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Stabilization mechanism of white kidney bean based milk through novel perspectives of endogenous starch

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ABSTRACT

Plant-based milk (PBM) substitutes have attracted worldwide attention, but the development is restricted by poor stability and limited categories. White kidney bean (WKB) is one potential healthy material for PBM production. Here, we aimed to obtain optimal WKB cultivars first, and further investigated stabilization mechanism in aspect of its endogenous starch. Among the investigated cultivars, three cultivars were selected as the most suitable for producing WKB emulsions. Native starch of the stable cultivars exhibited higher pasting temperature, less peak, trough, and final viscosity. With enzymatic hydrolysis, starch of stable cultivars showed higher solubility index while less swelling power, and less short range order. The observations were further proved through microscopy observation and correlation analysis between starch properties and WKB milk stability attributes. These results contribute to understand molecular mechanism for improving WKB milk stability from perspective of endogenous starch, and provide valuable information for raw materials selection with typical starch characteristics.

1. Introduction

Plant-based milk (PBM) substitutes have gained increased interest worldwide due to its balanced nutrients profile and popularity of vegetarianism. PBM is typically made from soybeans, legumes, nuts, cereals, and other plant seeds. Kidney bean (*Phaseolus vulgaris* L.) is one most important and widely cultivated legume, and the planting area is only second of soybean (Canton, 2021). Kidney bean is rich in carbohydrates (39 %–47 %, w/w DW, dry weight basis), proteins (22 %–27 %, w/w DW), and other bioactive compounds, while free of lactose and cholesterol (Wang, Chen, et al., 2020). Up to now, there is only one soley well know product of kidney bean, *i.e.* α-amylase inhibitor, for its ability to block starch hydrolysis, increase satiety, and assist weight loss (Houghton et al., 2023). The abundant nutrients of kidney bean make it also a promising candidate for PBM production. However, it is just because the rich nutrients and variance among different cultivars that raised necessity to select the optimal kidney bean cultivar and explore underlying stabilizing mechanism.

Stability of PBM is highly dependent on process conditions and nutrients quality of raw materials (Qamar et al., 2019). Researches have found that different homogenization methods can reduce sediment formation, or to prevent coalescence and flocculation through adding polysaccharides, such as guar gum, pectin, locust bean gum, and β-glucan in PBM (Kulczyk et al., 2023). Besides, protein is the most recognized component that influence PBM stability. For example, the stability of oat PBM was found to be highly correlated with protein concentration in oat seeds, and higher protein content could induce more sediment (Zhou et al., 2023). Other than concentration, protein composition and properties also showed significant effects on PBM stability, which was proven to be dependent on mung bean cultivars in a recent study (Dai et al., 2024). Starch is the most abundant component in kidney bean, with a content of about 45 % (Shevkani et al., 2022). However, the effect of starch on kidney bean PBM stability has never been reported. Therefore, starch content and physicochemical properties were mainly studied in associated with stability of kidney bean PBM.

Starch property is one determining factor for influencing food quality. For instance, gelatinization and swelling are unique starch characteristics, and especially affect palatability, structure, and texture of starch-based food (Chakraborty et al., 2022). Viscosity of starch is important for maintaining stability and acceptability of PBM, and could be influenced by gelatinization and swelling process of starches. During gelatinization, starch granules undergo phase transition from order to disorder, irreversibly disrupting the semi-crystalline structure of starch. In general, viscosity of starch paste increased fast first as gelatinization

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progress, followed by rapid decline according to Rapid Visco Analyzer (RVA) curve (Santamaria et al., 2022). Gelatinization degree are dependent on starch crystal type, size, shape, amylose content, and molecular weight of amylose/amylopectin, which largely influences emulsions texture (Li, 2022). Swelling behavior describes a process for starch to absorb water and become swollen. New hydrogen bonds form with water entering the amorphous region of starch granules, during which, more amylose are leached, accompanied by viscosity increase in suspension system, such as PBM (Jia et al., 2023). Furthermore, such starch swelling property could be decreased via enzymatic treatment to meet manufacturing needs (Jia et al., 2023).

There is only limited information available about the role of starches in raw materials play in white kidney bean milk stability. Furthermore, how enzymatic hydrolysis of starches in white kidney bean milk processing affect the emulsion stability still remaines to be studied. To this end, we first distinguished the stabilized kidney bean milk from 8 different cultivars by sensory evaluation and physical stability, and analyzed the key sensory attributes that most contributed to emulsion acceptability through principal component analysis. After we found that both white kidney bean milk and starch differed in viscosity between the more and less stable cultivars, we further investigated starches content and properties before and after enzymatic treatment that was involved in kidney bean milk production. Starches content including total starch, amylose, and resistant starch were all determined. Solubility index, swelling power, short-range order, and micro-structure of native and enzyme hyzrolyzed starches were also tested. These findings provide theoretical support for understanding the role of starch play in the stability of white kidney bean milk, and give guidance for selecting kidney bean cultivars with specific starch properties for production of stable and clean lable kidney bean milk.

2. Materials and methods

2.1. Materials

White Kidney Bean 20,210,165 (WK1), Japanese White (WK2), Long Kidney Bean 20 (WK3), NY6 (WK4), Long Kidney Bean 8 (WK5), Long 17–4197 (WK6), and 20,210,183 (WK7) were harvested in Heilongjiang Province, China. Suyang white kidney bean (WK8) was harvested in Liaoning Province, China. Amylase, glycosylase, protease, and cellulase were purchased from Novozymes Biotechnology Co., Ltd. (Beijing, China).

2.2. Preparation of white kidney bean (WKB) milk

WKB milk was prepared with referring to Zhou et al. (Zhou et al., 2023). After rinsing, WKBs (30 g) were soaked at 60 °C for 1 h and blanched at 90 °C for 5 min, followed by blending with deionized water (1/10, w/v) containing 0.05 % amylase (v/v, enzyme activity of 150,000 U/mL). Then, enzymes formula including 0.4 % glycosylase (v/v, 100,000 U/mL), 0.4 % cellulase (v/v, 3500 U/g), and 0.04 % protease (v/v, 50,000 U/g) was added and incubated at 55 °C for 1 h with stirring. The enzymes formula was optimized from preliminary tests. The mixture was then filtered through a 200-mesh, dispersed for 1 min by adding 2 % soybean oil, and homoginized at 40 MPa (Scientz-150, Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China). WKB milk was obtained with sterilization at 100 °C for 30 min.

