Research Article

Effect of Particle Size on Physicochemical, Functional, and Antioxidant Properties of Defatted Rice (*Oryza sativa* L.) Bran

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ABSTRACT

This study investigated the influence of particle size on the physicochemical, functional, and antioxidant properties of defatted rice bran (DRB), an agricultural by-product rich in valuable nutrients and antioxidants with potential applications for food and nutraceutical industries. DRB was fractionated into three different particle size groups: 250 μm, 150 μm, and <75 μm. Physicochemical parameters were assessed, namely, color, water activity (aw), moisture content, crude protein, total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF). Functional properties were also measured, such as via the water solubility index (WSI) and water absorption index (WAI). Antioxidant components and activity of DRB fractions were also evaluated. Results showed that finer particle sizes exhibited a significantly increased proportion of SDF and a decreased proportion of IDF ($p<0.05$) without impacting on TDF. Furthermore, smaller particle sizes significantly enhanced total phenolic content, antioxidant activity, improved lightness (L*) value, and WSI ($p<0.05$). These findings suggest that particle size reduction in DRB can optimize its dietary fiber proportion as well as functional and antioxidant potential, thus offering implications for its application in food product formulations.

Keywords: Defatted rice bran, Particle size, Dietary fiber, Total phenolic content, Antioxidant activity

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บทความวิจัย

ผลของขนาดอนุภาคต่อสมบัติทางเคมีกายภาพ สมบัติเชิงหน้าที่ และสมบัติในการต้านอนุมูลอิสระของรำข้าวสกัดไขมัน (*Oryza sativa* L.)

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของขนาดอนุภาคต่อสมบัติทางเคมีกายภาพ สมบัติเชิงหน้าที่ และสมบัติในการต้านอนุมูลอิสระของรำข้าวสกัดน้ำมัน ซึ่งเป็นผลพลอยได้จากการเกษตร อุดมไปด้วยสารอาหารที่มีคุณค่าและสารต้านอนุมูลอิสระและมีศักยภาพในการใช้ในอุตสาหกรรมอาหารและอาหารเสริม โดยแบ่งรำข้าวสกัดน้ำมันเป็น 3 กลุ่มตามขนาดอนุภาค ได้แก่ 250 µm 150 µm และ 75 µm จากนั้นวิเคราะห์สมบัติทางเคมีกายภาพ ได้แก่ สี ปริมาณน้ำอิสระ ปริมาณความชื้น โปรตีน ใยอาหารทั้งหมด ทั้งชนิดไม่ละลายน้ำและชนิดละลายน้ำ รวมถึงสมบัติเชิงหน้าที่ เช่น ดัชนีการละลายน้ำและดัชนีการดูดซึมน้ำ นอกจากนี้ยังประเมินปริมาณสารต้านอนุมูลอิสระและฤทธิ์ในการต้านอนุมูลอิสระ ผลการศึกษาพบว่า อนุภาคขนาดเล็กแสดงให้เห็นถึงสัดส่วนของใยอาหารชนิดละลายน้ำเพิ่มขึ้น และสัดส่วนของใยอาหารชนิดไม่ละลายน้ำลดลงอย่างมีนัยสำคัญ ($p<0.05$) โดยไม่กระทบต่อปริมาณใยอาหารทั้งหมด ขนาดอนุภาคเล็กลงยังเพิ่มปริมาณฟีนอลิกทั้งหมด ฤทธิ์ในการต้านอนุมูลอิสระ ค่าความสว่าง (L*) และดัชนีการละลายน้ำ ผลการวิจัยนี้ชี้ให้เห็นว่า การลดขนาดอนุภาคของรำข้าวสกัดน้ำมันสามารถเปลี่ยนแปลงสัดส่วนของชนิดใยอาหาร ปรับปรุงสมบัติเชิงหน้าที่ และเพิ่มฤทธิ์ในการต้านอนุมูลอิสระได้ ซึ่งทำให้มีศักยภาพในการนำไปใช้ในสูตรผลิตภัณฑ์อาหารได้อย่างเหมาะสมยิ่งขึ้น

