Physicochemical properties of tiger nut (*Cyperus esculentus* L) polysaccharides and their interaction with proteins in beverages

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A B S T R A C T

This study aimed to extract tiger nut polysaccharides (TNPs) by the cellulase method which were graded using the DEAE-cellulose ion exchange method to obtain neutral (TNP-N) and acidic (TNP-A) polysaccharide classes. Analysis of the physical structures and monosaccharide compositions of TNP-A (3.458 KDa) and TNP-N (10.640 KDa) revealed lamellar and dense flocculent structures, with both primarily containing the monosaccharides glucose, galactose, and arabinose (Glc, Gal, and Ara). Single-factor and orthogonal tests were used to select three hydrocolloids, and the optimal ratio of the composite hydrocolloids was determined. Peanut protein drinks with a centrifugal sedimentation rate of 9.71% and a stability factor of 69.28% were obtained by adding 2.78% polysaccharide extract, 0.1% monoglyceride, and peanut pulp at a ratio of 1:15.5 g/mL. Polysaccharide protein drinks are more stable than commercially available protein drinks, with nutritional parameters either comparable to or better than those of the non-polysaccharide protein drinks.

1. Introduction

Tiger nut (*Cyperus esculentus* L), an annual plant of the grass family from African and Mediterranean coastal countries, has been promoted by the Chinese government as a substitute for soybean due to its richness in oils, polysaccharides, proteins, and other nutritional elements (Codina-Torrella, Guamis, Zamora, Quevedo & Trujillo, 2018; Zhang, Wang, Song, Yu, Wang & Zhao, 2022). Tiger Nuts, also known as yellow nuts, rush nuts, and earth almonds, are cultivated worldwide (Marchyshyn, Budniak, Slobodianiuk & Ivasiuk, 2021). They have been reported to regulate the intestinal flora (Moral-Anter, Campo-Sabariz, Ferrer & Martín-Venegas, 2020) and treat non-communicable diseases such as diabetes (Cunningham, Stephens & Harris, 2021), obesity (Aoun, Darwish & Hamod, 2020), and neurological disorders (Suganya & Koo, 2020). These crops exhibit great potential in health and pharmacotherapy. Studies have demonstrated that tiger nuts are a valuable source of stable vegetable oils that are rich in monounsaturated fatty acids and phytosterols and other high-value-added compounds (Roselló-Soto, Poojary, Barba, Lorenzo, Mañes & Moltó, 2018). They possess a fatty acid composition similar to olive oil but exhibit even higher nutritional value (Adel, Awad, Mohamed & Iryna, 2015).Tiger nuts have a high straight chain starch content and viscosity properties similar to those of natural starches; however, their gelatinous texture properties (hardness,

elasticity, bonding, and chewiness) are better than those of maize and sweet potato starches (Akonor, Tortoe, Oduro-Yeboah, Saka & Ewool, 2019; Builders, Anwunobi, Mbah & Adikwu, 2013; Li, Fu, Wang, Ma & Li, 2017). Dietary fibre content of tiger nuts is higher (8.26%–15.47%) than that of other tuber plants (Sánchez-Zapata, Fernández-López & Angel Pérez-Alvarez, 2012).

Polysaccharides in tiger nuts exist in two forms—starch, a pure polysaccharide composed of glucose (Shuai, Dejian, Yujun & Wu, 2022), and heteropolysaccharides composed of sucrose, fructose, and glucose linked in an unknown order. A study revealed a tiger nut polysaccharide (TNP) content of 15–25% of heteropolysaccharides linked in an unknown order that possessed a unique structure and can be easily modified thereby exerting a positive effect on the treatment of many degenerative diseases by lowering blood lipids and regulating immunity (Razola-Díaz, Verardo, Martín-García, Díaz-de-Cerio, García-Villanova & Guerra-Hernández, 2020). Most studies have been predominantly focused on their physicochemical properties and optimal extraction processes. TNPs are a water-soluble polysaccharide derived from tiger nuts, which belong to the Salicaceae family. These are primarily composed of glucose, mannose, galactose, and rhamnose, of which glucose is the main monosaccharide. Studies have revealed that TNPs are primarily composed of linear and branched chains, with branched chains containing functional groups such as glycoside, acyl, and acetyl.

TNPs are resistant to digestion by enzymes in the human body. They are incorporated in a natural way in foods with small amounts of fibre (biscuits, drinks, dairy products and meat products) (Babiker, Özcan, Ghafoor, Juhaimi, Ahmed & Almussallam, 2021; Verdú, Barat, Alava & Grau, 2017). Studies have shown that oleaginous bean polysaccharide can inhibit the generation of free radicals, enhance the activity of antioxidant enzymes and improve antioxidant capacity by regulating various oxidative stress-related signaling pathways (Hernández-Olivas, Asensio-Grau, Calvo-Lerma, García-Hernández, Heredia & Andrés, 2022). Second, the regulation of blood glucose by TNPs has also received wide attention. It is found that TNPs can lower blood glucose levels by improving insulin sensitivity, inhibiting the activity of glycolytic and absorptive enzymes, and reducing the absorption of glucose in the digestive tract, with the effect of preventing and treating a variety of diabetes-related diseases (Chen, Zhang, Cai, Wang, Liao & Liu, 2022). The physiochemical properties of these polymers mean that they can be exploited for the sensorial and textural enhancement of a variety of food and beverage products (Lynch, Zannini, Coffey, & Arendt, 2018).

Tiger nuts are also widely utilized in the human diet due to their excellent physiological activity. In Spain and other Mediterranean countries, the delicious milk drink "horchata" is obtained from their crushed and rehydrated tubers (Selma-Royo, García-Mantrana, Collado & Perez-Martínez, 2022). Their sweetness and flavor render them suitable for snacks and fermented milk drinks as they promote probiotic growth and shorten fermentation time (Kizzie-Hayford et al, 2021). The addition of durum wheat semolina as an auxiliary has been reported to improve the structural, cooking, rheological, and nutritional properties of egg pasta (Martín-Esparza, Raigón, García-Martínez & Albors, 2021; Martín-Esparza, Raigón, Raga & Albors, 2018). TNP is a good natural sweetener, so it can be added to protein drink to make this drink more delicious and healthy (Carvalho Barros et al. 2020), and it can improve the stability of the drink as an additive (Sun, Weixuan et al., 2021).

