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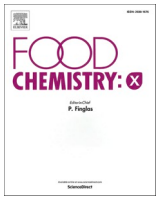

Structural-functional analysis of modified kudzu starch as a novel instant powder: Role of modified technology

Ruidi He [a], Chuanlai Du [a], Songnan Li [b], Li Guo [c], Kaiyue Wang [a], Liping Yang [a,*]

[a] Key Laboratory of Whole Grain Nutrition and High Value Utilization in Anhui Province, School of Food Engineering, Anhui Science and Technology University, 9 Donghua Road, Fengyang 233100, China
[b] Joint International Research Laboratory of Agriculture and Agri-Product Safety of the Ministry of Education of China, Institutes of Agricultural Science and Technology Development, Yangzhou University, 48 Wenhui East Road, Yangzhou 225009, China
[c] State Key Laboratory of Biobased Material and Green Papermaking, School of Food Sciences and Engineering, Qilu University of Technology, Shandong, Academy of Sciences, Jinan, China

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ABSTRACT

Adopting effective methods to modify starch structure for enhanced functional properties has become a pivotal pursuit within the realm of food science. This study investigated the physicochemical and structural properties of ball milling-modified kudzu starch (BS), extrusion puffing-modified kudzu starch (ES), alcohol-alkali-modified kudzu starch (ANS), urea-alkali-modified kudzu starch (UNS), pullulanase-modified kudzu starch (PS), and extrusion puffing-pullulanase-modified kudzu starch (EPS). The d (0.5) value increasing from 10.54 μm (NS) to 83.99 μm (ANS). The Small-angle X-ray scattering (SAXS) characteristic curve of other modified kudzu starch disappeared except for the UNS. The solubility of EPS was the highest, ranging from 73 % to 80 %, significantly higher than that of NS (0 %–1 %). The agglomeration rates of ES and EPS were 0.3 % and 0.6 %, respectively, at a stirring time of 30 s. indicating favorable hydration properties. Flavonoids content in ES increased to 0.1825 mg/g. Moreover, the resistant starch content of modified kudzu starch was increased, ranging from 58.50 %–86.87 %. This study is expected to provide a scientific foundation for selecting optimal modification methods for the production of instant kudzu powder.

1. Introduction

Kudzu, a perennial leguminous vine, was first documented as a medicinal herb in Shennong's Classic of the Materia Medica during the Western Han Dynasty. Rich in isoflavonoids, it boasts exceptional properties as an antioxidant (Chen et al., 2012), anti-inflammatory (Xu et al., 2013), and estrogen supplement (Ahn et al., 2019), among others. Kudzu starch is the primary constituent of kudzu, and is widely consumed in the form of kudzu brewing powder. However, its drawbacks, such as a high pasting temperature and poor hydration properties, pose challenges for consumption. Therefore, optimizing the physicochemical properties of kudzu starch by addressing these shortcomings is imperative. Improvement of starch properties is usually achieved by modification, which will provide kudzu starch with versatility and diversity such as high transparency, low pasting temperature, high resistant starch content and instant solubility, making it an important part of new food products for the future.

Physical, chemical, and enzymatic modifications can broaden the functionality of starch. Ball milling and extrusion puffing modification methods are widely employed to physically modify starch. Ball milling modification alters starch structure through mechanical energy, tailoring its properties, while extrusion puffing modification disrupts starch structure through mechanical energy, high temperature, or both (Zhang et al., 2019). As chemical modification methods, the alcohol-alkaline and urea-alkaline modification improved the properties of starch, but no introduction of chemical groups was found after modification (Qian et al., 2019). This may be due to the change in the arrangement of molecular chains affected by intermolecular hydrogen bonding interactions or Van der Waals' forces during the modification process, which affects the structure and properties of starch. Therefore, these two modifications are safer compared to other chemical modifications. In alcoholic-alkaline modification, sodium hydroxide is used to paste the starch, while alcohol is used to maintain the integrity of the starch granules (Kaveh et al., 2020). In 1994, Chen and Jane successfully

* Corresponding author.
E-mail address: 18788892719@163.com (L. Yang).

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prepared cold-water-soluble starch using the alcoholic-alkaline modification method, which could be widely used for the preparation of instant foods. In urea-alkaline modification, the addition of sodium hydroxide enhances starch solubility, while the addition of urea promotes pasting at room temperature, delaying the retrogradation of starch paste. Chen et al. (2019) successfully prepared granular cold-water-soluble maize starch with favorable solubility and a low glass transition temperature using urea-alkaline solution. Pullulanase, a debranching enzyme, selectively cleaves 1,6-α-D-glucosidic bonds, yielding numerous short linear glucan chains. Liu et al. (2015) prepared debranched corn, wheat, pea, and potato starch using pullulanase, improving the solubility and water-holding capacity of starch. In extrusion puffing-pullulanase modification, starch molecules degrade and partially debranch during extrusion puffing. Thus, using extruded starch as the substrate for debranching by pullulanase may significantly improve debranching efficiency (Liu et al., 2022).

The complex structure of starch significantly influences its functional properties. Modifications can alter the structure and properties of starch granule and have been widely investigated. Moreover, the same modification method may yield differing effects on the structure and properties of starches from different sources. For instance, Castanha et al. (2020) treated cassava, maize, and potato starches with ozone under identical conditions, observing similar results for cassava and potato starches but contrasting results for maize starch. At present, the modification of kudzu starch is mostly physical modification, and only its agglomeration and boiling water blending properties have been studied, which has certain limitations (Wang et al., 2020; Wu et al., 2022). Therefore, it is imperative to comprehensively study the effects of different modifications on kudzu starch for developing nutritious, convenient and high-quality kudzu starch.

Based on the aforementioned background, in this study, we investigated the impact of physical modifications (ball milling, extrusion puffing), chemical modifications (alcoholic-alkaline, urea-alkaline), enzyme modification (pullulanase), and dual modification (extrusion puffing-pullulanase) on the structure and physicochemical properties of kudzu starch. The objectives were: (i) to comparatively analyze the effects of various modifications on the structural properties of kudzu starch, aiming to broaden its potential applications in the future; (ii) to elucidate the relationship between modified starch structure and properties, offering a theoretical framework for preparing starch with specific attributes; and (iii) to identify, via comparative analysis, a modification most suitable for producing instant kudzu powder to meet the current market demand for healthy and convenient food products.

