Journal of Oleo Science
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doi : 10.5650/jos.ess23123
J. Oleo Sci. **73**, *(2) 147-161 (2024)*

Evaluation of Quality Properties of Brown Tigernut (*Cyperus esculentus* L.) Tubers from Six Major Growing Regions of China: A New Source of Vegetable Oil and Starch

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Abstract: Tigernut has been recognized as a promising resource for edible oil and starch. However, the research on the quality characteristics of tigernut from different regions is lagging behind, which limits the application of tigernut in food industry. Tigernut tubers were obtained from six major growing regions in China, and the physicochemical properties of their main components, oil and starch, were characterized. Tigernut tubers from Baoshan contained the most oil (30.12%), which contained the most β-carotene (130.4 μg/100 g oil) due to high average annual temperature. Gas chromatography analysis and fingerprint analysis results indicated that tigernut oil (TNO) consists of seven fatty acids, of which oleic acid is the major component. Changchun TNO contained the least total tocopherols (6.04 mg/100 g oil) due to low average annual temperature. Tigernut tubers from Chifeng (CF) contained the most starch (34.85%) due to the large diurnal temperature range. Xingtai starch contained the most amylose (28.4%). Shijiazhuang starch showed the highest crystallinity (19.5%). Anyang starch had the highest pasting temperature (76.0°C). CF starch demonstrated superior freeze-thaw stability (syneresis: 50%) due to low mean annual precipitation. The results could be further applied to support tigernut industries and relevant researchers that looks for geographical origin discrimination and improvements on tigernut quality, with unique physicochemical and technological properties.

Key words: tigernut, oil, starch, physicochemical properties, climatic environment

1 Introduction

Tigernut (*Cyperus esculentus* L.) is grown for its tubers, which accumulate large amounts of oil[1]. It is widely planted in temperate, subtropical, and tropical regions such as China, France, and the Congo[2]. Tigernut was introduced to China in 1950, and now is cultivated in the provinces of Jilin, Hebei, Henan, and Yunnan for sustainable utilization of wasteland and mudflats[3].

Tigernut oil (TNO), is approximately 28% by dry weight of tigernut tubers. It is comparable to olive oil and walnut oil in containing a particularly high level of monounsaturated fatty acids (>65%)[4]. TNO mainly consists of five fatty acids: palmitic, oleic, linoleic, stearic and *n*-heneicosanoic acid[5]. Tigernut starch (TS) is approximately 30% by dry weight of the tubers, which is higher than traditional oil crops (e.g., soya, peanuts)[1, 6]. Compared to potato and maize starch, TS has equivalent flow properties but stronger compatibility, better integration, and superior freeze-thaw stability[7]. In addition, tigernut tubers have abundant beneficial trace components, such as tocopherols (15-30 mg/100 g), β-carotene (50-160 μg/100 g) and phytosterol (300-360 mg/100 g)[2]. Thus, oil and starch extracted from tigernut have potential applications.

There are mainly three varieties of tigernut being grown, which are black, brown and yellow[5]. Varietal differences have a strong influence on the quality of tigernut. The brown tigernuts have been reported to contain the most oil (35.43%), ash (4.25%) and crude protein (9.70%)[8]. Brown TNO contains the most stearic acid (6.26%), *n*-heneicosanoic acid (0.51%) and total unsaturated fatty acids

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Accepted November 14, 2023 (received for review July 7, 2023)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

(82.05%)[5]. Black TNO contains the most palmitic acid (16.70%), oleic acid (77.71%) and total saturated fatty acid (22.78%). Yellow TNO contains the most linoleic acid (12.77%)[8]. Higher pasting properties (peak viscosity: 879.3 Brabender Units (BU), setback viscosity: 363.2 BU) were found in black TS, while yellow TS exhibits greater adhesion, cohesion, excellent freeze-thaw stability, and enhanced breakdown viscosity (556.3 BU)[9]. These studies on the differences in the composition of tigernuts have compared different tigernut varieties. At present, different growing regions of brown variety, the main tigernut variety in China, exhibit a wide span of climatic environments, which leads to differences in the quality characteristics of batches from different regions being the strongest influencing factor limiting the industrial utilization of tigernut[10]. However, there is inadequate information on the differences in the compositions of the same tigernut variety grown in different regions.

In this study, the physicochemical characteristics of brown tigernuts grown in six major growing regions in China were analyzed. Oil and starch were isolated and characterized. It is hoped that specific information about regional differences will promote efficient utilization of tigernut in the food and other industries.

2 Materials and Methods

2.1 Selection of brown tigernuts

Brown tigernuts were selected and collected from major growing regions of Anyang (AY), Baoshan (BS), Changchun (CC), Xingtai (XT), Chifeng (CF) and Shijiazhuang (SJZ) in China. The specific geographical parameters (e.g., longitude and latitude, average annual temperature and mean annual precipitation) of six regions are shown in Table 1. All tigernut tubers were washed to remove any adhering dirt and dried in an electric thermostaticdrying oven (DHG-9146A, Shanghai Jing Hong Laboratory Instrument Co., China) set at 40℃ for 12 h. The reagents used in this study were all analytically pure in accordance with international standards.

2.2 Extraction of TNO

Tigernut tubers were firstly crushed and sieved (40 meshes) to yield a medium-sized flour. Then, the flour was mixed with *n*-hexane at a ratio of 1:5 (m/v) with continuous stirring at 350 rpm and 25℃ for 48 h, followed by filtration with a Büchner flask. The residue was defatted tigernut flour. The filtrate was then centrifuged (3500 rpm, 5 min) at 25℃. The supernatant was rotary evaporated (R-1001VN, Zhengzhou, China) at 40℃ to remove hexane, leaving the oil. The obtained TNO was kept at 4℃ until analyses.

2.3 Isolation of TS

The defatted tigernut flour was then mixed with 0.4% NaOH water solution in a proportion of 1:5 (m/v) to remove the protein. The sediment was washed with deionized water, and the aqueous leachate was centrifuged at 4000 rpm for 10 min. After centrifugation, the protein-rich layer located at the top of the precipitate was recovered by manual scraping[11]. The above operation was repeated 3-5 times until the starch coating was free from visible impurities. The precipitate was washed by immersing in an aqueous solution of ethanol (70%, w/v) to remove the low molecular weight non-starch fraction. The precipitate was de-watered at 35℃ for 24 h before analysis.

2.4 Macroscopic physical properties of tigernut tubers

The shapes of 100 tubers from each region were recorded, and the length and diameter were measured using vernier calipers. The thousand-grain weight (the weight of 1000 tubers expressed in grams) and bulk weight (the weight of tubers per 1 dm^3) of tigernut tubers were determined[12].

2.5 Content of the main components

Before analysis, tubers were ground in a laboratory grinder, and passed through a 40-mesh sieve. Crude fat content was measured by the Soxhlet extractor method as documented in ISO 659.2009. Moisture and crude protein levels were determined using AOAC (2000) protocols 930.15 and 990.03, respectively. Total starch content was measured following the enzymatic method documented by

Table 1 The growing areas, conditions of six different tigernut tubers.