The current study explores the wide range of application prospects of the usage of TNPs in industry and healthcare. With the increasing demand for improved health and quality of life, it is believed that TNP will have broader development prospects in the future.

2. Materials and methods

2.1. Materials

Peeled tiger nuts were supplied by the Jilin Academy of Agricultural Sciences, and chemically pure anhydrous ethanol was purchased from Sinopharm Chemical Reagents Co. Ltd. Petroleum ether, cellulase (food grade), glucose, phenol, concentrated sulfuric acid, dextrose, bovine serum albumin (BSA), Thomas Brilliant Blue, hydrochloric acid, and chloroform were purchased from Beijing Chemical Factory. Sulfamic acid, m-hydroxyphenyl, anhydrous methanol, and acetonitrile were purchased from Shanghai Maclean Biochemical Technology Co., Ltd. Analytically pure PMP (1-phenyl-3-methyl-5-pyrazolone) was purchased from the Shanghai Pharmaceutical Group. Spectrally pure potassium bromide was purchased from the Tianjin Damao Chemical Reagent Factory. Sodium chloride was purchased from Tianjin Guangfu Science and Technology Development Co. Xanthan gum, CMC (Carboxymethyl Cellulose), carrageenan, pectin, and monoglycerides were obtained from Henan Wanbang Industrial Co.

2.2. Methods

TNP extracted through the cellulase method contains different polysaccharide fractions that require compositional analysis (Wang, Wu, Wu, TeYu, Liu & Kang, 2023). The neutral and acidic polysaccharides were separated through ion exchange chromatography to obtain the desired sugar fractions (Nakamura, Furuta, Maeda, Takao & Nagamatsu, 2002; Xing et al., 2014). Given the health benefits of TNP and peanut protein, we used single-factor and orthogonal tests to obtain the best

ratio of composite hydrophilic gum; thereafter, we conducted single-factor and response surface tests with the centrifugal sedimentation rate and stability coefficient as reference indicators to investigate if TNP can replace some stabilizers to improve the stability of peanut protein drinks and determine the optimum process conditions. The experimental procedure is presented in Fig. 1.

2.2.1. Ion exchange chromatography

The polysaccharide content (2 g) was accurately weighed, fixed to 100 mL and centrifuged at 8,000 rpm for 5 min, and the supernatant was then added to a DEAE-cellulose ion exchange column. After the sample fully infiltrated the cellulose column, it was eluted with dH_2O, concentrated, and then lyophilized to obtain TNP-N. The sample was eluted with a 0.5 M NaCl solution to yield TNP-A. The samples containing polysaccharides were collected, desalted through dialysis, concentrated, and then lyophilized (Tang, Liu, Yin & Nie, 2020).

2.2.2. Chemical composition of the polysaccharides

2.2.2.1. Determination of total polysaccharides.
We used the phenol–sulphuric acid method for the determination of polysaccharides owing to its high stability and operability (Xu et al., 2019).

2.2.2.2. Determination of glyoxylate content.
The glyoxylate content in the polysaccharides was determined using a previously described method (Yanbo, Liyuan, Yang, Ciru & Jun, 2022). Optical density (OD) was measured at 525 nm, and three parallel tests were performed. A standard curve was used to calculate the glyoxylate content of the polysaccharides.

2.2.2.3. Determination of protein content.
The proteins were quantified by the Thomas Brilliant Blue method (Zhang et al., 2009).

2.2.2.4. Determination of ash content.
To determine the ash content, the muffle sintering method was utilized (Gruber, Seidl, Zanetti & Schnabel, 2021) and the crucible was then cauterized for 10 h. The three sugars were weighed and placed separately in a crucible. The crucible was cooled to room temperature, weighed, and the ash content was calculated.

2.2.2.5. Determination of monosaccharide composition.
One milligram sample of polysaccharide was accurately weighed and placed in an acid hydrolysis vial, and 1 mL of hydrochloric acid/methanol solution (2 mol/L) was added. The vial was sealed with N_2, hydrolyzed (80 °C, 12 h) and dried, and 1 mL of trifluoroacetic acid (2 mol/L) was added. The sample was dried after hydrolysis reaction (120 °C, 1 h). Standard samples of glucose and arabinose were accurately measured to 100 μL at 1 mg/mL, configured into standard solutions, and placed in acid hydrolysis bottles. After drying the sugar samples and standards, NaOH and PMP-methanol solutions were added to fully dissolve the sugar samples and standards, and the monosaccharide composition was determined by referring to the method of preparing sugar samples and monosaccharide standards and the method of detecting monosaccharide composition by high-performance liquid chromatography (HPLC) (J. Yan et al., 2016).

2.2.2.6. Determination of molecular mass distribution.
The molecular mass of TNP-N and TNP-A were determined and analyzed through LC-10Avp HPLC using a 0.2 M NaCl solution and filtered through a 0.22 μm membrane (Schieppati, Patience, Campisi & Patience, 2021).

