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The identification of thresholds of starch phosphate and amylose levels on multi-scale structures and functional properties of potato starch

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ABSTRACT

Both amylose content (AC) and starch phosphate content (SPC) play important roles in determining the functional features of starch in potato (*Solanum tuberosum* L.) tubers. However, the relative contribution of these two factors on starch properties has not been determined. The SPC, AC, and various multi-scale structures and functional properties of 13 natural potato starches with varying SPC (ranging from 449 ppm to 1004 ppm) and AC (from 18.8 % to 27.8 %) were investigated. It was found that SPC is closely correlated with the length and proportion of branch chains, and potato starches with high SPC tended to have a lower proportion of amylose. An elevation in SPC significantly enhanced the surface layer ordering of starch granules, while an increase in AC restricted the expansion of starch granules. With a critical SPC of 700 ppm, when the SPC of potato starch surpassed this threshold, phosphate played a predominant part in conferring the functional properties to the potato starch. An increase in SPC boosted the swelling capacity and lessened short-term retrogradation and strength of the starch gel. Conversely, when the SPC fell below this threshold, the influence of AC became more prominent. A higher AC promoted short-term retrogradation and strength of the starch gel. The results of this study further elucidates the subtle interplay between AC and SPC, providing deeper insights into the relationship between phosphate and amylose content and the structure and function of potato starch.

1. Introduction

Potato is the most important non-grain food crop consumed as staple food at present and is expected to increase its consumption in the near future due to increasing global population and food security issues (Ahmad, Zhang, Rasheed, Xu & Bao, 2022). Starch, comprising 70–90 % of dry mass is the predominant component of potato tubers (Nayak, De J. Berrios & Tang, 2014). Typical starch granules are formed in a semi-crystalline nature and have a molecular structure of two α -glucan types: the primarily α -1,4 glucosidic linked linear polymer amylose (AM) and highly branched α -1,4 - α -1,6 branch glucosidic linked amylopectin (AP). Amylose content (AC) in normal potato starches typically falls between 15 % and 30 % (Karlsson, Leeman, Björck & Eliasson, 2007; Liu, Tarn, Lynch & Skjoldt, 2007; Pérez & Bertoft, 2010).

The AC is one of many factors affecting the multi-scale structure of starch granules, thereby impacting the functional features of the starch, for example, the viscosity, texture, and digestibility (Cai et al., 2014; Lin et al., 2016; Zhong et al., 2020) and the various quality characteristics of starch-based products (Han et al., 2017). Besides AC, phosphorylation of the AP molecules provides a key additional factor impacting the functional properties of potato starches (Blennow & Engelsen, 2010). Potato starch possesses inimitable physicochemical characteristics relative to starches from other plant sources. Notably, it contains phosphate monoester groups mainly on the amylopectin fraction, forms large granules, and demonstrates high purity (Ahmed et al., 2018). These attributes enable potato starch to form relatively clear, viscoelastic pastes upon heating in water and following cooling. Additionally, potato starch lacks internal lipids (Mishra & Rai, 2006) and produces clear gels

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following gelatinization (Alvani, Qi, Tester & Snape, 2011). These properties are primarily owing to the presence of phosphate monoesters (N. Singh, Kaur, Sandhu, Kaur & Nishinari, 2006) and marked deficiency in lipids (Mishra et al., 2006).

AC and phosphate substitution are well known to be significant features of starches, influencing their physicochemical properties and functionality (Kozlov, Blennow, Krivandin & Yuryev, 2007; Parker & Ring, 2001). Phosphate monoesters in potato starch are mostly located in the so-called B-chains (relatively long chains) of amylopectin, and hence, high starch phosphate ester content (SPC) is usually in association with a high level of long unit chains in the amylopectin (Blennow, Engelsen, Munck & Møller, 2000; Karim et al., 2007; Takeda & Hizukuri, 1982). However, in high-amylose starches from different crop sources, there is a negative correlation between AC and the content of long branch chains (Tian et al., 2024). Lu, Donner, Yada and Liu (2012) reported that starch granules with higher SPC exhibit a more ordered structure, whereas Ding et al. (2023) argued that both AC and SPC in the starch decrease the structural ordering of starch. Amylose increases the rigidity of starch granules and limits granule swelling (Lii, Tsai & Tseng, 1996). However, the presence of phosphate groups enhances the hydration capacity of starch granules, making the granules more prone to swelling (Noda et al., 2004). A high level of phosphate groups present in starch to a certain extent inclines to inhibit its retrogradation (Jane, Kasemsuwan, Chen and Juliano (1996). However, some reports indicated that phosphate in potato starch granules had a positive relationship with the degree of retrogradation (Hopkins & Gormley, 2000). The above research results indicate that amylose and phosphate have opposing effects on the amylopectin chain length distributions (CLD), pasting properties, and retrogradation characteristics. Thygesen, Blennow and Engelsen (2003) indicated that retrogradation of potato starch depends on a subtle interrelationship of AC and SPC. Finally, SPC, more than AC, negatively affects potato starch granule exo-hydrolytic degradation (Wickramasinghe, Blennow & Noda, 2009). Previous studies have primarily focused on the impact of individual variables on starch structure and properties, but it is evident that there is a more complex balanced relationship between the effects of AC and SPC on starch. Lu et al. (2012) found that phosphate content plays a dominant role in some physicochemical properties of starch when the AC was above a threshold level. However, there is currently no data supporting such a threshold for SPC. To date, no clear relationships between AC, SPC, fine molecular structures and functionality of potato starches have been established.

To elucidate the relationship between SPC, AC, and the structure and functionality of potato starch, and to identify possible threshold values for the interplay between SPC and AC, 13 natural potato starches with varying SPC (ranging from 449 ppm to 1004 ppm) and AC (from 18.8 % to 27.8 %) were investigated with respect to their molecular structure, crystalline structure, lamellar structure, and granule size, and also analyzed along with respect to their thermal properties, pasting properties, textural properties, and rheological properties. Principal component analysis (PCA) and Pearson correlation analysis disclosed major relationships between different SPC, AC and the multi-scale structures and functionalities of potato starch. The results of this study elucidates subtle interplays between AC and SPC, providing deeper insights into the relationship between phosphate and amylose content and the structure and function of potato starch. This understanding will facilitate the development of potato varieties tailored to various needs through the breeding-assisted regulation of AC and SPC.

2. Material and methods

2.1. Plant materials

A total of 13 potato accessions with different SPCs were employed in this study (Table 1). All accessions were grown simultaneously under the same conditions in early March 2021 and harvested in mid-June 2021 at the campus farm of Zhejiang University in Hangzhou, China. Cultivation

Table 1
The phosphate contents of potato samples.

Group	Sample code	phosphate content ^a (ppm)
HPPS	SP85	1003.6 ± 0.4a
	SP45	928.0 ± 2.2b
	SP98	901.1 ± 0.9c
	SP07	876.8 ± 4.8d
	SP63	801.6 ± 1.9e
	SP69	773.4 ± 2.2f
	SP30	720.8 ± 3.2g
LPPS	SP87	644.1 ± 2.4h
	HP22	639.9 ± 2.8hi
	SP86	631.5 ± 4.5i
	HP210	622.3 ± 3.5j
	SP100	508.7 ± 1.8k
	SP34	448.8 ± 4.4l

^a Values are mean ± SD. Values with different letters in the same column indicate significant difference at $p < 0.05$.

of these 13 potato accessions was followed the standard field management practices.

2.2. Starch isolation

Potato tuber starch was extracted as follows (Singh & Singh, 2001). Tubers were washed, peeled, and chopped into 2–3 cm chunks, soaked in distilled water with sodium bisulfite (0.04 g/L) for 30 min to prevent browning, homogenized for 3 min. Starch granules were filtered through a 212 μm sieve (Jiufeng, Hebei, China), settled, washed in distilled water 5 times until clear and the suspension was centrifuged at 3500 rpm for 15 min at 4 °C using a centrifuge (Cence, Changsha, China). The starch granules were washed twice with 95 % ethanol and finally with absolute ethanol for 30 min, re-centrifuged, dried at 37 °C for 36 h, and gently ground and passed through a 212 μm mesh sieve.