2. Materials and methods

2.1. Materials

Kudzu starch was provided by Anhui Gelaixiang (Chuzhou, China). Sodium hydroxide, anhydrous ethanol, urea, pullulanase, α-amylase (14 U/mg), amyloglucosidase (10 U/mg), and 3,5-dinitrosalicylic acid (DNS) were purchased from McLean (Shanghai, China). Planetary Ball Mill (SFM-1, ShenZhen, China), extruder (Model 70 extruder, JiNan, China).

2.2. Sample preparation

BS, ES, ANS, UNS, PS, EPS were prepared as outlined in our previous study (He et al., 2024). All samples were passed through a 120-m sieve and stored in a desiccator at room temperature for later use (See Fig. 1).

2.3. Chain length distribution

Pre-treatment of kudzu starch was performed according to the approach of Nishi et al. (2001). Starch (10 mg) was dissolved in 5 mL water in a boiling water bath for 60 min. Sodium azide solution (10 μL 2 % *w/v*), acetate buffer (50 μL, 0.6 M, pH = 4.4), and isoamylase (10 μL, 1400 U) were added to the starch dispersion, and the mixture was incubated in a water bath at 37 °C for 24 h. The hydroxyl groups of the debranched glucans were reduced by treatment with 0.5 % (*w/v*) of sodium borohydride under alkaline conditions for 20 h. The preparation about 600 μL was dried in vacuo at room temperature and allowed to dissolve in 30 μL of 1 M NaOH for 60 min. Then, the solution was diluted with 570 μL of distilled water. The chain length distribution in kudzu starch was analyzed by high-performance anion-exchange chromatography (HPAEC), the data were acquired on an ICS5000 (ICS5000+, Thermo Fisher Scientific, Massachusetts, USA) and processed using a chromeleon 7.2 CDS (Thermo Scientific).

2.4. SAXS

SAXS tests were performed using a SAXS system (Bruker AXS, Karlsruhe, Germany). Initially, 50 mg of a sample was placed in 350 μL of purified water at room temperature overnight and then centrifuged at 25 °C, 5000 rpm, for 15 min to remove the supernatant prior to testing. Subsequently, the instrument was operated at 50 mA and 40 kV, employing Cu Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The average repeat distances for amorphous and crystalline flakes were calculated using the following formula:

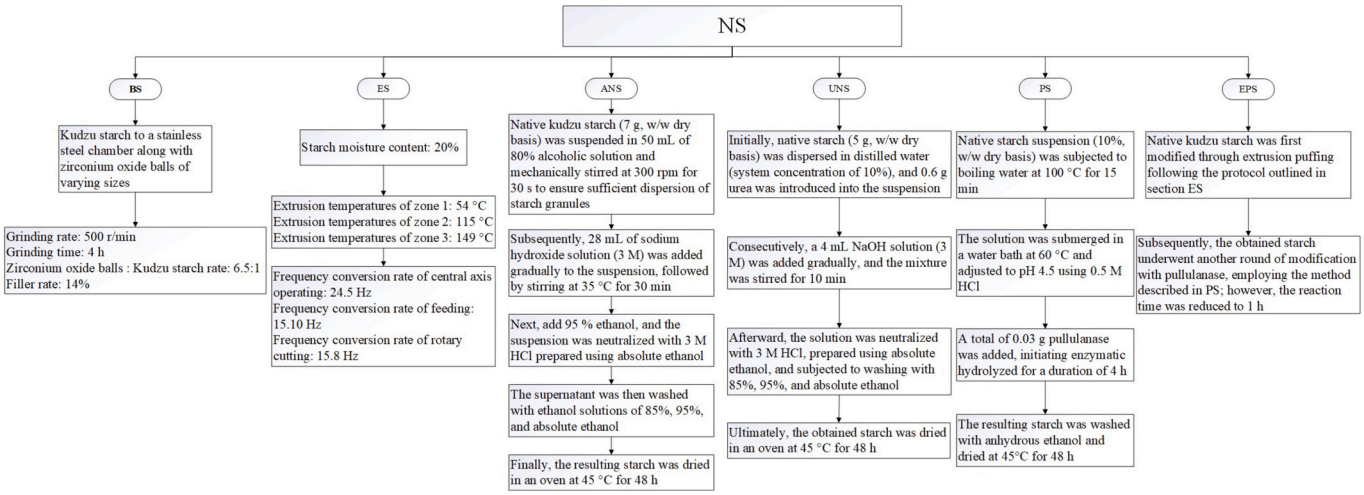

Fig. 1. Methods for the preparation of BS, ES, ANS, UNS, PS and EPS.

$$d = \frac{2\pi}{q} \quad (1)$$

where d is the laminar repetition distance (nm) and q is the scattering vector (nm^{-1}).

2.5. Particle size distribution

The particle size distribution of starch samples was determined using a laser particle sizer (Mastersizer 2000 System, Wales, UK), following the mixing of starch and anhydrous ethanol at a concentration of 0.05 mg/mL. The refractive indices of the starch samples and anhydrous ethanol were 1.53 and 1.33, respectively. The average particle size of the starch samples was recorded as the d (0.5) value.

2.6. Thermal analysis

The thermal properties of both native and modified kudzu starches were assessed using a differential scanning calorimeter (DSC) (Model DSC 3, Zurich, Switzerland). Three milligrams of starch samples were placed into aluminum crucibles, combined with 9 μL of distilled water, and sealed. Prior to analysis, samples were allowed to equilibrate at room temperature for 12 h. Instrument parameters were configured following the methodology outlined by Yang et al. (2016).

2.7. Paste clarity

The paste clarity of both the native and modified kudzu starches was assessed utilizing the methodology outlined by Yang et al. (2017). Starch paste clarity, expressed as a light transmittance (%), was determined using the spread spectrum photometer (SP-752, Shanghai, China) at 650 nm.