Origin	Longitude and latitude	AAT (℃)	DTR (℃)	MAP (mm)	Planting date	Harvesting date
Anyang	114° 35′ 24.82″ E, 36° 10′ 34.42″ N	11-21	9.6	849.7	April 2021	August 2021
Baoshan	99° 16′ 71.33″ E, 25° 11′ 18.02″ N	13-24	8.8	568.5	April 2021	August 2021
Changchun	125° 32′ 45″ E, 43° 88′ 68.41″ N	2-12	8.6	860.3	May 2021	September 2021
Xingtai	114° 50′ 88.53″ E, 37° 06′ 82″ N	10-20	9.0	751.6	April 2021	August 2021
Chifeng	118° 52′ 58.14″ E, 42° 15′ 23.50″ N	2-14	10.2	512.6	June 2021	October 2021
Shijiazhuang	114° 50′ 24.61″ E, 38° 04′ 54.74″ N	8-20	9.4	787.4	April 2021	August 2021

AAT (average annual temperature); DTR (diurnal temperature range); MAP (mean annual precipitation).

Liu *et al.*[13]. Total carbohydrates were determined by the phenol-sulfuric acid method[14].

2.6 Characterization of oil

2.6.1 Physicochemical properties

Colour of the TNO samples was analyzed following the protocol documented by Sunil *et al.*[15]. Acid value, peroxide value, iodine value, and saponification value were determined using methods specified by the International Organization for Standardization (ISO)[6].

2.6.2 Nutritional composition

2.6.2.1 Fatty acids

The fatty acid composition was measured by gas chromatography following the protocol by Guo *et al.*[6] using methylation. The qualitative and quantitative analysis of fatty acids was based on standard curves established using standard chemicals.

2.6.2.2 β-carotene

β-carotene levels were determined following the protocol by Naebi *et al.*[16]. The oil samples were diluted in *n*-hexane, and the absorbance was acquired at 450 nm on a spectrophotometer UV-vis (UV-6000PC, Shanghai, China).

2.6.2.3 Tocopherols

Tocopherols were quantified on a high-performance liquid chromatograph (puriFlash® XS 420, Montlucon, France) equipped with an XBridge® BEH Amide 5 μm column according to the method of Dąbrowski *et al.*[17]. For analysis, a certain amount of oil was weighed and dispersed into chromatographic-grade hexane. For the analytical procedure, *n*-hexane and isopropanol were mixed at 99:1 (v/v) to prepare the mobile phase, and the excitation and detection wavelengths were 298 and 325 nm, respectively.

2.6.2.4 Chlorophyll

The chlorophyll level was obtained by spectrophotometer UV-vis at 670 nm. A sample of oil was weighed, diluted to a 30% solution with cyclohexane, then tested[18]. The specific calculation formula is provided below:

$$\text{Chlorophyll (mg/kg)} = \frac{\text{Abs}_{670} \times 10^6}{613 \times 100 \times \text{D}}$$

Where Abs_{670} denotes absorbance at 670 nm; 613 denotes the coefficient of specific extinction in cyclohexane; D denotes the spectrophotometer cell thickness (1 cm).

2.6.3 Oxidative stability

The anti-oxidation capacity of TNOs was studied following the protocol documented by Barbosa *et al.*[19]. A volume of the oil sample was weighed and then measured using a 743 Rancimat apparatus (Herisau, Switzerland).

2.7 Characterization of TS

2.7.1 Physicochemical properties

2.7.1.1 Scanning electron microscopy (SEM)

Samples were mounted on stubs, coated with gold, then observed using a ZEISS SEM (SIGMA 500, Germany) with a low voltage of 3.0 kV to avoid sample damage from the electron beam.

2.7.1.2 Particle size distribution

Particle size distribution was measured by a laser particle size analyzer (Malvern Mastersizer 3000, Malvern, UK). The starch samples were diluted with deionized water to achieve 80% light transmission before analysis.

2.7.1.3 Amylose

The amylose content of TS was estimated using a starch assay kit (K-AMYL), following the protocol documented by Liu *et al.*[13].

2.7.2 Structural properties

2.7.2.1 Fourier transform infrared spectroscopy (FT-IR)

The short-range ordered structures of starch were measured following the protocol documented by Bai *et al.*[20]. All samples were scanned using an infrared spectrometer (NEXUS 470, Nicolet Corporation, USA) against a background of KBr slices in the range of 4000 to 400 cm^{-1}.

2.7.2.2 X-ray diffraction (X-RD)

A method documented by Wang *et al.*[21]. was used to obtain X-ray diffractograms (X 'Pert Pro MPD, Nalytical, Netherlands) of starch. The operating conditions were set to a scanning speed of 1°/min and a scanning area of 5 to 40° (2θ).

2.7.2.3 ^{13}C NMR

A pre-adjusted moisture sample of starch was measured by a Bruker AVANCE NEO NMR spectrometer to obtain a spectrum. The double helix, single helix, and amorphous regions of the samples were quantified using PeakFit software[22].

2.7.3 Functional characteristics

2.7.3.1 Pasting properties

The pasting behavior of the six starches was studied with the Rapid Viscosity Analyzer (RVA, Perten 4500, Hagersten, Sweden). Each starch sample (3.0 g) was mixed in an aluminum can containing 25 mL of distilled water. The slurry was pasted according to the heating procedure recorded by Du *et al.*[23].

2.7.3.2 Thermal properties

The thermal characteristics of the starch samples were analyzed by reference to the protocol documented by Du *et al.*[23]. The thermal characteristics (i.e., melting enthalpy (ΔH), conclusion temperatures (T_c), peak temperatures (T_p), and onset temperatures (T_o) of the samples were obtained by preparing an aqueous starch solution in a container at 0.33 mg/μL and subjecting it to a heating procedure in the range of 25-100℃.

2.7.3.3 Freeze-thaw stability

The determination of freeze-thaw stability was based on the method of Phogat *et al.*[24]. The dehydration ratio is the ratio of the water separated in the centrifuge tube to the weight of the original gel. Briefly, the aqueous starch solution (6%, w/w) was heated (95℃, 30 min) in a water bath for pasting. After cooling to room temperature, the

samples were stored at 4℃ for 16 h and then frozen at −24℃ for 22 h. The samples were left to stand at 25℃ for 2 h. The water separated from the starch paste by centrifugation (3000 rpm, 15 min) was weighed. The freeze-thaw cycle was repeated 5 times at −24℃.

2.8 Statistical analysis

All results were reported as means ± SD (standard deviation). All reported values were tested for discrepancy and variance using SPSS 20.0 (SPSS Inc. Chicago, USA). A post-hoc comparison of means was also carried out using the Duncan test ($p < 0.05$). All analyses were performed in triplicate.

3 Results and Discussion

3.1 Physical properties of tigernut tubers

As seen in Fig. 1, the overall color of the six tigernut tubers was brown, varying slightly from yellowish brown to grayish brown. There were four types of shapes: round, irregularly round, oval, and elongated. The tubers had a wrinkled epidermis. Table 2 presents the physical properties, in terms of tuber length, diameter, thousand-grain weight, and bulk weight of six tigernut tuber samples. AY, BS, CC, XT, CF and SJZ tubers had lengths in the range of 1.1-1.3 cm. The length of the BS and SJZ tubers was significantly longer, at 1.3 cm. The diameter of AY, BS, CC, XT, CF, and SJZ tubers was in the range of 1.2-1.8 cm. A considerable difference was found in the thousand grain weight of tigernut tubers from the six regions, with the following order: SJZ (773.5 g) > AY (523.9 g) > BS (438.0 g) > CF (428.5 g) > XT (356.1 g) > CC (323.9 g). In addition, there were remarkable differences in the bulk weight of tigernut tubers; they varied in the following order: BS (725.0 g/L) > SJZ (667.5 g/L) > AY (604.4 g/L) > CF (578.6 g/L) > XT (568.2 g/L) > CC (494.3 g/L). These differences may be caused by different climatic conditions (e.g., average annual temperature and mean annual precipitation).

