ORIGINAL ARTICLE



Effects of drying on the structural characteristics and antioxidant activities of polysaccharides from *Stropharia rugosoannulata*

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Abstract We investigated the structural characteristics and antioxidant activities of two types of neutral polysaccharides and two types of acidic polysaccharides from Stropharia rugosoannulata under different drying methods. Fresh S. rugosoannulata were processed with freeze-vacuum drying (FVD) and hot-air drying (HAD). Polysaccharides from the dried S. rugosoannulata (SRP) were purified using a DEAE-52 cellulose column to obtain two types of neutral SRPs (FSRP-1 and HSRP-1) and two types of acidic SRPs (FSRP-2 and HSRP-2). We found that drying can affect the structural characteristics and antioxidant activities of SRPs. Varied monosaccharide compositions were found in FSRP-1, FSRP-2, HSRP-1 and HSRP-2, and HAD-treated SRP had more glucose and less galactose. The $(1 \rightarrow 6)$ - α -D-Galp linkage was the primary chain in FSRP-1 and HSRP-1, whereas the $(1 \rightarrow 3)$ - β -D-Glcp was the backbone structure in FSRP-2 and HSRP-2. Our results thus suggest that hot air drying changed the β configuration in polysaccharides. FSRP-1, FSRP-2, HSRP-1 and HSRP-2 had positive ferric ion reducing antioxidant power and scavenging activities on ABTS⁺ and hydroxyl radicals, whereas HSRP exhibited a stronger antioxidant activity than that of FSRP. Hot-air dried S. rugosoannulata

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could therefore be recommended as a suitable candidate for use in the preparation of antioxidant polysaccharides as functional foods.

Keywords *Stropharia rugosoannulata* · Polysaccharides · Drying · Characterization · Antioxidant activity

Introduction

Stropharia rugosoannulata is a type of valuable edible mushroom that is recommended by the United Nations Food and Agriculture Organization (FAO) for cultivation in developing countries (Song et al. 2009). S. rugosoannulata is a grass-rotting edible mushroom that has been rapidly cultivated in China in recent years. S. rugosoannulata has high nutritional value and pharmacological activities (Yan et al. 2004). In addition, it has been reported that S. rugosoannulata confers medicinal benefits such as bacteriostatic activity, antitumor and antioxidant activity and reducing endoplasmic-reticulum (ER) stress (Wu et al. 2013, 2012; Luo et al. 2006). The functional activities of S. rugosoannulata are related to its chemical compounds including polysaccharides, steroids, flavone and lectins (Zhang et al. 2014; Wu et al. 2011; Yan et al. 2020). Among these compounds, polysaccharide is the primary bioactive component, and many types of bioactivity polysaccharides have been separated from S. rugosoannu*lata* (Liu et al. 2020a).

However, fresh *S. rugosoannulata* mushrooms are highly perishable due to their high water content and high respiration rate. A drying process is often used to reduce the moisture content of *S. rugosoannulata* in order to extend its shelf life. Among various drying techniques, hot air drying is one of the most popular and frequently used drying methods for fruits and vegetables dehydration (Yu et al. 2020). With the sublimation of ice from frozen products, freeze-vacuum drying minimizes losses of flavor and nutritional composition and keeps the organoleptic properties of the initial fresh products due to the absence of liquid water and the low temperature used in the dehydration process, which hinder most of deterioration and microbiological reactions (Rezvankhah et al. 2020). So in current work, hot air drying was employed to dry S. rugosoannulata and compared with freeze dried samples. Freeze-vacuum and hot-air drying techniques are widely used to produce dried mushrooms, and it has been reported that the bioactivities of polysaccharides were influenced by drying methods (Ahmadi et al. 2019). For example, polysaccharides from Inonotus obliquus mushroom dried with the freeze drying method are reported to have better antioxidant ability (Ma et al. 2013). Moreover, Liu et al. (Liu et al. 2020b) reported that polysaccharides from freeze dried shiitake mushroom showed stronger antioxidant activities, and polysaccharides from hot-air dried mushrooms exhibited higher immunomodulatory activity. However, the effects of drying on the structural characteristics and antioxidant activities of polysaccharides from S. rugosoannulata are unknown.