2.8. Hydration properties

2.8.1. Solubility, swelling power, and water absorption index

A starch suspension (5 g dry basis in 100 g distilled water) was stirred for 30 min at the indicated temperatures (25, 35, 45, and 55 °C) and thereafter centrifuged at 5000 rpm for 15 min. The resulting supernatant was poured into a pre-weighed plate and subsequently dried at 115 °C for 4 h. The weight of the supernatant was then recorded. The solubility (%), swelling power (g/g), and water absorption index of the samples were calculated using the methodology outlined by Núñez-Bretón et al. (2024) and Wang et al. (2016).

$$\text{Solubility} = \frac{W_2}{W_1} \times 100 \quad (2)$$

$$\text{Swelling power} = \frac{W_3}{W_1 \times (100 - \text{Solubility})} \quad (3)$$

$$\text{Water absorption index} = \frac{W_3}{W_1} \quad (4)$$

W_1, W_2 and W_3 denote the weight of the kudzu starches, the weight of dissolved solids in the supernatant, and the wet weight of the precipitate, respectively.

2.8.2. Aggregation rate

The aggregation rate of kudzu starches was determined following the method outlined by Wang et al. (2020) with slight modifications. 10 g of a starch sample was weighed and added to 100 mL of boiling water. The mixture was stirred with a glass rod for 30, 60, 90, 120, 150, and 180 s. The resulting paste was then passed through a 20-m sieve and washed with cold water. The agglomerate on the sieve was dried to a constant weight. The agglomeration rate (%) was calculated using the following formula:

$$\text{Agglomeration rate} = \frac{\text{Weight of the agglomerates after drying}}{\text{Weight of the starch sample}} \times 100 \quad (5)$$

2.9. Bulk density

The bulk density was assessed following the protocol outlined by Wani et al. (2014). Added starch to a 10 mL graduated cylinder. Subsequently, the graduated cylinder was gently tapped until the sample reached a height corresponding to the 10 mL mark on the scale. Then the mass of the filled sample was measured. The Bulk density is the mass per unit volume of the sample (g/mL).

2.10. Sedimentation rate

The concentration of 2 % starch emulsion to the 95 °C water bath for 30 min, cooled to room temperature, weighed the total weight of starch paste, after placed for 12 h, centrifuged at 5000 rpm for 20 min, weighed the weight of the supernatant, sedimentation rate (%) is calculated as follows:

$$\text{Sedimentation rate} = \frac{\text{The total weight of starch paste}}{\text{The weight of the supernatant}} \times 100 \quad (6)$$

2.11. Determination of flavonoid content

The sample was weighed 0.1 g, added 1 mL of extract (60 % ethanol) and extracted by ultrasonication (power 300 W, temperature 60 °C, extraction 30 min. 12,000 rpm, 25 °C, centrifugation 10 min, the supernatant was taken, and the extract was fixed to 1 mL. Subsequently, the extract was assayed using the kit (Plant Flavonoids Content Assay, Solarbio Biochemical Assay Division, Beijing, China). And Flavonoid content (mg /g) was calculated according to the following formula:

$$\text{Flavonoid content} = \frac{x \times V}{W} \quad (7)$$

where x is the sample concentration; V is the volume of the extract; and W is the sample mass.

2.12. In vitro digestibility

The in vitro digestibility of kudzu starches was assessed following Ding et al.'s method, with slight modifications (Ding et al., 2018). Hundred milligrams of a kudzu starches were combined with 15 mL of sodium acetate buffer (0.2 mol/L, pH = 5.2) in a 250 mL conical flask through vortexing. The mixture was then placed in a boiling water bath and stirred continuously for 30 min. Afterward, the kudzu starches were equilibrated in an oscillating water bath at 37 °C for 20 min before the addition of 10 mL of a mixture of porcine pancreatic α-amylase and amyloglucosidase. Kudzu starches were withdrawn at intervals of 0, 20, and 120 min, respectively, followed by immediate termination of the enzyme reaction through the addition of ethanol to the hydrolysate. The glucose content was quantified using the DNS method.

2.13. Statistical analysis

All analyses were conducted in triplicate. Experimental data are expressed as mean ± standard deviation. Data were analyzed using one-way analysis of variance. Statistical significance was defined at $P < 0.05$. In addition, principal component analysis (PCA) was used to assess the structural and physicochemical properties of the kudzu starches, facilitating visualization of differences among the native and the six types of modified kudzu starches, as well as their similarities and differences in these properties. All statistical analyses were performed using IBM Statistics SPSS 25 (Chicago, USA).

3. Results and discussion

3.1. Chain length distribution

The distribution of chain lengths, referring to the number of monomeric units in a chain, significantly impacts the properties of starch-based products. The length distribution of starch chains can be classified according to the degree of polymerization into A-chains (DP 6–12), B1-chains (DP 13–24), B2-chains (DP 25–36) and B3-chains (DP ≥ 37). Table 1 illustrates the chain length distributions in the native and modified kudzu starches. For NS, the A, B1, B2, and B3 chains constituted 24.19 %, 50.16 %, 12.80 %, and 12.85 %, respectively. No significant difference in the chain length distribution was observed between UNS and NS, potentially accounting for the minimal variation in their measured properties. Alcoholic-alkaline modification significantly altered the chain length distribution, with A, B1, B2, and B3 chains at 35.28 %, 41.81 %, 12.14 %, and 10.77 %, respectively. This may be due to the disruption of the internal structure of kudzu starch by sodium hydroxide infiltration, leading to increased degradation of long and short chains. BS displayed a decrease in the A-chain and an increase in the B-chains, indicating a change in the chain length distribution of kudzu starch. We speculate that this may be associated with the partial pasting of starch during the modification process and the subsequent rearrangement of starch molecules upon cooling. Extrusion puffing treatment did not significantly affect amylopectin chain length distribution in kudzu starch, consistent with the findings of Sun et al. (2021). Dual modified kudzu starch has a higher proportion of A-chains than ES and PS, which may be due to the fact that dual modification increases the production of small molecule fragments, thus increasing the number of short starch chains (Hii et al., 2012).