คำสำคัญ: รำข้าวสกัดน้ำมัน, ขนาดอนุภาค, ใยอาหาร, ปริมาณฟีนอลิกทั้งหมด, ฤทธิ์ต้านอนุมูลอิสระ

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INTRODUCTION

Rice (*Oryza sativa* L.) is well-known as an important economic crop with approximately 776 million tons being produced globally in 2022[1]. The main by-product from the rice milling process is rice bran, which amounts to roughly 80 million tons annually[2]. Rice bran has been used mainly to produce edible oil because it is rich in valuable fat and has a good composition of essential fatty acids and bioactive compounds (γ-oryzanol, tocopherol, and tocotrienols) as natural antioxidants[3].

During the industry's rice bran oil extraction process, defatted rice bran (DRB) is a by-product that is produced in large quantities and has been used as an ingredient in livestock feed or discarded as waste. DRB possesses a high nutritional value and potential for utilization in supplemental or functional food products because of its rich source of dietary fiber (DF), proteins, vitamins, and minerals[4,5]. In addition, DRB contains natural bioactive compounds, such as phenolic substances[6]. It also contains a high amount of total dietary fiber (TDF), mainly insoluble dietary fiber (IDF), consisting of cellulose and hemicellulose[2,4]. Convincing evidence supports the significance of DF for human health, especially in preventing chronic non-communicable diseases, such as obesity, diabetes, and cancer, through its potential roles in promoting satiety, reducing energy intake, delaying glucose diffusion, enhancing insulin sensitivity, accelerating defecation time, and reducing carcinogen concentration in the intestine[7]. DRB contains protein with a complete profile of essential amino acids[8]. The predominant phenolic compounds in DRB are phenolic acids, particularly ferulic acid and p-coumaric acid[5], responsible for their antioxidant, anti-inflammatory, and anti-cancer effects[6]. DRB has been proven to enhance gut health in rats by promoting the growth of intestinal microbiota and increasing the production of crucial metabolites, short-chain fatty acids (acetate, propionate, butyrate), from intestinal bacterial fermentation of DF[9]. These metabolites are associated with maintaining intestinal barrier integrity and immunomodulatory as well as having anti-inflammatory and anti-cancer properties[9]. Furthermore, DRB has been demonstrated to impede colorectal cancer development by reducing chronic inflammation and delaying tumorigenesis[5].

Despite its various health benefits, utilizing DRB in food products is challenging due to its coarse texture and low functional attributes. Factors such as processing methods, extraction techniques, and particle size can influence physical, functional, and nutritional qualities[10]. Particle size reduction is critical in determining physicochemical and functional properties, such as color, DF, and solubility. Previous studies have reported that reducing particle size could improve color[11-13], modify IDF into SDF[14], and enhance solubility[13]. In addition, reducing particle size improves the bioaccessibility of bioactive compounds, mainly phenolic acids, leading to enhanced antioxidant activity[14,15]. However, research is scarce on the influence of particle size reduction on the physicochemical, functional, and antioxidant properties of DRB. Understanding these critical parameters is essential for optimizing DRB as a functional ingredient in food formulations. Consequently, this study investigated differences in particle size and changes in the physicochemical, functional, and antioxidant properties of DRB. This study highlights the potential benefits of particle size reduction, which can enhance the application of DRB in food and nutraceutical industries.

MATERIALS AND METHODS

Materials

Food-grade DRB powder was obtained from the Thai Edible Oil Co., Ltd (Bangkok, Thailand). It was produced from the extraction of heat-treated full-fat rice bran obtained after the milling of a mixture of Thai brown rice (*Oryza sativa* L.), with n-hexane as solvent extraction.

Preparation of defatted rice bran

A powder grinder (Spring Green Evolution, Bangkok, Thailand) was used to grind the DRB powder, which was then divided into three particle size fractions: 250 µm, 150 µm, and 75 µm. This powder was vibrated using a vibratory sieve shaker (ANALYSETTE 3 PRO, FRITSCH, Germany) and passed through a 60-mesh sieve (Endecotts, London, UK) to obtain 250 µm DRB, passed through a 100-mesh sieve to obtain 150 µm DRB, and finally passed through a 200-mesh sieve to obtain 75 µm DRB. Each size of the DRB sample was prepared and stored at 4 °C until required for further analysis.