2.2.2.7. Scanning electron microscopy and infrared spectroscopy.
The ultrastructures of TNP, TNP-N, and TNP-A were investigated through scanning electron microscopy. The dried samples were placed on a sample stage, sprayed with gold, and their morphologies were analyzed
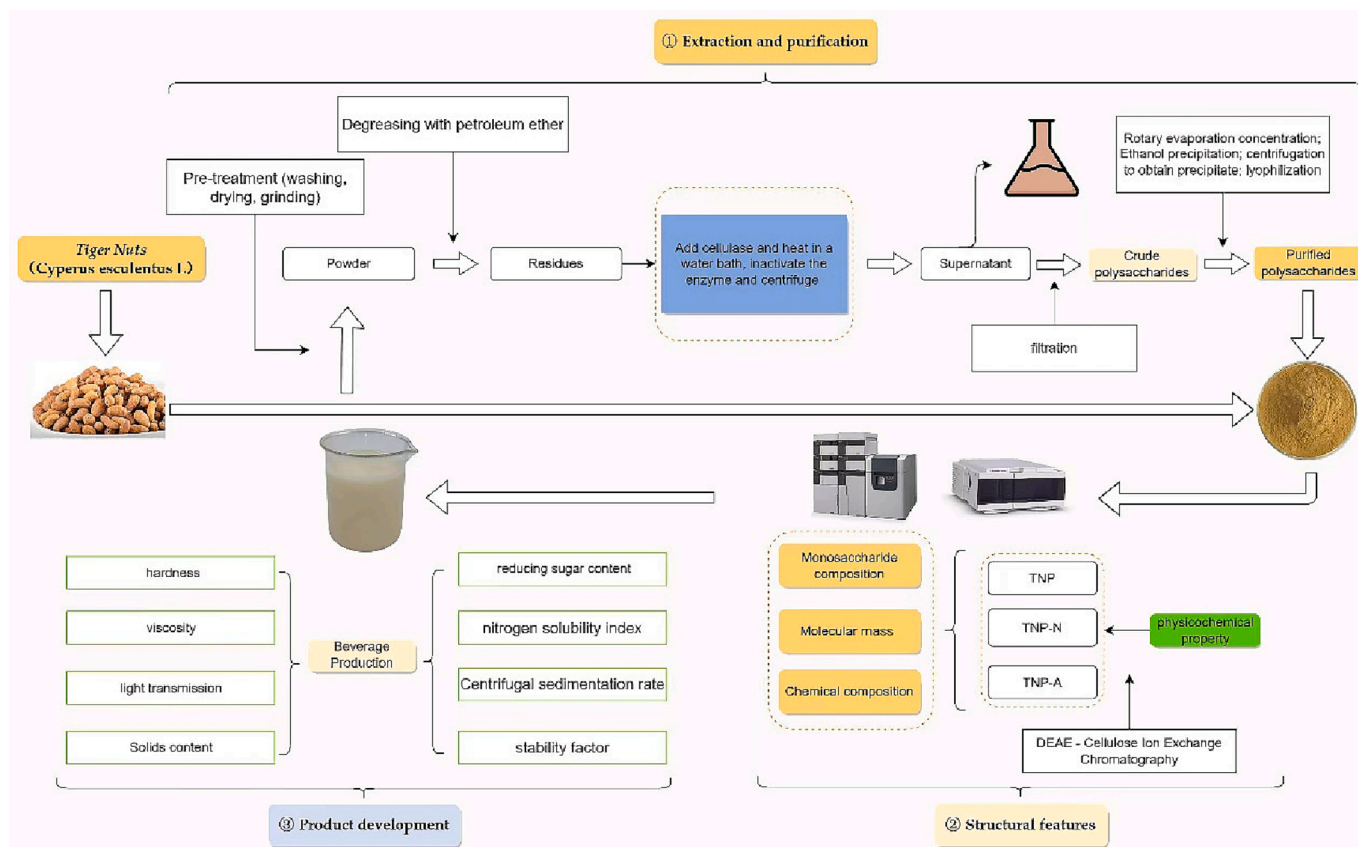
Fig. 1. Flow chart for the study of the physical and chemical properties of tiger nut polysaccharides and development of protein beverages.

at 100X and 1000X magnifications. TNP, TNP-N, and TNP-A were each weighed to 1 mg and then ground in a mortar. The three sugars were homogenously mixed with powdered KBr and pressed into thin slices. The infrared spectra of the three sugars were scanned within the wavelength range of 500–4000 cm^{-1} (Nuerxiati, Mutailipu, Abuduwaili, Dou, Aisa & Yili, 2021; Sun, Sun, & Juzenas, 2017).

2.2.3. Composite hydrocolloid experimental design

2.2.3.1. Single-factor test.
The volume of each peanut protein drink was 50 mL. Three of the four hydrophilic colloids were selected as the most suitable colloids for peanut protein drinks and their dosages were determined. Sodium carboxymethyl cellulose, xanthan gum, carrageenan, and pectin each were added at concentrations of 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, and 0.16%. The stability of the peanut protein beverage was determined after cooling at a temperature of 55 °C for 25 min.

2.2.3.2. Orthogonal test.
L$_9(3^4)$ was used for the orthogonal addition of sodium carboxymethylcellulose, xanthan gum, and pectin, and optimum hydrocolloid ratios were obtained.

2.2.3.3. Determination of centrifugal sedimentation rates and stability coefficients.
After shaking the peanut protein drink, the sample was accurately weighed and centrifuged at 6000 rpm for 20 min, and the lower precipitate (mL) was weighed after centrifugation.

Centrifugal sedimentation rate $(\%) = (m_1/m_0) \times 100\%$

m_0: sample mass; m_1: mass of the lower precipitate after centrifugation.

A smaller centrifugal sedimentation rate is indicative of better stability. After the peanut protein drink was shaken well, a 10 g sample was accurately weighed and centrifuged. After centrifugation, the whey

phase (0.5 mL) was diluted with water at a volume ratio of 1:15 by volume and the OD A_1 was measured at 570 nm, shaken and diluted as above and OD A_0 was measured. The stability factor was calculated, wherein a higher stability factor indicates better stability.

Stability factor $(\%) = (A_1/A_0) \times 100$

2.2.3.4. Determination of the nitrogen solubility index.
The peanut protein was diluted 10 times with distilled water and centrifuged for 10 min at 6000 rpm. The protein content in the supernatant and the sample were determined using the Kormas blue method. The ratio of the two indicated the nitrogen solubility index. An average of three parallel tests was obtained.

2.2.3.5. Determination of reductive polysaccharide content.
The reduced sugar content of the peanut protein beverage was calculated using the standard curve according to the method described by Geng et al (2023). The equation of the standard curve was

$y = 1.986x - 0.0679$ (R$^2 = 0.999$).

3. Results and discussion

3.1. Polysaccharide composition

3.1.1. Determine the content of total polysaccharide, glyoxylic acid, protein and ash

The OD of TNP, TNP-N, and TNP-A measured at 490 nm were used in the standard curve (y = 0.041x + 0.1193, R^2 = 0.9989), and the total sugar content in TNP, TNP-N, and TNP-A were calculated to be 83.62 %, 65.73%, and 47.03%, respectively. The test indicated that all three

sugars from tiger nuts contained high sugar content and the isolated TNPs were pure.