3.2. SAXS analysis

SAXS analysis revealed the semi-crystalline layer of starch, which typically comprises amorphous zones and alternating crystals. The average repetition distance of a semi-crystalline sheet is commonly determined by the position of scattering peaks at q values of 0.6 nm^{-1}. In addition, the intensity of scattered light in the SAXS pattern correlates with the degree of ordering within its semi-crystalline structure. Fig. 2 illustrates the double logarithmic pattern of SAXS for both the native and modified kudzu starches. The peak center position (q) and laminar repetition distance (d) values for kudzu starch are presented in Table 1. Scattering peaks were observed only for the NS and UNS, at 0.66 nm^{-1} and 0.65 nm^{-1}, respectively. The decrease in intensity of the scattering peaks for urea-alkaline-modified kudzu starch indicates a decrease in the degree of ordering within the semi-crystalline structure (Tan et al., 2015). The laminar repetition distance for the NS and UNS was 9.52 nm and 9.64 nm, respectively. The increase in the d-value of UNS indicates potential void formation between starch molecules during the urea-alkaline modification process. This possibly also accounts for the higher solubility of UNS in cold water. The characteristic laminar peaks associated with BS, ES, ANS, UNS, PS, EPS were not observed, suggesting disruption of the semi-crystalline layer structure. A similar finding has been reported by Lopez-Rubio et al. (2008).

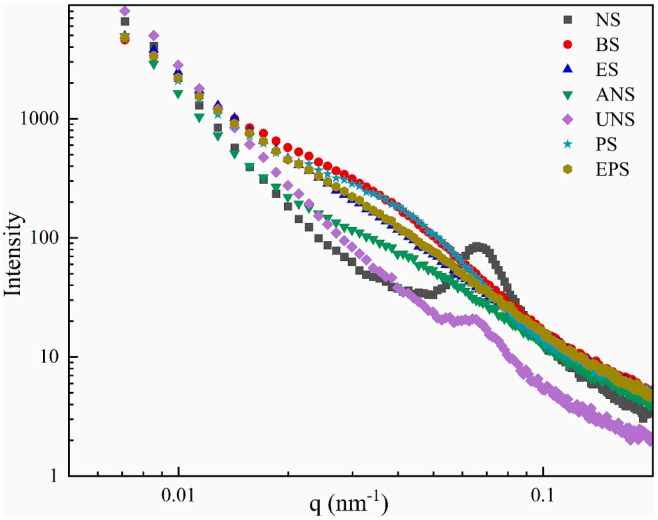

Fig. 2. SAXS curves of the kudzu starch sample.

3.3. Particle size distribution

The size of starch granules significantly influences both the pasting and functional properties of starches. Table 1 illustrates the average particle sizes of the native and modified kudzu starches. The d (0.5) values of modified kudzu starch increased to varying extents. Following starch modification, the d (0.5) values of BS, ES, ANS, UNS, PS, EPS increased by 11.13, 19.96, 73.45, 6.01, 16.38, and 7.64 μm, respectively. The increase in average particle size post-modification may have resulted from fragment agglomeration during the modification process (Soe et al., 2020). In contrast, the increase in d (0.5) values of ANS may be due to the swelling of starch granules facilitated by the addition of sodium hydroxide, in addition to the aforementioned factors. The d (0.5) of UNS was much lower than that of ANS, which was only 16.55 μm. We speculate that it may be due to the introduction of urea which greatly increased the hydrogen bond breakage.

3.4. Thermal properties

The thermal parameters of the native and modified kudzu starches are shown in Table 2 and Fig. 3. The onset pasting temperature, critical pasting temperature and ΔH of native kudzu starch were 71.20 °C, 81.34 °C and 5.4 J/g, respectively, while those of UNS starch were 73.27 °C, 83.79 °C and ΔH of 6.99 J/g, respectively. No DSC characteristic curves were detected for the other modified kudzu starches, and the values of the pasting temperatures and enthalpy changes were not observed. This validated the significant disruption of the modified kudzu starch granules, and the percentage of remaining crystalline structure

Table 1
Chain length distribution (A, B1, B2 and B3-chain), lamellar structure parameters (q and d) and d (0.5) of the kudzu starch samples.

Sample	Chain length distributions of starch (%)				q (nm^{-1})	d (nm)	d (0.5) (μm)
	A(DP6–12)	B1(DP 13–24)	B2(DP 25–36)	B3(DP ≥ 37)			
NS	24.19	50.16	12.80	12.85	0.66	9.52	10.54
BS	23.50	50.59	12.76	13.14	ND	ND	21.67
ES	24.48	49.55	13.11	12.86	ND	ND	30.50
ANS	35.28	41.81	12.14	10.77	ND	ND	83.99
UNS	24.10	50.48	12.76	12.66	0.65	9.64	16.55
PS	24.41	50.93	12.81	11.85	ND	ND	26.92
EPS	25.09	49.34	13.00	12.57	ND	ND	18.18

DP, degree of polymerization; ND, not detected.

Table 2
Thermal properties and bulk density of the kudzu starch samples.

Sample	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Bulk density (g/mL)
NS	71.3 ± 0.14^{b}	77.11 ± 0.06^{b}	81.45 ± 0.16^{b}	5.47 ± 0.10^{b}	0.90 ± 0.00^{a}
BS	ND	ND	ND	ND	0.71 ± 0.00^{c}
ES	ND	ND	ND	ND	0.76 ± 0.00^{b}
ANS	ND	ND	ND	ND	0.62 ± 0.00^{e}
UNS	73.11 ± 0.26^{a}	78.94 ± 0.23^{a}	83.86 ± 0.07^{a}	8.18 ± 0.08^{a}	0.55 ± 0.00^{g}
PS	ND	ND	ND	ND	0.56 ± 0.00^{f}
EPS	ND	ND	ND	ND	0.64 ± 0.00^{d}

To: onset temperature; Tp: peak temperature; Tc: critical temperature; ΔH: gelatinization enthalpy; ND: not detected.

