Research paper

Differential roles of C-3 and C-6 phosphate monoesters in affecting potato starch properties

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ABSTRACT

The effects of starch phosphate monoester content (SPC), namely C-3 (C3P) and C-6 phosphate monoesters (C6P), on the starch properties were investigated using four potato starches with varied SPC/C3P/C6P and two non-phosphorylated maize starches with a similar range of amylose content (AC) as controls. The starch property results showed that a higher SPC is associated with lower turbidity, storage and loss modulus after storage, and water solubility, but higher swelling power (SP) and pasting viscosities. These findings suggested that SPC inhibited molecular rearrangement during storage and starch leaching during heating, and enhanced swelling and viscosities due to increased hydration and water uptake caused by the repulsion effect of phosphate groups and a less ordered crystalline structure. Increased SPC also resulted in lower resistant starch (RS) content in a native granular state but higher RS after retrogradation. Pearson correlations further indicated that SPC/C3P/C6P were positively correlated with peak ($r^2 = 0.925$, 0.873 and 0.930, respectively), trough ($r^2 = 0.994$, 0.968 and 0.988, respectively), and final viscosities ($r^2 = 0.981$, 0.968 and 0.971, respectively). Notably, SPC, mainly C3P, exhibited a significantly positive correlation with SP ($r^2 = 0.859$) and setback viscosity ($r^2 = 0.867$), whereas SPC, mainly C6P, showed a weak positive correlation with RS after retrogradation ($r^2 = 0.746$). However, SPC had no significant correlations with water solubility, turbidity and rheology properties, which were more correlated with AC. These findings are helpful for the food industry to select potato starches with desired properties based on their contents of SPC, C3P, or C6P.

1. Introduction

Potato starch has wide applications in food industries, such as a thickener, gelling agent emulsifier, colloidal stabilizer, and water-holding agent [1]. This is attributed to its high content of starch phosphate monoesters (SPC), long amylopectin (AP) chains, high-molecular-weight amylose (AM), and large and smooth granules [2]. Normal potato starches (NPS) naturally contain 15% to 30% of AM and 0.03% to 0.12% of SPC, depending on the botanic sources and growth environments [3,4]. Both contents of AM (AC) and SPC are key factors determining potato starch properties. AM has been well documented to be an inhibitor of starch swelling and is easier to leach out when heating and faster to reassociate to form networks during cooling, contributing to the short-term retrogradation and strong gels [5–7]. In addition, starch with a high AC is highly resistant to thermal treatments and enzymatic hydrolysis, making it a great source of resistant starch (RS) [8,9]. However,

the effects of SPC on the starch properties lack a thorough and detailed investigation.

SPCs are preferably located in the long chains of AP [2,10,11], and longer AP chains are more likely to accommodate more phosphate monoesters [3]. In addition, the majority (~70%) of the phosphate groups in starches are on the C-6 positions of the glucosyl units (C-6 phosphate monoesters, C6P), catalyzed by the glucan water dikinase 1 (GWD1), while the rest are on the C-3 positions (C-3 phosphate monoesters, C3P), catalyzed by the glucan water dikinase 3/phosphoglucan water dikinase (GWD3/PWD) [12–14]. Although low in amount, SPC has remarkable effects on the starch properties [2]. Potato starches with higher SPC had significantly higher swelling power and viscosities [15]. Elevating SPC also led to higher gelatinization temperatures [4,16] and higher clarity but lower gel hardness and adhesiveness [17]. In addition, phosphorus content of potato starch has been reported to be positively correlated with swelling power and peak viscosity (PV), and negatively

correlated with water solubility but had little effect on the retrogradation [18]. Another report found a weak negative correlation between the degree of phosphorylation and retrogradation in potato starches [19]. The different findings in the two studies are likely attributed to the different cultivars and methods (DSC enthalpy *vs.* LF NMR relaxation curves) used to characterize retrogradation and how the different genotypes were generated and selected. Notwithstanding, there is limited documentation on the impacts of SPC on *in vitro* starch digestibility, with only a few reported instances [20].