3.2 Contents of the main components

The main components (e.g., moisture, protein, oil, starch) in tigernut tubers were statistically analyzed ($p < 0.05$), and the results are presented in Fig. 2. The moisture content ranged from 4.57% to 10.06%. All samples contained less than 14% moisture, which favors long term storage and will greatly reduce the potential risk of food safety problems caused by mould and other microorgan-

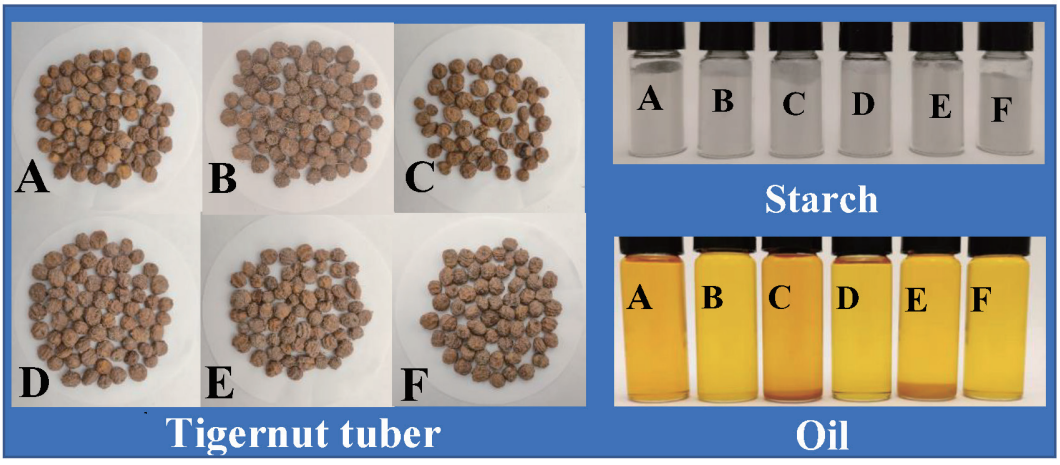


Fig. 1 Photos of whole tigernut tubers, extracted oils and starches.
A-F in order from Anyang (AY), Baoshan (BS), Changchun (CC), Xingtai (XT), Chifeng (CF), and Shijiazhuang (SJZ) of tigernut tubers, oil and starch samples.

Table 2 Physical properties of tigernut tubers from six regions.

Sample	AY	BS	CC	XT	CF	SJZ
Length (cm)	1.1 ± 0.1^{a}	1.3 ± 0.1^{a}	1.2 ± 0.0^{a}	1.1 ± 0.0^{a}	1.1 ± 0.1^{a}	1.3 ± 0.0^{a}
Diameter (cm)	1.2 ± 0.1^{bc}	1.8 ± 0.0^{a}	1.3 ± 0.0^{b}	1.3 ± 0.1^{b}	1.8 ± 0.2^{a}	1.5 ± 0.2^{ab}
Thousand grain weight (g)	523.9 ± 2.6^{b}	438.0 ± 1.6^{c}	323.9 ± 2.9^{f}	356.1 ± 0.8^{e}	428.5 ± 1.5^{d}	773.5 ± 1.2^{a}
Bulk weight (g/L)	604.4 ± 1.0^{c}	725.0 ± 0.4^{a}	494.3 ± 0.7^{f}	568.2 ± 1.6^{e}	578.6 ± 1.2^{d}	667.5 ± 1.9^{b}

AY (tigernut tubers from Anyang); BS (tigernut tubers from Baoshan); CC (tigernut tubers from Changchun); XT (tigernut tubers from Xingtai); CF (tigernut tubers from Chifeng); SJZ (tigernut tubers from Shijiazhuang). Mean ± standard deviation, different superscript letters indicate statistically significant differences in the same row of data ($p < 0.05$).

isms. XT tubers contained the most moisture (10.06%), while BS tubers contained the lowest value (4.57%). This can be explained by the different contents of soluble sugars due to their hydrophilic properties, which affect the water holding capacity of the tigernut tuber matrix during the drying procedure[25]. Oil contents in the six samples differed significantly ($p<0.05$) within the range of 19.06%-30.12%. BS tubers contained the highest (30.12%), while CC tubers contained the lowest (19.06%). There is a significant positive correlation between oil content and average annual temperature. We speculate that higher temperatures will promote oil accumulation[26]. The crude protein content of the six tubers ranged from 4.47% to 6.54%. There is a significant negative correlation between protein content and diurnal temperature range. This may be because diurnal temperature range inhibit an increase in protein content[27].

The total content of starch in the six tubers ranged from 24.42% to 34.85%. This is generally consistent with the content (26%-30%) reported by Liu *et al.*[13]. There was a significant negative correlation between starch content and average annual maximum and average annual minimum temperature. The total starch content in CF tubers were 34.85%, which was higher than the other five regions. This can be explained by high average annual temperature that inhibits carbohydrate accumulation during the growing season[28]. From the principal component analysis, it can be concluded that the most abundant components in all tigernut tuber samples were starch, oil, non-starch carbohydrates (sucrose, glucose and fructose), moisture and protein (Fig. 2B).

3.3 Physicochemical properties of TNOs

The physicochemical properties like the acid value (AV), peroxide value (PV), saponification value (SV), iodine value (IV) and chromatic value (CV) are commonly used to measure the quality and stability of edible oils. Those physicochemical properties show differences due to climatic conditions (e.g., average annual temperature and mean annual precipitation)[29]. As shown in Table 3, the red and