The objective of this study was thus to investigate the impact of different drying methods on the structural properties and antioxidant activities of SRPs. The antioxidant activities of SRPs were determined by ferric ion reducing antioxidant power (FRAP) and scavenging capacities against ABTS⁺ and hydroxyl radicals.

Materials and methods

Dried S. rugosoannulata preparation

Fresh *S. rugosoannulata* mushrooms purchased from Chengdu (Sichuan Province, China) were separately dehydrated by freeze-vacuum drying and hot-air drying processes. One group of mushroom samples was treated with a freeze dryer (SCIENTZ-30ND, Ningbo Xinzhi Instrument Factory, Ningbo, China) for 48 h, while the other group of samples was prepared using a drying oven (Changzhou Yineng Instrument Factory, Changzhou, China) for 6 h at 50 °C. After the drying process, the two types of dried mushrooms had a moisture content of approximately 10% in wet basis.

Extraction and purification of SRPs

The dried mushrooms were treated with petroleum ether to remove any crude fat. 500 mL of distilled water was then added to 20 g of defatted sample for the extraction of polysaccharides using the microwave assisted extraction method (Liu et al. 2016). Next, the polysaccharide was precipitated with anhydrous ethanol and deproteinated by the Sevag method. Finally, the crude polysaccharide samples were obtained by lyophilization for 48 h.

The crude polysaccharide (200 mg) was dissolved in deionized water and subjected to a DEAE-52 column (3.6×20 cm) (Liu et al. 2020b). The column was then stepwise eluted with deionized water and a 0.3 M sodium chloride solution. According to the absorbance of 490 nm (DuBois et al. 1956), the first fraction eluted with distilled water was collected followed by collection of the second fraction that was eluted with 0.3 M NaCl. Finally, the obtained polysaccharides samples were dialyzed for 24 h and freeze-dried for further study.

Monosaccharide analysis

FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were hydrolyzed for the measurement of monosaccharide compositions using our reported method (Liu et al. 2020a). Briefly, each sample (10 mg) was hydrolyzed for 4 h with 4.0 M trifluoroacetic acid at 120 °C. Next, 1-phenyl-3-methyl-5pyrazolone (PMP) was added to react with the hydrolysate. The derivatives of monosaccharides were then determined by a high performance liquid chromatography system (HPLC, Agilent, United States) with a C18 column (SHI-SEIDO, 4.6 mm × 250 mm × 5 um) at a wavelength of 245 nm. Solvent A was phosphoric solution (0.1 M) with a pH value of 6.9, and solvent B was acetonitrile. The ratio of solvent A to B was 0.82: 0.18 with a flow rate of 1.0 mL/min.

Fourier-transform infrared spectra (FT-IR) analysis

FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were mixed with potassium bromide powder to prepare pellets for the detection of FT-IR spectra using a Nicolet Nexus 470 spectrometer (Thermo Nicolet, United States). The spectra of SRPs were recorded in the range of $4000-400 \text{ cm}^{-1}$ (Liu et al. 2016).

NMR spectra analysis

FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were dissolved in D_2O for the detection of ¹H NMR and ¹³C NMR spectra using a Bruker Avance Neo NMR spectrometer (Bruker Corporation, United States) at 600 MHz (Liu et al. 2020b). The spectra data were recorded at 25 °C by standard Bruker software.

Antioxidant activity evaluation

ABTS⁺ radical scavenging capacity assay

The scavenging capacities of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 on ABTS^{•+} were determined with our reported method (Liu et al. 2020b). The ABTS^{•+} solution (180 μ L) was first mixed with various concentrations of sample solutions (20 μ L). The mixed solution was kept at room temperature for 5 min and then the absorbance was assayed at 734 nm. The scavenging capacity of SRPs on ABTS^{•+} was measured using a scavenging rate (%) with the Eq. (1):

Scavenging rate (%) = $[1 - (A_1 - A_2)/A_0] \times 100$ (1)

Here, the absorbance of the control (deionized water) is A_0 , the absorbance of the ABTS^{•+} sample is A_1 , and the absorbance of the blank sample (without ABTS^{•+}) is A_2 .