C3P is a unique feature as phosphorylation at this position is rarely seen in nature and is thus expected to have particular functionalities on starch properties [21]. C6P is reported to be preferably located in the amorphous regions, while C3P is equally distributed among the semi-crystalline and amorphous regions of starch granules, respectively [22,23]. In addition, our previous study indicated that a higher SPC led to more long AP chains with DP > 24 and fewer short AP chains with DP ≤ 24. Moreover, potato starch with a higher SPC is less ordered and more flexible in the lamellar structure, attributed to the fact that C3P can prevent helical formation and disorient the parallel alignment of the double-helical lamellae [3]. Based on the above statements, we hypothesized that C3P had unique functionalities on starches compared to C6P. For example, C3P may be the main reason for the high swelling power, as C3 phosphorylation exerts a steric hindrance for the double-helical crystallinity, resulting in a less ordered structure, thereby facilitating the water uptake during heating [3,24]. However, how SPC/C3P/C6P affects the properties of potato starches and whether the modelled difference in C3P and C6P phosphorylation can be translated to validate effects on the starch functional properties remains to be reported.

To reveal how SPC affects the starch properties and to test the hypothesis above, four native types of potato starches with different SPC (3–74 nmol/mg starch), C3P (1–22 nmol/mg starch) and C6P (2–52 nmol/mg starch) were selected as model systems with two non-phosphorylated maize starches as controls to blank out of the effect of AC. Moreover, their multi-structures were already reported in our previous study [3]. The multi-properties, including turbidity, swelling power, and water solubility, pasting and rheology properties, and *in vitro* digestibility, were further investigated, and the relationships between the SPC/C3P/C6P/multi-structures and these properties were established using Pearson correlation and thoroughly discussed. These findings can provide insights into choosing proper potato starches with desired properties in food and other industries from specific SPC or distributions.

2. Materials and methods

2.1. Materials

Four native potato starches with different SPC (3–74 nmol/mg starch), C3P (1–22 nmol/mg starch), and C6P (2–52 nmol/mg starch) were selected in this study. The four potato starch types were extracted from Dianella wild type (normal potato starch, NPS), Dianella RNAi GWD1 line (asGWD, low phosphate potato starch, LPPS), Dianella dual RNAi starch branching enzyme I and II line (asSBE, high phosphate potato starch, HPPS), and Kuras RNAi GBSS line (waxy potato starch, WPS) as described before [25,26]. The SPCs for the WPS, NPS, LPPS and HPPS were 25.0, 24.0, 3.1 and 73.8 nmol/mg starch, respectively, with C3Ps being 3.7, 9.9, 0.9 and 21.8 nmol/mg starch, and C6P being 21.3, 14.1, 2.2 and 52.0 nmol/mg starch, respectively. Two types of maize starch with different ACs (0.1% and 30.5%) but no detectable phosphate monoesters were used as controls. The molecular, helical, crystalline, and lamellar structural parameters of these six starch samples have already been reported in our previous study [3] and summarized in Table S1. Pancreatin (Cat. No. P7545, activity 8 × USP) and amyloglucosidase (Cat. No. A7095, activity 260 U/mL) were bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

2.2. Turbidity

The turbidity of potato and maize starches was measured as described previously with a slight modification [27]. Starch (10 mg) was suspended in 1 mL of MilliQ water, and then the starch suspension was heated at 100 °C for 1 h in an Eppendorf Thermomixer with consistent shaking at 500 rpm. After cooling to room temperature, absorbance at 640 nm of these gelatinized starches was measured directly and every two days after storage at 4 °C until the fifth day.

2.3. Swelling power and water solubility

Swelling power and water solubility were determined as described in our previous report [28] with some modifications. Briefly, 20 mg of starch was suspended in 2 mL MilliQ water and incubated at 95 °C for 1 h in an Eppendorf Thermomixer under 500 rpm of shaking. After cooling to room temperature, the swollen materials were recovered by centrifugation at 13,000 *g* for 10 min, and the supernatant was carefully collected and dried at 120 °C overnight. The swollen materials and dried supernatants were weighed and denoted as m_1 and m_2, respectively. Swelling power and water solubility were calculated:

$$\text{Swelling power (g/g)} = \frac{m_1 - 20}{20}$$

$$\text{Water solubility (\%)} = \frac{m_2}{20} \times 100$$

2.4. Pasting and rheological properties

The pasting properties were determined from 1 g of starch suspended in 20 mL water (5%, *W/V*) using a Rapid Visco Analyzer (RVA, Newport Scientific, Warriewood, Australia) with ICC Standard Method No. 162 as reported in [29]. The procedure was: holding at 50 °C for 1 min, followed by heating to 95 °C over 3 min 42 s, then holding at 95 °C or 2.5 min, final cooling to 50 °C over 3 min 48 s and holding at 50 °C for 2 min, with an initial mixing speed of 960 rpm for 10 s, followed by 160 rpm for the rest of time. The recorded pasting parameters were collected using Thermocline software for Windows (Perten Instruments, Hägersten, Sweden).