2.3. Size-exclusion chromatography (SEC)

Chain length distributions (CLD) of the debranched potato starch samples were analyzed using a size-exclusion chromatography (SEC)-triple detector array (SEC-TDA) system (Viscotek, Malvern, UK), as described (Zhong et al., 2021, 2020). Briefly, the SEC had Shodex GS-520HQ/GS-320HQ columns linked to a TDA302 detector. A starch suspension in water (5 mg/mL) was gelatinized at 100 °C for 1 h, debranched with isoamylase and pullulanase (Sigma, St. Louis, MO, USA) (0.8 U) at 40 °C for 3 h, and the reaction terminated at 99 °C for 10 min. Following centrifugation, 50 μL supernatant (1 mg/mL) was injected onto the columns, and separated using ammonium formate (10 mM) at 0.4 mL/min flow rate and 60 °C. Data was processed with OmniSec Software 4.7. Pure maize AM and AP before and post debranching were used as standards.

2.4. X-ray diffraction

X-ray diffraction of potato starch was carried out in a Nano-inXider instrument (Xenocs SAS, Grenoble, France) (Zhong et al., 2021). Samples equilibrated at a relative humidity of ~90 % for 7 days were sealed in mica films (5–7 μm) for analysis. The radially averaged intensity was measured at a scattering angle (2θ) from 5° to 35° after mica background subtraction. The relative crystallinity was determined using PeakFit software (Zhong et al., 2021).

2.5. Starch granule size analysis

The size distribution of starch granules was analyzed by a particle size analyzer (Mastersizer 3000, Malvern, UK). The starch-water

suspension underwent stirring at a speed of 2000 rpm, and the measurement commenced once the obscuration of the suspension attained 10 %. Please explain the D [3,2] and D [4,3] parameters here.

2.6. Phosphate contents

The quantification of SPC in the starch was carried out mainly as described (Morrison, 1964) with specific adaptations. Approximately 20 mg of starch was dispersed in 250 μ l 70 % (w/w) perchloric acid (HClO₄) and incinerated at 250 °C for 25 min until completely charred. To clarify the solution, 50 μ l 30 % (w/v) hydrogen peroxide (H₂O₂) was added, followed by gentle boiling for 2 min. After cooling, the volume was diluted to 2 ml with water. Hundred μ l aliquots were analyzed placed in 96-well microtiter plates with 200 μ l the color reagent 0.75 % (w/v) ammonium molybdate tetrahydrate [(NH₄)₆M₀₇O₂₄·4H₂O], 3 % (w/v) iron (II) sulfate heptahydrate (FeSO₄·7H₂O), and 0.75 % (w/v) sodium dodecyl sulfate in 0.375 M sulfuric acid. The absorbance was measured at 750 nm using a Model 680 XR Microplate Reader (Bio-Rad, US).

2.7. Pasting and textural properties

The pasting properties of potato starch samples were measured with a Model 4500 Rapid Visco Analyzer (RVA, Perten Instrument, Hägersten, Sweden). A mixture consisting of 2 g dry starch and 26 g distilled water was prepared in the sample can of the RVA. The "Standard 1" program was used. The viscosities were reported in rapid visco units (RVU).

Post RVA testing, the starch gels formed were preserved in the RVA canister, sealed with Parafilm™, and stored at 4 °C for 24 h. The hardness, cohesiveness and chewiness were calculated from data using a texture analyzer (TA.XTC-18, Shanghai Bosin Industrial Development Co., Shanghai, China), following a two-cycle testing procedure as described by Bao, Shen, Sun and Corke (2006).

2.8. Thermal properties

The thermal properties of the starches were measured using a Differential Scanning Calorimeter (DSC) Q20 (TA Instruments, New Castle, DE, USA), following the protocols described in Ahmed et al. (2018).

2.9. Rheological properties

The rheological behavior of starch gels was characterized using a rheometer (DHR-2, TA Instrument, New Castle, DE, USA) equipped with a plate-plate geometry (40 mm diameter, 0.1 mm gap). Prior to testing, the starch solution (6 %) was fully gelatinized at 100 °C, and then cooled at room temperature for 1 h. A plastic dropper was utilized to dispense the same number of drops for each sample. Subsequently, the samples were allowed to equilibrate at 25 °C for 2 min. Shear viscosity tests were performed in flow ramp mode, and increasing shear rate was set from 0.01 to 100 1/s at 25 °C. Frequency sweep was conducted with an angular frequency ranging from 1 to 100 rad/s at a constant deformation of 0.1 % strain at 25 °C.

2.10. Statistical analysis

Each test was performed in at least two replicates. The Analysis of Variance (ANOVA), along with Tukey's range test, was executed using SAS software (version 8, from SAS Institute Inc., Cary, NC, USA). Additionally, Pearson correlation analysis, specifically for the potato starch data, was carried out utilizing the R software version 4.3.2.

3. Results and discussion

To investigate which factor, SPC, AC or AP structure, plays a more important role in determining starch functionality, the SPC (Table 1),

CLDs (Table 2), starch granule size distribution, relative crystallinity and FTIR ratios (Table 3), thermal properties, and pasting properties (Table 4), along with textural and rheological properties (Table 5), were measured for the thirteen selected potato starches. Principal component analysis (PCA) was conducted to identify potential relationships among these parameters (Tables 1–5). Surprisingly, the analysis revealed that the parameters tended to be distributed according to the SPC (Fig. 1). The first and second components explained 56.6 % of the total variation, demonstrating the importance of these two components for the variation in the data set.

The SPC of the 13 potato starches ranged from 449 ppm to 1004 ppm (Table 1), encompassing the range of reported SPC values for potato starch (Kim, Wiesenborn, Orr, & Grant, 1995; Morrison et al., 2001; Noda et al., 2007). Based on the PCA results (Fig. 1), the potato starches were clearly divided into two groups: high-phosphate potato starches (HPPS, SPC > 700 ppm) and low-phosphate potato starches (LPPS, SPC < 700 ppm). All subsequent discussions on the multi-scale structures, particle sizes, crystal structures, and functionalities of potato starches are based on these two groups.

3.1. Chain length distributions (CLDs) analyzed by SEC

The weight-based CLD profiles exhibited three populations (Fig. 2), which revealed three major structural components consistent with previous findings (Yu et al., 2019): short amylopectin (AP1) chains of DP 6–36, long amylopectin (AP2) chains of DP 37–200, and amylose (AM) chains with DP >200. Table 2 summarized the difference in peak positions, average chain lengths (ACLs), and relative contents (RCs) of these three starch components. The relative content of amylose (RC_{AM}, i.e. AC) of HPPS ranged from 18.6 to 20.9 % were lower than that of LPPS ranging from 20.4 to 27.8 %. However, there was no difference in AC among the seven HPPS, while among the six LPPS, there was significant difference in AC. The relative content of short amylopectin (RC_{AP1}) of HPPS, which varied from 52.5 % to 62.2 %, were significantly higher than that of LPPS varying from 48.6 % to 55.1 %. No significant difference in RC_{AP2} was found between HPPS and LPPS. In the HPPS group, RC_{AP1} increased with SPC, while RC_{AP2} decreased with SPC. However, within the LPPS group, RC_{AP1} and RC_{AP2} were uncorrelated with SPC. The average chain lengths of short branches (ACL_{AP1}, DP 10.8–11.6) and long branches (ACL_{AP2}, DP 56.3–65.2) in the HPPS group were both higher than the ACL_{AP1} (DP 10.1–10.6) and ACL_{AP2} (DP 54.0–57.8) in the LPPS group. Additionally, the peak positions of short branches (Peak_{AP1}) and long branches (Peak_{AP2}) in HPPS group were also higher than those in LPPS group. However, no significant difference in ACL_{AM} and Peak_{AM} was found between HPPS and LPPS. Moreover, there were no significant differences in ACL_{AP1}, ACL_{AP2}, Peak_{AP1} and Peak_{AP2} in either HPPS or LPPS. However, the ACL of amylopectin in SP85, which had the highest SPC (1004 ppm), was significantly higher than the other samples. In the HPPS group, ACL_{AM} decreased significantly with increased SPC.

