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Modified Bamboo Shoots Flour Derived from the Ampel Gading Bamboo (*Bambusa vulgaris* Schrad var. Striata): Physicochemical Properties and Potential Applications as a Thickening Agent

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Abstract

Modified flour is widely used in the food industry to enhance viscosity and texture. Previous research has investigated fermenting Bamboo Shoots Flour from Ampel Gading Bamboo which is rich in fiber. Physical process combination, like temperature changes, and chemical modifications using acids or bases, may alter the flour's gel-forming properties, thereby expanding its applications, including as a thickening agent. The objective of this study is to evaluate the physicochemical properties and potential applications of Modified Bamboo Shoots Flour (MBFS) as a thickening agent. The analysis demonstrated that MBSF comprises 28.41% carbohydrates, with 4.88% crude fiber and 18.68% starch, featuring 4.74% amylose and 13.94% amylopectin (wet basis). Additionally, it contains 28.10% protein and 11.17% fat (wet basis), maintaining the characteristic form of MBSF. Scanning Electron Microscope (SEM) evaluation revealed the presence of ovate-shaped, rough and irregular surface starch granules. Heating a 2% MBSF suspension to 100°C increases viscosity, solubility, and swelling power. Low acidity (pH 10) enhances swelling power without affecting viscosity significantly. Both low acidity and heat treatments enhance the thickening properties of the MBFS. This study offers fundamental insights into the physical and chemical characteristics of MBFS, thereby facilitating its potential application in final products.

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Introduction

The annual increment, around 10-12%, in the quantity and value of imported food additives for Indonesia's food and beverage industry is notable. Extensive research spanning several decades has been conducted on diverse hydrocolloid sources employed as food additives.[1,2,3] Thickeners are deliberately incorporated to impact specific attributes of food quality. Presently, Indonesia's regulatory framework approves 59 types of thickeners for circulation and use in food processing, including pectin, cellulose, dextrin, enzyme-modified starch, acid, and alkaline modified starch.[4,5,6] Modified starches are derived through various methods such as high-pressure heat application, enzymatic, and chemical modifications.[5,6] Enzymatically modified starches are achieved through fermenting carbohydrate-rich raw materials utilizing culture or spontaneous fermentation, for instance, the production of fermented cassava flour and modified bamboo shoots starch.[5,6,7,8,9]

Bamboo shoots stand as one of the widely favored vegetables in Central Java, Indonesia, commonly utilized fresh or fermented as fillers in spring rolls.[9,10] Typically sourced from the Ampel Gading bamboo variety, fermented bamboo shoots (FBS) exhibit the capacity to enhance dissolved dietary fibers, mitigate cyanide acid (HCN), and improve texture and digestibility.[9,11] Recognized for its nutritional and functional attributes, fermented bamboo shoots serve as a wholesome dietary option.[12,13] Previous investigations have indicated that Fermented Bamboo Shoots Flour (FBSF) is rich in proteins, minerals, and insoluble fibers, rendering it unsuitable as a thickening agent but suitable as animal feed.[11]

Fermented bamboo shoots are used in traditional Indian medicine (Ayurveda) for various medicinal purposes[13] and extend into non-food industries, contributing to the production of bioethanol, bio-methane, food fibers, carbohydrates, and serving as a raw material for extracting potassium minerals.[13,14] Numerous studies worldwide have explored the health benefits of both fermented and unfermented bamboo shoot flour, its potential as a substitute for wheat in cookie production, and its role in altering the nutritional composition of frozen products.[15,16,17] Reports indicated the utilization of fermented bamboo shoots flour (FBSF) derived from Petung bamboo (*Dendrocalamus asper*) in cookie dough preparation, while a combination of FBSF and swamp tuber, originating from South Kalimantan, Indonesia, demonstrated suitability as a coating for various fried products.[15] Acceptable supplementation of cookies with bamboo shoot flour is up to 6%.[16] FBSF, abundant in proteins, lipids, minerals, and insoluble fibers, holds promise as animal feed due to its low acid detergent fibers (ADF) and neutral detergent fibers (NDF) content.[11]. Fermented Bamboo Shoots (FBS) as a substitution or supplementation material in food product manufacturing remains highly promising. A synergistic approach to processing targeted at generating modified flour holds potential for broadening its applicability in food products, with a particular emphasis on its role as a thickening agent. The integration of physical modifications, characterized by temperature variations, alongside chemical alterations utilizing acids and bases, may exert influence on the gelling properties inherent to modified fermented flour. This combined approach offers a comprehensive strategy for optimizing the functional attributes of flour to meet the diverse requirements encountered in food formulation and processing. Through the integration of various flour modification processing techniques, this study aims to evaluate the physicochemical properties and potential applications of Modified Bamboo Shoots Flour as a thickening agent in food products.