The dynamic rheological analysis of starch gels prepared by RVA as above was analyzed using a Discovery HR-3 Rheometer (TA Instruments, New Castle, USA) at room temperature. The starch gels were measured directly and after storage at 4 °C for 7 days. Frequency sweeps were carried out from 0.01 to 100.00 Hz. The storage and loss modulus were selected at 1 Hz in the linear viscoelastic region.

2.5. In vitro digestibility

The *in vitro* digestion of native granular, and retrograded starch (99 °C heating for 1 h followed by storage at 4 °C for 1 day) was analyzed using the Zhang's method as reported [30] with some modifications. In brief, starch samples (100 mg) were suspended in 15 mL sodium acetate buffer (0.1 M, pH 5.2) and equilibrated at 37 °C for 30 min under 500 rpm of shaking (Eppendorf Thermomixer). Then, pancreatin (150 × USP) and amyloglucosidase (3.5 U) in 2.5 mL sodium acetate buffer were added to initiate the hydrolysis. Aliquots (100 μL) were taken out after 20 and 120 min and immediately mixed with 1 mL of 96% aqueous ethanol to terminate the reaction. The concentration of the released glucose was determined spectrophotometrically with the GOPOD kit (K-GLUC; Megazyme, Wicklow, Ireland) at 510 nm. Rapidly digestible starch (RDS) digested within the first 20 min, slowly digestible starch (SDS) digested within 20 to 120 min, and RS undigested were quantified using following equations.

$$RDS\ (\%) = (G20 - FG) \times 0.9$$

$$SDS\ (\%) = (G120 - G20) \times 0.9$$

$$RS\ (\%) = 100 - RDS - SDS$$

where G20 (%) is glucose released after 20 min, G120 (%) is glucose released after 120 min, and FG (%) is free glucose in the starch samples.

2.6. Statistical analysis

All experiments were performed at least in triplicates, and the results were expressed as means ± standard deviations. Pearson's correlations and Variance (ANOVA) analysis with Duncan's test were conducted using SPSS 25.0 software (SPSS, Inc. Chicago, IL, USA).

3. Results and discussion

3.1. Effects of starch phosphate monoesters on the turbidity

Turbidity is one of the most important starch paste properties, particularly in food applications [31]. Turbidity changes in the starch paste during storage are an indication of starch retrogradation [32,33], which is a recrystallization process caused by the hydrogen bond formation between hydroxyl groups on adjacent AP and/or AM molecules during cooling and storage [34]. The turbidity changes for potato and maize starch gels stored at 4 °C for 0 to 5 days, as shown in Fig. 1, indicated that increased AC in maize starch systems (comparing waxy maize starch (WMS) and normal maize starch (NMS)) was related to higher turbidity for fresh gels. AM is regarded as the leading provider of short-term retrogradation during cooling (several hours to 1 day), while AP is responsible for the long-term retrogradation during storage (several days), as AM with a smaller molecular weight undergoes more rapid recrystallization than AP [35]. Interestingly, there was no significant difference in the turbidity for fresh potato starch gels with increased AC (comparing WPS and NPS) or SPC (comparing LPPS and HPPS), indicating the complex effects of the presence of both AC and SPC.

After storage at 4 °C, NMS showed the most significant increases in turbidity from 0 to 5 days, and its turbidity at each point was significantly higher than those of WMS ($P < 0.05$), indicating faster molecular rearrangement and higher long-term retrogradation of NMS due to its higher AC. The second rapidly increased turbidity with increasing storage time was observed in LPPS, followed by the HPPS, suggesting a slower molecular rearrangement for potato starches with higher SPC, consistent with our previous report [19]. WPS and NPS presented a similar slow increase in the turbidity in the first three days; however,

after five days of storage, NPS with a higher AC had a significantly higher turbidity than WPS ($P < 0.05$).