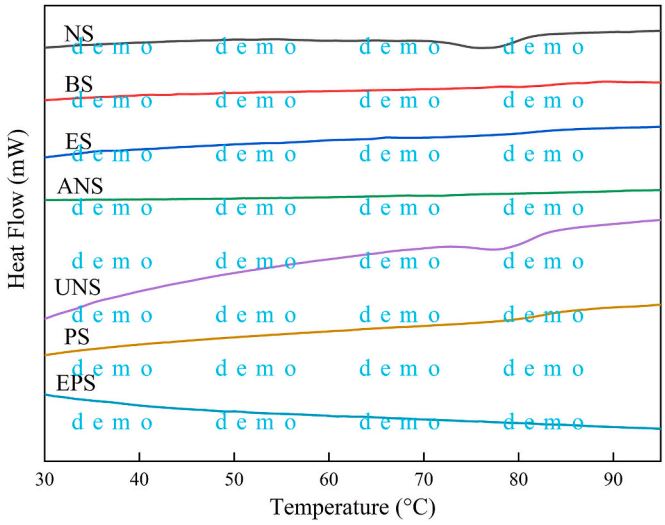

Fig. 3. DSC profile of the kudzu starch sample.

was too minute. Therefore, it can't be detected by the DSC. Notably, the DSC characteristic peak for UNS shifted to a higher temperature range compared to the NS, while its solubility in cold water was notably higher than that of the NS. We speculate that this may be due to the enlargement of kudzu starch granules during the modification process, resulting in a slower water absorption speed and necessitating a higher temperature for gelatinization.

3.5. Paste clarity

The clarity of starch paste is a characteristic index of starch-based products (Fig. 4). Except for that of the UNS and PS, the paste clarity of the other four modified kudzu starches exceeded that of the NS. ES exhibited the highest paste clarity at 53.93 %, while PS demonstrated the lowest at 16.20 %. The enhanced clarity may be due to the degradation of starch molecules during the modification process, which increased the presence of hydrophilic groups in the starch, thereby facilitating binding between kudzu starch molecules and water molecules and the irreversible destruction of the starch molecules (Liu et al., 2014). Given its superior paste clarity, ES can be applied in the production of transparent shrimp dumplings, iced mooncakes, punch powder, and vermicelli in the food industry, thereby enhancing the commercial value of NS. The low clarity of UNS and PS may stem from disruption of the crystalline regions within starch granules during the modification process since the molecular chains are prone to re-aggregation upon cooling, resulting in diminished paste clarity. In addition, the clarity of starch pastes is influenced by variety of factors, such as starch granules, swelling capacity, amylose content, amylose/

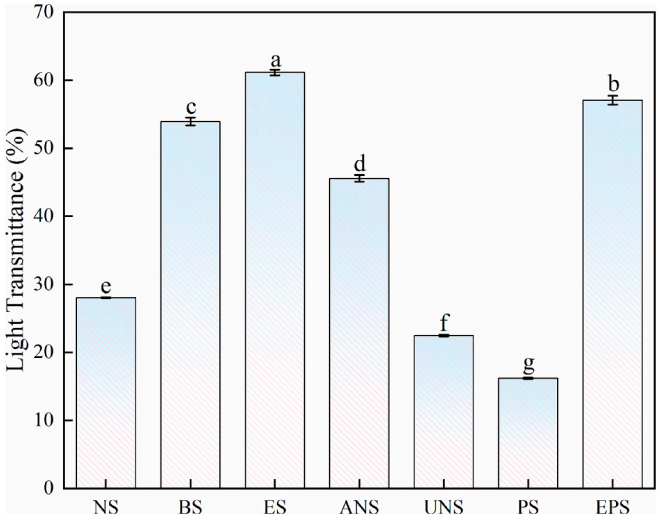

Fig. 4. Paste clarity of the kudzu starch samples.

amylopectin ratio, and the high refraction of light by residues of swollen starch granules (Zhang et al., 2011).

3.6. Hydration properties

Hydration property is one of the key properties for the development of starch-based products. In this study, the hydration properties of native and starch modification of kudzu starch were determined at 25, 35, 45 and 55 °C (Table 3).

3.6.1. Solubility

The solubility of modified kudzu starches was significantly higher than that of NS. The solubility of BS ranged from 28.79 % to 53.11 % at 25 °C to 55 °C. During the ball milling process, the kudzu starch granules were subjected to physical forces, exposing the hydrophilic groups in the starch molecules, thereby improving the solubility of BS at lower temperatures. The solubility of ES varied the most substantial alterations at lower temperatures compared to other modified kudzu starches, with an increase exceeding 70 %. This may be due to the high temperature, high pressure and shear force in the extrusion puffing process that promoted starch pasting and degradation, and disrupted inter- and intramolecular hydrogen bonding of kudzu starch (Wang et al., 2020). In ANS, the high concentration of sodium hydroxide disrupted internal hydrogen bonding and the double helix structure of starch, leading to enhanced solubility at lower temperatures. Pullulanase, a debranching enzyme, hydrolyzed kudzu starch, increasing the abundance of short chains and the site of action of water molecules, consistent with the findings of previous studies (Ge et al., 2021). Compared with PS and EPS showed higher solubility at lower temperatures. On the one hand, ES exhibited more loose internal packing, which facilitated the entry of water molecules. On the other hand, this structure was more susceptible to enzymatic degradation by pullulanase, yielding more short chains and exposed hydroxyl groups, thus solubility is higher than that of PS. This finding was corroborated by the chain length distribution analysis.

3.6.2. Swelling power

Swelling power is related negatively with solubility (Singh et al., 2004). The swelling power of physically, chemically and enzyme modified starches varied and the enzyme modification contributed more to the improvement of starch swelling power. Juarez-Arellano et al. (2021) showed that lower energy inputs result in higher solubility and swelling power during the ball milling modification process. The swelling power of the modified kudzu starches was increased, except for ES and EPS. The swelling power of physically, chemically and enzyme

Table 3
Hydration properties (Solubility, Swelling power and Water absorption index) of the kudzu starch samples.