Determination of physicochemical properties

The proximate composition of the samples was analyzed following the methods of the Association of Official Analytical Chemists (AOAC), 2019[16]. Moisture content was determined using the hot air oven method (AOAC 925.10). Crude protein content was estimated using the Kjeldahl method (AOAC 992.23), and total nitrogen content was multiplied by a factor of 6.25 to calculate protein content. TDF, IDF, and SDF were determined using enzyme gravimetric methods, as described by the AOAC 985.29, 991.42, and 993.19 methods, respectively, and the method of Wunjuntuk et al.[17] Water activity (a_w) of DRB fractions was measured using a water activity meter (AQUALAB 4TE, METER group, Pullman, WA) at 25 °C of the temperature. Color of DRB fractions was measured using a colorimeter (ColorFlex EZ, HunterLab, Reston, VA, USA) and expressed with a CIE Lab system in terms of L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) values.

Determination of water solubility index (WSI) and water absorption index (WAI)

The water solubility index (WSI) and water absorption index (WAI) of DRB fractions were determined following the method of Kraithong et al.[18] with slight modifications. Approximately 1 g of each sample was suspended in 10 mL of distilled water and mixed with a vortex mixer for 1 min. The suspension was then centrifuged at 3000 rpm (Allegra X-15R, Beckman, Germany) for 10 min. The supernatant was decanted, and the wet sediment was then weighed to determine WAI. The WSI was calculated from the supernatant's 105 °C overnight dried solids. The WAI and WSI of each sample were calculated using the following equations:

$$\text{WSI (\%)} = \text{Weight of dried supernatant (g)} / \text{Initiate Weight of sample (g)} \times 100$$

WAI (g/g) = Weight of wet sediment (g) / Initiate Weight of the sample (g)

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was assessed using the Folin-Ciocalteu reagent assay[19]. DRB samples (3 g) were extracted with 50% N, N-dimethylformamide (DMF; 25 mL) at room temperature in a water bath for 18 h. Following centrifugation (130 g / 10 min) of the extracted solution, the supernatant was used to determine TPC. The extracted samples (25 µL) were mixed with the 10% Folin-Ciocalteu reagent (125 µL) and 0.5 M aqueous sodium hydroxide solution (100 µL), then incubated in the dark at room temperature for 15 min. The absorbance of the mixed solution was completed using a microplate reader at 750 nm. Gallic acid was served as a standard by diluting with 50% DMF to 10-80 µg/mL. TPC value was expressed in milligram gallic acid equivalents (GAE) per 100 g sample or mg GAE/100 g.

Sample extraction for determination of antioxidant activity

The DRB samples were extracted using a 50% acetone solution prepared with deionized water (v/v). For each extraction, a sample (0.5 g) was mixed with the acetone solution (50 mL) and agitated on a mechanical shake at 400 rpm at room temperature for 1 h. The mixture was centrifuged (4,400 g / 15 min), and the supernatant was collected for subsequent analysis of antioxidant activities.

Determination of antioxidant activity by DPPH free radical scavenging assay

DPPH radical scavenging activity was measured using the spectrophotometric method[19] with some modifications. The supernatant from the extracted sample was diluted to different concentrations using absolute methanol. Each diluted sample solution (1 mL) was added with 0.2 mM DPPH radical solution in methanol (1 mL). Each diluted sample solution was added with absolute methanol (1 mL) instead for blank samples. The mixed solutions were kept in the dark at room temperature for 45 min. Thereafter, absorbance was measured at 517 nm with a UV-Vis spectrophotometer (Helios Beta, Thermo Fisher Scientific, Waltham, MA, USA). The 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), a hydrophilic vitamin E analog, served as a standard by dissolving Trolox powder in deionized water, with a standard curve established over a concentration range of 6.25 to 100 µmol. DPPH value was expressed as millimoles of Trolox equivalents (TE) per 100 g of sample or mmol TE/100 g.