3.2. Effects of starch phosphate monoesters on the swelling power and water solubility

The extent of swelling and solubility reflect the strength of interactions between starch chains within the amorphous and crystalline regions [36]. A 95 °C (close to the temperature for a daily life boiling cooking situation) of heating was selected to present the full swelling and water-soluble capacity of these starches. Swelling and water solubility of these six starch samples after 1 h of heating at 95 °C, as shown in Fig. 2, varied from different samples, ranging from 21.9 to 63.9 g/g and 1.5%–44.0%, receptively.

All potato starches showed higher swelling powers than maize starches, which is attributed to the high hydration capacity due to the presence of SPC on AP chains. The starch swelling is mainly caused by the water uptake of AP, and the repulsion between phosphate groups on adjacent AP chains weakened the bonding extent either within the amorphous (C6P or C3P) or crystalline region (C3P), thereby increasing the hydration [15,22,37]. An increase in the AC in the maize system led to a lower swelling power and water solubility, consistent with the well-known effects of AM on restricting starch swelling and maintaining the structure of swollen starch granules [6,38,39]. Interestingly, in the potato system, the swelling power and water solubility of NPS were 2.5 times higher but 10 times lower, respectively, than those of WPS. As reported in our previous study [3], WPS and NPS had a similar chain length distribution and SPC content but different phosphate distributions; *e.g.*, NPS (9.9 nmol/mg starch) had higher C3P than WPS (3.7 nmol/mg starch). Hence, the higher swelling power of NPS is likely due to the effects of C3P. Except for the repulsion effect on weakening the binding, C3P can disturb the double helical formation and disorient the parallel alignment of the double-helical lamellae, thereby resulting in a less ordered and loose crystalline lamellae, as NPS presented lower peak scattering intensity (I_{max}) but higher thickness of crystalline lamellae (d_c) than WPS [3]. The less ordered and loose crystalline lamellae can enhance the affinity between water molecules and starch chains in the crystalline region, increasing water uptake [5]. Similarly, increased SPC (especially the C3P) (comparing LPPS and HPPS) also resulted in 2.4 times higher swelling power but 9 times lower water solubility, further indicating that SPC/C3P can enhance the starch swelling but inhibit starch leaching [4,18,20]. These data also demonstrated our hypothesis that C3P mainly contributes to higher swelling.
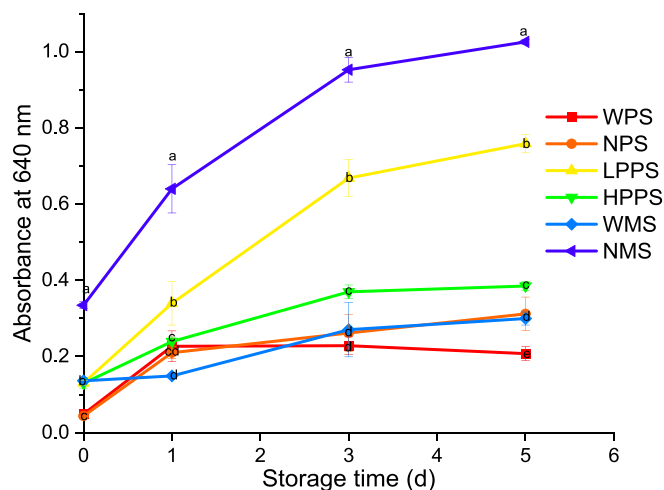

Fig. 1. The turbidity changes for potato and maize starch gels stored at 4 °C for 0 to 5 days (Different lowercase letters for each storage time are significantly different at $P < 0.05$).
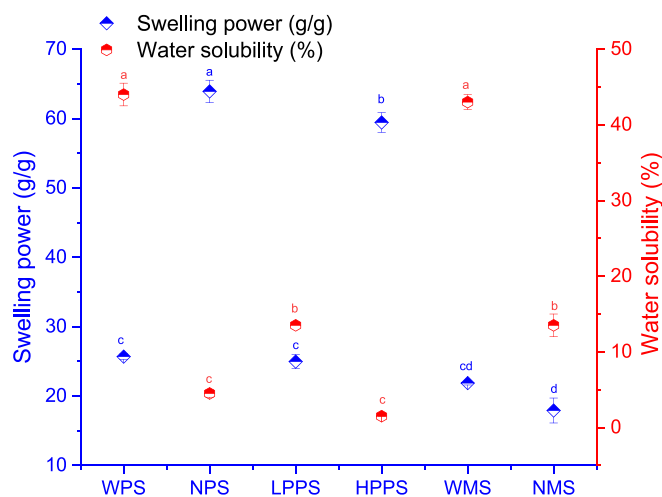

Fig. 2. The swelling power and water solubility of potato and maize starches (Different lowercase letters in the same color are significantly different at $P < 0.05$).