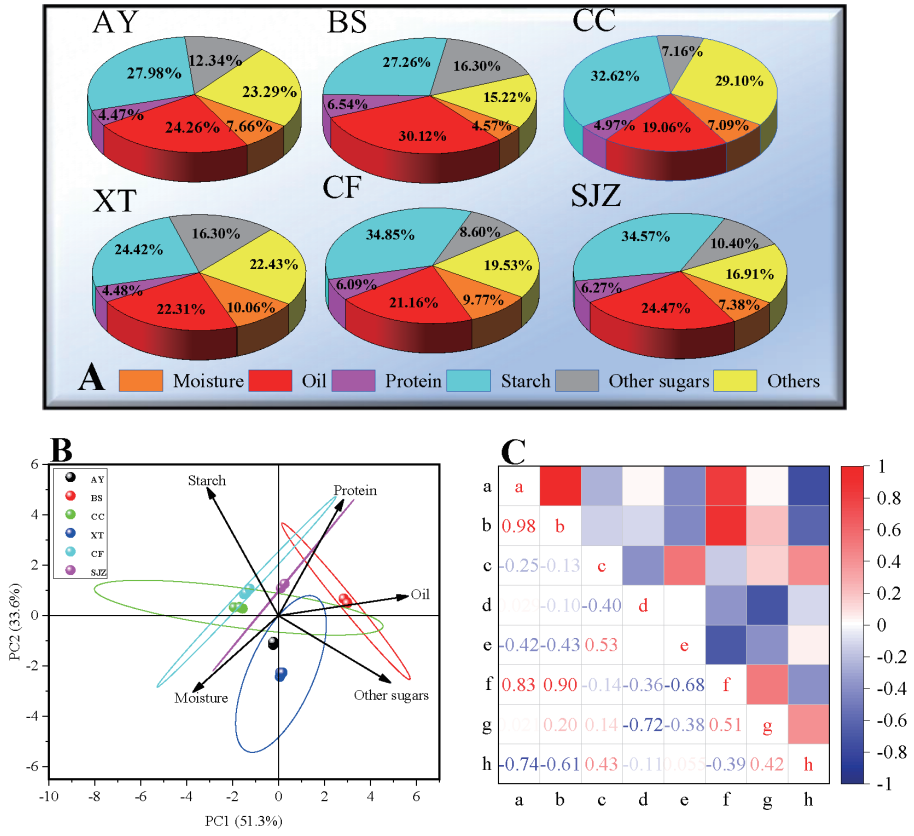


Fig. 2 A: The content of the main components of tigernut tubers from different regions; B: Principal component analysis graph; C: Correlation analysis between component content and geographical factors.
AY (tigernut tubers from Anyang); BS (tigernut tubers from Baoshan); CC (tigernut tubers from Changchun); XT (tigernut tubers from Xingtai); CF (tigernut tubers from Chifeng); SJZ (tigernut tubers from Shijiazhuang). a: Average annual maximum temperature; b: Average annual minimum temperature; c: Mean annual precipitation; d: Diurnal temperature range; e: Moisture content; f: Oil content; g: Protein content; h: Starch content.

Table 3 Physicochemical characteristics of TNOs.

Properties		AY	BS	CC	XT	CF	SJZ
Color	Red	5.1 ± 0.1^{b}	3.2 ± 0.2^{d}	6.3 ± 0.1^{a}	2.7 ± 0.1^{e}	3.7 ± 0.1^{c}	2.9 ± 0.1^{de}
	Yellow	71.4 ± 0.4^{ab}	71.0 ± 0.1^{ab}	72.1 ± 0.3^{a}	69.5 ± 0.5^{c}	70.5 ± 0.5^{bc}	70.2 ± 0.2^{bc}
Acid value (mg KOH/g)		2.45 ± 0.09^{b}	0.64 ± 0.14^{e}	3.46 ± 0.57^{a}	2.71 ± 0.10^{b}	2.08 ± 0.08^{c}	1.54 ± 0.02^{d}
Peroxide value (meq/kg oil)		2.42 ± 0.01^{b}	3.40 ± 0.27^{ab}	4.70 ± 0.58^{a}	3.52 ± 0.15^{ab}	2.62 ± 0.14^{b}	2.36 ± 0.01^{b}
Saponification (mg KOH/g oil)		158.4 ± 5.8^{b}	172.9 ± 3.8^{a}	160.8 ± 0.2^{b}	167.2 ± 1.6^{ab}	164.5 ± 0.4^{ab}	167.0 ± 2.6^{ab}
Iodine value (g I_2/ 100 g oil)		73.1 ± 1.5^{a}	70.4 ± 1.5^{bc}	71.7 ± 0.1^{ab}	73.1 ± 0.5^{a}	69.4 ± 0.6^{c}	67.0 ± 4.4^{d}
Induction period (h)		19.7 ± 0.4^{c}	17.1 ± 0.2^{d}	11.6 ± 0.3^{e}	16.8 ± 0.2^{d}	29.4 ± 0.5^{a}	24.4 ± 0.3^{b}

AY (oil from tigernut tubers of Anyang); BS (oil from tigernut tubers of Baoshan); CC (oil from tigernut tubers of Changchun); XT (oil from tigernut tubers of Xingtai); CF (oil from tigernut tubers of Chifeng); SJZ (oil from tigernut tubers of Shijiazhuang). Mean ± standard deviation, different superscript letters indicate statistically significant differences in the same row of data ($p < 0.05$).

yellow values of the TNOs differed considerably in the six samples. CC TNO presented the largest red (6.3) and yellow values (72.1), while XT TNO presented the lowest red (2.7) and yellow values (69.5). This difference may be due to the presence of pigments such as lutein, chlorophyll and carotenoids that migrate from the oilseeds, and the pigment contents affect the overall colour of the samples[30].

AV is an index of the content of free fatty acid in an oil sample. The AVs of the TNOs in Table 3 showed a range of 0.64-3.46 mg KOH/g. The values are below the maximum permissible AV for oils (<4 mg KOH/g) established by the Codex Alimentarius Commission[31]. The highest (3.46 mg KOH/g) was found in CC, while the lowest (0.64 mg KOH/g) was found in BS.

PV is commonly used as an indicator of oil quality and stability, and measures the extent of oil rancidification during storage[32]. Oils with a peroxide value of less than 6 meq/kg oil are considered fresh and of good quality. As shown in Table 3, the PVs for the six oil samples ranged from 2.36 meq/kg oil to 4.70 meq/kg oil, indicating relatively good quality. SJZ had the lowest PV (2.36 meq/kg oil).

SV is an indicator of average molecular mass of fatty acid in the oil sample, indicating the deterioration of the oils. As shown in Table 3, the SV of TNO from different origins ranged from 158.4 mg KOH/g oil to 172.9 mg KOH/g oil, which is comparable to the values (174.4-176.7 mg KOH/g oil) of TNO from Tumushuke (Xinjiang Province, China) reported by Guo *et al.*[6]. In this study, reasons for the lower SV of the TNOs could be: ① Higher proportions of high molecular weight fatty acids in the TNOs obtained by the solvent extraction process[33, 34]; ② Higher content of unsaponifiable matter (e.g., sterol, hydrocarbons, fatty alcohols, etc.) in the TNOs[35]; ③ Higher contents of monoacylglyceride and/or diacylglycerol in the TNOs[36]; ④ Influences of the growing conditions and variety on the enrichment of the above-mentioned components in tigernut samples[31]. The highest SV (172.9 mg KOH/g) was found in BS. This suggests that BS TNO either has a higher average molecular mass of fatty acid or that the oil has more ester bonds.

IV measures the degree of unsaturation in vegetable oil[6]. It determines the stability of oils to oxidation. As shown in Table 3, the IV for the six oil samples ranged from 67.0 g I_2/100 g to 73.1 g I_2/100 g. The highest IV (73.1 g I_2/100 g) was found in XT and AY. This suggests that XT and AY oil have a high level of unsaturation, which is thus detrimental to the oxidative stability of the oil.

3.4 Nutritional composition of TNOS

3.4.1 Fatty acid composition

Fatty acids are important components that influence the nutritional properties of edible oils. The results are shown in Table 4. Seven fatty acids, i.e., stearic acid (C18:0), oleic acid (C18:1), palmitic acid (C16:0), palmitoleic acid (C16:1), linoleic acid (C18:2), arachidic acid (C20:0) and linolenic acid (C18:3) were identified. Oleic acid (69.43%-73.58%) was found to be the major component in all the TNOs, followed by palmitic acid (12.79%-15.99%), linoleic acid (9.18%-13.13%) and stearic acid (1.99%-3.75%). This is generally consistent with the content reported by Guo *et al.*[6]. The content of monounsaturated fatty acid (69.52%-73.75%) in all the TNOs is comparable with the that in olive oil[37]. If these differences can be explained by the influence of geographical parameters, such as altitude, latitude and longitude, then they might be used to determine the geographical origins of tigernut tubers[38].