Determination of antioxidant activity by Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was conducted using the spectrophotometric method[20]. Extracted samples (1 mL) were mixed with a freshly prepared FRAP reagent composed of 300 mM acetate buffer (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in a 10:1:1 v/v/v ratio. The mixed solution was incubated at 37°C for 4 min. Trolox served as a standard by dissolving Trolox powder in deionized water, with a standard curve established over a concentration range of 6.25 to 100 µmol. The absorbance of the test solution was completed at 593 nm using a spectrophotometer and compared to the Trolox standard. FRAP value was expressed as micromoles of TE per 100 g of sample or µmol TE/100 g.

Determination of antioxidant activity by Oxygen Radical Absorption Capacity (ORAC) assay

The oxygen radical absorbance capacity (ORAC) assay was performed to measure the capacity of antioxidant compounds in test samples to inhibit peroxyl radicals induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)[21]. AAPH was used as the peroxyl radical generator. Trolox was used as a standard by dissolving Trolox with 75 mM phosphate buffer (pH 7.2). The Trolox standard curve was established over a 6.25 to 100 µmol concentration range. Reaction mixed solutions, consisting of 4.19 µM fluorescein solution in 75 mM phosphate buffer pH 7.2 (3 mL), were mixed with either an extracted sample (0.5 mL), a Trolox standard solution, or a blank (75 mM phosphate buffer, pH 7.2). The mixtures were pre-incubated at 37 °C for 10 min, followed by the addition of 153 mM AAPH solution (0.5 mL), and then the immediate decrease in fluorescence was measured using a spectrofluorometer at an excitation wavelength of 493 nm and an emission wavelength of 515 nm. ORAC value was expressed as micromoles of TE per 100 g of sample or µmol TE/100 g.

Statistical Analysis

All experiments were conducted in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) post-hoc test to determine significant differences (p<0.05) between the sample fractions. The statistical software SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used for the analysis.

RESULTS

Effect of particle size on physicochemical properties of DRB

The proximate composition of DRB at different sizes is presented in **Table 1**, including moisture content, crude protein, TDF, IDF, and SDF. As the particle size reduced from 250 to 75 µm, a slight but significant (p<0.05) increase in protein content occurred from 16.85 to 17.66%. Although no significant difference in TDF was observed with decreasing particle size, IDF showed a significant reduction, decreasing from 20.31% to 18.03%, and SDF significantly increased from 4.05% to 6.01%. Furthermore, no significant difference in moisture content and a_w were observed, ranging from 6.65% to 6.91% and 0.33 to 0.34, respectively, with the different particle sizes.

For color change, the L^* increased significantly (p<0.05) at 64.00 when the DRB size was reduced to 75 µm, while no significant difference in L^* was observed between the particle sizes of 250 µm and 150 µm. Additionally, as the particle size reduced from 150 to 75 µm, *a** and *b** values significantly decreased from 1.58 to 1.21 and 13.21 to 11.90, respectively.

Effect of particle size on WSI and WAI of DRB

The functional properties of DRB were determined by WSI and WAI assay. As shown in **Table 2**, the WSI values of DRB exhibited a significant increase, rising from 9.77% to 10.16%, with the reduction of particle sizes from 250 to 75 µm. In contrast, the WAI values decreased significantly (p<0.05) from 3.12 to 2.95 g/g when particle sizes of DRB decreased. Thus, the WSI of the DRB with the particle size of 75 µm was higher but lower in WAI.

Effect of particle size on antioxidant activity of DRB

The antioxidant activity of DRB at different particle sizes was evaluated using the *in vitro* DPPH, FRAP, and ORAC assays, as shown in **Table 3**. With decreasing particle sizes from 250, 150, and 75 µm, a significant increase in DPPH radical scavenging activities was observed, with values of 28,372, 29,560, and 30,465 mmol TE/100 g, respectively. Similarly, the FRAP assay indicated that the antioxidant power of DRB increased significantly with smaller particle sizes (*p*<0.05), with DRB at 75 µm exhibiting the highest FRAP value at 2,609 µmol TE/100 g. Furthermore, the ORAC assay demonstrated a higher antioxidant capacity at 75 µm DRB, with a value of 20,007 µmol TE/100 g, but no significant difference was observed between the particle sizes of 250 and 150 µm.