Apart from the differences between the HPPS and LPPS groups, there were also variations among the individual samples within each of these groups. Interestingly, distinct characteristics emerged within each group. SPC within the HPPS group had no significant relationship with ACL, but had a significant positive correlation with RC_{AP1}, and negative correlation with RC_{AP2}, showing that higher SPC was associated with more short amylopectin chains of DP 6–36. Previous researches reported that for potato starches with similar AC, SPC was positively correlated with RC_{fb2}, and negatively correlated with RC_{fa} and RC_{fb1}, showing that higher SPC was associated with more long AP chains with DP ranging from DP 25 to DP 36 (Ding, Blennow & Zhong, 2024, 2023; Hanashiro, Abe & Hizukuri, 1996). The activity of the starch phosphorylase Glucan, Water-Dikinase 1 (GWD1) was reported to be high on long AP chains (DP ≥ 22) (Mikkelsen, Baunsgaard & Blennow, 2004). Hence, the high SPC can be related to this higher activity on existing long AP chains. Alternatively, it is plausible that the high SPC potatoes had higher

Table 2
Chain length distributions (CLDs) of potato starch analyzed by SEC.^a

Group	Sample code	RC _{AP1} (%) ^b	RC _{AP2} (%)	RC _{AM} (%)	ACL _{AP1} (DP)	ACL _{AP2} (DP)	ACL _{AM} (DP)	Peak _{AP1} (DP)	Peak _{AP2} (DP)	Peak _{AM} (DP)
HPPS	SP85	62.2 ± 0.8a	19.0 ± 0.5f	18.8 ± 0.3ef	11.60±0.04a	65.2 ± 0.5a	2485±62ef	15.3 ± 0.0abc	44.4 ± 0.4abc	5337±115c
	SP45	56.3 ± 0.6bc	23.6 ± 1.0cde	20.2 ± 0.3def	11.02±0.09bc	57.7 ± 0.6bcd	2729 ±217de	15.5 ± 0.2abc	43.0 ± 0.8a-d	4715±203d
	SP98	57.3 ± 1.1b	22.3 ± 0.7e	20.4 ± 0.4c-f	10.80±0.16bcd	58.3 ± 1.6b	3239±11bc	15.2 ± 0.2abc	41.9 ± 0.9cd	6646±136a
	SP07	53.2 ± 0.6cd	25.9 ± 0.6abc	20.9 ± 0.0cd	10.76±0.03cd	56.6 ± 0.7b-e	3305±56bc	15.8 ± 0.6ab	45.4 ± 0.2a	6415±180a
	SP63	52.7 ± 0.9d	26.7 ± 0.8ab	20.7 ± 0.1cde	10.81±0.05bcd	57.6 ± 0.3bcd	3060±40c	15.8 ± 0.1ab	44.0 ± 0.9abc	5379±419c
	SP69	52.5 ± 0.4d	26.9 ± 0.9a	20.7 ± 0.5cde	10.89±0.01bcd	56.3 ± 0.2b-e	3436±74b	16.0 ± 0.3a	44.5 ± 0.1ab	5818±245b
	SP30	57.3 ± 1.3b	24.2 ± 0.9b-e	18.6 ± 0.4f	11.19±0.01ab	58.1 ± 1.3bc	3462±37b	15.4 ± 0.2abc	42.4 ± 0.2bcd	6582±15a
LPPS	SP87	52.6 ± 0.3d	25.3 ± 0.9abc	22.1 ± 0.6c	10.05±0.22f	54.0 ± 0.3e	3023 ±127cd	15.0 ± 0.2abc	42.0 ± 0.6bcd	5491 ±154bc
	HP22	49.2 ± 1.8e	24.9 ± 0.3a-d	25.9 ± 1.5b	10.30±0.02ef	54.8 ± 0.2de	2586±56ef	14.9 ± 0.3abc	40.9 ± 0.6d	4416±75d
	SP86	54.6 ± 0.1bcd	25.1 ± 0.4a-d	20.4 ± 0.5c-f	10.62±0.29cde	54.9 ± 1.3de	3004±81cd	14.9 ± 0.3abc	42.3 ± 0.1bcd	5371±66c
	HP210	48.6 ± 1.6e	23.6 ± 0.8cde	27.8 ± 0.8a	10.63±0.26cde	57.8 ± 1.4bcd	2329±85f	14.8 ± 0.7bc	40.9 ± 1.8d	4472±167d
	SP100	55.1 ± 0.8bcd	22.8 ± 0.7de	22.1 ± 0.1cd	10.60±0.11cde	55.2 ± 0.8cde	3151 ±143bc	14.6 ± 0.1c	40.6 ± 0.5d	5264±34c
	SP34	53.1 ± 1.1cd	26.0 ± 0.9abc	20.9 ± 0.2cd	10.52±0.09de	55.2 ± 0.6cde	3842±21a	15.2 ± 0.1abc	42.5 ± 0.4bcd	6572±5a

^a Values are mean ± SD. Values with different letters in the same column indicate significant difference at $p < 0.05$.

^b CLD profiles of debranched starch contain three peaks: short AP (AP1, DP 6–36), long AP (AP2, DP 37–200) and AM chains (DP > 200); PeakX: the DP of the peak of the fraction X; ACLX: the average CL of the fraction X; RCX: the relative content of the fraction X; DP: degree of polymerization.

Table 3
Granule size distribution, relative crystallinity and FTIR ratios of potato starches.^a

Group	Sample	D [3,2] (µm) ^b	D [4,3] (µm) ^b	Small granules (%)	Large granules (%)	Crystallinity (%)	1022/995	1045/1022
HPPS	SP85	21.25±0.01b	39.55±0.01c	4.14 ± 0.00g	95.86±0.00d	24.57±0.20ab	0.603±0.002bc	0.805±0.003abc
	SP45	20.86±0.02c	39.76±0.03b	3.62±0.00j	96.38±0.00a	21.78±0.00 cd	0.582±0.001e	0.828±0.004ab
	SP98	19.45±0.02g	38.47±0.06e	4.25±0.02e	95.75±0.02f	22.67±1.86bc	0.610±0.005b	0.777±0.018cde
	SP07	19.79±0.27f	36.34±0.01g	4.18±0.00f	95.82±0.00e	19.77±1.05de	0.594±0.000cd	0.789±0.010a-d
	SP63	22.59±0.01a	41.29±0.00a	3.81±0.00h	96.19±0.00c	21.47±1.50cd	0.603±0.001bc	0.769±0.000cde
	SP69	19.61±0.02fg	37.51±0.02f	4.27±0.00e	95.73±0.00f	19.62±0.16de	0.599±0.000c	0.775±0.010cde
	SP30	20.55±0.00d	39.46±0.00d	4.15±0.00g	95.85±0.00d	25.81±0.09a	0.599±0.000c	0.768±0.025cde
LPPS	SP87	20.13±0.01e	37.44±0.01f	3.76±0.00i	96.29±0.00b	22.73±0.18bc	0.585±0.004de	0.832±0.018a
	HP22	17.76±0.00i	33.70±0.00j	4.91±0.00b	95.10±0.00i	18.54±0.00e	0.602±0.001bc	0.785±0.014bcd
	SP86	19.18±0.03h	35.35±0.04i	3.77±0.00i	96.29±0.00b	23.12±1.20abc	0.587±0.003de	0.807±0.018abc
	HP210	16.99±0.01j	32.22±0.02k	4.78±0.00d	95.22±0.00g	24.62±0.35ab	0.592±0.003cd	0.810±0.010abc
	SP100	16.13±0.00k	31.54±0.01l	5.15±0.01a	94.85±0.01j	19.96±0.31de	0.624±0.008a	0.740±0.007e
	SP34	17.79±0.01i	35.56±0.02h	4.82±0.01c	95.18±0.01h	23.19±0.21abc	0.611±0.001b	0.759±0.002de

^a Values are mean ± SD. Values with different letters in the same column indicate significant difference at $p < 0.05$.

^b D[3,2]: surface-weighted mean diameter; D[4,3]: volume-weighted mean diameter.