2.3. Extraction of WKB starch

WKB flour was mixed with 0.04 mol/L NaOH at a ratio of 1:8 (w/v) and extracted at 30 °C for 2 h. The mixture was centrifuged at 1800 *g* for 20 min and the supernatant was discarded. Then the mixture was mixed with deionized water at a ratio of 1:8 (v/v) and left at room temperature for 4 h. The supernatant was then filtered through 100-mesh and centrifuged to discard the supernatant. The precipitation was washed with water until the starch was free of impurities. Finally, it was dried at 45 °C for 10 h (Zou et al., 2020). The purity was determined by Megazyme total starch assay kit (AACC Method 76.13), and all of them were around 90 %.

Total starch content was determined by Megazyme total starch assay kit (K-TSTA). Amylose content was determined by Megazyme gibberellin assay kit (K-TSTA). Resistant starch content was determined by Megazyme resistant starch assay kit (K-TSTA).

2.4. Enzymatic hydrolysis of WKB starch

According to the starch content and amount of amylase added in WKB milk production, the extracted WKB starch was mixed with deionized water and hydrolyzed with amylase at 90 °C for 5 min. The hydrolyzed starch mixture was applied for solubility, swelling, and apparent viscosity analysis. Starch was lyophilized in prepareation for short-range order and microscopic morphologyanalysis.

2.5. Sensory evaluation

Sensory evaluation of WKB milk was conducted by 10 trained panelists using a 0–10 points hedonic scale. Sensory attributes included texture, color, aroma, mouthfeel and overall acceptability as shown in Table 1 (Dai et al., 2024). Each participant signed an informed consent form prior to the start of the experiment, and all participants were aware of and had consumed commercially available plant-based milks. The

Table 1

Sensory evaluation attributes and standards for white kidney bean milk.

Attributes	Evaluation Standards	Score
Texture (10 points)	Uniform system, no visible separation	7–10
	Relatively uneven system, slight wall-cling or separation	4–7
	Unstable system, obvious separation	0–4
Color (10 points)	Uniform color, milky white	7–10
	Slightly dull, nearly milky white	4–7
	Dull and tend to be gray	0–4
Aroma (10 points)	White kidney bean aroma and strong	7–10
	Normal white kidney bean aroma with little beany smell	4–7
	Heayy beany smell	0–4
Mouthfeel (10 points)	Smoothy and no graininess	7–10
	Slightly graininess	4–7
	More graininess	0–4
Overall acceptability (10 points)	Better overall feeling	7–10
	Ordinary overall feelings	4–7
	Poor overall feeling	0–4

recruited participants were also trained of the processes required for the experiment and basics of sensory evaluation.

2.6. Physical stability analysis

Physical stability was determined using an analytical centrifuge (LUMiSizer®, LUM GmbH, Berlin, Germany). Separation profile was obtained with rotation speed at 903 g, temperature 25 °C, time 7650 s, and time interval 30 s. Separation rate was determined when plotting transmissivity over time with an intercept of 0. Sediment and creaming height were calculated of the last profile by subtracting positions with ≤20 % transmittance at the bottom of tube or meniscus, respectively. To characterize separation of the emulsion under accelerated gravity for a given time, instability index was taken between 0 and 1. Value of 0 indicated the highest stability, implying no transfer or separation of the phase, and 1 indicated the lowest stability, implying complete separation of the phase (Kori et al., 2021).

2.7. Rheological analysis

The rheological properties of WKB milk and enzymatic hydrolyzed starch were determined using a rheometer (MARS iQ Ai, Thermo, Carlsbad, CA, USA). A CC27 rotor was selected. The measurement was conducted within a shear rate range of 0 to 100 s^{-1} at 25 °C for 3 min (Huang et al., 2022).

2.8. Gelatinization properties

Rapid Visco Analyzer (RVA) was applied for gelatinization properties analysis of WKB flour (Pan et al., 2019). The procedure was set as follows: The initial temperature was 50 °C, and after equilibration for 1 min, it was heated to 95 °C at a rate of 12 °C/min and stirred for 2.5 min. Then the temperature was cooled to 50 °C at a rate of 11.7 °C/min, and held for 1.5 min. Viscosity parameters including pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown (BD) and setback (SB) were determined.

2.9. Solubility index and swelling power

Starch (dry weight M_0) mixture was made according to the concentration of starh in WKB milk. The mixture was incubated in water bath at 90 °C for 30 min, and centrifuged at 1000 *g* for 20 min after cooled to room temperature (Kumar et al., 2018). The supernatant was transferred into an aluminum cup and dried to constant weight at 105 °C, weighed as soluble starch (M_1). The lower sediment was weighed as swollen starch (M_2). Solubility index and swelling power were calculates as:

$$\text{Solubility index } (\%) = M_1/M_0 \times 100$$

$$\text{Swelling power } (g/g) = M_2/[M_0 \times (1 - M_1/M_0)]$$

2.10. Fourier transform-infrared spectroscopy (FT-IR)

The infrared (IR) spectra of starch was determined with an infrared spectrometer (Spectrum Two FTIR spectrometer, PerkinElmer) (Wang et al., 2017). Briefly, 2.0 mg of dry starch sample and 100 mg of potassium bromide powder were weighed, quickly ground for 1 min and then pressed into thin slices. The slices were scanned with a spectral resolution of 4 cm^{-1} within a scanning range of 4000–400 cm^{-1}. Spectra in range of 1200–800 cm^{-1} were analyzed by data processing by OMNIC software (OMNIC 9.2).

2.11. Scanning electron microscopy

Scanning electron microscope (SEM) was used to observe the surface morphology of starch granules. A portion of starch sample was taken and placed on double-sided conductive adhesive, and then it was sprayed with gold and photos were taken under SEM with a magnification of 800× (Yan et al., 2022).

2.12. Statistical analysis

All experiments were conducted in three replications. The results were expressed as mean ± standard deviation (SD), and were analyzed by one-way analysis of variance (ANOVA). Significance was considered when $p < 0.05$ using SPSS software (SPSS Statistics 17). Principal component analysis (PCA) of data with emulsion stability indicators was conducted with SPSS.

3. Results and discussion

3.1. Sensory evaluation of white kidney bean (WKB) milk

Sensory evaluation of WKB milk made from 8 cultivars were conducted by 10 trained panelists. Attributes of texture, color, aroma, mouthfeel, and acceptability of emulsions were evaluated based on a 10-point hedonic scale (Table 2A). Compared to WK4-WK8, there were no visible separation of WK1-WK3, and showed more uniform emulsion system. WK1-WK4 showed milky white in color, while WK5-WK8 were slightly dull. WK1-WK3 milk showed more smoothy and no graininess, had more pleasant WKB aroma, and better overall feeling than WK4-WK8.