The OD of TNP-Z and TNP-A that were measured at 525 nm were used in the equation (y = 0.0996x + 0.1988, R^2 = 0.997), and the results demonstrated that the glucuronic acid content of TNP-Z and TNP-A was 70.36% and 20.15%, respectively.

The OD of TNP, TNP-N, and TNP-A that were measured at 595 nm were calculated using the equation y = 0.0095x + 0.2264 (R^2 = 0.9975). The protein contents of TNP, TNP-N, and TNP-A were 11.98%, 10.23%, and 15.24%, respectively.

The ash contents of the TNP, TNP-N, and TNP-A were 1.7%, 8.7%, and 15.3%, respectively.

3.1.2. Determination of monosaccharide composition

The monosaccharide compositions and ratios of TNP, TNP-N, and TNP-A are listed in Table 1.

The molar ratios of the monosaccharides in TNP were Man:GlcA:Rha: GalA:Glc:Gal:Xyl:Ara = 0.3:0.38:0.3:0.23:93.83:2.74:0.38:1.85, indicating that TNPs primarily contained Glc, Gal, and Ara. The molar ratios of the monosaccharides in TNP-N were Man:GlcA:Rha:GalA:Glc:Gal:Xyl: Ara = 1.28:0.57:1.01:0.57:88.43:5.58:0.4:2.16. Thus indicating that the main monosaccharides were Glc, Gal, and Ara. The monosaccharides in TNP-A and their molar ratios were Man:GlcA:Rha:GalA:Glc:Gal:Xyl:Ara = 2.33:1.95:2.14:2.98:33.22:32.77:2.3:22.31. The results revealed that the monosaccharide compositions of the three sugars were similar and consisted primarily of Glc, Gal, and Ara in varying concentrations. Its composition is similar to that of plant polysaccharides such as codonopsis polysaccharide and okra polysaccharide, and its antioxidant and hypolipidemic capacity can be investigated in the next study (Pan et al., 2022; Su et al., 2022).

3.1.3. Determination of molecular mass

Fig. 2(a) presents the HPLC standard curve, whereas Fig. 2(b) and 2 (c) indicate the molecular mass distributions of TNP-N and TNP-A, respectively. The standard curve is y = −0.4524x + 9.5477 (R^2 = 0.9945). The retention times of TNP-N and TNP-A were 12.2 min and 13.286 min, respectively. The molecular mass of TNP-N and TNP-A were calculated as 10.640 KDa and 3.458 KDa. From the above molecular mass, it can be assumed that TNP-A will have better entanglement properties with CMC, improving the original rheological properties and solids content of the mixture (Horinaka, Chen & Takigawa, 2018).

3.1.4. Scanning electron microscope analysis

Fig. 2(d-f) present the microstructures of TNP, TNP-A, and TNP-N at 1000× magnification, respectively. Electron microscopy scans reveal that there are clear differences in structure and shape among the three sugars found in tiger nuts. As presented in Fig. 2(d), the TNP surface is irregularly shaped overall, with multiple pores, available in blocks and strip molecules. Fig. 2(e) indicates that the surface of TNP-A is relatively uneven and rough with a scattered flake structure. As presented in Fig. 2 (f), the appearance of TNP-N was smoother, and the flocculent form was denser when magnified 1000×.

3.1.5. Infrared spectral analysis

The IR spectra of TNP, TNP-A and TNP-N are presented in Fig. 2(g).

The spectra of TNP, TNP-N, and TNP-A were generally similar with the absorption peaks near 3,414.75 cm^{-1} indicating the stretching vibration of the O—H group that causes strong interactions between the

polysaccharide chains. Due to the stretching vibration of the C—H bond, a characteristic absorption peak for polysaccharides was formed near 2,932.91 cm^{-1}. The absorption peak near 1,655.42 cm^{-1} was revealed to be an asymmetric absorption peak of C=O (Yan et al., 2010). The absorption peak at 1,417.09 cm^{-1} is a C—H variable angle vibration absorption peak. The characteristic absorption peak near 1,077.24 cm^{-1} indicates the presence of a procyclic ring for TNP, and the absorption peak of TNP-A at 525.04 cm^{-1} indicates the presence of alpha-linked glycosyl residues (Chen et al., 2023). The analysis revealed that both TNP-N and TNP-A were effectively separated.

3.2. Results and analysis of composite hydrocolloid experiments

3.2.1. Results and analysis of a single-factor test

As presented in the Fig. 3(a), with the gradual increase in CMC the centrifugal sedimentation rate of the peanut protein drinks first decreased, and then increased. The stability factor also peaked and then decreased. When CMC was added at 0.12%, the stabilizer dissolved well in the protein drink without agglomeration, and the system acquired a stable state. Therefore, CMC can be effectively added to the peanut protein drink at 0.12% concentration. From 3.1.6 above, it can be seen that TNP-A is the main binding agent for CMC in TNPs, and its content will affect the stability of the solution.

As presented in the Fig. 3(a1), the stability coefficient of the peanut-based protein drinks first increased and then decreased as the amount of xanthan gum was increased, whereas the centrifugal sedimentation rate decreased to minimum and then increased. These findings indicated that xanthan gum can be effectively added to peanut protein beverages at 0.08% concentration as it exhibited good hydrophilic effect, high beverage stability, easy dispersion within the system, and no gel phenomenon, and it can be added to the peanut protein beverage. When xanthan gum was added at 0.08%, the beverage stability was good.

As can be observed in the Fig. 3(a2), under varying carrageenan concentrations, the centrifugal sedimentation of peanut-based protein drinks first decreased, then increased sharply and decreased. The stability coefficient increased sharply and then ultimately decreased, exhibiting an unstable trend. When carrageenan solubility was low, gel clusters were readily formed, and the carrageenan would not dissolve completely under heating conditions, so it did not significantly enhance the stability of the protein drinks. These findings indicate that carrageenan is ineffective as a stabilizer for peanut-based protein drinks.