GWD1 activity. Phosphate monoesters have been identified on the branched chains of starch, particularly near the branching points of amylopectin (Wikman, Larsen, Motawia, Blennow & Bertoft, 2011). Phosphate groups are regularly not found in amylose supposedly due to the inadequate branching points in amylose. Consequently, variation in SPC exerted a minimal influence on the structure or content of amylose. However, the elevated SPC in HPPS was significantly related to the decreased ACL_{AM}, and when comparing HPPS with LPPS, it is evident that the AC of HPPS group was notably lower than that of LPPS. The AM fraction in HPPS may consist of so-called "amylose-like" molecules, which possess relatively more branches than regular AM and as a consequence are susceptible to a certain degree of phosphorylation (Zhong et al., 2022). In LPPS, the content and CLD of amylose and amylopectin had no correlations with SPC. This suggests that once the

phosphate level decreased below a certain threshold, its impact on starch chain structure diminished.

3.2. Granular size and crystalline structure

The potato starch granules were divided into small granules (granule size ranging from 0.5 to 10 µm) and large granules (granule size from 10 to 100 µm) according to the granule size distribution (Fig. 3). To investigate the effects of SPC on the granular size of potato starches, the relative content of small granules and large granules of HPPS and LPPS groups were analyzed (Table 3). D [3,2] and D [4,3] of the seven HPPS ranged from 19.5 to 22.6 µm and 36.3 to 41.3 µm, and D [3,2] and D [4,3] of the six LPPS ranged from 16.1 to 20.1 µm and 31.5 to 37.4 µm.

Compared with the proportion of small granule in LPPS, the

Table 4
Thermal properties and pasting properties of potato starches.^a

Group	Sample	To (°C) ^b	Tp (°C)	Tc (°C)	ΔH (J/g)	PV (RVU)	HPV (RVU)	BD (RVU)	CPV (RVU)	SB (RVU)
HPPS	SP85	61.99±0.50f	65.34±0.12bc	78.07±0.29abc	14.16 ±0.06ab	671.2 ± 11.8b	221.3 ± 0.8d	449.9 ± 12.6cd	267.0 ± 0.1ef	45.7 ± 0.9ab
	SP45	62.63±0.04de	66.83±0.33ab	71.80±0.50e	15.63±0.50a	814.7 ± 2.8a	218.7 ± 1.3d	596.0 ± 4.0a	250.2 ± 2.8ef	31.5 ± 4.1b
	SP98	60.43±0.03g	64.75±0.55d	78.62±0.09ab	14.62 ±1.62ab	661.3 ± 4.5b	218.1 ± 5.5d	443.3 ± 1.1d	254.8 ± 6.2ef	36.8 ± 11.7ab
	SP07	63.35±0.25bc	66.90±0.32ab	79.89±0.06a	13.83 ±0.01ab	396.6 ± 14.5fg	272.0 ± 0.8bc	124.9 ± 15.1h	320.8 ± 2.4cd	49.1 ± 1.8ab
	SP63	63.13±0.00cd	67.00 ±0.02abc	74.96±2.88cde	15.32 ±0.09ab	550.2 ± 7.7c	301.5 ± 3.5b	248.7 ± 11.1e	344.6 ± 1.6c	43.2 ± 1.9ab
	SP69	63.69±0.08ab	67.18±0.01ab	75.82±1.59bcd	13.43 ±0.52ab	510.3 ± 14.8cd	294.2 ± 5.5bc	216.1 ± 9.3f	340.0 ± 3.8cd	45.8 ± 1.8ab
	SP30	63.49 ±0.01abc	66.86±0.38ab	76.37 ±0.23abcd	12.99 ±0.49ab	461.2 ± 7.1de	335.7 ± 2.0a	125.5 ± 5.1h	388.9 ± 0.8b	53.3 ± 1.3ab
LPPS	SP87	62.34±0.03ef	65.48 ±0.02abc	78.98±0.54ab	13.81 ±1.54ab	713.4 ± 8.3b	205.1 ± 1.1d	508.3 ± 7.3b	243.2 ± 1.1f	38.1 ± 0.0ab
	HP22	62.68±0.01de	64.20±1.95d	74.07±1.58de	12.88 ±0.21ab	451.0 ± 10.3ef	289.1 ± 7.8bc	161.9 ± 18.1g	338.1 ± 7.8cd	49.0 ± 0.0ab
	SP86	62.73±0.04de	65.61 ±0.12abc	74.01±0.15de	14.15 ±1.39ab	693.9 ± 11.4b	215.6 ± 3.6d	478.3 ± 7.8bc	255.8 ± 3.7ef	40.2 ± 0.1ab
	HP210	63.93±0.01a	67.41±0.02a	76.66 ±0.09abcd	13.27 ±0.34ab	385.3 ± 52.2g	349.1 ± 35.8a	36.2 ± 16.3i	442.4 ± 24.1a	93.3 ± 59.9a
	SP100	60.49±0.09g	64.21±0.01d	74.72±0.05cde	12.97 ±0.20bc	410.3 ± 4.3efg	216.8 ± 2.3d	193.4 ± 2.1f	271.2 ± 6.3e	54.3 ± 4.0ab
	SP34	60.53±0.07g	65.76 ±0.02abc	77.82±0.10abc	13.81 ±0.15ab	356.5 ± 5.8g	262.3 ± 3.7c	94.2 ± 2.1h	318.3 ± 4.5d	56.0 ± 0.8ab

^a Values are mean ± SD. Values with different letters in the same column indicate significant difference at $p < 0.05$.

^b To: gelatinization onset temperature; Tp: gelatinization peak temperature; Tc: gelatinization conclusion temperature; ΔH: gelatinization enthalpy. PV: peak viscosity; HPV: minimum viscosity; BD: breakdown; CPV: final viscosity; SB: setback.

Table 5
Texture properties and rheological properties of potato starches.^a

Group	Sample	Hardness (gf)	Cohesiveness	Chewiness	G ^b	G''	G*	tan(δ)	K (Pa·s ⁿ)	n	R ²
HPPS	SP85	19.1 ± 0.9d	0.43±0.01de	3.0 ± 0.1d	91.4 ± 1.1de	19.2 ± 0.3g	93.4 ± 1.1de	0.21 ±0.00cd	56.2 ± 0.6de	0.16 ±0.01ab	0.998
	SP45	22.2 ± 1.8cd	0.44±0.01b-e	3.7 ± 0.6d	49.8 ± 1.5f	14.7 ± 0.1h	51.9 ± 1.4f	0.29±0.01a	21.1 ± 0.4f	0.16 ±0.02ab	0.998
	SP98	20.1 ± 2.7cd	0.41±0.02e	3.0 ± 0.1d	85.0 ± 6.2de	19.0 ± 1.0g	87.1 ± 6.3de	0.22±0.01c	50.1 ± 0.2e	0.20±0.01a	0.999
	SP07	22.6 ± 1.6cd	0.50 ±0.01abcd	7.2 ± 1.2bcd	147.3 ± 2.0bc	27.0 ± 0.1ef	149.7 ± 2.0bc	0.18 ±0.00ef	64.9 ± 1.6cd	0.16 ±0.02ab	0.999
	SP63	30.3 ± 1.1b	0.52±0.01a	11.9 ± 0.5b	157.4 ± 6.3b	29.4 ± 0.6de	160.1 ± 6.3b	0.19±0.00e	72.9 ± 3.0bc	0.20±0.02a	0.999
	SP69	18.9 ± 0.6d	0.52±0.02ab	5.9 ± 0.9cd	230.4 ± 29.7a	38.37±2.7c	233.6 ± 29.7a	0.17±0.01f	102.7 ± 9.2a	0.15 ±0.00ab	1
	SP30	23.7 ± 1.7cd	0.52±0.01ab	8.7 ± 1.7bc	224.0 ± 9.1a	42.5 ± 0.2b	228.0 ± 8.9a	0.19±0.01e	72.7 ± 5.5bc	0.18 ±0.03ab	0.999
LPPS	SP87	20.6 ± 0.2cd	0.46±0.04a-e	3.8 ± 0.7d	62.7 ± 2.7ef	16.4 ± 0.9gh	64.8 ± 2.9ef	0.26±0.00b	25.9 ± 4.3f	0.08±0.02c	0.997
	HP22	25.5 ± 1.0bc	0.53±0.00a	9.6 ± 0.6bc	165.8 ± 5.0b	31.9 ± 0.5d	168.9 ± 5.0b	0.19±0.00e	79.0 ± 3.1b	0.17±0.03b	0.999
	SP86	20.4 ± 1.6cd	0.43±0.02de	3.5 ± 0.9d	64.2 ± 3.6ef	17.4 ± 0.9gh	66.5 ± 3.7ef	0.27±0.00b	24.3 ± 3.0f	0.07±0.00c	0.998
	HP210	46.3 ± 0.9a	0.44±0.05cde	16.2 ± 3.0a	241.2 ± 9.6a	47.1 ± 0.6a	245.79±9.6a	0.20 ±0.01de	59.1 ± 3.1de	0.12 ±0.03bc	0.998
	SP100	23.5 ± 1.9cd	0.48±0.04a-e	7.0 ± 2.6cd	115.6 ± 4.0cd	24.4 ± 0.2f	118.1 ± 4.0cd	0.21 ±0.01cd	78.6 ± 2.2b	0.15±0.01b	0.999
	SP34	23.2 ± 3.2cd	0.51±0.02abc	8.5 ± 1.5bc	145.4 ± 10.4bc	31.8 ± 2.0d	148.9 ± 10.6bc	0.22±0.00c	61.8 ± 0.9cde	0.08±0.04c	0.998