To gain deeper understanding of the sensory attributes that mainly raised sensory evaluation difference among 8 WKB milk samples, principal component analysis (PCA) was applied with 5 evaluated variables (Zhao et al., 2023). As shown in Table 2B, the first two PCs were selected, and these two PCs could explain 96.189 % of the total information and variance. The contribution rate of PC1 and PC2 accounted for 91.940 % and 4.249 % of the total variance, respectively. The contribution rate of each sensory attribute to PC1 and PC2 was listed as a component matrix in Table 2C. Among all attributes, texture (X1) mainly reflected PC1 with contribution rate of 2.242, and aroma (X3) mainly reflected PC2 with contribution rate of 0.380. The results

Table 2A
Sensory evaluation of white kidney bean milk made by different cultivars.

Cultivars	Texture	Color	Aroma	Mouthfeel	Acceptability
WK1	7.63 ± 0.48^{a}	8.25 ± 0.66^{a}	7.25 ± 0.43^{ab}	7.75 ± 0.97^{ab}	7.75 ± 0.97^{a}
WK2	8.19 ± 0.50^{a}	7.88 ± 0.78^{ab}	7.00 ± 0.71^{abc}	7.88 ± 0.33^{a}	7.50 ± 0.50^{ab}
WK3	7.50 ± 0.50^{a}	8.13 ± 0.60^{a}	7.38 ± 0.70^{a}	8.25 ± 0.66^{a}	8.13 ± 0.60^{a}
WK4	3.88 ± 1.27^{c}	7.50 ± 1.00^{ab}	6.38 ± 1.22^{bcd}	6.63 ± 0.86^{d}	6.75 ± 0.83^{bc}
WK5	5.75 ± 0.83^{b}	6.88 ± 0.78^{bc}	6.25 ± 0.97^{cd}	6.88 ± 0.78^{bc}	6.31 ± 0.66^{cd}
WK6	2.00 ± 1.66^{d}	5.38 ± 1.11^{d}	6.88 ± 0.78^{abc}	6.38 ± 1.32^{d}	5.88 ± 0.60^{d}
WK7	4.13 ± 0.78^{c}	6.88 ± 0.93^{bc}	5.88 ± 0.60^{d}	6.31 ± 1.03^{d}	6.00 ± 0.87^{cd}
WK8	3.65 ± 0.68^{c}	6.20 ± 1.10^{cd}	6.90 ± 0.91^{abc}	6.20 ± 0.79^{d}	5.70 ± 0.42^{d}

Different letters in the same column stand for significant difference ($p < 0.05$).

Table 2B
Initial eigen values and cumulative contribution rate.

Principal component	Initial eigen values	Contribution rate (%)	Cumulative contribution rate (%)
1	7.266	91.940	91.940
2	0.336	4.249	96.189
3	0.254	3.214	99.403
4	0.046	0.588	99.991
5	0.001	0.009	100.000

Table 2C
Component matrix of white kidney bean sensory attributes.

Attributes	Principal component 1	Principal component 2
Texture (X1)	2.242	−0.220
Color (X2)	0.916	−0.045
Aroma (X3)	0.272	0.380
Mouthfeel (X4)	0.763	0.224
Acceptability (X5)	0.861	0.301

suggested that texture in particular was the main sensory attribute of WKB milk.

Sensory attributes of WKB milk were standardized (S) as SX1, SX2, SX3, SX4, and SX5 and listed in Table 2D. According to PCA equation (Zhao et al., 2023), PC1 and PC2 here were therefore expressed as:

$$Y1 = 0.832SX1 + 0.340SX2 + 0.101SX3 + 0.283SX4 + 0.329SX5 \tag{1}$$

Table 3
Stability index of white kidney bean milk of different cultivars.

Cultivars	Separation Rate (%/h)	Sediment (mm)	Creaming (mm)	Instability Index
WK1	1.49 ± 0.05^{h}	2.78 ± 0.08^{f}	3.43 ± 0.13^{e}	0.05 ± 0.00^{h}
WK2	3.82 ± 0.03^{f}	3.23 ± 0.13^{e}	3.76 ± 0.11^{d}	0.13 ± 0.01^{f}
WK3	3.30 ± 0.01^{g}	2.19 ± 0.02^{g}	0.53 ± 0.01^{f}	0.11 ± 0.00^{g}
WK4	14.21 ± 0.07^{c}	5.50 ± 0.02^{a}	3.96 ± 0.08^{cd}	0.62 ± 0.02^{c}
WK5	18.58 ± 0.39^{a}	4.08 ± 0.03^{c}	4.73 ± 0.02^{a}	0.68 ± 0.00^{b}
WK6	10.55 ± 0.30^{d}	5.19 ± 0.02^{b}	4.40 ± 0.22^{b}	0.50 ± 0.02^{d}
WK7	18.04 ± 0.26^{b}	3.81 ± 0.07^{d}	3.99 ± 0.14^{cd}	0.72 ± 0.00^{a}
WK8	8.84 ± 0.06^{e}	5.27 ± 0.08^{b}	4.16 ± 0.19^{bc}	0.45 ± 0.00^{e}

Different letters in the same column stand for significant difference ($p < 0.05$).

Table 2D
Standardization of sensory attributes of white kidney bean milk. Ranking of different milk was made according to PC1 (Y1), PC2 (Y2), and comprehensive scores (Y).

Cultivars	Texture (SX1)	Color (SX2)	Aroma (SX3)	Mouthfeel (SX4)	Acceptability (SX5)	Y1	Y2	Y	Ranking
WK1	1.011	1.115	0.985	0.890	1.072	1.91	1.07	1.80	2
WK2	1.260	0.740	0.504	1.046	0.803	1.90	0.62	1.77	3
WK3	0.956	0.990	1.225	1.511	1.474	2.15	1.71	2.05	1
WK4	−0.647	0.365	−0.696	−0.506	−0.002	−0.63	−0.44	−0.60	5
WK5	0.182	−0.259	−0.937	−0.196	−0.471	−0.24	−0.98	−0.26	4
WK6	−1.477	−1.758	0.264	−0.817	−0.941	−2.33	0.07	−2.14	8
WK7	−0.537	−0.259	−1.657	−0.894	−0.807	−1.21	−1.63	−1.18	6
WK8	−0.747	−0.934	0.312	−1.034	−1.129	−1.56	−0.42	−1.45	7

