograph (magnification 1000x) of fresh (A-D) and dried (E-H) red bean starch in the following order: native starch, 4, 5, 6 hours duration of HMT

reallocation of moisture in the granules and providing a wider range of applications for modified starch (Niu et al., 2020; Zhao et al., 2020). The influence of HMT on solubility and swelling power varies across different starch sources, as reported by the distinct behaviors of mung bean, cassava, rice, potato, pinhão, and banana (Fonseca et al., 2021; Mathobo et al., 2021). The impact of HMT on the morphology of red bean starch granules was observed using light and Scanning Electron Microscope, with magnifications of 100x and 1000x, as shown in Figures 1 and 2.

For native starch from fresh red bean, the granules predominantly showed round or elliptical shapes. The greatest and shortest length recorded was 43.26 µm and 7.7 µm, while the maximum width was 34.47 µm. This typical morphology was subjected to significant alterations on HMT. After 4 hours of HMT, the granules primarily transformed into polyhedral and irregular shapes with a reduction in size and visible agglutination. The extension of the HMT duration to 5 hours led to further morphological changes, resulting in more pronounced polyhedral shapes and increased agglutination, with a continued decrease in granule size. At 6 hours, these changes persisted, with granules showing similar sizes and characteristics as observed in the 5-hour treatment.

For native starch derived from dried red bean, the granules were mostly round or elliptical, with a maximum and minimum length of 44.14 µm and 9.3 µm, respectively. The maximum width was observed to be 32.64 µm and HMT process induced alterations in starch granules. After 4 hours, the granules appeared oval with indentations and showed signs of agglutination but the size remained similar to the native form. Increasing HMT duration to 5 hours resulted in more polyhedral and irregular shapes, alongside greater agglutination, with the granule size remaining similar. At 6 hours of HMT, a significant decrease in size was reported, with granules showing a smaller, polyhedral, and irregular shape, accompanied by considerable agglutination.

Morphological changes observed in red bean starch granules due to HMT are balanced with the existing literature. According to Gani et al. (2016), native bean starch granules typically show round, elliptical, or irregular shapes, with smooth surfaces. The observations of native starch from fresh and dried red beans are consistent with the descriptions. HMT process led to significant alterations in granule morphology, such as surface indentation and formation of grooves, similar to changes observed in other research on mung bean starch and various cultivars (Gani et al., 2016; Li et al., 2011).

Ashwar et al. (2015) reported that starch granules could have polyhedral or irregular shapes with variable sizes, a natural variability attributed to climate, agronomic conditions, and processing. Morphological transformations observed in starch granules could be attributed to external factors, as well as the intrinsic properties of starch (Ashwar et al., 2015). The loss of the granular structure and development of a continuous network post-treatment was also reflected, particularly in granules treated for longer durations.

The form of starch granules is influenced by the location of the hilum, as discussed by Letviany et al. (2012). The variations in shape from elliptical to kidney-like forms could be related to structural aspects of starch granules (Letviany et al., 2012). BeMiller and Huber (2015) reviewed numerous research on HMT-modified starch and discovered major structural changes, such as enhanced mobility of chains, molecular degradation, and morphological alterations such as surface cracking and partial gelatinization (BeMiller & Huber, 2015).

Soltovski et al. (2018) and Lacerda et al. (2015) observed alterations in the size, roughness, and morphology of potato, sweet potato, and avocado starch. These modifications, including clumping of granules and alterations in size and texture, resonate with observations in red bean starch (Lacerda et al., 2015; Soltovski et al., 2018). Bartz et al. (2017) and Wattananapakasem et al. (2021) also reported morphological changes in potato and germinated-black rice starch following HMT. Therefore, HMT significantly impacts starch granule morphology (Bartz et al., 2017; Wattananapakasem et al., 2021).

Morphological changes lead to significant alterations in thickening properties. These changes can lead to a lower swelling power, altered gelatinization behavior, decreased viscosity, and potentially less stable thickened products. Morphological results are similar to swelling power and solubility index, where larger and more regular granules show higher swelling and solubility indexes.

Dried bean starch has oval shapes with indentations after 4 hours of treatment, while fresh bean starch morphology becomes polyhedral and irregular. The two types of starch show a decrease in size and a more irregular structure upon further treatment. Drying the bean initially makes the structure less prone to prolonged heat treatment. This alteration affects the final product since smaller and more irregular granules provide a creamier texture and less thickness when used as a thickening agent.

The reviewed literature reports the critical roles played by treatment time, moisture content, and starch source in dictating the functional and morphological transformations in starch due to HMT. Therefore, red bean starch granules show pronounced morphological alterations with significant variations in WAC and OAC, swelling power, and solubility index as a function of the duration of HMT. These changes suggest the potential for modifying the functional properties of starch in various food applications through controlled HMT processes.

CONCLUSION

In conclusion, this research reported the impacts of HMT on red bean starch, showing significant changes in WAC and OAC, swelling power, and solubility index with varying durations of HMT. Morphologically, starch granules transitioned from round or elliptical to more polyhedral and irregular shapes, showing the impact of HMT on starch structure. These results suggested that HMT had value in optimizing the functional properties of red bean starch but required further insight into the optimal treatment duration. Even though valuable insights were provided, further research could explore the applicability of the results in different food processes, such as the function of a thickening agent in sauces, soups, or desserts.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest concerning the publication of this research.

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