Materials and Methods

Materials

Bamboo shoots of the Ampel Gading var. (*Bambusa vulgaris* Schrad var. Striata) obtained from bamboo farmers in Demak (Central Java, Indonesia) were validated by the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University Yogyakarta with a certificate No.01479/S.Tb/III/2021. Culture *Lactobacillus plantarum* FNCC-0027 (CCRC 12251) was obtained from the Food and Nutrition Culture Collection (FNCC) Microbiology Lab. Gadjah Mada University. The chemicals used for analysis were sulfuric acid (H_2SO_4), potassium sulfate (K_2SO_4), boric acid (H_3BO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl), petroleum ether, glucose, acetic acid, and potassium iodide (KI) obtained from Sigma-Aldrich (Missouri, USA).

Sample Preparation of Modified Bamboo Shoots Flour

Fresh bamboo shoots measuring between 20 and 23 cm in height were dissected to separate the blade and sheath parts, obtaining the edible part of bamboo shoots (EPBS). The EPBS underwent thorough washing with running water until completely clean. Subsequently, the bamboo shoots were boiled in a water bath at 80±3 ^{o}C for 30 minutes.[17] Post-boiling, the boiled bamboo shoots (BBS) were removed, drained, and sliced into 0.2-0.4 mm thick pieces using a slicer. These slices were then subjected to fermentation in a 5% salt solution with the addition of *Lactobacillus plantarum* FNCC-0027 starter (10 mL/L of mixture) for a fermentation period of 14 days within a fermenter set at 28±2 ^{o}C and 80-90% relative humidity in a sealed air fermenter. The fermented bamboo shoot slices were pulverized using a chopper to obtain a slurry. The resultant slurry was filtered using a filter cloth to extract the filtrate. Sedimentation of the filtrate was conducted for 12 hours, followed by decantation to separate the solid sediment. The obtained sediment underwent washing and separation processes involving two cycles of water filtration and settling. Subsequently, the clean sediment was dried in an oven at 45 ^{o}C for 20 hours, ensuring a moisture content of 8-10% at the completion of the drying process. The resulting dried sediment was identified as modified bamboo shoots flour (MBSF).

Preparation of MBSF Gel at Various Temperatures

The preparation of MBSF gel was conducted following the methodology outlined in[2] with slight modifications. A solution containing 2% MBSF was heated in a water bath at various temperatures corresponding to specific treatments (60, 70, 80, 90, and 100°C) for 15 minutes under continuous stirring. Subsequently, heating of the MBSF gel was ceased, allowing the gel to cool down to room temperature (28-30°C). Once cooled, the viscosity of the gel was measured using a Brookfield viscometer.

Preparation of MBSF Gel at Different Acidity Levels

The preparation of MBSF gel at varying acidity levels was conducted following the procedure outlined in.[18] with minor adaptations. A mixture comprising 1.5% MBSF in solvents with different acidity levels (ranging from pH 2 to pH 10) was heated in a water bath at 90°C for 15 minutes while continuously stirring. Subsequently, the heating of the MBSF gel was terminated, allowing the gel to cool to room temperature (28-30°C). After reaching room temperature, the viscosity of the gel was measured using a Brookfield viscometer.

Viscosity Measurement

The measurement of gel viscosity was conducted following the method described in[2] with minor adjustments. In brief, each sample, comprising 100 mL, was placed in a 250 mL glass beaker, and three spindles were utilized, setting the shear rate at 200 rpm. Triplicate measurements were performed for each treatment.