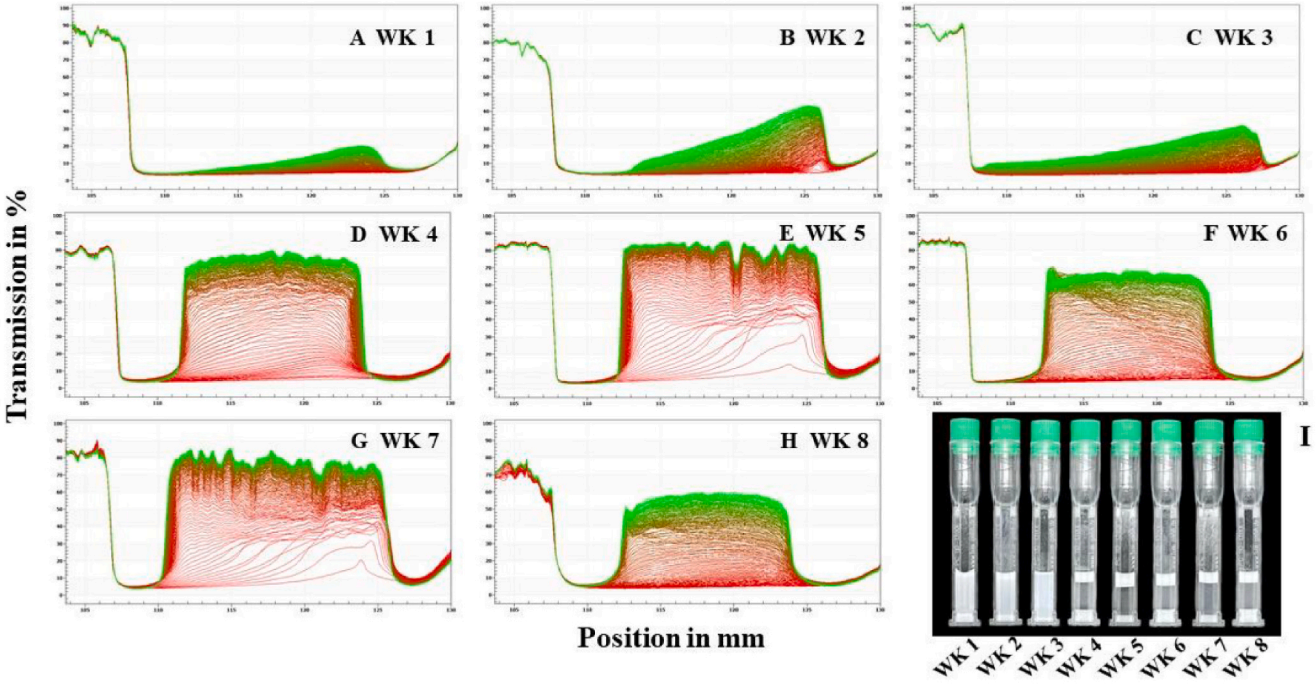

Fig. 1. Separation profiles of different white kidney bean milk with analytical centrifuge (LUMisizer) (A-H). Stability status of different white kidney bean milk after centrifugation with LUMisizer (I).

$$Y2 = -0.380SX1 - 0.078SX2 + 0.656SX3 + 0.386SX4 + 0.519SX5 \quad (2)$$

$$Y = 0.919Y1 + 0.042Y2 \quad (3)$$

The ratio of each PC contribution rate to the cumulative contribution rate of PC1 and PC2 were taken as weight to get the comprehensive eq. (3). The evaluation scores of each WKB milk were therefore calculated as Y, and WK3 got the highest score, indicating the best emulsion stability and acceptability, followed by WK1, WK2, WK5, WK4, WK7, WK8, and WK6.

3.2. Physical stability

Physical stability of WKB milk was measured with full-function stability analyzer, which allows to track milk stability in the process of centrifugation. The transmittance rate was recorded as light line from red to green during infrared light scanning, and a wider spectrum indicated less stability of the sample (Qin et al., 2023). As illustrated in Fig. 1A-H, the physical stability varied dramatically among different cultivars. The light spectrum of WK1, WK2, and WK3 were relatively narrow, and no clear separation contour was observed. In contrast, WK4, WK5, WK6, WK7, and WK8 showed a much wider spectrum, and larger variance of transmittance rate than WK1, WK2, and WK3. The observtions may imply better stability of WK1-WK3 than WK4-WK8. Consistent with the separation profiles in Fig. 1A-H, separation rate and instability index of WK1-WK3 were significantly lower than those of WK4-WK8 (Table 3). WK1 had the lowest separation rate of 1.49 %/h and the lowest instability index of 0.05, indicating that the system was relatively stable. The separation rate of WK4-WK8 were within 8.84 to 18.58 %/h, which were significantly higher than those of WK1-WK3. WK7 had the highest instability index of 0.72, indicating less stable emulsion system. In addition, the height of sediment and creaming layer of WK1 to WK3 were within 2.19 to 3.23 mm to 0.53 to 3.76 mm, respectively, which were much lower than those of WK4 to WK8 with 3.81 to 5.50 mm to 3.96 to 4.73 mm, respectively. These might lead to more clear phase separation of WK4 to WK8 than WK1 to WK3 as shown in Fig. 1I. It may imply that cultivars of WK1 to WK3 had higher potential to produce homogeneous and stabilized WKB milk.

3.3. Apparent viscosity

The apparent viscosity of WKB milk decreased as the increase of shear rate within 0 s^{-1} to 70 s^{-1} and tended to be flat after 10 s^{-1} (Fig. 2). Such shear-thinning behavior and low flow characteristics implied that all 8 WKB milk belong to pseudoelastic non-Newtonian fluids. Within 0 s^{-1} to 10 s^{-1}, WK7 showed the highest apparent viscosity, followed by WK4, WK8, WK6, WK5, WK3, WK2, and WK1. Among these samples, WK1, WK2, and WK3 with lower viscosity were more stable than those with higher viscosity (Fig. 2A). Similar tendency was obtained with WKB starch with enzymatic treatment as shown in Fig. 2B. Consistent with our observation, Dai et al. (2024) also reported that mung bean milk groups with lower viscosity and smaller particle size showed higher stability (Dai et al., 2024). Novel technologies such as ultrasound has also been developed to reduce viscosity to improve plant based milk quality (Gregersen et al., 2019). One possible mechanism for reducing viscosity might be the disruption of large-sized aggregates that induced unstable emulsion (Gregersen et al., 2019).