Hydroxyl Radical Scavenging Capacity Assay

The scavenging capacities of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 on hydroxyl radical were assayed using the method reported by Jiang et al. (2014). Various concentrations of the sample solution (50 μ L) were mixed with FeSO₄ (50 μ L 9 mM), salicylic acid (50 μ L 9 mM), and H₂O₂ (50 μ L 20 mM). Next, the reaction was kept at 37 °C for 1 h and the absorbance was measured at 510 nm. The scavenging capacity of SRPs on hydroxyl radicals was determined as a scavenging rate (%) with the Eq. (2):

Scavenging rate(%) =
$$[1 - (A_1 - A_2)/A_0] \times 100$$
 (2)

Here, the absorbance of the control (deionized water) is A_0 , the absorbance of sample and hydroxyl radical is A_1 , and the absorbance of the sample blank (without hydroxyl radical) is A_2 .

Ferric ion reducing antioxidant power (FRAP) analysis

The FRAP values of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were measured using a reported method (Benzie and Strain 1996). First, 10 mM of 2,4,6-Tris (2-pyridyl)-s-triazime (TPTZ) solution, 20 mM FeCl₃ solution, and 300 mM acetate buffer (pH 3.6) were mixed at a volume ratio of 10:1:1 to prepare the FRAP working solution. Next, the FRAP working solution (180 μ L) was mixed with 5 μ L sample solution and incubated for 5 min at room temperature, and various concentrations of FeSO₄ were used as the control group. The incubation solution was then determined at an absorbance of 593 nm. The FRAP values of the four types of SRPs were expressed as equivalent concentrations of FeSO₄ (mM Fe²⁺/mg).

Statistical analysis

All analyses were performed in triplicate, and the results were given as mean \pm standard deviation. The variance analysis was performed using one-way analysis of variance with an assay of differences (Duncan's test, P < 0.05).

Results and discussion

Purification of SRPs

The crude polysaccharide from S. rugosoannulata was purified using a DEAE-52 cellulose column based on the acidic group levels in samples (Liu et al. 2016). As shown in Fig. 1, two main polysaccharide fractions from FVD treated mushrooms were separately collected with deionized water and 0.3 M of NaCl solution. Similar results were also reported in the purification of polysaccharides from four kinds of mushrooms (Yan et al. 2019). The neutral polysaccharide fraction eluted with distilled water was named FSRP-1. However, the acidic polysaccharide fraction eluted by 0.3 M NaCl solution was named FSRP-2 (Fan et al. 2012a). A similar result for HAD treated mushrooms is shown in Fig. 1. The two separated fractions named HSRP-1 and HSRP-2 were collected. Next, through concentration, dialysis and lyophilization, the four types of SRPs including FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were obtained for further study.



Fig. 1 Elution profile of SRP on DEAE-52 chromatography column with gradient of NaCl solution (0 and 0.3 M)

Monosaccharide compositions analysis of SRPs



 $(t_R = 34.985 \text{ min})$ were detected in the four types of SRPs. The results indicated that FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were all heteropolysaccharides. Liu et al. (2020a) also determined that glucose, galactose and mannose were the primary monosaccharide components in the SRPs. In addition, the monosaccharide compositions of mushroom



polysaccharides were mainly reported to be glucose, galactose and mannose (Morales et al. 2019).