Determination of MBSF Solubility

The solubility (%) of MBSF was assessed using the method outlined by Pham *et al.* [18] with slight modifications. Initially, 0.5 g of MBSF was suspended in 30 mL of distilled water within a 50 mL test tube. The suspension was then heated in a thermostatically controlled water bath for 30 minutes at varying temperatures, ranging from 60 to 100°C in 10°C intervals. Subsequently, the test tube was rapidly cooled to room temperature before centrifugation at 2500 rpm for 30 minutes. The supernatant obtained was transferred into an aluminum cup and dried at 120°C for 4 hours. The solubility of MBSF was calculated using the following formula:

Solubility (%)= (MBSF dissolved in supernatant (g))/(Dry MBSF weight) (g)×100%

Determination of Swelling Power

Swelling power refers to the increase in volume and weight of MBSF following its exposure to water and subsequent heating. The swelling power of MBSF was determined following the methods outlined by Pham *et al.*,[18] with slight modifications. Initially, a sample of MBSF weighing 0.2 g was dispersed in 5 mL of distilled water and then heated incrementally from 60 to 100°C at 10°C intervals. After maintaining this temperature for 10 minutes, the heated sample was rapidly cooled to room temperature and subsequently centrifuged at 2500 rpm for 15 minutes. The supernatant was carefully removed, and the swelling power was calculated as the weight of the sediment using the following formula:

Swelling power (%)= (MBSF post weight (g))/(Dry MBSF weight) (g)×100%

Determination of Chemical Composition, SEM, and Color Analysis

The proximate analysis of MBSF was conducted following the pertinent standard procedure outlined in.[19] The amylose content was quantified in accordance with the methodology described by,[20] while the determination of starch content was performed using the method outlined in AOAC 920.44. The assessment of starch granule morphology and the molecular composition of MBSF were examined using scanning electron microscopy (SEM/EDX Mapping). Additionally, colorimetric measurements were obtained using a Chromameter Minolta CR 400.

Statistical Analysis

The physicochemical properties data of MBSF were acquired from three replicates and are presented as mean ± standard deviation. Statistical analysis was conducted to ascertain significant differences ($p < 0.05$) among the obtained results, utilizing ANOVA followed by Duncan's multiple range test. All statistical analyses were performed using SPSS 23.0 (SPSS Inc., USA).

Results and Discussion

Chemical Composition and SEM Profile

Extraction of modified bamboo shoots flour (MBSF) by filtration and sedimentation methods obtained a yield of 0.37 ± 0.02% as crude starch. The chemical composition of modified flour from fermented bamboo shoots is shown in Table 1. The starch content of MBSF was 18.68 ± 0.11%, which is composed of amylose 4.74 ± 0.04 % and amylopectin 13.94 ± 0.14% respectively. The amylopectin fraction is much larger than the amylose (3:1), as is the composition of starch in general. Amylopectin, a branched polysaccharide, contributes to the gelatinization process and the formation of gels. Amylose, a linear polysaccharide, also plays a role in the thickening properties of starch. The ratio between amylose and amylopectin determines the texture of the gelatinized starch, with a higher amylose content resulting in firmer gels. However, the MBSF contains relative high protein (28.10 ± 0.05 %), lipid (11.27 ± 0.06%), and ash (24.00 ± 0.02 %), so does not meet the standard as starch flour.[21] Referring to the national tapioca industry standard, SNI 3451:2011 concerning tapioca, which requires a minimum starch content of 75%, a maximum of 0.4% crude fibers and maximum moisture content of 14%.[22]

Scanning with an electron microscope shows the shape of MBSF granules is ovate, rough and irregular surface, that may provides more surface area for interaction with liquids, enhancing the thickening properties of the flour (Figure 1). This is because a rough surface increases the contact area between the flour and the liquid, allowing for more efficient absorption and swelling of the starch granules. MBSF as a food additive is a minerals source of 24% (Table 1), consisting of 4.3% Na_2O, 0.7% P2O5, 0.7% SO_3, 5.32% Cl, 0.95% K_2O, 0.64% Ca0, 1,05% CuO and Ag_2O in trace. From Fig. 1. It appears that the starch molecules are spaced out, this confirms that the starch obtained is still in the form of crude starch (Table 1).