3.3. Effects of starch phosphate monoesters on the pasting properties

Pasting profiles in Fig. 3A indicated that increased SPC (comparing LPPS and HPPS) led to significantly higher viscosities during the RVA analysis, consistent with previous data [25]. However, the effects of elevating AC on the viscosities are less prominent in both potato and maize systems, showing relatively similar patterns between WPS and NPS and between WMS and NMS. To further evaluate the exact pasting differences among these six starches, peak (PV), final (FV), trough (TV), breakdown (BDV), and setback (SBV) viscosities were characterized from the RVA profiles and shown in Fig. 3B.

The swelling degree and granules integrity determine the viscosity of starch paste [40]. PV indicates the swelling extent or water-binding capacity of starch granules, and TV and BDV are related to the disintegration of granules and paste stability, while the FV and SBV reflect short-term retrogradation of starch molecules, mainly AM [34,41]. Increased AC in both potato (comparing WPS and NPS) and maize (comparing WMS and NMS) systems is related to lower PV and BDV, higher FV and SBV but similar TV, suggesting that higher AC resulted in less starch swelling but higher short-term retrogradation degree. AM inhibits swelling and leaches during heating, and these leached amylose molecules rapidly reorganize and form networks during cooling, which contribute to SBV and FV [42]. A negative correlation between the AC and PV or BDV and a positive correlation between the AC and FV or SBV were also reported in previous studies [43,44]. It is noteworthy that increased SPC (comparing the LPPS and HPPS) contributed to significantly higher all these pasting parameters, indicating the effect of SPC on enhancing pasting viscosities [4,20,25]. In addition, almost all potato starches showed higher values in these five parameters than two maize starches due to the presence of phosphate groups.

3.4. Effects of starch phosphate monoesters on the rheology properties

The storage and loss modulus are important rheology properties, which indicate the strength of gels and the viscosity of a starch paste, respectively [28]. The rheology properties of starch gels at 1 Hz in Table 1 indicated that increased AC in both potato (comparing WPS and NPS) and maize (comparing WMS and NMS) systems and higher SPC (comparing LPPS and HPPS) in potato system led to significantly higher storage and loss modulus for fresh gels, suggesting stronger and more stable gel networks in starches with higher AC or SPC during cooling [25], consistent with higher SBV value of HPPS than LPPS (Fig. 3B). After storage at 4 °C for 7 days, NMS and NPS showed increases in both modulus compared to fresh one, and their storage and loss modulus are significantly higher than those of corresponding waxy ones, further demonstrating the AC is an essential contributor to strong and stable gels [6,7]. However, an increase in SPC (comparing the LPPS and HPPS) led to a slower increase in both modulus and the storage and loss modulus of LPPS were 4 and 3 times higher than those of HPPS after 7 days of storage, indicating that SPC is an inhibitor for the formation of strong and stable gel network during storage [19]. Considering the findings from sections 3.1 and 3.3, where an increase in SPC resulted in decreased turbidity after 1–5 days of storage but an increase in SBV, it is plausible that SPC inhibits long-term retrogradation while promoting short-term retrogradation. However, further investigation is required to substantiate this hypothesis.