As shown in Table 1, the dominant monosaccharide of the four types of SRPs was all glucose. However, FSRP-1, FSRP-2, HSRP-1 and HSRP-2 had different molar ratios for the four kinds of monosaccharides. FSRP-1 and HSRP-1 (neutral polysaccharides) had molar ratios of 1:7.70:25.39:2.46 and 1:4.34:31.02:1.53, respectively.The molar ratios in FSRP-2 and HSRP-2 (acidic polysaccharides) were 1: 6.20:19.19:3.55 and 1:5.63:13.79: 0.37, respectively. A decrease in glucose and an increase in mannose levels were found in the acidic polysaccharides. Compared with the FVD treatment, higher levels of glucose and lower levels of galactose were found in the HAD treated SRPs. Similar results were also reported in bamboo polysaccharides (Chen et al. 2019). The degradation of galactose might be related to the higher temperature or oxygen content in the HAD process. Our results indicated that the monosaccharide ratios of polysaccharides were influenced by drying pretreatments. The monosaccharide compositions in other mushroom polysaccharides were also reported to be changed by drying methods. Ma et al. (2013) also reported that the treatments of freeze drying, hot air drying and vacuum drying changed the monosaccharide (rhamnose, arabinose, mannose, galactose and glucose) compositions of polysaccharides from Inonotus obliquus mushroom. Due to the different modes of water loss in S. rugosoannulata during FD (sublimation) and HD (evaporation) process, the bound water of the SRPs may be different (Huang et al. 2021a), which may affect the monosaccharide compositions.

FTIR spectroscopy measurements of SRPs

The infrared spectra of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 are shown in Fig. 3. The absorption peaks at 3410 cm^{-1} were related to the hydroxyl groups in SRPs. The two peaks of 2925 cm⁻¹ and 2360 cm⁻¹ were due to the C-H stretching vibrations in SRPs (Chen and Huang 2019). The broad absorption bands with strong intensities at 1640 cm⁻¹ were shown for C = O (Yang et al. 2020), and the weak peaks around 1540 cm⁻¹ indicated that FSRP-1, FSRP-2, and HSRP-1 had combined proteins. The C-O stretching vibration of carboxyl groups was shown at

Table 1Monosaccharidecomposition of polysaccharidesfrom dried S. rugosoannulata



Fig. 3 FT-IR spectrum of FSRP-1, FSRP-2, HSRP-1, and HSRP-2

about 1421 cm⁻¹. The vibration of S = O shown at 1150 cm⁻¹ was the presence of sulfates in FSRP-1, FSRP-2 and HSRP-1 (Wang et al. 2018). The two peaks around 1077 cm⁻¹ and 1045 cm⁻¹ were due to the galactose and glucose in the SRPs. This result was consistent with the monosaccharide compositions analysis (Table 1). Yang et al. (2018) also reported that the band at approximately 1070 cm⁻¹ showed the presence of galactose. The diagnostic absorption peak at about 803 cm⁻¹ would suggest that FSRP-2 and HSRP-1 had α -type glycosidic linkages of galactose (Shi et al. 2017). Based on the FT-IR analysis,

Sugar components (molar ratio)	Polysaccharide samples			
	FSRP-1	FSRP-2	HSRP-1	HSRP-2
Arabinose	1	1	1	1
Galactose	7.70	6.20	4.34	5.63
Glucose	25.39	19.19	31.02	13.79
Mannose	2.46	3.55	1.53	2.37

the preliminary structures of SRPs were not changed by different drying methods. Similar results of polysaccharides from *Hohenbuehelia serotina* (Li et al. 2016) and *Medicago sativa* L. (Shang et al. 2021) were also reported.