However, the direct correlation between viscosity and emulsion stability seems to be controversial. For example, there were researches demonstrated that relatively high viscosity could increase emulsion stability through thickening or gelling properties, and creating three-dimensional network to prevent particles migration. These were commonly observed when polysaccharides such as β-glucan, pectin, inulin, or starch were applied (Huang et al., 2021). As reported, a higher concentration of starch could improve the emulsion stability *via* increasing its viscosity (Hedayati et al., 2020), implying a different

Table 4

The content of total starch, amylose, amylopectin, and resistant starch in different white kidney bean cultivars.

Cultivars	Total Starch (%)	Amylose (%)	Amylopectin (%)	Resistant Starch (%)
WK1	40.00 ± 0.82^{b}	14.40 ± 0.31^{b}	25.60 ± 0.31^{b}	31.67 ± 1.34^{a}
WK2	33.78 ± 0.53^{e}	11.11 ± 0.44^{d}	22.67 ± 0.44^{d}	29.54 ± 1.12^{bc}
WK3	38.7 ± 0.09^{bc}	14.24 ± 0.18^{b}	24.46 ± 0.18^{c}	32.30 ± 0.65^{a}
WK4	36.85 ± 0.55^{cd}	13.07 ± 0.53^{c}	23.78 ± 0.53^{c}	28.81 ± 0.74^{c}
WK5	39.48 ± 1.24^{b}	15.12 ± 0.50^{a}	24.36 ± 0.50^{c}	32.33 ± 0.79^{a}
WK6	39.36 ± 0.36^{b}	13.83 ± 0.12^{b}	25.43 ± 0.12^{b}	31.27 ± 1.15^{ab}
WK7	43.47 ± 1.83^{a}	15.68 ± 0.09^{a}	27.79 ± 0.09^{a}	28.56 ± 0.81^{c}
WK8	35.36 ± 0.29^{de}	12.69 ± 0.09^{c}	22.67 ± 0.09^{d}	27.56 ± 0.44^{c}

Different letters in the same column stand for significant difference ($p < 0.05$).

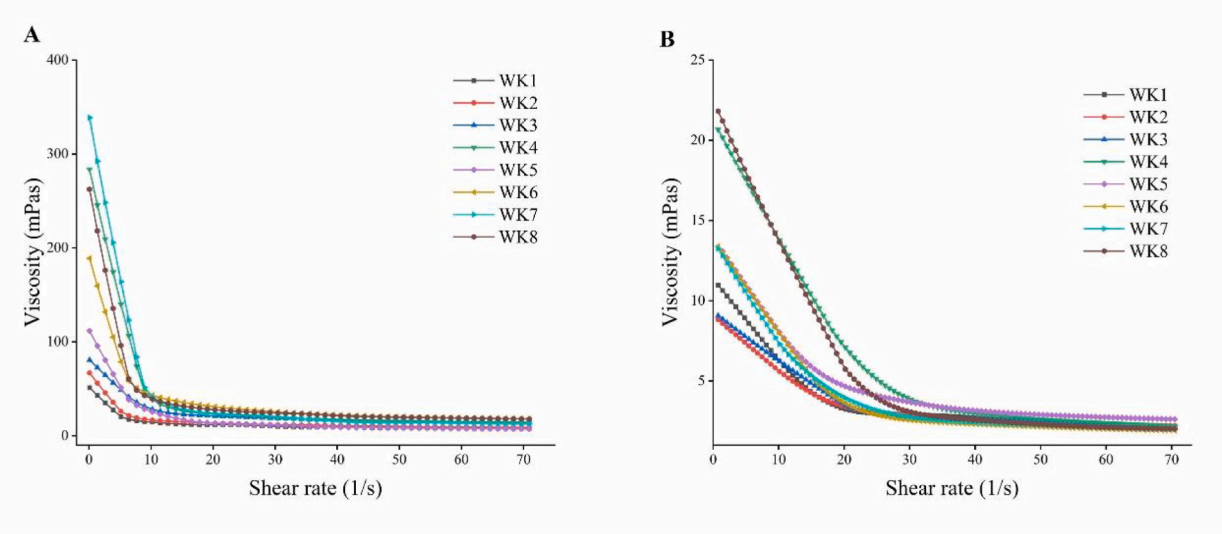

Fig. 2. Apparent viscosity of white kidney bean milk (A) and white kidney bean starch with enzymatic treatment (B).

stabilization mechanism. According to Stokes' law, both viscosity and particle size are key factors for influencing emulsion stability of non-Newtonian fluids (He et al., 2001). Here, we found that WK4-WK8 samples exhibited high apparent viscosity but relatively low emulsion stability. Starch granule, being the most abundant component in kidney bean, is also the major large-sized solid particles causing aggregation and instability of plant based milk systems (Aydar et al., 2020). It may indicate that WK4-WK8 starches were more prone to aggregate to form large size particles under high viscosity, resulting in more sediment and leading to less stable emulsion (Dai et al., 2024; Wang, Yang, et al., 2020). Additionally, in WKB milk manufacturing process, the hydrolysis of starch may breakdown the large-sized aggregates, reduce the emulsion viscosity, and eventually affect the stability (Huang et al., 2021). Therefore, we hypothesized that the content and physicochemical properties of kidney bean starch might play an important role in the stability of the final product.

3.4. Starches content in WKB

First, the content of total starch, amylose, amylopectin, and resistant starch were quantified as listed in Table 4. The total starch content of different cultivars was in the range of 33.78 %-43.47 %, which was consistent with the range (25 %-45 %) reported by Aydar et al. (2020). Although there were significant differences of total starch content among 8 cultivars, no clear difference was observed between the more stable samples of WK1-WK3 and the less stable samples of WK4-WK8. Amylose and amylopectin content act as one important factor for emulsification and stabilization properties of starches, and high amylose content could increase viscosity of acidified milk gels (Mu et al., 2021). Here, WK4-WK8 contained a slightly higher amylose concentration range of 12.69 %-15.68 % than WK1-WK3 of 11.11 %-14.40 %, which might contribute to the higher viscosity of WK4-WK8 than WK1-WK3. Like regular starches, the content of amylopectin of all WKB samples was higher than amylose. Like wise, the content range of resistant starch of WK1-WK3 remained similar to WK4-WK8. The observations may indicate that the content of starches in WKB was not the main factor for influencing the stability of WKB milk. Therefore, physicochemical properties of WKB starch were investigated as follow.