Table 1. Comparison of the physicochemical properties of defatted rice bran at three different particle sizes

Physicochemical properties	**Particle size (µm)**		
	250	**150**	**75**
Moisture content (%) ^{ns}	6.91 ± 0.11	6.86 ± 0.06	6.65 ± 1.08
Crude protein (%)	16.85 ± 0.14^{b}	17.10 ± 0.23^{b}	17.66 ± 0.13^{a}
Total dietary fiber, TDF (%) ^{ns}	24.36 ± 0.15	24.06 ± 0.31	24.04 ± 0.21
Soluble dietary fiber, SDF (%)	4.05 ± 0.16^{c}	5.17 ± 0.12^{b}	6.01 ± 0.12^{a}
Insoluble dietary fiber, IDF (%)	20.31 ± 0.29^{a}	18.89 ± 0.39^{b}	18.03 ± 0.11^{c}
Water activity (a_w) ^{ns}	0.33 ± 0.01	0.34 ± 0.00	0.33 ± 0.00
L*	62.31 ± 0.08^{b}	62.25 ± 0.06^{b}	64.00 ± 0.02^{a}
a*	1.57 ± 0.06^{a}	1.58 ± 0.03^{a}	1.21 ± 0.02^{b}
b*	13.39 ± 0.03^{a}	13.21 ± 0.06^{a}	11.90 ± 0.03^{b}

Values are means ± standard deviation (n=3)

Different letters ($^{a-c}$) in the horizontal data set indicate a statistically significant difference (p < 0.05)

The letter(^{ns}) indicates no statistically significant difference.

Table 2. Comparison of the functional properties of defatted rice bran at three different particle sizes

Functional properties	Particle size (μm)		
	250	150	75
Water solubility index (%)	9.77 ± 0.07[c]	9.94 ± 0.08[b]	10.16 ± 0.09[a]
Water absorption index (g/g)	3.04 ± 0.03[b]	3.12 ± 0.02[a]	2.95 ± 0.02[c]

Values are means ± standard deviation (n=3)

Different letters ([a-c]) in the horizontal data set indicate a statistically significant difference ($p < 0.05$)

Table 3. Comparison of the antioxidant properties of defatted rice bran at three different particle sizes

Antioxidant components and activities	Particle size (μm)		
	250	150	75
TPC (mg GAE/100 g)	483.78 ± 4.36[c]	509.75 ± 7.28[b]	542.47 ± 8.05[a]
DPPH (mmol TE/100 g)	28,372.23 ± 423.40[c]	29,559.93 ± 217.76[b]	30,465.19 ± 307.09[a]
FRAP (μmol TE/100 g)	2,352.74 ± 31.01[c]	2,465.74 ± 35.14[b]	2,609.42 ± 55.92[a]
ORAC (μmol TE/100 g)	17,595.52 ± 287.02[b]	17,746.37 ± 774.56[b]	20,007.46 ± 701.70[a]

Values are means ± standard deviation (n=3)

Different letters ([a-c]) in the horizontal data set indicate a statistically significant difference ($p < 0.05$)

DISCUSSION

This study investigated the influence of DRB particle size on physicochemical, functional, and antioxidant properties. This study's findings indicate that reducing DRB particle size affected physicochemical attributes, especially DF composition, protein, and color profile. In addition, WSI and WAI, as functional properties of DRB, were altered as particle size declined. Notably, improved TPC and antioxidant activity were also observed after particle reduction.