Parameter		Sample						
		NS	BS	ES	ANS	UNS	PS	EPS
25 °C	Solubility (%)	0.01 ± 0.01^{e}	28.79 ± 0.73^{c}	73.12 ± 2.41^{a}	56.27 ± 1.48^{b}	16.61 ± 0.86^{d}	19.17 ± 2.05^{d}	71.78 ± 0.18^{a}
	Swelling power (g/g)	2.00 ± 0.03^{d}	6.60 ± 0.33^{b}	0.93 ± 0.01^{e}	2.33 ± 0.20^{d}	4.55 ± 0.59^{c}	8.11 ± 0.45^{a}	0.80 ± 0.04^{e}
	Water absorption index	2.00 ± 0.03^{d}	6.58 ± 0.33^{b}	0.93 ± 0.01^{e}	2.32 ± 0.20^{d}	4.54 ± 0.58^{c}	8.09 ± 0.45^{a}	0.80 ± 0.05^{e}
35 °C	Solubility (%)	0.01 ± 0.13^{f}	29.53 ± 0.15^{d}	80.30 ± 1.18^{a}	65.34 ± 0.17^{b}	11.87 ± 0.19^{e}	35.63 ± 0.17^{c}	79.39 ± 0.20^{a}
	Swelling power (g/g)	2.11 ± 0.35^{d}	7.73 ± 0.43^{a}	1.20 ± 0.11^{e}	2.75 ± 0.62^{d}	4.73 ± 0.01^{c}	5.82 ± 0.39^{b}	0.75 ± 0.16^{e}
	Water absorption index	2.11 ± 0.04^{d}	7.71 ± 0.43^{a}	1.19 ± 0.11^{e}	2.73 ± 0.62^{d}	4.73 ± 0.01^{c}	5.80 ± 0.38^{b}	0.75 ± 0.16^{e}
45 °C	Solubility (%)	1.43 ± 0.07^{f}	36.22 ± 0.37^{e}	78.29 ± 1.63^{a}	65.30 ± 0.06^{b}	14.93 ± 0.25^{d}	43.32 ± 6.74^{c}	80.68 ± 1.52^{a}
	Swelling power (g/g)	1.99 ± 0.14^{cd}	6.53 ± 0.15^{a}	1.52 ± 0.46^{de}	2.68 ± 0.04^{c}	4.42 ± 0.12^{b}	6.16 ± 0.57^{a}	0.88 ± 0.08^{e}
	Water absorption index	1.99 ± 0.14^{cd}	6.51 ± 0.15^{a}	1.51 ± 0.56^{de}	2.66 ± 0.04^{c}	4.41 ± 0.12^{b}	6.15 ± 0.58^{a}	0.87 ± 0.85^{e}
55 °C	Solubility (%)	2.11 ± 0.53^{f}	53.11 ± 0.44^{c}	79.40 ± 1.65^{a}	69.85 ± 0.11^{b}	17.39 ± 0.41^{e}	46.51 ± 2.12^{d}	80.19 ± 0.05^{a}
	Swelling power (g/g)	2.14 ± 0.05^{c}	4.88 ± 0.13^{b}	1.28 ± 0.21^{c}	1.80 ± 0.01^{c}	4.50 ± 0.18^{b}	6.39 ± 1.39^{a}	0.91 ± 0.13^{c}
	Water absorption index	2.14 ± 0.05^{c}	4.86 ± 0.12^{b}	1.27 ± 0.21^{c}	1.79 ± 0.01^{c}	4.50 ± 0.18^{b}	6.36 ± 1.39^{a}	0.90 ± 0.13^{c}

modified starches varied and the enzyme modification contributed more to the swelling power of kudzu starch.

3.6.3. Water absorption index

The water absorption index of modified kudzu starch ranged from 0.80 to 8.09. All modifications increased water absorption by kudzu starch, except for extrusion puffing and extrusion puffing-pullulanase modifications. Several studies have demonstrated that extrusion puffing modification increases the water absorption by starch (Gandhi et al., 2021). However, Qi et al. (2023) found that extrusion puffing modification decreases water absorption by starch, aligning with our findings. Two reasons account for reduced water absorption index due to extrusion expansion: increased broken starch granules as a result of high temperature, pressure, and shear during the modification process, and dextrinization exceeding pasting. Among the modified starches that exhibited increased water absorption index, PS exhibited the highest water absorption index, which can be attributed to the water solubility promotion of starch by enzymatic hydrolysis. The increase in the water absorption index of BS may be due to the increased surface area and hydrophilic groups of starch following the ball milling process (Ahmad et al., 2020). The water absorption index of UNS was higher than that of ANS, possibly because the introduction of urea hydrate hindered the regeneration of starch, ensuring sufficient water absorption by starch.

3.6.4. Agglomeration rate

The lower the agglomeration rate of starch, the greater the development potential for instant kudzu powder development (Wang et al., 2020). Fig. 5 are shown the agglomeration rates of native and modified kudzu starches for various stirring durations (30 s, 60 s, 90 s, 100 s, 120 s, 150 s, and 180 s). The agglomeration rate of all the samples decreased as the stirring duration increased. The agglomeration rate of ES and EPS was nearly zero. For a stirring duration of 30 s, NS exhibited notably higher agglomeration rates compared to modified kudzu starches. NS and UNS suspensions displayed a lot of agglomerates encapsulating the powder after stirring for 180 s, while BS, ANS and PS showed a small number of fully pasted agglomerates. This may be due to the looser structure of starcher resulting from ball milling, extrusion puffing, alcoholic-alkaline, pullulanase and extrusion puffing-pullulanase modifications, which facilitated the rapid permeation of water into the starch molecules, resulting in a lower rate of agglomeration. Overall, hydration properties play a vital role in determining suitable modifications for obtaining starch with the desired properties. For example, starches exhibiting low swelling power and high solubility (ES and EPS) are more appropriate for applications demanding high-temperature cooking (vermicelli and rice vermicelli).

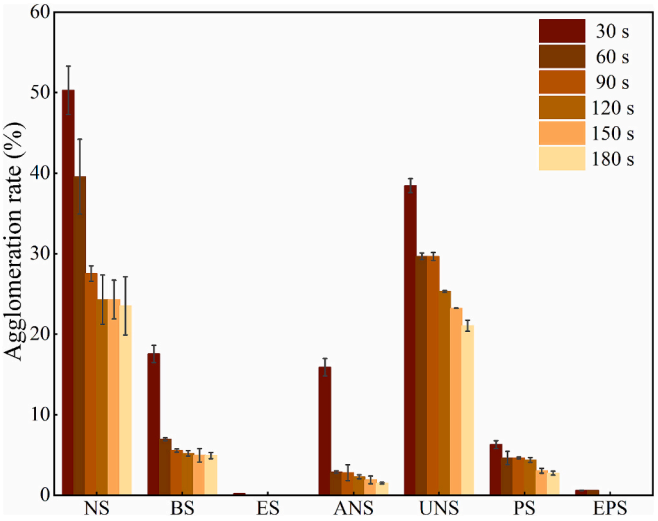

Fig. 5. The agglomeration rate of the kudzu starch samples.