Table 1: Chemical composition of MBSF

Sample	Content
Moisture (%)	8.20 ± 0.03
Lipid (%)	11.27 ± 0.06
Protein total (cf = 6.25)	28.10 ± 0.05
Carbohydrate (*by difference*)	28.41 ± 0.03
Crude fiber (%)	4.88 ± 0.05
Starch (%)	18.68 ± 0.11
Amylose (%)	4.74 ± 0.04
Amylopectin (%)	13.94 ± 0.14
Calorie (kkal)	280.81 ± 0.52
Color (L)	73.82±0.025
Color (a*)	0.12 ± 0.015
Color (b*)	18.23 ± 0.04
Bulk density (g/cm^3)	0.76 0.03

Note: Values are mean ± standard deviation

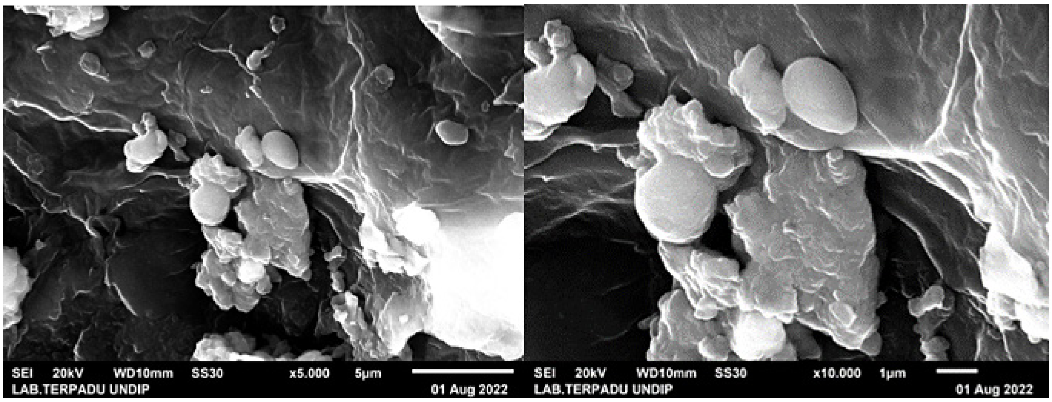

Fig. 1: Modified bamboo shoots flour granule morphology scanning as result of scanning with SEM/EDX at 5.000 x (left) and 10.000 x (right) magnification.

Psychochemical Properties of MBSF Gel by Changes in Heating Temperature

Changes in the Gel Viscosity

The gel viscosity of 2% flour suspension at 5 heating temperature (60-100oC) is shown in Table 3. The heating temperature had a significant effect on the resulting gel viscosity ($p<0.05$). The increasing suspension heating temperature resulted in an increasingly viscous gel. The higher the heating temperature, the more color will be generated.

Table 2: Physicochemical gel of MBSF at various heating temperature

Sample	Viscosity (cP)*	Solubility (%)*	Swelling power (%)*
T1/ 60 ºC	9. 50 ± 1.00^{a}	1.02 ± 0.06^{a}	17.33 ± 0.42^{a}
T2/ 70 ºC	10.00 ± 0.50^{a}	1.77 ± 0.08^{b}	21.99 ± 0.44^{b}
T3/ 80 ºC	11.16 ± 0.29^{b}	2.42 ± 0.67^{c}	23.07 ± 1.82^{c}
T4/ 90 ºC	13.00 ± 0.50^{c}	3.07 ± 0.12^{d}	25.15 ± 1.99^{d}
T5/ 100 ºC	13.83 ± 0.28^{c}	3.91 ± 0.26^{e}	27.71 ± 2.54^{e}

*Numbers followed by different superscript letters in the same columns indicate there were a significant difference between treatments ($p<0.05$), n=3.

Heat is used to break the bond between starch molecules, so that the broken starch granules bind more water on the amorphous side and cause the starch suspension to become more viscous.[23] The maximum viscosity of the 2% MBSF suspension which is 13-13.8 cP., is equivalent to the viscosity of 2% sweet potatoes starch.[18] The MBSF gel viscosity assay using the Perten rapid analyzer method (Model RVA 4, Newport Scientific, Australia) obtained equivalent data. However, the viscosity of MBSF is much lower than that of Konjac flour (*A. oncophyllus*) 1.5% of 12x103 cP.[2] This may be related to the level of purity of MBSF (Table 1).