With a decline in particle size, and while there was no impact on TDF, remarkable changes in the composition of DF, classified into IDF and SDF based on their solubility in water[7], were found. Specifically, IDF content decreased and SDF increased, indicating a conversion from an insoluble to soluble form, possibly caused by the degradation of IDF, such as hemicellulose, cellulose, and lignin, which were broken down into SDF and other small molecules after grinding process[22]. These results align with those of previous studies on wheat bran[23] and hull-less barley bran[24], which reported that reducing particle size to finer particles decreased IDF content and correspondingly increased SDF content. Furthermore, the finest DRB particles with the highest lightness value were found, which can be attributed to the increased surface area that allowed for higher reflection of light and thus an increase in brightness[15]. In contrast, the a* and b* values declined as the particle size reduced, which may be due to loss of pigmentation during the grinding and sieving processes[25]. Comparable results have been reported in previous studies on wheat bran[11] and foxtail millet bran[26]. The smaller particles suggest their potential for use in producing food products due to low intense color, which results in lower disrupting color, a crucial factor influencing consumer choice of foods[25]. Moisture content and a_w are critical parameters for food stability and shelf life[27]. The three sizes of DRB particles were within the limits of safe storage by minimizing microbial growth potential in terms of both moisture content (6.65-6.91%) and a_w values (0.33-0.34) and without significant changes due to particle size reduction.

The functional properties of all sizes of particles were different. Water solubility is an essential functional quality of food materials. Usually, WSI was used to determine the quantity of free polysaccharides or other compounds released from the particles to water[28]. The WSI of DRB powder increased as particle size was reduced. This phenomenon may be due to the degradation of DRB structure, starch, or IDF into free polysaccharides or water-soluble components that can be dissolved after adding excess water. Alternatively, it can be associated with increased surface area, resulting in an exposed more polar and water binding site on the DRB powder, thus leading to higher solubility as particle size declined [29]. These results are consistent with prior studies on rice bran[30] and oat bran[31] which suggest that DRB's increased water solubility may improve textural properties and the consistency of liquid-based foods and provide better thickening and stabilizing properties, making it suitable for applications in beverages, soups, and sauces.

On the other hand, water absorption capacity for smaller sized DRB particles decreased, as was determined by using WAI as a parameter representing the portion of water absorbed by granules[28]. The decrease in WAI with particle size reduction could be due to the disruption of the spatial network structure of the fiber, which is responsible for bounding water by breaking the long cellulose chains into various short cellulose fragments, resulting in the release of water held by the network. In addition, the porosity of DRB is reduced with declining particle size, leading to a lower capacity of pores to hold water[30].

Most phytochemicals in DRB were phenolic compounds, which consisted of free form and bound form covalently conjugated to the structural carbohydrates, fibers, and proteins of the cell walls [32]. After reducing particle size, TPC increased in the smaller DRB particles. Moreover, DPPH radical scavenging activity, FRAP assay, and ORAC assay presented a similar trend as TPC, with the finest DRB particles. These findings are consistent with trends observed in several previous studies[12,14,15,33]. The increased TPC and antioxidant activities of DRB might be attributed to the fact that an increase in the surface area of smaller particles would favor the dissolution of free-form phenolics or antioxidants, thus yielding higher phenolic compound during extraction[12]. In addition, the disruption of the cell wall structure by grinding broke the protein and fiber matrix structure and thus increased the availability of bound-form phenolics embedded in the matrix[12]. However, this study lacked an analysis of particle size distribution, crucial for understanding sample variability and its effects on the functional properties and bioavailability of bioactive compounds of DRB.

CONCLUSION

This study indicates that the reduction of particle size of DRB powder profoundly impacts its physicochemical, functional, and antioxidant properties. Finer fractions exhibited increased SDF, improved color, improved water solubility, and enhanced antioxidant capacity. The applications of different DRB particle sizes in food products can enhance their fiber content. Finer DRB particle sizes can be utilized in products like beverages, baked goods, and nutraceuticals, potentially reducing the gritty texture associated with coarse DRB and improving consumer acceptance. Further research should explore changes in morphology, additional functional activities, and nutrient bioavailability of DRB-enriched food products to optimize the utilization of by-products, contributing to both economics and the sustainability of the cereal industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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