3.7. Bulk density

Bulk density is related to the degree of loose starch structure (Agnes et al., 2017). Following modification, kudzu starches exhibited a notable decrease in bulk density, ranging from 0.55 g/mL to 0.76 g/mL, representing a reduction of about 10 % or more (Table 2). It might be caused by different degrees of structural disruption of the modified kudzu starch, this corresponds to our previous findings (He et al., 2024). These alterations likely contributed to the decrease in bulk density of the modified kudzu starches. In addition, the hydration properties of starch are closely associated with its bulk density (Olagunju et al., 2020).

3.8. Sedimentation rate

Sedimentation refers to the phenomenon of insolubility, aggregation or crystallization of gelatinized starch molecules after being placed for a period of time, which is an important indicator of aging properties of starch. Fig. 6 shows the Sedimentation rate of native and modified kudzu starch. After modification, the sedimentation rate of starch followed BS < ANS < UNS < PS < NS < EPS < ES. Among them, ES and EPS sedimentation rate were significantly reduced from 49.05 % (NS) to 2.88 % and 4.5 %, respectively. This indicates that extrusion and double modification can effectively improve the stability of starch, which can

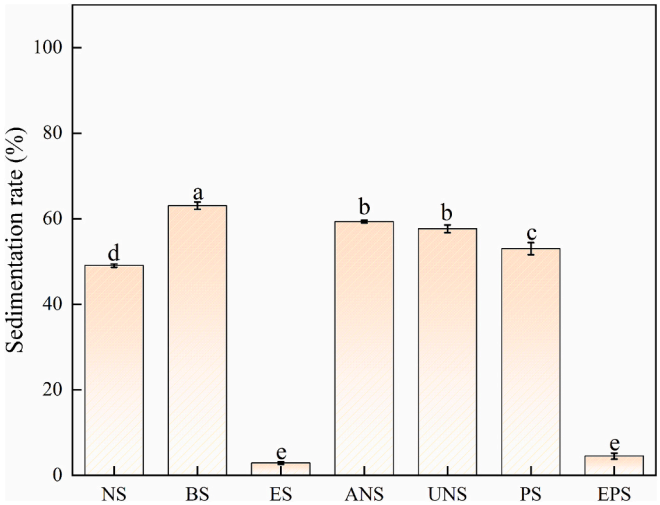

Fig. 6. The sedimentation rate of the kudzu starch samples.

be rehydrated quickly and maintain a better texture when developing instant food products. While the sedimentation rate of BS, ANS, UNS and PS increased to different degrees, which indicated that ball milling, alcohol-alkali, urea-alkali, pullulanase modification tended to make NS more susceptible to aging.

3.9. Flavonoid content

Kudzu is rich in flavonoids that have immune-improving, antioxidant, as well as alcohol- and liver-protecting properties (Zhu et al., 2023). However, their low bioavailability greatly inhibits their development. As shown in Fig. 7, the flavonoid content decreased in BS compared to NS, while no flavonoids were detected in ANS, UNS and PS. This may be due to the use of the same solvent (ethanol) as the bioactives in the elution of modified starch, resulting in the loss of bioactives. Whereas, the flavonoid content in ES increased rather than decreased. This may be due to the rupture of the cell wall matrix during extrusion, the release of bioactive substances, and the improvement of the extractability and solubility of the flavonoid bioactivities (Wang et al., 2014). At the same time, the high frequency of screw speed shortened the residence time of starch in the extrusion barrel, thus reducing the possibility of decomposition of bioactivities due to high temperatures, the same phenomenon observed in the study of Tiznado et al. (2013). Therefore, the nutritive value of ES is better able to satisfy the demand for nutrients and has a health-promoting effect.

3.10. In vitro digestion

Fig. 8 illustrates the in vitro digestive characteristics of native and modified kudzu starches. There were significant differences in the digestive properties between the modified starches and NS. Regarding the modified kudzu starches, the ready digestible starch (RDS) ranged from 13.87 % to 69.59 %, the slowly digested starch (SDS) ranged from 18.19 % to 61.51 %, and the resistant starch (RS) ranged from 12.22 % to 44.56 %, while the RDS, SDS, and RS of NS were 69.59 %, 18.19 %, and 12.22 %, respectively. The results of the agglomeration rate indicated that, all modified kudzu starches exhibited agglomeration phenomena, except for ES and EPS. These phenomena inhibited the action of amylase to some extent, resulting in an increased RS content of kudzu starches. The higher SDS and RS in modified kudzu starch can help in the development of low glycemic index foods for specific populations. The large increase in resistant starch content in BS was attributed to the formation of a more compact structure of starch during ball milling, which is difficult to be eroded by enzymes. The increased RS content of ES could be attributed to the formation of carbon dots due to the Maillard reaction that occurs during extrusion at elevated temperatures, and its structure is more resistant to heat and decomposition. In addition, the interaction of hydrogen bonding between flavonoids and starches is responsible for its higher RS starch content. Radhika Reddy et al. (1993) showed that long chains could provide support to the starch structure and make starch less susceptible to enzymatic digestion. This may explain the increased SDS and RS in BS and UNS. While the proportion of long chains decreased in ANS and PS, but their SDS and RS content increased, which indicated that starch digestion is affected by multiple factors. PS may exhibit a concentrically arranged structure after modification, which is one of the reasons for the increase in its SDS and RS content (Li et al., 2019). Meanwhile, the RDS content of EPS was lower than that of ES, possibly due to a higher proportion of short linear chains at DP 9–11 in EPS. Higher DP 9–11 have been shown to enhance the likelihood of order orientation and rearrangement of short linear chains, facilitating the formation of a more robust enzyme-resistant structure (Li et al., 2019).