Solubility of MBSF

Flour solubility expresses the amount of flour (g) dissolved in the supernatant (100 g of solvent) due to the amylose fraction leaching and dissociating and coming out of the granules during swelling, then the is recovered after supernatant is dried. The statistical analysis showed that the heating temperature had a significant effect of solubility ($p<0.05$), shown in Table 2. The solubility of MBSF was lower than the solubility of both Yam and sweet potato starch. The solubility of both flour at heating temperature of 40-90 ºC according to Pham *et al.*[18] between 2-20% and 1-5% respectively. The solubility of modified cassava flour was 3.32 (g/g).[24] Heat causes the interaction of the amylose and amylopectin fraction in the dissociated polysaccharide structure, causing an increase in water-soluble amylose.[18,24] Solubility is perceived because the attractiveness of the solute fraction is greater than the solvent.[23] The amylose fraction of 4.74±0.04% (Table 1) as the soluble fraction which is much smaller than the amylopectin of 13.94%, was equivalent to the MBSF solubility at 90-100 oC of 3.9 % (Table 2).

Swelling Power of MBSF

In general, the heating temperature had a significant effect on the swelling power ($p<0.05$). The higher the heating temperature (60-100 ^{o}C) causes a 60% increase in swelling power. This is in line with what was stated by Pham *et al.*[18] that heating at 40-90 oC increases the swelling power of sweet potato starch (2-14 g/g) and Taro starch (2-12 g/g). The swelling power of MBSF (Table 2) is very low when compared to the swelling power of others type of starch flour. It was added that the swelling power of modified cassava flour was 941-2718%,[24] while sweet potato starch was 200-1400% and Taro starch was 200-1200%.[18] The low gel swell ability of starch is due to the low starch amylose content.[25] Another factor is thought to be due to the low level of MBSF purity. The swelling power of MBSF was correlative (r=0.92) with the viscosity gel obtained and the correlative (r=0.96) with its viscosity value. Meanwhile according to Pham *et al.*,[18] swelling power is positively correlated to viscosity but does not to solubility.

Changes in Color of MBSF Gel

Color analysis of thickening agents is important because it can influence the appearance of the final product upon their application. Heating causes a change in the color of the flour suspension to become opaque at first, but as the temperature increases and the fraction of flour granules gelatinizes, a clearer gel is formed.[24] Such a phenomenon was not seen in heating the 2% MBSF suspension (Table 3).

Table 3: Changes in color of MBSF gel

Sample	Lightness (L*)	Redness (a*)	Yellowness (b*)
T1/ 60 ºC	72.71 ± 4.03^{d}	-2.21 ± 0.37^{a}	2.10 ± 0.69^{a}
T2/ 70 ºC	66.15 ± 2.05^{c}	-1.79 ± 1.20^{a}	5.66 ± 0.28^{b}
T3/ 80 ºC	57.69 ± 2.85^{b}	-1.35 ± 1.08^{ab}	12.01 ± 3.62^{cd}
T4/ 90 ºC	56.57 ± 0.52^{b}	1.01 ± 0.63^{c}	14.73 ± 0.40^{d}
T5/ 100 ºC	41.21 ± 1.37^{a}	-0.10 ± 0.08^{b}	16.74 ± 0.30^{d}

*Numbers followed by different superscript letters indicate there was a significant difference between treatments ($p<0.05$), n=3

Heating 2% MBSF at 60-100oC showed an increase in turbidity. The higher the heating temperature, the obtained gel color tends to be more yellow and not bright (Table 3). It is suspected that the fraction of broken starch granules-causing amylose and amylopectin leaching – followed by water absorption (swelling) is relatively low compared to the non-starch fraction. So the accumulation of water trapped in the broken starch granules (gelling) is not sufficient to provide a transparent effect of light.[18,24] On the other hand, MBSF contains 28.10% protein and 11.27% lipid which is thought to contribute to the discoloration of the obtained gel.

Physicochemical Properties of MBSF Gel by Changes in Acidity

To determine the effect of the degree of acidity (pH 2-10) on physicochemical properties of MBSF gel, heating 1.5% MBSF suspension in acetic acid solution (pH 2-6) and sodium hydroxide (pH 8-10) solution with heating at 90 oC/15 minutes was performed. The viscosity, turbidity and swelling power of modified bamboo shoot flour formed can be seen in Table 4.