AY TNO was selected as the control oil for the fatty acid fingerprint similarity analysis. The fatty acid fingerprint similarity of six TNOs ranged from 99.70% to 100%, showing that the fatty acid composition varied little between different regions. The fatty acid fingerprint similarity of all TNOs was not found to be significantly different.

3.4.2 Tocopherol content

Tocopherols are natural antioxidant found in vegetable-based oils. Tocopherol (AH) traps peroxyl radical (LOO·) and converts it to hydroperoxyde product (LOOH) and tocopheroxyl radical (A·) (LOO· + AH→LOOH + A·). The A· reacts with another LOO· to form non radical inactive

Table 4 Fatty acid composition of six TNOs.

	AY	BS	CC	XT	CF	SJZ
C16:0 (%)	12.79 ± 0.06^{a}	15.99 ± 0.72^{a}	13.73 ± 0.01^{a}	13.37 ± 0.08^{a}	13.65 ± 0.05^{a}	15.31 ± 0.67^{a}
C16:1 (%)	0.17 ± 0.01^{c}	0.09 ± 0.02^{cd}	0.11 ± 0.01^{cd}	0.47 ± 0.01^{a}	0.07 ± 0.01^{d}	0.35 ± 0.05^{b}
C18:0 (%)	2.70 ± 0.13^{abc}	2.13 ± 0.02^{bc}	1.99 ± 0.01^{c}	3.56 ± 0.06^{ab}	2.11 ± 0.06^{bc}	3.75 ± 0.06^{a}
C18:1 (%)	73.58 ± 0.69^{a}	69.43 ± 0.57^{c}	69.92 ± 0.01^{bc}	71.93 ± 0.34^{ab}	70.64 ± 0.80^{bc}	70.39 ± 1.10^{bc}
C18:2 (%)	9.66 ± 0.61^{c}	11.43 ± 0.12^{b}	13.13 ± 0.11^{a}	9.74 ± 0.17^{c}	12.45 ± 0.59^{ab}	9.18 ± 0.17^{c}
C20:0 (%)	0.71 ± 0.11^{a}	0.48 ± 0.01^{d}	0.44 ± 0.09^{e}	0.51 ± 0.01^{d}	0.57 ± 0.10^{c}	0.63 ± 0.13^{b}
C18:3 (%)	0.39 ± 0.01^{e}	0.45 ± 0.05^{c}	0.68 ± 0.04^{a}	0.42 ± 0.05^{d}	0.51 ± 0.08^{b}	0.39 ± 0.01^{e}
SFA (%)	16.20 ± 0.08^{c}	18.60 ± 0.71^{ab}	16.16 ± 0.11^{c}	17.44 ± 0.15^{bc}	16.33 ± 0.21^{bc}	19.69 ± 1.33^{a}
MUFA (%)	73.75 ± 0.68^{a}	69.52 ± 0.55^{c}	70.03 ± 0.01^{bc}	72.40 ± 0.35^{ab}	70.71 ± 0.79^{bc}	70.74 ± 1.15^{bc}
PUFA (%)	10.05 ± 0.61^{c}	11.88 ± 0.12^{b}	13.81 ± 0.10^{a}	10.16 ± 0.17^{c}	12.96 ± 0.59^{ab}	9.57 ± 0.18^{c}
UFA (%)	83.80 ± 0.08^{a}	81.40 ± 0.67^{bc}	83.84 ± 0.11^{a}	82.56 ± 0.19^{ab}	83.67 ± 0.21^{a}	80.31 ± 1.33^{c}
Fatty acid fingerprint similarity (%)	100	99.80	99.70	99.90	99.90	99.90

AY (oil from tigernut tubers of Anyang); BS (oil from tigernut tubers of Baoshan); CC (oil from tigernut tubers of Changchun); XT (oil from tigernut tubers of Xingtai); CF (oil from tigernut tubers of Chifeng); SJZ (oil from tigernut tubers of Shijiazhuang). SFA, Saturated fatty acids; UFA, Unsaturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. Mean ± standard deviation, different superscript letters indicate statistically significant differences in the same row of data ($p < 0.05$).

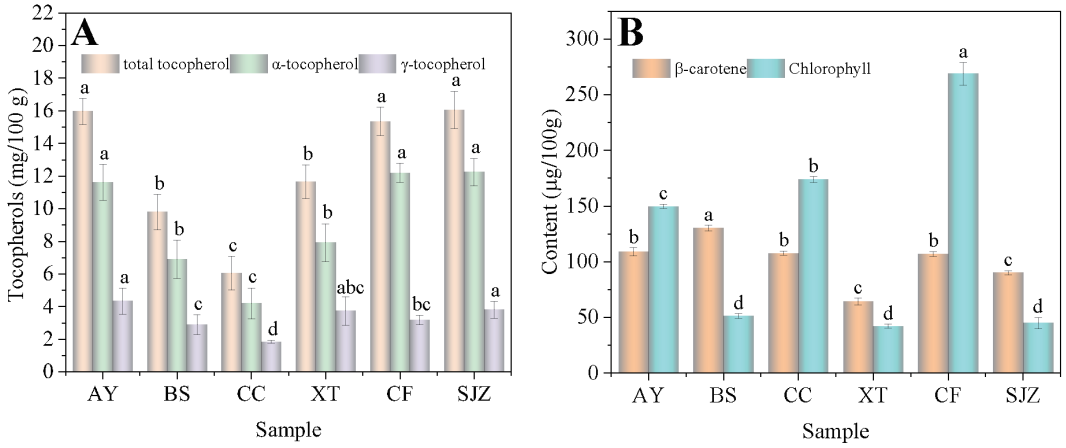


Fig. 3 Functional component content in six TNOs.
A: Tocopherol compounds of tigernut oils; B: β-carotene, and Chlorophyll compounds of tigernut oils. AY(oil from tigernut tubers of Anyang); BS(oil from tigernut tubers of Baoshan); CC(oil from tigernut tubers of Changchun); XT (oil from tigernut tubers of Xingtai); CF(oil from tigernut tubers of Chifeng); SJZ(oil from tigernut tubers of Shijiazhuang).

product(A· + LOO·→inactive product). α-tocopherol with the alkoxyl radicals(LO·)to inhibit the decomposition of the hydroperoxides and decrease the formation of aldehydes(LO· + AH→LOH + A·)[39]. As shown in Fig. 3A, the total tocopherol levels in the six TNOs ranged from 6.04 mg/100 g oil to 16.05 mg/100 g oil. α-Tocopherol was identified as the major component; it is considered as the most important chain-breaking antioxidant in preserving oil quality. The lowest total tocopherol content was found in the CC TNO(6.04 mg/100 g oil). This difference may be due to the fact that the synthesis of tocopherols during photosynthesis in plants is influenced by different climatic conditions[40].

3.4.3 β-carotene and chlorophyll content

β-carotene is a powerful antioxidant naturally presents in many plant-based oils, such as palm oil and fresh groundnut oils[41]. As shown in Fig. 3B, the β-carotene contents in all TNOs varied from 64.1 μg/100 g oil to 130.4

μg/100 g oil. Among these oils, β-carotene content was highest in the BS TNO (130.4 μg/100 g oil), while it was lowest in XT TNO (64.1 μg/100 g oil). Previous research has reported that β-carotene content is positively associated with annual temperature but negatively associated with mean annual precipitation, and that temperatures of 18-26℃ is suitable for β-carotene synthesis[42].