Table 5
Gelatinization properties of white kidney bean were analyzed by Rapid Visco Analyzer (RVA).

Cultivars	PT (°C)	PV (cP)	TV (cP)	FV (cP)	BD (cP)	SB (cP)
WK1	84.09 ± 0.03^{a}	1284 ± 39.19^{ef}	1220 ± 5.66^{e}	1880 ± 24.66^{e}	68 ± 29.93^{b}	664 ± 20.40^{d}
WK2	84.08 ± 0.01^{a}	1308 ± 42.71^{e}	1160 ± 20.40^{ef}	1876 ± 57.41^{e}	140 ± 20.40^{a}	708 ± 35.33^{cd}
WK3	84.17 ± 0.04^{a}	1156 ± 22.63^{f}	1092 ± 25.92^{f}	1788 ± 29.39^{e}	60 ± 9.80^{b}	696 ± 9.80^{cd}
WK4	82.2 ± 0.08^{d}	1740 ± 29.39^{bc}	1724 ± 14.97^{b}	2672 ± 28.28^{b}	16 ± 22.63^{bc}	948 ± 29.39^{b}
WK5	82.48 ± 0.10^{cd}	1456 ± 14.97^{d}	1432 ± 5.66^{d}	2352 ± 33.94^{d}	20 ± 11.31^{bc}	920 ± 31.50^{b}
WK6	82.87 ± 0.62^{c}	1620 ± 68.59^{c}	1600 ± 85.60^{c}	2368 ± 152.21^{cd}	20 ± 24.66^{bc}	772 ± 91.04^{c}
WK7	83.38 ± 0.04^{b}	1824 ± 157.68^{ab}	1824 ± 122.38^{ab}	2548 ± 165.22^{bc}	0 ± 42.71^{c}	728 ± 50.28^{cd}
WK8	80.17 ± 0.13^{e}	1924 ± 11.31^{a}	1872 ± 9.80^{a}	2968 ± 39.60^{a}	48 ± 19.60^{bc}	1096 ± 31.50^{a}

Different letters in the same column stand for significant difference ($p < 0.05$). PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BD, breakdown; SB, setback.

3.5. Gelatinization properties of WKB starch

Gelatinization properties of WKB starch were shown in Table 5. Pasting temperature (PT) of starch gives information of the minimum temperature should reach to be cooked. PT value of the more stable samples of WK1-WK3 was ranged within 84.08–84.17 °C, which were significantly higher than WK4-WK8 with PT value in the range of 80.17–83.38 °C ($p < 0.05$). The higher PT of WK1-WK3 may indicate that WK1-WK3 had a more ordered crystalline structure and were more resistant to swelling and rupturing (Kaur et al., 2004).

In general, swelling is one important functional property of starch, which refers to the process of starch to absorb water and increase the starch emulsion viscosity, thus to modify the texture quality and nutritional value of starch-based food (Vamadevan & Bertoft, 2020). Peak viscosity (PV) corresponds to the degree of swelling of starch granules during gelatinization process. Here, PV values of the more stable WK1-WK3 were significantly lower ($p < 0.05$) than those of the less stable WK4-WK8 samples. The results may suggest that WK4-WK8 were more prone to swelling due to higher water absorption and more starch dissolution, which would lead to the formation of more precipitates, and showed adverse effect on emulsion stability (Jia et al., 2023). Although PV here did not show any significant correlation with swelling property of WKB starch ($p > 0.05$) (Fig. 5), it did show significant positive correlation with emulsion sediment and instability index ($p < 0.05$), and negative correlation with texture ($p < 0.05$), emulsion mouthfeel and acceptability ($p < 0.01$). Similar results were observed in trough viscosity (TV) and final viscosity (FV) values that stable WK1-WK3 were also significantly less than WK4-WK8 ($p < 0.05$). TV and FV also showed positive correlation with emulsion sediment and instability index ($p < 0.05$), and negative correlation with texture ($p < 0.05$), emulsion mouthfeel and acceptability ($p < 0.01$). The observations corresponded to the results of apparent viscosity of WKB based milk (Fig. 2), *i.e.,* the lower viscosity, the more stability of the emulsion. Cultivars with less viscosity inhibit large particles flocculation, reduce precipitates, which would contribute to a more evenly distributed system and enhance the stability (Wang et al., 2022).

Although WK1-WK3 showed higher breakdown (BD) value with a range of 60–140 cP than WK4-WK8 with 0–48 cP, significance was mainly observed between WK1 and all the other samples ($p < 0.05$). BD indicates the stability of WKB starch during heating process, and the highest BD of WK1 may suggest the least granule integrity (Luo et al., 2017). BD was negatively correlated with separation rate and instability index ($p < 0.05$) while positively correlated with texture ($p < 0.05$) (Fig. 5). Higher BD value may contribute to reduce emulsion separation rate and instability index, while improve the texture of WKB emulsion. As counterpart to BD, setback (SB) reflects the starch retrogradation degree during cooling after heating process, especially degree of amylose recrystallization and rearrangement. The SB value showed a significant positive correlation with the emulsion precipitates (Fig. 5). Lower SB indicates less retrogradation chance and less amylose rearrangement level (Pan et al., 2019). Here, SB values of WK1-WK3 were significantly lower than WK4-WK8 ($p < 0.05$), implying WK4-WK8 were more prone to retrogradation (Pan et al., 2019). It may suggest that WK4-WK8 had stronger gelling property and the starch granules were more easily clustered, and would be detrimental to the stability of WKB milk system.

3.6. Solubility and swelling properties of WKB with enzyme hydrolysis

Solubility index reflects starch leaching capacity during gelatinization (Kumar et al., 2018). Solubility index of native WKB starch was in the range of 9.98 %–13.80 % (Fig. 3). Although no clear difference was observed between the more stable samples WK1-WK3 and the less stable

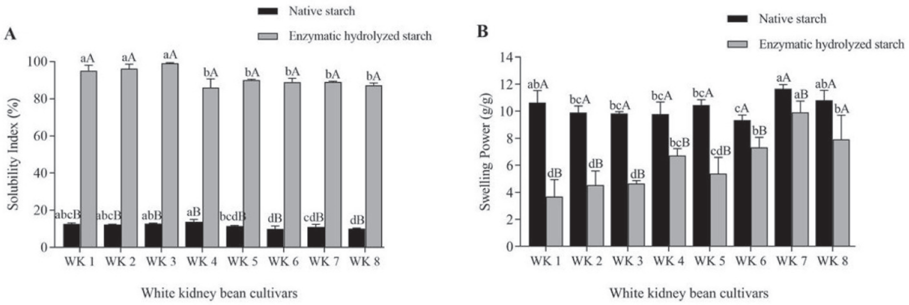

Fig. 3. Solubility index (A) and swelling power (B) properties of native and enzymatic hydrolyzed starch of different white kidney bean cultivars. Different lowercase letters stand for significant differences among different cultivars of the same treatment, *i.e.* native or enzymatic hydrolyzed starch ($p < 0.05$). Different uppercase letters stand for significant differences between different treatments of native and enzymatic hydrolyzed for the same cultivar ($p < 0.05$).