As presented in the Fig. 3(b), as the amount of pectin increased, the centrifugal sedimentation of the peanut protein drink first decreased to a minimum before increasing, and the stability coefficient tended to increase and then decrease. Pectin exhibits good solubility and dispersibility in the system and does not easily agglomerate. The product exhibited high stability at 0.08% concentration, indicating that pectin could be added to the peanut protein beverage as a stabilizer.

3.2.2. Results and analysis of orthogonal tests

To further improve the stability of peanut protein drinks, we combined the results of the single-factor test with the centrifugal sedimentation rate and the stability coefficient, and performed orthogonal tests on sodium carboxymethyl cellulose (A), xanthan gum (B), and pectin (C) for L9(3^4). The results are presented in Table 2.

The findings revealed that CMC, xanthan gum, and pectin significantly affected the centrifugal sedimentation rate and stability coefficients of the peanut protein drinks in the order B > C > A and C > A > B, respectively. Considering the inverse relationship between the centrifugal sedimentation rate and stability and the positive relationship between the stability coefficient and the stability of protein drinks, the best combination of hydrocolloid $A_2B_1C_3$ (0.12% CMC, 0.06% xanthan gum, and 0.1% pectin) resulted in the most stable peanut protein drink system under these conditions.

Table 1
Monosaccharide composition of polysaccharides.

	Man	GlcA	RHA	Gala	Glc	Gal	Xyl	Ara
TNP	–	–	–	–	93.83	2.74	–	1.85
TNP-N	1.28	–	1.01	–	88.43	5.58	–	2.16
TNP-A	2.33	1.95	2.14	2.98	33.22	32.77	2.3	22.31
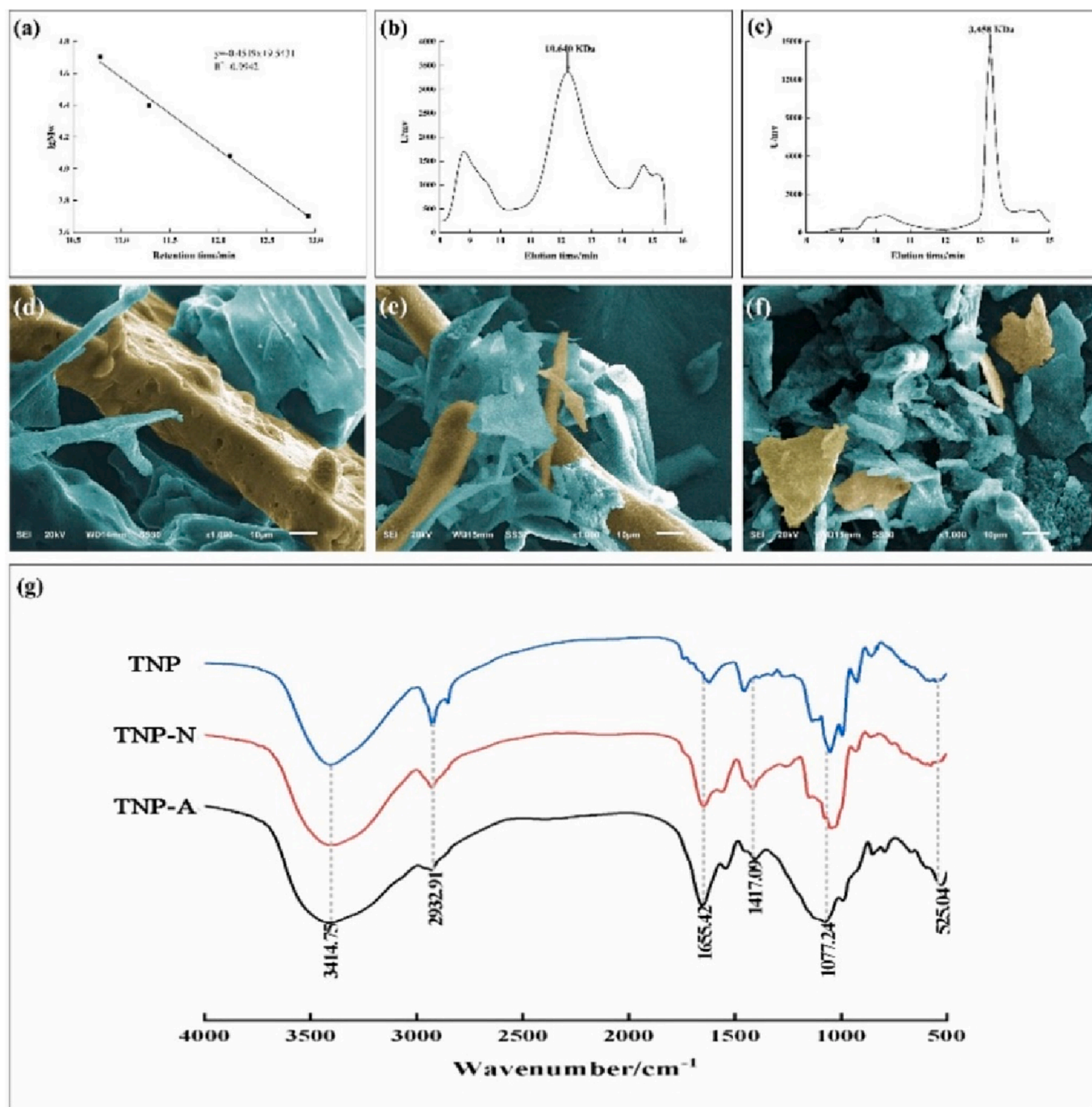
Fig. 2. (a) HPLC standard curve graph; (b) TNP-N Molecular mass distribution map; (c) TNP-A Molecular mass distribution map; (d) TNP scanning electron micrograph; (e) TNP-A scanning electron micrograph; (f) TNP-N scanning electron micrograph; (g) Infrared spectra of TNP, TNP-N, and TNP-A.