Chlorophyll is a natural pigment found in most plant-based oils. It is considered to be a factor that can reduce the quality of oils, as it makes the oils more susceptible to photo-oxidation, leading to low oxidative stability and the development of off-flavors[43]. As shown in Fig. 3B, the chlorophyll content of the six TNOs ranged from 44.8 μg/g oil to 268.9 μg/g oil. Chlorophyll content was highest in the CF TNO (268.9 μg/g oil), while it was lowest in XT TNO (44.8 μg/100 g oil). This difference can be explained by the different amounts of chlorophyll-binding protein accumulated in the matrix, which is affected by the climatic conditions (e.g., average annual temperature and mean annual precipitation)[44].

3.5 Oxidative stability of TNOS

The oxidation induction period (IP) of the six TNOs are presented in Table 3. Longer IP indicates greater antioxidant activity[6]. The longest IP obtained was 29.4 h for the CF TNO, followed by 24.4 h for the SJZ TNO. The shortest IP was 11.6 h for the CC TNO. This meant that CF TNO had the strongest antioxidant activity and CC TNO was with the worst oxidation stability. This can be attributed to the fact that having a higher content of antioxidant components (e.g., tocopherols) can effectively increase the IP of the samples[45].

3.6 Physicochemical properties of TS

3.6.1 Starch morphology and size analysis

SEM micrographs of starch granules of the six samples are shown in Fig. 4. The appearance of all TS was uniformly oval and smooth. However, at the same magnification,

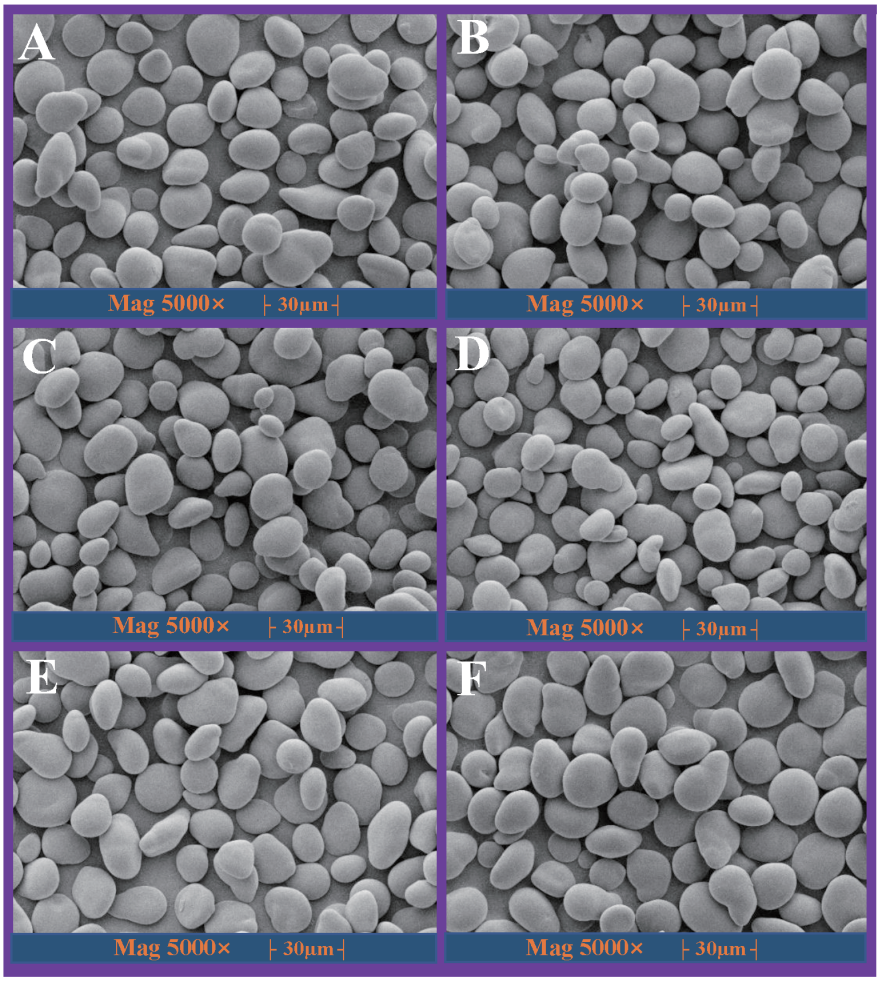


Fig. 4 SEM of the six tigernut starch samples.
A-F in order from Anyang (AY), Baoshan (BS), Changchun (CC), Xingtai (XT), Chifeng (CF), and Shijiazhuang (SJZ) of tigernut starch.

there were differences in particle size. Particle size ranged from 4 μm to 18 μm, and showed a normal distribution (Fig. 5B). The largest volume means diameter (D[4,3]) was found in AY starch, with a value of 9.84 μm (Table 5). This can be explained by that high average annual temperature suppresses small granule initiation, and the available substrate is diverted toward pre-existing large granules[46].

3.6.2 Amylose content

Amylose is one of the large molecules that make up starch; it plays a key role in some of the main functional properties (e.g., gel formation and starch regeneration) of starch[47]. Amylose content of the six TS ranged from 20.1% to 28.4%, as shown in Table 5. XT starch contained the most amylose (28.4%). There was no significant difference between the amylose content of XT starch and that of CC,

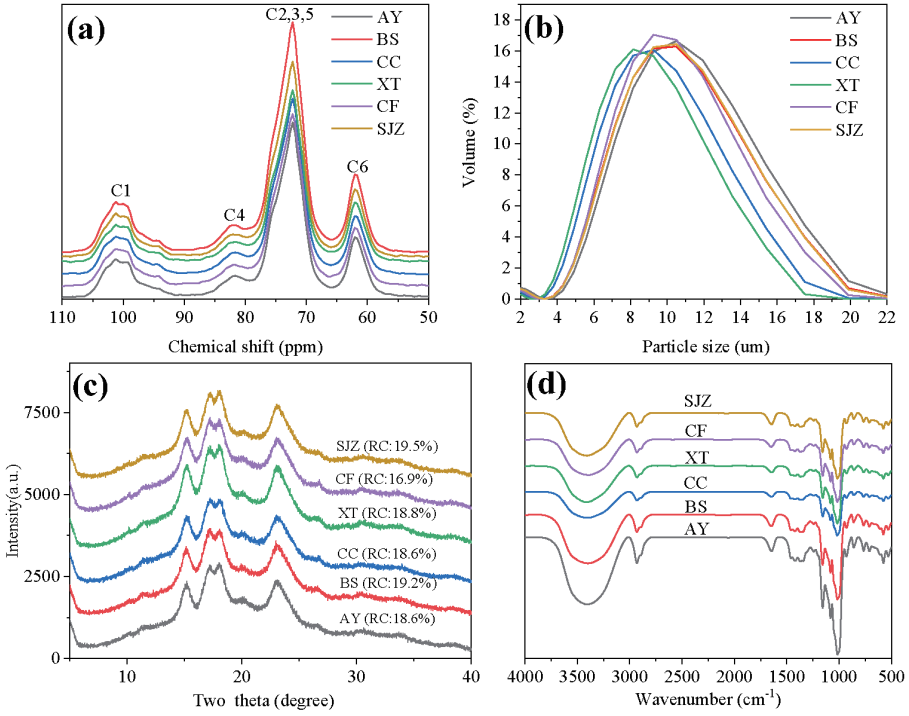


Fig. 5 ^{13}C CP/MAS NMR spectra (a), Particle size distributions (b), XRD spectra (c), and FT-IR spectra (d).
AY (starch from tigernut tubers of Anyang); BS (starch from tigernut tubers of Baoshan); CC (starch from tigernut tubers of Changchun); XT (starch from tigernut tubers of Xingtai); CF (starch from tigernut tubers of Chifeng); SJZ (starch from tigernut tubers of Shijiazhuang); RC (relative crystallinity).