WK4-WK8, solubility index of WK1-WK3 was significantly higher than WK6 and WK8 ($p < 0.05$). As expected, under enzymatic hydrolysis, starch solubility of all cultivars were dramatically improved to 86.05 %–99.13 % ($p < 0.05$). Solubility index of enzymatic hydrolyzed WKB starch of WK1-WK3 was significantly higher than WK4-WK8 ($p < 0.05$). According to correlation analysis (Fig. 5), both native starch and hydrolyzed starch were positively correlated with texture, mouthfeel, and acceptability of WKB milk, while negatively correlated with sediment, PV, TV and FV ($p < 0.05$). It could be explained that strategies to improve the solubility of starch could reduce viscosity and enhance emulsion stability (Mäkinen et al., 2015). This might also be attributed to the reduced molecular weight of starch by enzymatic hydrolysis, along with increased emulsifying ability.

Swelling power of native WKB starch was between 9.34 and 11.66 g/g, and no significant difference was observed between WK1-WK3 and WK4-WK8. However, with enzymatic hydrolysis, swelling power of all starches were significantly reduced to a range of 3.68–9.92 g/g (Fig. 3). As reported, enzymatic process itself could inhibit starch gelatinization, thus to reduce emulsion viscosity caused by starch swelling (Sethi et al., 2016). Interestingly, hydrolyzed starch swelling of stable samples WK1-WK3 were significantly lower than the less stable WK4-WK8. Such high swelling of WK4-WK8 implied more water absorption, and much higher volumetric concentration, which may indicate more particles sediment of WK4-WK8 starch granules (Feng et al., 2024). Accordingly, swelling power of hydrolyzed starch showed negative correlation with emulsion texture, mouthfeel and acceptability (Fig. 5). It can be explained by the higher starch viscosity as a result of stronger swelling of WK4-WK8 (Fig. 2B, starch viscosity result after Enzyme), and showed adverse impact on emulsion stability.

Table 6
Short-range order of white kidney bean starches before and after enzymatic hydrolysis.

Cultivars	Native starch		Enzymatic hydrolyzed starch	
	995/1022 cm^{-1}	1047/1022 cm^{-1}	995/1022 cm^{-1}	1047/1022 cm^{-1}
WK1	4.97 ± 0.05^{bA}	2.40 ± 0.02^{abA}	0.69 ± 0.02^{cB}	0.86 ± 0.01^{dB}
WK2	5.39 ± 0.20^{aA}	2.53 ± 0.01^{aA}	0.84 ± 0.09^{bcB}	0.90 ± 0.11^{dB}
WK3	5.09 ± 0.08^{bA}	2.40 ± 0.15^{abA}	0.95 ± 0.07^{abcB}	0.82 ± 0.09^{dB}
WK4	4.55 ± 0.04^{cA}	2.20 ± 0.02^{cA}	1.27 ± 0.17^{aB}	1.20 ± 0.03^{abcB}
WK5	4.57 ± 0.00^{cA}	2.34 ± 0.05^{bcA}	1.18 ± 0.13^{aB}	0.93 ± 0.05^{cdB}
WK6	4.43 ± 0.02^{cA}	2.27 ± 0.04^{bcA}	1.23 ± 0.28^{aB}	1.41 ± 0.31^{aB}
WK7	4.02 ± 0.13^{dA}	2.29 ± 0.01^{bcA}	1.11 ± 0.10^{abB}	1.23 ± 0.04^{abB}
WK8	4.44 ± 0.08^{cA}	2.20 ± 0.05^{cA}	0.83 ± 0.04^{bcB}	0.96 ± 0.01^{bcdB}

Different lowercase letters in the same column stand for significant difference among different white kidney bean cultivars ($p < 0.05$). Different uppercase letters stand for significant difference between native sand enzymatic hydrolyzed starch ($p < 0.05$).

3.7. *Short-range order of WKB starches after enzymatic hydrolysis*

To gain insight into short-range structure of WKB starches, FTIR was conducted before and after enzymatic treatment. Peaks of WKB starches appeared at wavelengths of 995, 1022, and 1047 cm^{-1} after being deconvoluted in the range of 800 and 1200 cm^{-1} (Xu et al., 2019). The ratio of bands intensity at 995/1022 cm^{-1} expressed molecular short-range double helix in starch crystallization region. And value of 1047/1022 cm^{-1} reflected the ratio of ordered crystalline region and amorphous region (Wang et al., 2017). As shown in Table 6, native starch of WK1-WK3 showed significantly higher 995/1022 cm^{-1} value than WK4-WK8, and higher 1047/1022 cm^{-1} than WK4 and WK8 ($p < 0.05$). It suggested that native starch granules of WK1-WK3 had higher degree of short range order and stronger helix in the crystallization region than WK4-WK8, and this might cause higher gelatinization temperature of WK1-WK3 (Feng et al., 2024).

With enzymatic hydrolysis, both 995/1022 cm^{-1} and 1047/1022 cm^{-1} were dramatically decreased ($p < 0.05$), indicating starch crystallinity reduction and helix structure unfolding due to starch granule disintegration. In this process, more amylose and highly branched amylopectin might be dissolved out and thereby to increase the starch solubility and decrease its viscosity (Cornejo-Ramírez et al., 2018). Here, WK1-WK3 showed even lower 995/1022 cm^{-1} and 1047/1022 cm^{-1} value than WK4-WK8. Accordingly, 995/1022 cm^{-1} of enzymatic hydrolyzed starch was positively correlated with emulsion separation rate, and instability index, while 1047/1022 cm^{-1} was negatively correlated with emulsion texture, mouthfeel, and acceptability. These may suggest that, compared to WK4-WK8, starches of the more stable cultivars, *i.e.* WK1-WK3 were more prone to enzyme hydrolysis, resulting more damage of crystalline region, and stronger helix unfolding. The weakened molecular interaction gave more access to water, and hydrolyzed starch molecules were more evenly dispersed and dissolved, thus to improve the stability of emulsion (Lee et al., 2008).