^a Values are mean ± SD. Values with different letters in the same column indicate significant difference at $p < 0.05$.

^b G': storage Modulus; G'': loss Modulus; G*: The complex modulus; tan(δ): the tangent of the phase angle δ between G' and G''; K: The consistency index in the power law model relating shear stress and shear rate; n: The flow behavior index in the power law model, indicating degree of non-Newtonian behavior; R²: The linear regression fit parameter in plots used to determine the power law indices.

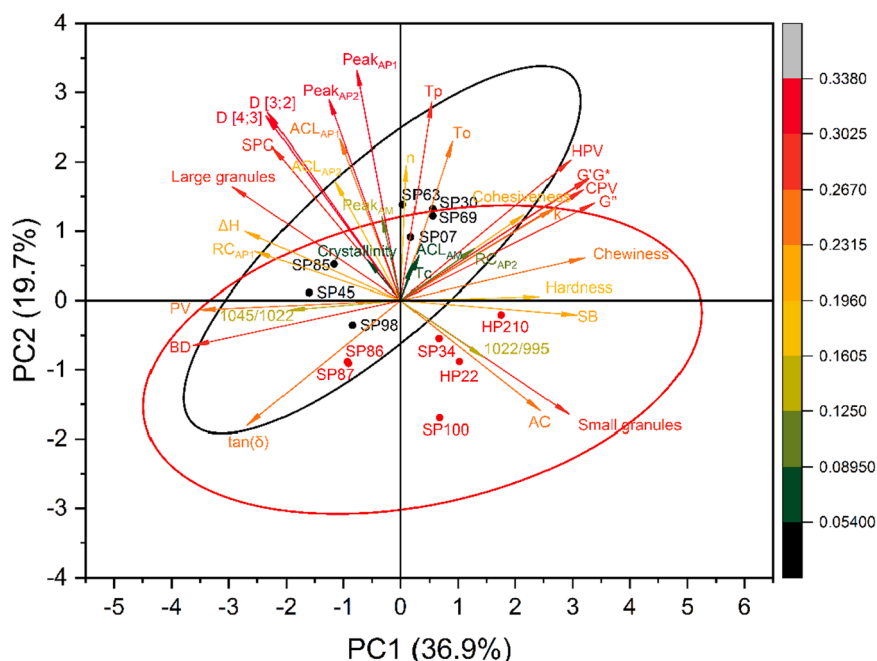


Fig. 1. Principal component analysis (PCA) of potato starches. The samples marked in black are potato starch with high SPC; The samples marked in red are potato starch with low SPC. Sample ID as in Table 1.

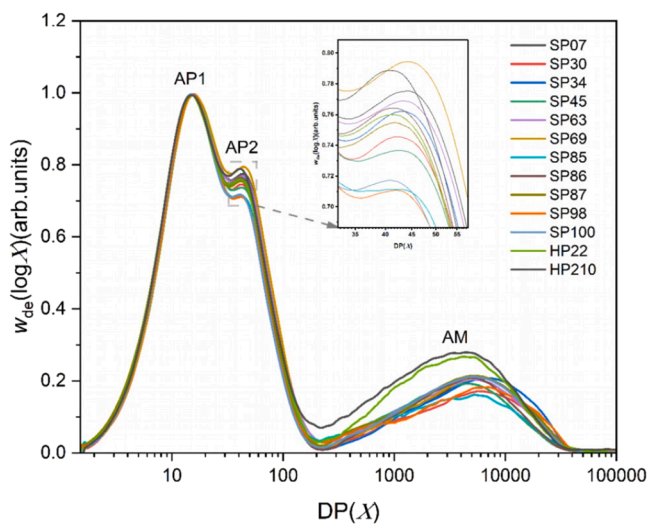


Fig. 2. Chain length distribution profiles of debranched starches determined by SEC. AP1, short amylopectin chains (DP 6–36); AP2: long amylopectin chains (DP 37–100; AM: amylose chains (DP >100); DP: degree of polymerization. Sample ID as in Table 1.

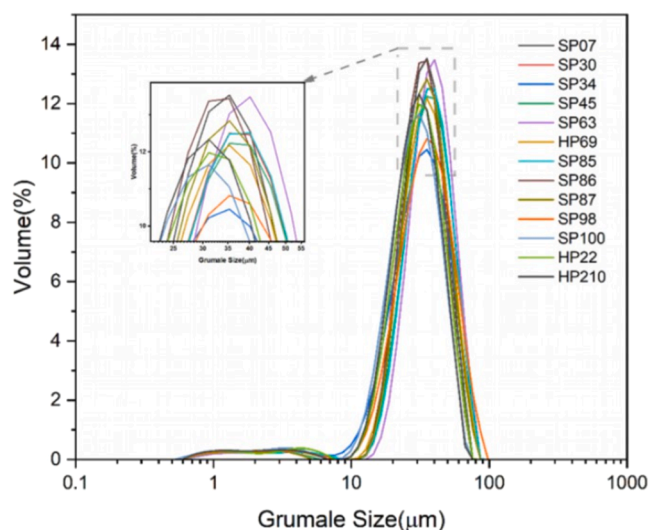


Fig. 3. Granule size distribution of potato starch granules. Sample ID as in Table 1.

proportion of small granules in HPPS decreased by 0.47 % on average. With increased SPC, potato starch granule size tends to increase. Small sized starch granules have been reported to have higher SPC (Chen, Schols & Voragen, 2003; Noda et al., 2005). However, Li et al. (2023) found that small granules exhibit a higher phosphate content per glucose equivalent but a lower phosphate content per unit of starch granule surface. The presence of two distinct starch granule sizes are typical in endosperm of temperate cereals such as wheat, due to the existence of small so called B-type starch granules appearing at later developmental stage (Langeveld, van Wijk, Stuurman, Kijne & de Pater, 2000). It was possible that in potatoes, a larger number of phosphate groups would be more conducive to the turnover process where smaller starch granules develop into larger ones.