Table 5 Relative crystallinity (RC), relative proportions of amorphous (RPA), single and double helix conformations from six samples of tigernut starch, measured by ^{13}C NMR.

Sample	AY	BS	CC	XT	CF	SJZ
D [4,3] (μm)	9.84 ± 0.06^{a}	9.52 ± 0.05^{b}	8.61 ± 0.09^{c}	8.23 ± 0.10^{d}	9.42 ± 0.05^{b}	9.63 ± 0.05^{ab}
Amylose (%)	22.6 ± 0.7^{b}	20.1 ± 2.0^{c}	27.4 ± 0.4^{a}	28.4 ± 0.7^{a}	26.1 ± 0.7^{ab}	26.2 ± 0.8^{ab}
RC by NMR (%)	41.7 ± 1.5^{bc}	43.9 ± 0.2^{ab}	40.0 ± 0.3^{c}	43.2 ± 1.2^{abc}	36.3 ± 0.9^{d}	46.3 ± 1.1^{a}
Double helix content (%)	57.6 ± 0.8^{a}	57.9 ± 0.5^{a}	58.1 ± 0.1^{a}	60.0 ± 0.6^{a}	58.8 ± 0.6^{a}	58.4 ± 0.7^{a}
Single helix content (%)	4.71 ± 0.11^{a}	4.32 ± 0.29^{a}	3.29 ± 0.02^{b}	3.28 ± 0.09^{b}	3.62 ± 0.07^{b}	3.51 ± 0.29^{b}
RPA (%)	7.68 ± 0.28^{ab}	7.46 ± 0.23^{b}	8.01 ± 0.22^{ab}	7.88 ± 0.04^{ab}	8.42 ± 0.18^{a}	7.66 ± 0.29^{ab}
1047 cm^{-1}/1022 cm^{-1}	0.96 ± 0.06^{a}	0.93 ± 0.16^{a}	0.99 ± 0.09^{a}	0.97 ± 0.07^{a}	0.95 ± 0.03^{a}	0.97 ± 0.01^{a}
995 cm^{-1}/1022 cm^{-1}	1.04 ± 0.07^{a}	1.03 ± 0.07^{a}	1.05 ± 0.06^{a}	1.09 ± 0.07^{a}	1.09 ± 0.11^{a}	1.20 ± 0.20^{a}

AY (starch from tigernut tubers of Anyang); BS (starch from tigernut tubers of Baoshan); CC (starch from tigernut tubers of Changchun); XT (starch from tigernut tubers of Xingtai); CF (starch from tigernut tubers of Chifeng); SJZ (starch from tigernut tubers of Shijiazhuang). Mean ± standard deviation, different superscript letters indicate statistically significant differences in the same row of data ($p < 0.05$).

CF and SJZ. The increase in amylose content was ascribed to the increased activity of the key enzyme that catalyzes amylose biosynthesis (granule-bound starch synthase), as a result of lower average annual temperature[48]. Furthermore, high amylose starch can be used as a raw material for products (e.g., biodegradable biofilms)[49]. These differences will help in the selection of suitable growing areas for the high-value use of tigernut starch.

3.7 Starch granule structure analyses

3.7.1 Solid-state ^{13}C NMR analysis

The molecular structural information of the starches provided by NMR is recorded in Fig. 5A and Table 5. The relative crystallinity values (RC:36.3%-46.3%) calculated from the NMR spectra were significantly higher than those calculated from the X-ray diffractograms (RC: 16.9%-19.5%). This situation can be attributed to the fact that NMR can measure the helices in both long-range and short-range ordered structures used to form crystals, but XRD can only measure the helices in the long-range ordered structures used to form crystals[50]. The double helix content of the six starches ranged from 57.6% to 60.0%. SJZ starch had the highest relative crystallinity (RC measured by ^{13}C NMR: 46.3%). All samples showed slight differences in the single helix content (3.28%-4.71%) and amorphous phase (7.46%-8.42%).

3.7.2 X-ray diffraction analysis

Crystallographic information for the six starches is depicted in Fig. 5C. A-type diffraction peaks (15°, 17°, 18° and, 23° of 2θ) were observed in all samples. This is consistent with the description by Liu *et al.*[3]. As shown, the relative crystallinity of six TS followed this order: SJZ (19.5%) > BS (19.2%) > XT (18.8%) > AY (18.6%) = CC (18.6%) > CF (16.9%). RC differences can be related to amylopectin and moisture content[51].

3.7.3 FT-IR analysis

The FT-IR spectra revealed information about the short-range ordered structure of the outer areas of the starch granules (Fig. 5D). As mentioned in the literature, the double helix conformation and ordered structure of starch (lotus seed resistant starch) molecules is represented by peaks between 950 cm^{-1} and 1065 cm^{-1} [52]. The absorption ratios of 1047/1022 cm^{-1} and 995/1022 cm^{-1} reflect the ratio between the ordered and amorphous structures of starch and the proportion of double helices in amylose, respectively[53]. As can be seen from Table 5, there were no statistically significant differences regarding the value of 1047/1022 cm^{-1} ration and the value of 995/1022 cm^{-1} ration of the six starch samples ($p > 0.05$), indicating that their short-range ordered structures do not differ significantly.

3.8 Functional properties of starch

3.8.1 Pasting properties

The pasting properties of starch play a key role in assessing various potential applications for its usage in the industry as a thickener or in making baked goods[54]. The results showed significant differences in all pasting parameters of the six starch samples (Table 6 and Fig. 6A). The paste temperature (76.0℃) of AY starch was highest, which suggested a more ordered double helix arrangement in its amylopectin. The lowest peak viscosity (4117 cP) was found in the XT starch. This can be related to higher content of amylose, because amylose prevents the expansion of the starch granules and the formation of insoluble complexes through gelation[43]. The lowest breakdown (BD) viscosity (1673 cP) was found in the AY starch. The lower BD points to the greater heat and shear resistance of this starch under high-temperature conditions[55]. The setback (SB) viscosity of starches illustrates interaction between leached amylose chains during the cooling cycle and the occur-

Table 6 Pasting properties of the six starch samples.