3.8. *Micro-structure of WKB starches after enzyme hydrolysis*

To better understand the association between WKB emulsion stability and starch micro-structures, SEM was applied to visualize microstructure difference of native WKB starch and after enzymatic hydrolysis. The more stable cultivars of WK1 and WK2 and less stable cultivars of WK5 and WK8 were selected as shown in Fig. 4. Native WKB starches of all cultivars showed elliptical or round particles with integral and smooth surface (Fig. A, C, E, G). After enzyme treatment, the left over starch fraction of WK1 and WK2 kept relatively smooth surface although enzyme treatment could dig holes around starch particles (Dura et al.,

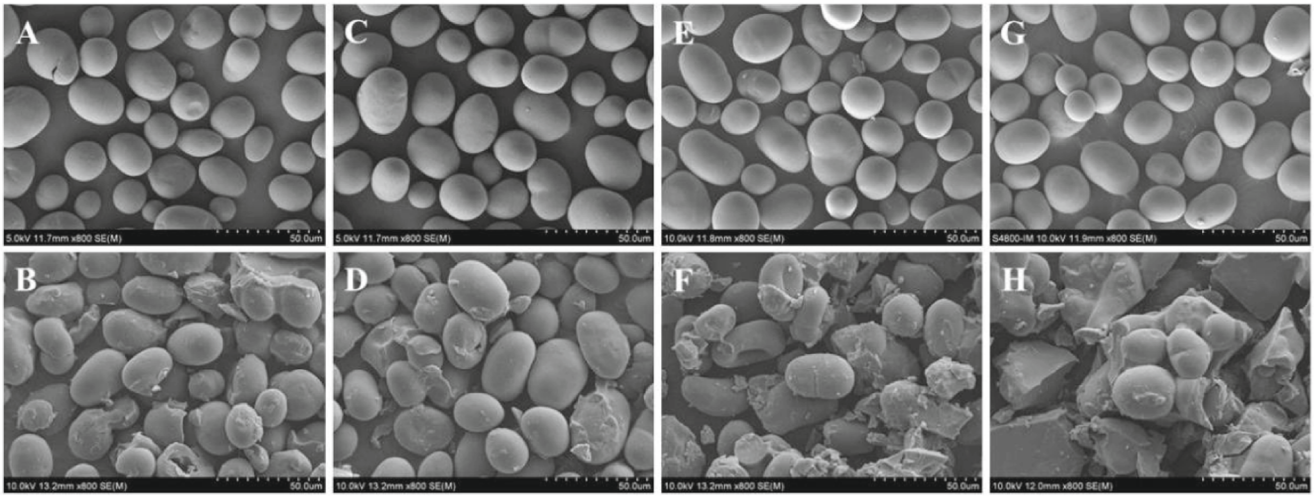

Fig. 4. Scanning electron micrographs (SEM, 800 × magnification) of native and enzyme hydrolyzed white kidney bean starches. Native white kidney bean starches of WK1 (A), WK2 (C), WK5 (E), and WK8 (G). Enzymatic hydrolyzed white kidney bean starches of WK1 (B), WK2 (D), WK5 (F), and WK8 (H).

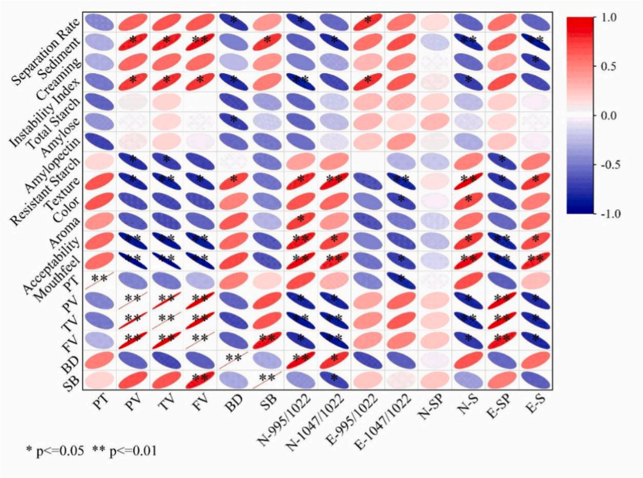

Fig. 5. Correlation analysis between different indicators. PT, pasting temperature; PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BD, breakdown; SB, setback. N-SP, swelling power of native starch; N-S, solubility index of native starch; *E*-SP, swelling power of enzymatic hydrolyzed starch; E-S, solubility index of enzymatic hydrolyzed starch.

2014). However, molecular adhesions were observed in WK5 and WK8, which might be due to more resistance to enzyme hydrolysis of the less stable WKB starches. The partially hydrolyzed starches aggregated as large particles, which raised heterogeneous plant emulsion.

4. Conclusion

To conclude, here we show that starch properties rather than the content determine the stability of white kidney bean milk. In our analysis, higher pasting temperature and less viscosity of native WKB starch contributed to lower separation rate and instability index of WK1, WK2, and WK3. Meanwhile, with enzyme treatment, WK1, WK2, and WK3 starches showed higher solubility index, contributing to more evenly dispersed and homogeneous emulsion. Whereas WK4, WK5, WK6, WK7, and WK8 starches were proved to be more resistant to enzymatic hydrolysis as indicated by higher swelling degree, and the resulting large granules tended to form precipitates. Molecular structure change with enzymatic hydrolysis further proved that starches of stable cultivars were more prone to crystalline damage, with more access to water, and more evenly dispersed to improve the stability. Microscopy observation and correlation analysis further supported above statements. These findings provide guidance for selecting raw materials for WKB milk production, and offer information for future seed breeding for white kidney bean milk procssing. Subsequent investigations could expand to explore the interactions between starch with other macromlecules such as proteins and dietary fibers by constructing model system, thereby to further improve the stability of white kidney bean milk.

Ethical statement

The study approved by the Institutional Review Board (or Ethics Committee) of China Agricultural University (protocol code CAUHR-2019006 and 29 January 2019), and was conducted in accordance with the Declaration of Helsinki. All participants read the study instructions carefully and all signed the informed consent form.

CRediT authorship contribution statement

Chunli Kong: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Yixuan Zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Yimei Hu:** Validation, Software, Methodology. **Caiping Duan:** Visualization, Software, Methodology. **Zheng Yan:** Writing – review & editing, Supervision, Resources, Project administration. **Sumei Zhou:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

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Data availability

Data will be made available on request.

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