The WAXS patterns of the selected potato starches (Fig 4) documented that all samples had the typical B-type crystalline polymorph with 7 peaks at diffraction angles (2θ) 5.78°, 14.81°, 17.25°, 19.51°, 22.34°, 23.97° and 26.41° typical for potato starch (Poza et al., 2018). The crystallinities varied from 18.0 to 25.8 %, and there was no significant difference between HPPS and LPPS. The surface structural order as deduced by ATR-FTIR was performed to provide information on chain folding and the ratio of ordered to amorphous fractions at the surface of the granules (Sevenou, Hill, Farhat & Mitchell, 2002; van Soest, Tournois, de Wit & Vliegenthart, 1995). The ratio of absorbance at 1045/1022 cm^{-1} indicates the degree of the short-range order structure in relation to the molecular and rotational vibrations of the starch matrix, and the 1022/995 cm^{-1} ratio to estimate the relative contents of amorphous and crystalline parts of the starch. No significant difference in either of these ratios and between the HPPS and LPPS groups were

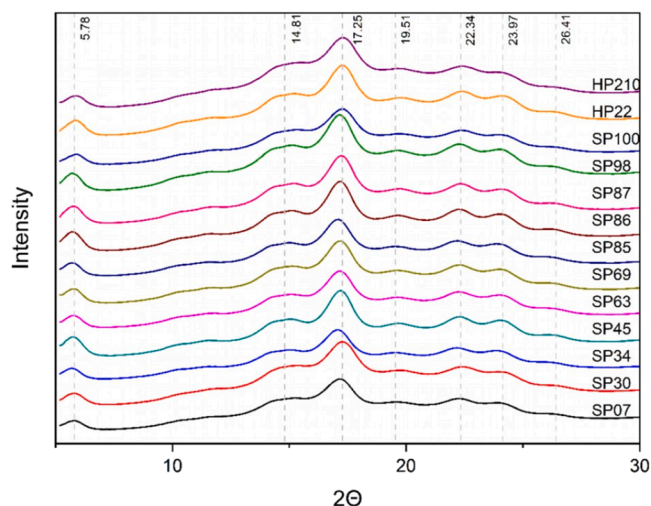


Fig. 4. X-ray diffraction (XRD) spectra of potato starches. Sample ID as in Table 1.

found (Table 3). However, separate analysis of the HPPS and LPPS demonstrated that, within each group, as the SPC increased, the surface structure of starch became more ordered, indicating that increase phosphate monoesters can contribute to a higher structural surface ordering of starch granules.

3.3. Thermal and pasting properties

The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and the enthalpy of gelatinization (ΔH) of all the potato starches as determined by DSC are shown in Table 4. The peak gelatinization temperatures of the potato starches varied from 64.2 to 67.4°C (Table 4). No significant differences in T_o , T_p and T_c were found between the HPPS and LPPS groups. However, the ΔH in the HPPS group was higher than that of the LPPS group. The T_p did not correlate with either SPC or AC. However, the higher enthalpy values observed within the HPPS group shows that high SPC requires more energy to disrupt the starch crystalline structure, suggesting a more stable, or higher fraction of crystalline structures. This finding aligned with the ATR-FTIR data (Table 4). Lu et al. (2012) also found that the T_p did not follow any trend with AC or SPC, but the ΔH increased with increasing SPC. However, other studies have also revealed that a higher degree of phosphorylation tends to disrupt the crystalline structure of starch (Karim et al., 2007; Wischmann et al., 2005). Concurrently, the degree of phosphorylation can influence the proportion of amylose and the CLD of amylopectin (Karim et al., 2007; Wischmann et al., 2005), both of which are factors that can potentially affect the gelatinization temperature and enthalpy. This might explain the absence of a consistent pattern in the changes observed in gelatinization temperature and enthalpy in this study.

The pasting parameters peak (PV), cold paste (CPV), hot paste (HPV) viscosities and breakdown (BD, =PV - HPV), and setback (SB, =CPV - HPV) parameters were calculated from the RVA profile (Table 4). PV indicates the swelling extent or water-binding capacity of fully swollen starch granules, HPV and BD relate to the disintegration of swollen granules and paste stability, respectively, and the CPV and SB indicate gelation of the starch paste, reflecting a starting point of retrogradation (Bao & Corke, 2002). There was significant difference in PV between the HPPS and LPPS groups. The average PV of the HPPS was 580.4 RVU and the average PV of six the LPPS was 501.3 RVU. No difference in the range and average values of CPV, HPV, BD, and SB was found between the HPPS and LPPS groups. However, these parameters exhibited interesting trends within their respective groups. In the HPPS group, PV and BD were both positively correlated with SPC, whereas HPV, CPV, and SB were negatively correlated SPC increased. Increased SPC notably

enhanced the swelling capacity as deduced by increased PV. However, SPC significantly decreased CPV and BD, indicating a reduction in the short-term gelling. Within the LPPS group, PV, CPV, HPV, BD, and SB did not systematically change with SPC. Conversely, HPV and CPV showed an increasing trend with higher AC. This indicated that, in the LPPS group, SPC was not the dominating factor affecting the viscosity characteristics of the potato starches. Instead, the AC had a more significant impact on pasting properties. As expected, increased AC led to a decrease in PV and BD of potato starch, while CPV and SB increased. Hence, elevation of AC diminished the swelling capacity of potato starch and enhanced the gelling ability of the starch gels.

3.4. Texture and rheological properties

In the HPPS group, no discernible pattern was observed in the hardness variation of the starch hydrogels. However, within the LPPS group, the hardness of starch hydrogels increased with an increased AC. Amylose molecules restrict starch swelling during gelatinization, thereby limiting starch leaching and resulting in a harder texture of the starch hydrogels (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016). The parameter chewiness provides information on the energy needed for mechanical disintegration prior to swallowing and the parameter cohesiveness describes the resistance of disrupting the internal structure of a given starch gel, and these two characteristics typically exhibit a strong correlation with viscosity (Lau, Tang & Paulson, 2000; Li & Gilbert, 2018). The cohesiveness and chewiness exhibited the similar trend as CPV in the RVA results, i.e. high SPC leading to a low cohesiveness and chewiness, whereas high AC leading to low cohesiveness and chewiness of the starch gels.

Rheological properties (Fig. 5, Table 5) are vital properties of starch gels. Two typical rheological experiments are usually applied to starch; static rheology for which the major parameters are the consistency coefficient (K) and the fluid index (n), and dynamic rheology, for which storage (elastic) modulus (G'), loss (viscous) modulus (G''), and loss angle tangent ($\tan \delta = G'' / G'$) are derived (Chen et al., 2022). Moreover, the shear-thinning behavior of a given gel system is estimated by fitting the viscosity profiles (Fig. 5E,F) to the Power Law equation. All samples presented shear-thinning behavior, indicated by a decrease in viscosity and an increase in shear stress with increased shear rate. The minimum and maximum values of K among the potato starch gels were 21.1 Pa·sⁿ and 102.7 Pa·sⁿ, corresponding to sample SP45 and SP69, respectively, both of which were high phosphate starches. The K presented no systematic variation with SPC. The flow index (n) was <1 (Table 5), suggesting that the gels exhibited shear-thinning behavior. Compared with HPPS, the n of LPPS decreased significantly, suggesting that more pronounced shear thinning behaviors in this group. Specifically, the n of the HPPS group ranged from 0.15 to 0.20 and the n of LPPS ranged from 0.07 to 0.17. The intermolecular aggregates can be readily formed in starches with high phosphate content through hydrogen bonding and polymer entanglements, hence, these starches displayed high viscosity at low shear rates. Such aggregates are disrupted rapidly with the application of high shear rates.

The storage moduli, G' , was notably higher than the loss moduli, G'' , for all starches and they both exhibited frequency-dependent behavior (Fig. 5A, Fig. 4B). $\tan \delta$ indicates whether the material has a solid (elastic) or fluid (viscous) characteristic. For $\tan \delta < 1$, the material is regarded to exhibit more elastic than viscous behaviour (Fig. 5C). The complex modulus, G^* , indicates a solid-like property, indicating the ability to counteract the compression deformation and the mechanical strength (Huang, 2018), as shown in the curve of G^* (Fig. 5D). The values of G' , G'' , and G^* at 38 rad/s for gels are also presented in Table 5. The variation of G' , G'' , and G^* within the HPPS and LPPS groups, showed no significant difference, which is consistent with the RVA results. However, interestingly, similar to the RVA results, the rheological data showed different trends within their respective populations. Within the HPPS group, increased SPC was significantly related to the decreased