3.11. PCA

PCA elucidated the similarities and differences among the various modified kudzu starches, as well as the relationship among the measured properties (Fig. 9). The distances between samples on the graph are proportionate to their dissimilarities. The first and second

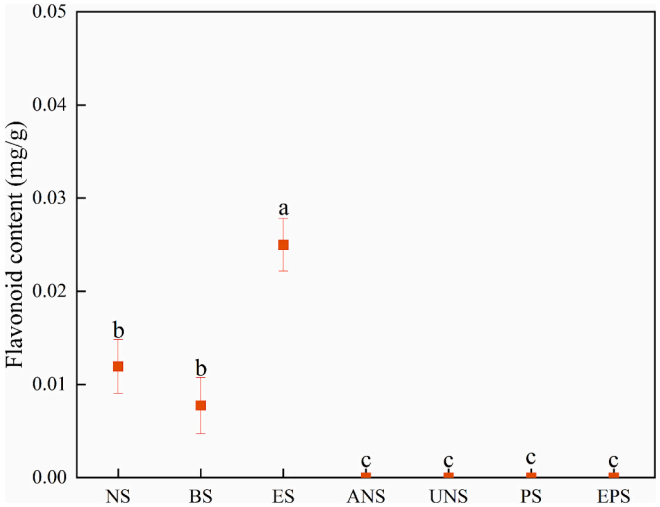

Fig. 7. The flavonoid content of the kudzu starch samples.

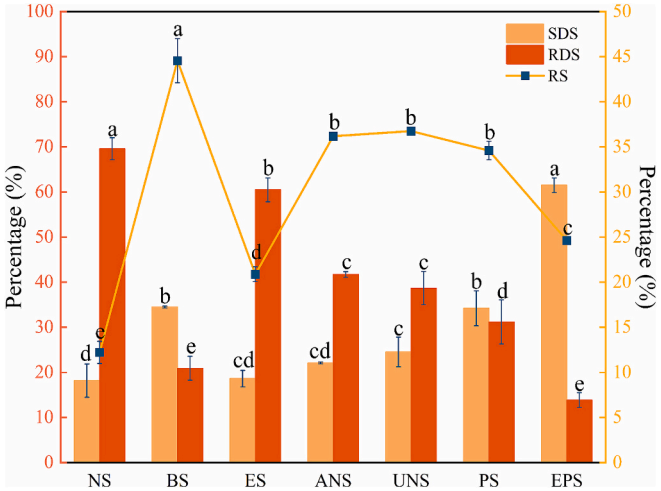

Fig. 8. The RDS, SDS, and RS contents of kudzu starch samples.

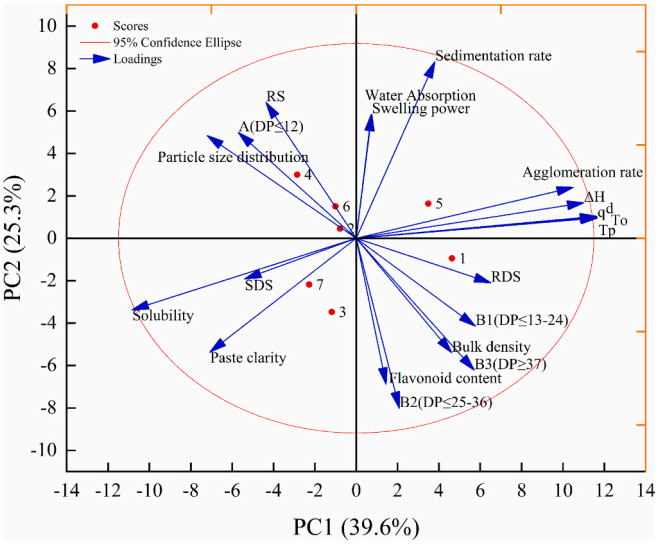

Fig. 9. The PCA of the kudzu starch samples. (1) NS; (2) BS; (3) ES; (4) ANS; (5) UNS; (6) PS; (7) EPS.

principal components accounted for 39.6 % and 25.3 % of the total variances, respectively. Solubility, agglomeration rate, lamellar structure (q, d), thermodynamic properties (To, Tp, ΔH), RDS content, and SDS content were pivotal factors for PC1 constructs. In contrast, paste clarity, sedimentation rate, flavonoid content, particle size, starch chain length distribution (A, B1, B2, and B3 chains), bulk density, swelling power, RS content, and water absorption index influenced the PC2 constructs. Attributes positioned closely on the graph exhibited high positive correlation (Cosine close to 1), while opposing curve directions signified negative correlation (Cosine close to -1). For example, the close proximity of agglomeration rate, q, d, and thermal properties indicated a strong positive correlation among them. RS has a strong negative correlation with bulk density and B-chains. The distance between sample points reflects the difference in their degree of similarity. Sample points that are closer together are more similar in the feature space. The six modified starches were widely distributed in the PCA plots, indicating effects of diverse modifications on the structural properties of kudzu starch. Compared to other modified kudzu starches, the urea base modification caused the least structural damage to kudzu starch, which the structure determines the properties. Therefore, the distance between UNS and NS is the closest in the PCA plot.

4. Conclusion

In this study, the physicochemical properties and structural characteristics of BS, ES, ANS, UNS, PS, EPS were systematically investigated. The results show that the modification treatment effectively affect the fine structure as well as solubility, sedimentation, transparency, in vitro digestive properties and nutritional properties of NS. At the same time, it provides options for expanding the application of NS. At the same time, modifications provided options for expanding the application of NS. Given that modified kudzu starch and native starch each have their own characteristics in terms of structure and properties, they can be used to produce products with a variety of properties. For example, the good hydration properties and anti-sedimentation demonstrated by ES and EPS are favorable for the development of products with high solubility requirements. While extrusion puffing-modified requires less energy than extrusion puffing-pullulanase-modified, and therefore, extrusion puffing is expected to be a more environmentally friendly and economical modification technique. Furthermore, extrusion puffing-modified kudzu starch presents excellent potential for the water-soluble pharmaceutical industry. This study not only expands the application of kudzu starch but also provides a scientific foundation for employing effective modification methods to modify the structure of kudzu starch and enhance its functional properties. In the future, our research should focus on how to preserve the functional components and enhance the nutritional value of modified kudzu starch for commercial food applications.

CRediT authorship contribution statement

Ruidi He: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Chuanlai Du:** Formal analysis. **Songnan Li:** Writing – review & editing, Formal analysis. **Li Guo:** Formal analysis. **Kaiyue Wang:** Formal analysis. **Liping Yang:** Writing – review & editing, Supervision, Resources, Funding acquisition.

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

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101912.

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