NMR Spectroscopy Analysis of SRPs

The NMR technique has been utilized in food systems to determine the chemical structure of polysaccharides (Yang and Yang 2020; Pizzoferrato et al. 2000). Figure 4 shows the ¹H NMR and ¹³C NMR spectra of FSRP-1, FSRP-2, HSRP-1 and HSRP-2. The typical peak distributions of polysaccharides (δ_H 3.4–5.4 ppm and δ_C 60–110 ppm) were found in SRPs (Wang et al. 2014). The strong signals in the $\delta_{\rm H}5.04$, 5.03 ppm and $\delta_{\rm C}93.18$ ppm regions of the FSRP-1 were due to the anomeric protons of α -D-Galp, whereas the β -D-Glcp generally occurred at the δ 4.84, 4.49, and 4.48 ppm shifts. HSRP-1 was detected to have three chemical shifts of $\delta 5.04$, 5.03 and 4.84 ppm, but the signals at $\delta 4.49$ and 4.48 ppm were not determined, indicating that the HSRP-1 had lower levels of β -D-Glcp than that of the FSRP-1. Huang et al. (2021b) also used the corresponding areas in the NMR spectrum to determine the levels of β -D-glucose. The signals in the δ_{C} 72.47, 72.11, 71.08, 69.65, and 60.47 ppm suggested that the primary chain in the both of neutral SRPs was $(1 \rightarrow 6)$ - α -D-Galp linkage (Maity et al. 2013). Moreover, FSRP-1 had side both chains of $(1 \rightarrow 6)$ - β -D-Glcp and $(1 \rightarrow 3)$ - β -D-Glcp, whereas HSRP-1 only had side chain of $(1 \rightarrow 3)$ - β -D-Glcp. After hot-air drying, the increased ratio of 1,3-link residues may be due to the fact that 1,3-links are more likely to formed during the high temperature drying process than 1,6-links (Gan et al. 2021). The α type of glycoside was also previously reported in mushroom polysaccharides (Tang et al. 2020). For FSRP-2, the strong signals at about $\delta_{\rm H}4.84$ ppm and $\delta_{\rm C}102.96$ ppm showed the β -D- Glcp, and the weak signal at δ_H 4.98 ppm showed the α -D-Galp, whereas the β -D- Glcp was the dominant glycoside bond. Similar ¹H NMR and ¹³C NMR chemical shifts were also determined in HSRP-2, while the signal at δ 4.84 ppm was stronger than that of FSRP-2, indicating that the HSRP-2 had higher levels of β -D-Glcp. The signals in the δ_{C} 75.52, 72.99, 69.43, 68.20, and 60.61 ppm suggest that the $(1 \rightarrow 3)$ - β -D-Glcp linkage was the primary chain in the acidic polysaccharides (FSRP-2 and HSRP-2), and both had side chains of $(1 \rightarrow 4)$ - α -D-Galp. In general, our results suggested that food drying pretreatments could chang the sugar configurations of SRPs. After the hot-air drying process, the neutral polysaccharides had a higher β configuration level, whereas the acidic polysaccharides had a lower one. The results were in agreement with Gan et al. (2021), who reported that the drying process affected the configurations of longan polysaccharides.

Effects of drying on antioxidant activities of SRPs

ABTS⁺ radical scavenging activity of SRPs

The ABTS^{•+} scavenging abilities of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 are shown in Fig. 5a. The scavenging rates of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 reached 84.62%, 79.01%, 83.86.52%, and 99.70%, respectively, at a concentration of 5 mg/mL. The results indicated that the four types of polysaccharides had positive ABTS^{•+} scavenging abilities. Among the four types of SRPs, HSRP-2 had the highest ABTS^{•+} scavenging activity which might be due to its higher level of β -D-Glcp compared to that of the other SRPs. It has also been reported that the scavenging capacities of polysaccharides were affected by the glucoside bond type (Shi et al. 2013). The IC_{50} values of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were determined at 1.02, 1.38, 0.89 and 0.93 mg/mL, respectively. The results indicated that HAD treated samples had stronger scavenging abilities than the samples treated by FVD. We suggested that the scavenging activity of SRPs on ABTS^{•+} was affected by drying pretreatments, and the hot-air drying process would thus improve the ABTS^{•+} scavenging abilities of SRPs. However, Wu et al. (2014) reported that polysaccharides from Agaricus blazei Murrill obtained by FD method showed higher scavenging activity on ABTS radicals than that of HD method.

Hydroxyl radical scavenging activity of SRPs

The hydroxyl radicals scavenging activities of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 are shown in Fig. 5b. Generally, FSRP-1, FSRP-2, HSRP-1 and HSRP-2 showed potential scavenging activities against hydroxyl radicals. The scavenging activities of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 increased to 50.58%, 62.69%, 52.18% and 97.55%, respectively, when the sample concentration reached 10 mg/mL. This result was lower than that of polysaccharides from shiitake mushrooms (Wang et al. 2015), but higher than that of polysaccharides from *Lepista* nuda mushrooms (Shu et al. 2019). Moreover, the IC_{50} values of FSRP-1, FSRP-2, HSRP-1 and HSRP-2 were 9.85, 5.38, 9.35, and 2.87 mg/mL, respectively. The hydroxyl radicals scavenging activities of SRPs was in the order of: HSRP-2 > FSRP-2 > HSRP-1 \approx FSRP-1. The results indicated that the neutral polysaccharides (HSRP-1 and FSRP-1) showed lower hydroxyl radicals scavenging activities that those of acidic polysaccharides (HSRP-2 and FSRP-2). Previous results also reported that the acidic polysaccharides from mushrooms had a higher antioxidant activity (Liu et al. 2016). Our results suggested that the scavenging capacity of acidic polysaccharides from S. rugosoannulata might be improved by hot-air drying