3.2.3. Analysis of centrifugal sedimentation rates and stability coefficients

3.2.3.1. Optimizing the concentration of each factor for peanut protein drinks.
As presented in the Fig. 3(b1), the centrifugal sedimentation rate of the peanut protein drinks decreased and then increased as the amount of the tiger nut polysaccharide extract increased, whereas the stability coefficient first increased and then decreased. When polysaccharide extract concentration was < 4%, no precipitation or stratification was observed in the protein drink, indicating high beverage stability. As presented in the Fig. 3(b2), the centrifugal sedimentation rate of the peanut protein drink decreased and then increased as the amount of added monoglyceride increased, whereas the stability factor increased

and then decreased. The findings indicated that monoglycerides can be added at a concentration of 0.1% to enhance beverage stability. As presented in the Fig. 3 (b3), the centrifugal sedimentation of the peanut protein drink decreased and then increased, whereas the stability coefficient increased and then decreased as the material-to-liquid ratio increased. At the material-to-liquid ratio of 1:15 (g/mL), the protein beverage system was highly stable without precipitation. Based on this, the material-to-liquid ratios of 1:10, 1:15, and 1:20 (g/mL) were selected for the response surface test.

3.2.3.2. Response surface optimization tests.
Tests were performed according to the design in 2.2.3.2 with centrifugal sedimentation rates and

A

B
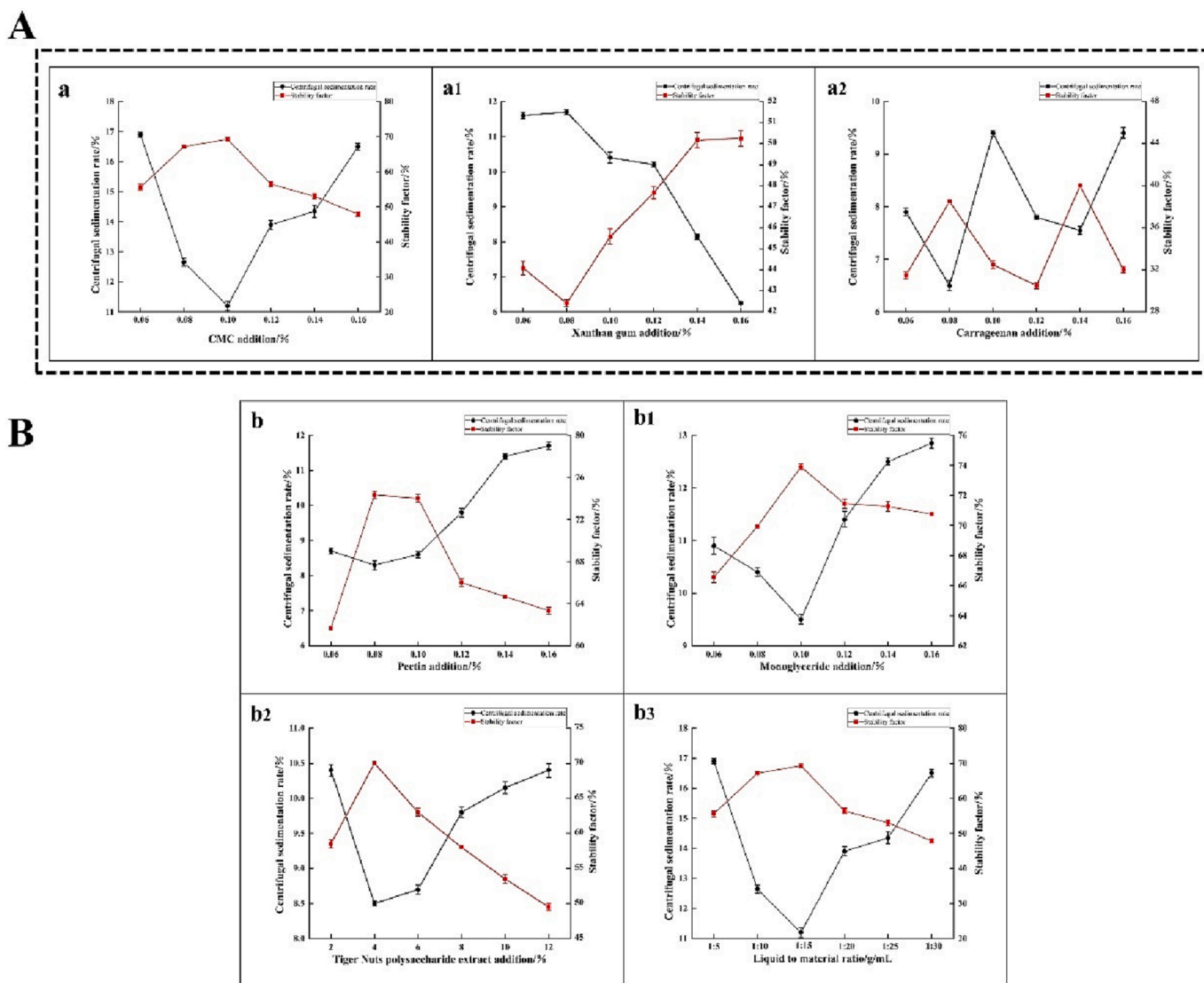
Fig. 3. (a) Effect of CMC on the stability of peanut protein drinks; (a1) Effect of xanthan gum on the stability of peanut protein drinks; (a2) Effect of carrageenan on the stability of peanut protein drinks; (b) Effect of pectin on the stability of peanut protein drinks; (b1) Effect of the of tiger nut polysaccharide extract on the stability of peanut protein drinks; (b2) Effect of monoglyceride addition on the stability of peanut protein drinks; (b3) Effect of peanut pulping material to liquid ratio on the stability of peanut protein drinks.

stability factors as response values.

The quadratic polynomial regression equation between the centrifugal sedimentation rate (R_Y) and the amount of tiger nuts polysaccharide extract added (A), the amount of monoglyceride added (B), and the peanut pulping material-to-liquid ratio (C) was as follows:

$R_Y = 9.62 + 0.25A + 0.34B - 0.073C + 0.86AB + 0.40AC - 0.43BC + 0.61A^2 + 1.04B^2 + 2.37C^2$.