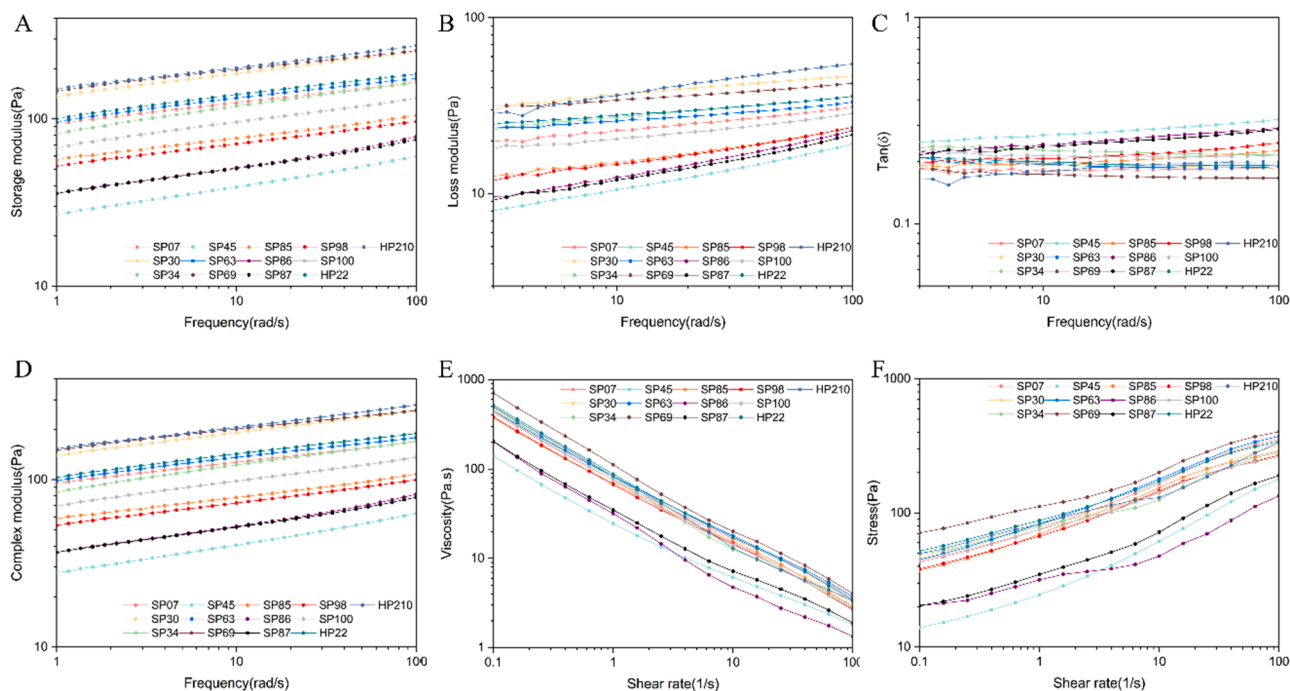


Fig. 5. Rheological properties of potato starches. (A) Elastic modulus. (B) Viscous modulus. (C) The tangent of the phase angle δ between G' and G'' . (D) Complex modulus. (E) Viscosity profiles over shear rate of 0.1–100 1/s. (F) Shear stress profiles over shear rate of 0.1–100 1/s. Sample IS as in Fig. 1.

G' from 230.4 Pa to 49.8 Pa, and decreased G'' from 42.5 Pa to 14.7 Pa demonstrating that, with increased SPC, both the viscosity and elasticity of a starch gel decreases. A similar trend for G^* ranging from 51.9 Pa to 233.6 Pa was observed. Thus, the resistance to deformation of a starch gel also decreased with increased SPC. Thygesen et al. (2003) found a weak negative correlation between the SPC and retrogradation. Such results indicate that high SPC inhibits retrogradation, leading to the formation of weaker starch gel networks. In the LPPS group, the storage modulus, G' , ranged from 62.7 Pa to 241.2 Pa, the loss modulus, G'' , from 16.4 Pa to 47.1 Pa, and the complex modulus, G^* , ranged from 64.8 Pa to 245.79 Pa. Although the distribution range was similar to that of the HPPS group, G' , G'' , and G^* did not vary with changes in SPC, but increased significantly with increased AC. This suggests that, when SPC is below a certain threshold level, amylose has a greater impact on gel formation, and below this threshold a higher proportion of amylose results in a stronger starch gel network.

3.5. Principal component analysis (PCA) and correlation analysis

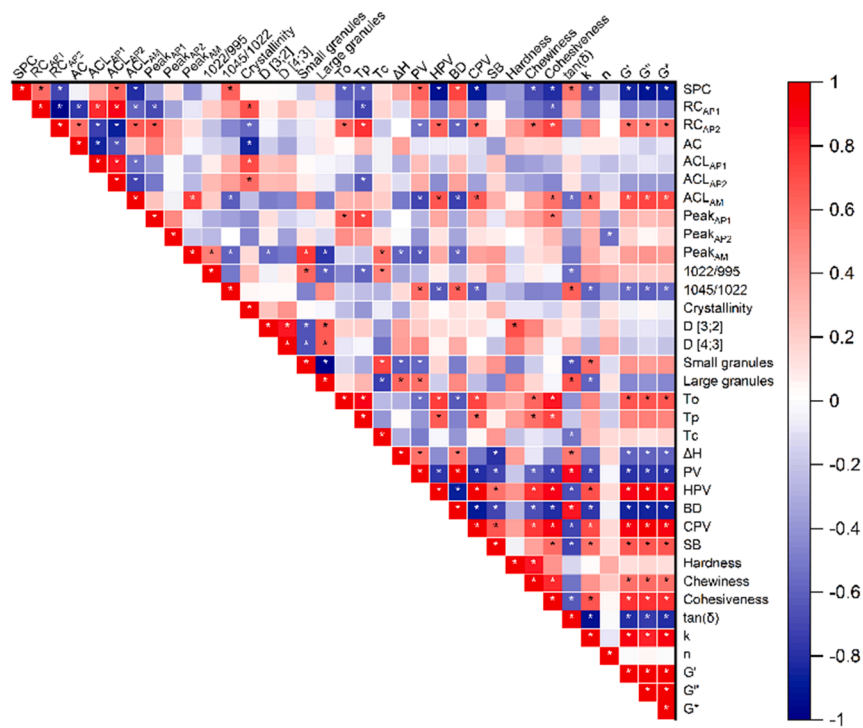
The first and second principle components explained 36.9 % and 19.7 % of total variance and they together explained 56.6 % of the total variation (Fig. 1). In the PCA landscape, close proximity indicated similarities in the analyzed parameters (Holtekjølén, Uhlen, Bråthen, Sahlström & Knutsen, 2006). The HPPS group was primarily distributed in the two north quadrants, while the LPPS were mainly concentrated in the two south quadrants, hence separated by the PC1 (Fig. 1), suggesting major differences in the structural characteristics, and influencing factors between these two populations. Based on the PC2 axis, the HPPS group was characterized by having high SPC, and high chain length parameters $Peak_{AP2}$, $Peak_{AP1}$, ACL_{AP1} and larger particle size, and the LPPS was characterized by having high AC and smaller particle size. Setting 700 ppm SPC as the critical value, when the SPC was greater than 700 ppm, the SPC played a dominant role in determining the starch functional properties. When the SPC was <700 ppm, the AC takes precedence in influencing starch characteristics. Based on the PC1 axis, as PV and BD increased, the data points moved further to the left, resulting in a negative PC1 score. Conversely, as HPV, CPV, SB, G' , G'' , G^* ,

hardness, chewiness, and cohesiveness increased, the data points shifted further to the right, yielding a positive PC1 score. SPC clustered in the north-west quadrant clustering together with PV and BD, while AC was situated in the south-east quadrant clustering with HPV, CPV, SB, G' , G'' , G^* , hardness, chewiness, and cohesiveness. This demonstrates that SPC and AC have opposing effects on the pasting properties, texture properties, and rheological properties of starch, in agreement with the previous reports (Blennow et al., 2005; Karim et al., 2007; Noda et al., 2004; Singh, McCarthy & Singh, 2006; Wischmann et al., 2005). Furthermore, SPC was clustered with ACL_{AP1} , ACL_{AP2} , $Peak_{AP1}$ and $Peak_{AP2}$, which might confirm that the phosphate groups are primarily bound to amylopectin. C-6 monoesters is the primary component of the phosphate esters, and these are predominantly bound to the amylopectin fraction (Li et al., 2023; Wikman et al., 2014), which is in agreement with the findings in this study.