pretreatment. However, the hydroxyl radical scavenging activity of polysaccharides from *Ganoderma lucidum* was reported to increase after freeze drying process (Fan et al. 2012b).

Ferric ion reducing antioxidant power (FRAP) of SRPs

The ferric ion reducing antioxidant powers (FRAP) of the four types of SRPs are shown in Fig. 5c. FSRP-1, FSRP-2,

Fig. 4 ¹H NMR (**a**) and ¹³C NMR (**b**) spectra of FSRP-1, FSRP-2, HSRP-1, and HSRP-2



Fig. 5 Antioxidant activity of FSRP-1, FSRP-2, HSRP-1, and HSRP-2. (a) $ABTS^+$ radical scavenging capacity; (b) Hydroxyl radical scavenging capacity; (c) FRAP abilities, different letters (a, b, c and d) indicate significant differences (P < 0.05)

HSRP-1 and HSRP-2 were determined with FRAP values of 28.96, 50.47, 43.66, and 75.12 mM Fe²⁺/mg, respectively. Our results indicated that the FRAP of the four types of SRPs decreased in the order of: HSRP-2 > FSRP-2 > FSRP-1 > FSRP-1 (P < 0.05). The results suggested that the FRAP activity of neutral polysaccharides was lower than that of the acid polysaccharides. Similar results wee also reported for *Ganoderma lucidum* polysaccharides (Shi et al. 2013). Compared with freeze-vacuum drying, the ferric ion reducing antioxidant power of SRPs was improved by the HD process.

Overall, FSRP-1, FSRP-2, HSRP-1 and HSRP-2 displayed potential antioxidant activities. HSRP-2, having a high β -D-Glcp level, showed the strongest antioxidant activity among the four types of polysaccharides. Our results indicated that hot-air drying process improved the antioxidant activities of polysaccharides from *S. rugosoannulata*, and acid polysaccharides would be the primary antioxidant polysaccharides. Similar results were also reported about polysaccharides from *Taraxacum mongolicum* (Li et al. 2021). The antioxidant activities changes of SRPs could be due to the varied enzyme activities in *S. rugosoannulata* during drying process, polysaccharides-related enzymes were activated by hot-air drying pretreatment (Chen et al. 2018).

Conclusion

In this study, four types of polysaccharides including two neutral polysaccharides (FSRP-1 and HSRP-1) and two acidic polysaccharides (FSRP-2 and HSRP-2) were purified from S. rugosoannulata treated by FVD and HAD. The monosaccharide composition, chain conformations, and antioxidant activities of polysaccharides from S. rugosoannulata were altered by food drying process. The neutral SRPs had a primary chain of $(1 \rightarrow 6)$ - α -D-Galp while the acidic SRPs had a primary chain of $(1 \rightarrow 3)$ - β -D-Glcp. Hot-air drying treatment enhanced the antioxidant activities of SRPs, especially the acidic SRPs. We would thus recommend preparing biological polysaccharides from dried S. rugosoannulata treated by the HAD process. However, the bioactive mechanism and structure-activity relationships of SRPs are envisaged by future in vivo experiments.

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Authors contributions QW: Methodology, Investigation, Data Curation. YZ: Methodology, Investigation, Data Curation. XF: Writing—Review & Editing. SAI: Writing—Review & Editing. WH: Data Curation, Resources. YL: Writing—Original Draft, Supervision, Resources.

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Declaration

Conflicts of Interest The authors declare no conflict of interest.

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