Sample	Pasting temperature (℃)	Viscosity (cP)				
		Peak	Trough	Final	Break down	Set back
AY	76.0 ± 0.1^{a}	4206 ± 1^{d}	2534 ± 1^{a}	3697 ± 1^{a}	1673 ± 0^{f}	1163 ± 0^{c}
BS	70.6 ± 0.3^{e}	6709 ± 2^{a}	2412 ± 0^{b}	3490 ± 1^{b}	4298 ± 2^{a}	1078 ± 1^{f}
CC	73.7 ± 0.2^{c}	4209 ± 1^{d}	1976 ± 1^{e}	3105 ± 0^{f}	2234 ± 0^{d}	1129 ± 1^{d}
XT	74.6 ± 0.2^{b}	4117 ± 1^{e}	2047 ± 1^{d}	3166 ± 1^{d}	2070 ± 0^{e}	1119 ± 0^{e}
CF	69.8 ± 0.3^{f}	4823 ± 1^{c}	2100 ± 1^{c}	3305 ± 0^{c}	2724 ± 1^{c}	1205 ± 1^{b}
SJZ	72.2 ± 0.2^{d}	4910 ± 1^{b}	1868 ± 1^{f}	3116 ± 1^{e}	3042 ± 0^{b}	1248 ± 0^{a}

AY (starch from tigernut tubers of Anyang); BS (starch from tigernut tubers of Baoshan); CC (starch from tigernut tubers of Changchun); XT (starch from tigernut tubers of Xingtai); CF (starch from tigernut tubers of Chifeng); SJZ (starch from tigernut tubers of Shijiazhuang). Mean ± standard deviation, different superscript letters indicate statistically significant differences in the same row of data ($p < 0.05$).

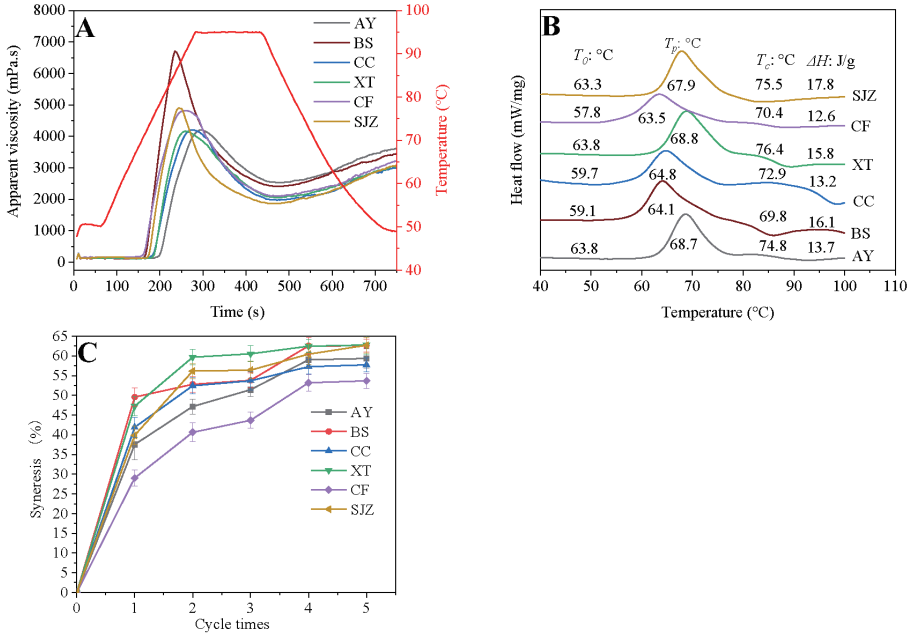


Fig. 6 A: Pasting properties, B: Thermal properties, C: Freeze-thaw stability.
AY (starch from tigernut tubers of Anyang); BS (starch from tigernut tubers of Baoshan); CC (starch from tigernut tubers of Changchun); XT (starch from tigernut tubers of Xingtai); CF (starch from tigernut tubers of Chifeng); SJZ (starch from tigernut tubers of Shijiazhuang).

rence of intact or fragmented granules in the amylose network. As can be seen, the BS starch showed the lowest SB (1078 cP), indicating that it had good cool paste stability at low temperature[50]. AY starch showed the highest final viscosity (3697 cP), indicating that it had a stronger tendency to gelling/retrogradation at low temperature. This can be related to restricted amylose leaching due to granule stiffness, reducing network strength[56]. These differences will help in the selection of suitable growing areas for tigernut food.

3.8.2 Thermal properties

Figure 6B depicts the thermal characteristics of the six starches. The transition temperatures (T_o, T_p, and T_c) and melting enthalpy (ΔH) of gelatinization differed significantly. The T_o (63.8℃) of XT starch was highest. This is because a starch with a more compact and arranged crystal structure or these double helices requires more energy to melt during gelatinization[57]. High T_o, indicates it is suitable for high-heat cooking such as stir frying.

The highest ΔT ($T_c - T_o = 13.2$℃) was found in CC starch. This is presumably because the crystalline areas of this sample were stable and uniform. The SJZ starch had the highest ΔH (17.8 J/g), indicating a higher retrogradation tendency, possibly due to its many short-chain structures for amylopectin. Shorter chains are more likely to align than longer chains, which contributes to the retrogradation of the starch[58]. These differences may be caused by growing conditions and plant physiological metabolism[59].

3.8.3 Freeze-thaw stability

Variation in the amount of water removed during freeze-thaw cycles for the six starch gels is shown in Fig. 6C. The syneresis ranged from 50% to 65% over five cycles. These results were smaller than those described by Kaur *et al.*[60] for bulk starches (maize, potato, etc.). This indicates that all TS have good freeze-thaw stability and can be considered for frozen food applications. The syneresis of CF starch (50%) was significantly lower than that of other TS ($p < 0.05$). This result suggests that this starch holds water well and does not readily retrograde. This is because the more branched structure of its amylopectin fraction[3], which can be increased by higher starch branching enzyme activities when mean annual precipitation is lower[61].

4 Conclusions

Brown tigernuts grown in six major growing regions in China were characterized in terms of their physicochemical properties. Oil and starch were isolated and tested. Significant differences ($p < 0.05$) were observed in composition and in properties of components, particularly starch. Statistical analysis results showed that the content of TNO, active ingredients in TNO (β-carotene, chlorophylls, tocopherols), total starch and amylose content in the TS were mainly influenced by average annual temperature. Average annual temperature was found to have a critical effect on the apparent characteristics (starch granule mor-

phology and size), structure (long-range and short-range ordered structure), pasting characteristics, and thermal properties of TS. The level of mean annual precipitation in different regions also affected freeze-thaw stability of TS, which exhibited significant differences. These results provide fundamental information about the main differences in components of the brown tigernuts in six major growing regions, which may be of relevance to farmers, industries and consumers searching for tigernut with specific physicochemical properties. The results could be further applied to support tigernut industries and relevant researchers that looks for geographical origin discrimination and improvements on tigernut quality, with unique physicochemical and technological properties.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Acknowledgements

This work was supported by a grant from the Key Project for Technological Breakthrough and Scientific Achievement in Henan, China (212102110082) and the "Double First-Class" Project for Postgraduate-Cultivating Innovation Platform Establishment Programme of Henan University of Technology (HAUTSYL2023PT09).

Author Contributions

Zhong-Wei Wu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing-original draft, Writing-review & editing. Hong-Rui Huang: Data curation, Formal analysis, Investigation, Methodology. Shu-Qiang Liao: Investigation, Formal analysis. Xiao-Shuang Cai: Formal analysis, Methodology, Visualization, Supervision, Writing-review & editing. Hua-Min Liu: Supervision, Methodology & writing-review. Yu-Xiang Ma: Visualization, Methodology. Xue-De Wang: Supervision, Funding acquisition.

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