The quadratic polynomial regression equation between the stability coefficient (R_Z) and the amount of tiger nuts polysaccharide extract added (A), the amount of monoglyceride added (B), and the peanut pulping material-to-liquid ratio (C) is as follows:

$R_Z = 69.15 + 0.48A + 2.10B + 4.31C + 2.98AB - 0.97AC + 2.40BC + 3.29A^2 - 9.88B^2 - 19.50C^2$.

As the regression analysis of the results of the response-surface test design, the model was significant ($p < 0.0001$), and the misfit term was not significant ($p = 0.4051 > 0.05$). The coefficients of determination R^2, R_{Adj}^2, and variation were 0.9777, 0.9490, and 3.05%, respectively,

and the precision was 15.765. This value was higher than 4, indicating that this model could predict the effect of various factors on the stability of polysaccharide peanut protein drinks. The effects of AB, A^2, B^2, and C^2 were highly significant ($p < 0.01$), whereas the effects of B and BC were significant ($p < 0.05$).

As presented in analysis of variance of regression equations for stability coefficients, the model was significant ($p < 0.0001$), whereas, the misfit term was not significant ($p = 0.1178 > 0.05$). The coefficients of determination R^2, R_{Adj}^2, and variation were 0.9880, 0.9726, and 3.56%, respectively, with a precision of 21.885, thus indicating that this model could predict the effects of various factors on the stability of polysaccharide peanut protein drinks. The effects of C, B^2 and C^2 were highly significant ($p < 0.01$), whereas those of B, AB, BC, and A^2 were significant ($p < 0.05$).

3.2.3.3. Response surface interaction analysis. The effects of the interaction of three factors, specifically the addition of polysaccharide extract (A), addition of monoglyceride (B), and ratio of peanut pulp to liquid (C), on the centrifugal sedimentation rate and the effects on the stability coefficient of peanut protein drinks are presented in the Fig. 4(A) and

Table 2
Orthogonal test table for centrifugal sedimentation rate and stability coefficients of peanut protein drinks $L_9(3^4)$.

number	Factors			Y	Z
	A CMC	B Xanthan gum	C Pectin	Centrifugal sedimentation rate	Stability factor
1	1 (0.1%)	1 (0.06%)	1 (0.06%)	9.760	61.743
2	1	2 (0.08%)	2 (0.08%)	15.163	57.466
3	1	3 (0.1%)	3 (0.1%)	10.710	65.940
4	2 (0.12%)	1	3	8.863	68.499
5	2	2	1	9.170	56.278
6	2	3	2	11.303	61.434
7	3 (0.14%)	1	2	10.987	55.836
8	3	2	3	11.410	58.345
9	3	3	1	12.363	57.265
K_{Y1}	11.878	9.87	10.431		
K_{Y2}	9.779	11.914	12.484		
K_{Y3}	11.587	14.459	10.328		
K_{Z1}	61.716	62.026	58.429		
K_{Z2}	62.07	57.363	58.245		
K_{Z3}	57.149	61.546	64.261		
R_Y	2.099	4.589	2.156		
R_Z	4.921	4.663	6.016		
Optimum combination	A_2	B_1	C_3		

Fig. 4(B), respectively.

As presented in the Fig. 4(A), the interaction among the amounts of added TNP extract, monoglycerides, and peanut beating stock ratio was significant. When one of these factors was held constant, the centrifugal sedimentation rate tended to decrease and then increase with the addition of another factor.

As presented in the Fig. 4(B), the interaction between the amount of added tiger nuts polysaccharide extract and monoglycerides and between the concentration of monoglycerides added and the ratio of peanut pulp to liquor was obvious, and the interaction between the factors led to a trend of increasing and then decreasing the stability coefficient of the peanut protein drinks.

Based on the above response surface optimization tests, the optimum production process conditions for the peanut protein beverage with TNP as a stabilizer were as follows: 2.78% oleosa bean polysaccharide extract addition, 0.1% monoglyceride addition, and 1:15.5 g/mL peanut pulp material-to-liquid ratio. Three parallel validation tests were conducted according to the abovementioned process conditions, ultimately resulting in a peanut protein drink with a centrifugal sedimentation rate of 9.71% and a stability factor of 69.28%. These results were in general agreement with the theoretical values.

3.2.4. Results and analysis of peanut protein drink quality analysis

Based on the optimum process parameters obtained in Section 3.2.3, peanut protein drinks and peanut protein drinks without added polysaccharides were prepared and compared to commercially available peanut protein drinks to verify if the quality of peanut protein drinks improved after the addition of TNPs. To compare the quality differences among the three peanut protein beverages we measured their stability indicators (centrifugal sedimentation rate, stability coefficient, and light transmission), nutritional composition (nitrogen solubility index, reducing sugar content, and solid content), and textural characteristics (hardness and viscosity). Among the three types of peanut protein drinks, the peanut protein drink with added polysaccharide from oleaginous beans had the lowest centrifugal sedimentation rate and a higher stability factor than the commercially available protein drinks.

The stability coefficient was lower than that of the commercially available protein drinks and higher than that of the protein drinks without polysaccharides. The light transmission rate of the protein drinks was better than that of the protein drinks with added polysaccharides.

The nitrogen solubilisation index of the polysaccharide peanut protein drink was slightly higher than that of the commercially available protein drinks. The nitrogen solubility index was slightly higher than that of the commercially available protein drinks. The reduced sugar content was higher than that of the commercially available protein drinks and the solids content was lower than that of the commercially available protein drinks. The solids content was lower than that of the commercially available protein drinks. The hydroxyl group in the polysaccharide of the oleaginous bean enhances the gel structure of the protein drink and allows the product to be more stable. The stability of the product can be improved. The analysis of the above physicochemical parameters led to the conclusion that the overall quality of the polysaccharides in the peanut protein drinks was similar to that of commercially available protein drinks and significantly better than protein drinks without added polysaccharides. This indicates that the polysaccharide protein drinks have better stabilisation than the regular protein drinks.

4. Conclusion

In this study, TNP was extracted from tiger nuts (*Cyperus esculentus* L.) and analyzed for its composition and application in protein drinks.