Correlation analysis was conducted separately for the HPPS potato starches (Fig. 6A) and LPPS potato starches (Fig. 6B) and all samples combined (Fig. S1). The correlation data for all samples showed that SPC had a significant positive correlation with RC_{AP1} , ACL_{AP1} and ACL_{AP2} , and a significant negative correlation with AC, which agreed with the PCA results (Fig. 1). Mikkelsen et al. (2004) postulated that the majority of the phosphate groups are preferentially bound at locations far from branch points, suggesting that longer AP chains have the potential to accommodate a greater number of phosphate groups. This hypothesis aligns with the findings, further supporting the notion that longer chains are better suited to harbor more phosphate monoesters. Consistent results were observed in the correlation analysis of the HPPS and LPPS groups separately. However, for the correlation results of the LPPS group, the correlation between SPC and the distribution of branch chain length was not significant. Therefore, it is speculated that the increase in SPC was mainly due to the increase in the length and proportion of branch chains, while the proportion of amylose decreased.

In terms of structural and physicochemical properties, there were distinct trends of variation between the HPPS and the LPPS groups, making the overall correlation analysis less accurate. Hence, the primary focus was directed towards the correlations found within each group separately. In each group, significant positive correlation existed

A



B

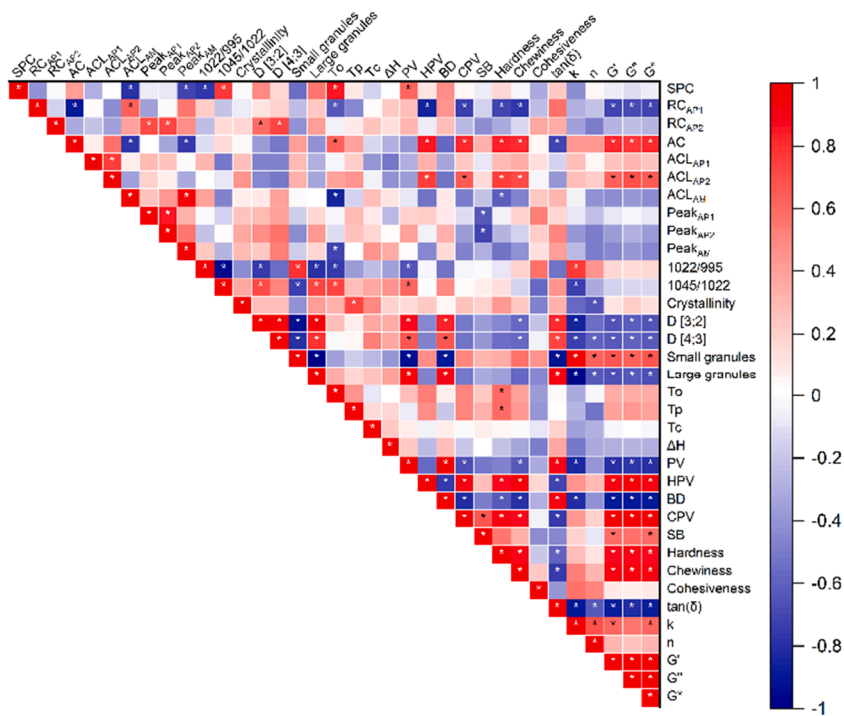


Fig. 6. Correlation analysis of structural and functional properties of HPPS (A) and LPPS (B).

between SPC and the FTIR ratio $1045/1022\text{ nm}^{-1}$, indicating that the presence of more phosphate groups contributed to a higher structural surface ordering of the starch granules. In rice starch, the ACL_{fb1} has shown a positive correlation with crystallinity and the relative content of the double helices (Zhong et al., 2021). Therefore, a higher SPC is associated with a longer ACL_{AP1} of starch, indicating that more chains are involved in the formation of double helices, which may appear in a crystalline order, thereby resulting in a high structural ordering.

Within the HPPS group, neither SPC nor AC was related to the starch granule size. However, in the LPPS group, a positive correlation was observed between SPC and both RC_{AP2} and starch granule size, while AC showed a negative correlation with these parameters. As mentioned earlier, the average particle size of HPPS was larger than that of LPPS, indicating that higher SPC and a greater number of AP2 chains can contribute to the formation of larger starch granules at low SPC. Nonetheless, in this process, amylose seemingly played an opposing role, limiting the enlargement of starch granules. When SPC exceeded a certain threshold, the effects of SPC, AC, and RC_{AP2} on starch particle size became insignificant. Alternatively, it could also be interpreted that, once potato starch granules reach a certain size, their growth is arrested.

In the HPPS group, SPC was positively correlated with PV and BD, and negatively correlated with HPV, CPV, K, G' , G'' , G^* , chewiness, and cohesiveness. Moreover, AC had no significant effect on these properties. However, in the LPPS group, SPC only demonstrated a significant positive correlation with PV. In contrast, AC had a significant positive correlation with HPV, CPV, G' , G'' , G^* , hardness, and chewiness, while RC_{AP1} exhibited an opposite correlation with AC. The PV is the maximum viscosity obtained from gelatinized, unbroken, starch granules during heating in water, which denotes the water-binding capacity and robustness of the swollen granules (Falade, Semon, Fadairo, Olanjuoye & Orou, 2014). The weak Coulomb repulsion generated by phosphate ions in potato starch causes the amylopectin molecules to unfold. This leads to a higher swelling capacity, larger swollen granules, and higher starch paste viscosity (Noda et al., 2004). The solvation effect caused by the Coulomb repulsion of negatively charged phosphate groups also affects the recrystallization of molecular chains in the starch gel, thereby inhibiting the short-term retrogradation of the starch gel. This is consistent with the finding of Jane et al. (1996) that a high starch phosphorylation tends to inhibit starch retrogradation. For SPC below 700 ppm, amylose played a dominant role in starch gelatinization and retrogradation. Amylose restricts the swelling of starch and reduces PV (Sasaki, Yasui & Matsuki, 2000; Tao, Li, Yu, Gilbert & Li, 2019). After complete gelatinization, during the short-term retrogradation phase, higher AM content leads to an increase in CPV and gel strength due to the faster retrogradation rate of AM compared to AP (Li, Gidley & Dhital, 2019). Additionally, in the LPPS group, ACL_{AP2} showed a significant positive correlation with HPV, CPV, G' , G'' , G^* , hardness, and chewiness, while ACL_{AM} demonstrated a significant negative correlation with these properties. Both short AM chains (AM1) and long AP chains (AP2) have the ability of rapid gelation (Clark, Gidley, Richardson & Ross-Murphy, 1989; H. Singh, Lin, Huang & Chang, 2012), which is consistent with these findings.

4. Conclusion

The SPC, AC, and numerous multi-scale structures and functional properties of 13 natural potato starches selected for a wide range of SPC (ranging from 449 ppm to 1004 ppm) and AC (from 18.8 % to 27.8 %) were investigated. The SPC in potato starch had a positive correlation with the length and proportion of branch chains. HPPS starches tended to have a lower AC, and an increase in AC tended to restrict the expansion of starch granule size. Hence, the characteristics of potato starch are determined by a delicate balance between AC and SPC. This study identifies a critical threshold of 700 ppm for SPC. When the SPC of potato starch exceeds this threshold, phosphate monoesters play a predominant role in determining the functional properties of potato starch.

Conversely, when the SPC falls below this threshold, the influence of AC becomes more prominent. The results of this study will further elucidate the subtle interplay between AC and SPC, providing deeper insights into the relationship between phosphate and amylose content and the structure and function of potato starch. This understanding facilitates the development of potato varieties tailored to various needs through the regulation of AC and SPC.

CRedit authorship contribution statement

Yaqi Hu: Writing – original draft, Investigation, Formal analysis. **Daraz Ahmad:** Writing – original draft, Investigation. **Li Ding:** Investigation. **Haroon Rasheed:** Investigation. **Andreas Blennow:** Investigation. **Jacob Judas Kain Kirkensgaard:** Writing – review & editing, Investigation. **Jinsong Bao:** Writing – review & editing, Writing – original draft, Supervision, 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.carpta.2025.100676](https://doi.org/10.1016/j.carpta.2025.100676).

Data availability

Data will be made available on request.

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