Table 4: Physicochemical properties of MBSF gel by changes in acidity

Sample	Viscosity (cP)	Abs. (λ=650 nm)	Swelling power (%)
pH 2	6.83±0.57[a]	2.32±1.0[ab]	14.8±1.0[d]
pH 4	6.66±0.28[a]	2.40±0.9[a]	18.8±1.0[c]
pH 6	7.66±0.28[a]	2.21±0.6[b]	21.1±1.0[b]
pH 8	7.16±1.04[a]	2.42±0.5[a]	17.3±0.7[c]
pH 10	6.66±0.28[a]	2.32±0.5[ab]	25.3±1.1[a]
Ref./pH6	56.16±7.14*	0.24± 0.0*	140.0±5.0*

Numbers followed by different superscript letters indicate there was a significant difference between treatments (p<0.05), n=3. *Ref. is tapioca suspension at 1.5%, pH 6.

Changes in the Gel Viscosity

The degree of acidity had no significant effect on the viscosity of MBSF gel (p>0.05). The MBSF gel value was quite low (6.66-7.16 cP), lower than the viscosity of 1.5% tapioca gel (pH6) of 56.16 ± 7.14 cP. This is in line with Akesowan,[2] which stated that differences in degree on acidity do not significantly affect the viscosity of the Konjac flour gel obtained.

Changes in the Turbidity of MBSF Gel

Acidity of the solvent significantly affected the turbidity of the MBSF gel obtained (p< 0.05) (Table 4). The level of turbidity is inversely proportional to clarity, and it is affected by temperature, heating time and degree of acidity. When the heating temperature increases, the turbidity decreases.[24] According to Noranizan *et al.*[1] the clarity of the paste is affected by penetration and trapping of water in the matrix, causing the starch granules to expand and increasing the light transmitting properties. Data from Table 4 shows that the clarity of the MBSF gel (pH 6-7) is the clearest. This is different from what was conveyed by Diniyah *et al*[24] that the increasing acidity of the solvent causes the turbidity in the modified cassava flour suspension to decrease. In addition, the turbidity of the modified cassava flour suspension decreased when the acidity mixture increased. When compared to the clarity of the tapioca gel (OD = 0.24), the MBSF clarity is quite low (OD = 2.21-2.40). This is thought to be related to the level of sample purity.

Changes in Swelling Power of MBSF Gel

The swelling properties of MBSF at various degrees of acidity (90 oC/pH 2-10) are shown in Table 4. In general, acidity has a significant effect on the swelling power (p< 0.05). The swelling power increases slightly, along with increasing degree of acidity. In the acidic range (pH 2-8), the swelling power did not change much but increased drastically in the basic zone. The swelling power during acid treatment, the hydrogen bonds between adjacent starches polymers are disrupted, therefore the amorphous regions are eroded, resulting in lower swell ability.[24] Pham *et al.*,[18] stated that acid treatment causes partial hydrolysis, thereby reducing swelling properties. The swelling ability of tapioca was 140 ± 5.0 %, while the swelling ability of MBSF was much lower. This is due to the low purity of MBSF, which contains 18.68±0.11 % starch and 4.74 ±0.04 % amylose, while standard tapioca contains at least 75 % starch.

Conclusions

The modified bamboo shoots flour (MBSF), obtained through the filtration and settling of bamboo shoots slurry, still retained crude starch with a yield of 0.37 ± 0.02%. The chemical characteristics of MBSF consisted of 28.41% carbohydrate, comprising 4.88% crude fiber; and 18.68% starch, which consists of 4.47% amylose and 13.94% amylopectin (wet basis). Additionally, it contains 28.10% protein and 11.17% lipid content (wet basis). Meanwhile, the physical characteristics of MBSF subjected to varying temperature treatments ranging from 60 to 100°C exhibited notable changes in viscosity, swelling, solubility, and color of the resultant gel. While modifications to acidity levels did not significantly impact viscosity, they did influence swelling properties, color, and solubility of the MBSF gel. The viscosity of the resulting MBSF gel was relatively low, measuring below 16 cP, which is lower than expected for a thickening agent. In order to optimize its efficacy as a thickening agent, further research and development are necessary.

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Conflict of interest

The authors declare no conflict of interest.

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