TNPs were graded by DEAE-cellulose ion exchange chromatography to obtain neutral sugar grade TNP-N and acidic sugar grade TNP-A from tiger nuts by 15.18% and 18.97%, respectively. The chemical and monosaccharide compositions of TNP, TNP-N, and TNP-A were analyzed, and the morphological structures and functional groups of the three sugars were assessed using electron microscopy and infrared spectroscopy. The chemical composition indicated that TNP, TNP-N, and TNP-A are pure polysaccharides, whereas the monosaccharide composition revealed that TNP, TNP-N, and TNP-A are primarily composed of Glc, Gal, and Ara, respectively. The infrared spectra of TNP, TNP-N, and TNP-A were typically similar, and all possessed characteristic absorption peaks of polysaccharides with the absorption peak near $3,414.75 \ cm^{-1}$ corresponding to the stretching vibration of O—H and the characteristic absorption peak of polysaccharides forming near $2,932.91 \ cm^{-1}$. The molecular mass of TNP-N and TNP-A were determined by HPLC, and the molecular mass of TNP-N and TNP-A were 10.640 KDa and 3.458 KDa, respectively. The optimum ratio of hydrocolloids was 0.12% sodium carboxymethyl cellulose, 0.06% xanthan gum, and 0.1% pectin. The optimum process was obtained by the addition of 2.78% TNP extract, 0.1% monoglyceride, and 1:15.5 g/mL peanut pulp as factors, and the centrifugal sedimentation rate and stability coefficient served as indicators in the single-factor and response surface tests. The peanut protein drink possessed a centrifugal sedimentation rate of 9.71% and a stability factor of 69.28%. The peanut protein drink that was produced according to this optimal process was compared to a non-polysaccharide peanut protein drink and a commercially available peanut protein drink for a comprehensive quality analysis. TNP can lead to better stability in protein drinks and its inclusion in beverages is feasible.

CRediT authorship contribution statement

Te Yu: Conceptualization, Methodology, Software. **Qiong Wu:** Project administration, Funding acquisition. **Jiaming Wang:** Supervision. **Bin Lang:** Visualization, Investigation. **Xusheng Wang:** Data curation, Writing – original draft. **Xinzhu Shang:** Writing – review & editing.

A

B
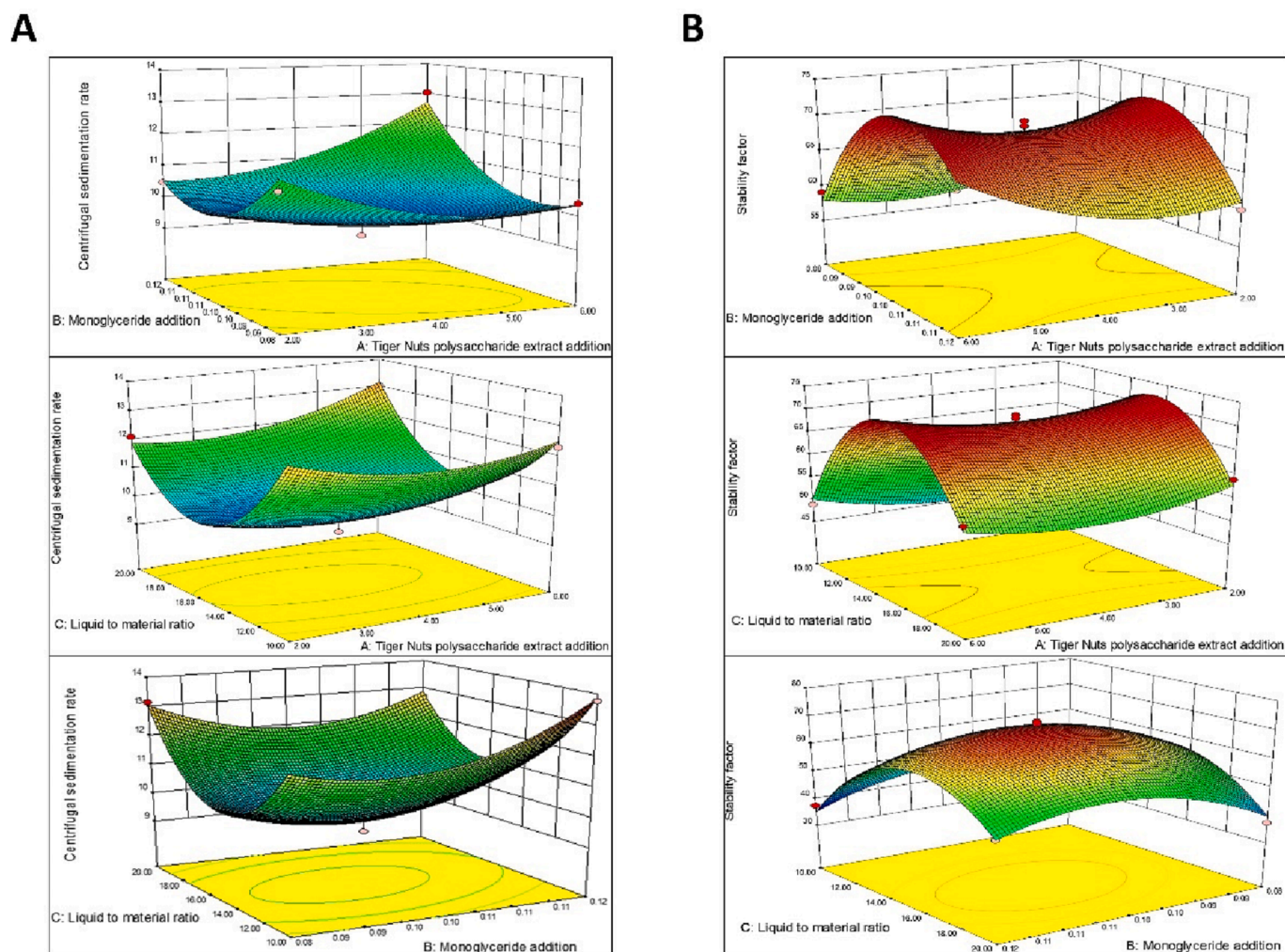
Fig. 4. Response surface graph of the influence of factor interaction on centrifugal sedimentation rate; (A) Response surface graph of the influence of various factors on the stability coefficient.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100776.

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