3.5. Effects of starch phosphate monoesters on the in vitro digestibility

In vitro digestibility, including contents of RDS, SDS and RS using the Englyst method is presented in Table 2. Accordingly, RDS undergoes
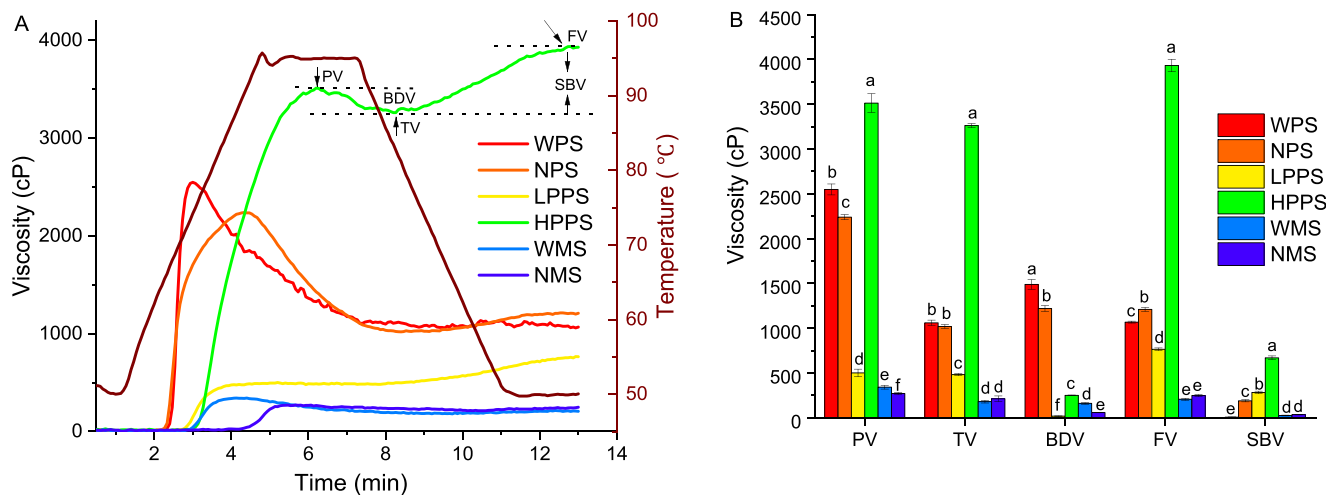

Fig. 3. RVA profiles (A) and parameters (B) of potato and maize starch samples (PV, peak viscosity; TV, trough viscosity; BDV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity. Different lowercase letters for the same viscosity are significantly different at P < 0.05).

Table 1
Storage and loss modulus of potato and maize starch gels stored at 4 °C for 0 and 7 days.[a]

Starch varieties	Fresh gels		Gels stored for 7 days	
	Storage modulus (Pa)	Loss modulus (Pa)	Storage modulus (Pa)	Loss modulus (Pa)
WPS	7.6 ± 0.6[E]	4.1 ± 0.3[D]	6.5 ± 0.5[E]	3.6 ± 0.2[E]
NPS	17.2 ± 0.1[D]	8.5 ± 0.3[C]	62.1 ± 3.9[D]	11.8 ± 0.4[D]
LPPS	54.9 ± 7.8[B]	22.9 ± 2.8[B]	2782.3 ± 749.1[A]	303.7 ± 79.5[A]
HPPS	195.6 ± 10.2[A]	57.1 ± 9.6[A]	673.4 ± 3.0[B]	93.1 ± 4.5[B]
WMS	2.5 ± 0.0[E]	1.9 ± 0.0[E]	3.0 ± 0.2[E]	2.1 ± 0.1[E]
NMS	27.3 ± 0.1[C]	5.8 ± 0.3[D]	320.4 ± 19.2[C]	31.0 ± 2.0[C]

Note: [a] Values are means ± SD. Values with different superscript uppercase letters in the same column are significantly different at P < 0.05.

Table 2

In vitro digestibility of native granular and retrograded potato and maize starch samples.[a]

Starch varieties	Native granular starch			Retrograded starch		
	RDS (%) [b]	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
WPS	22.5 ± 1.0[C]	15.0 ± 1.7[C]	62.5 ± 0.7[D]	71.0 ± 1.3[B]	8.7 ± 0.2[A]	20.3 ± 1.6[B]
NPS	16.8 ± 0.8[D]	12.6 ± 0.8[CD]	70.6 ± 1.7[C]	81.7 ± 1.8[A]	3.0 ± 0.7[B]	15.4 ± 2.6[C]
LPPS	11.8 ± 0.6[E]	6.9 ± 0.8[E]	81.2 ± 1.4[A]	76.8 ± 0.4[AB]	6.1 ± 1.7[A]	17.1 ± 1.3[BCE]
HPPS	13.7 ± 0.5[E]	10.4 ± 0.1[D]	76.0 ± 0.4[B]	65.9 ± 1.0[C]	6.9 ± 0.7[A]	27.3 ± 1.7[A]
WMS	60.0 ± 1.0[A]	33.0 ± 0.0[B]	7.0 ± 1.0[E]	79.3 ± 1.8[A]	1.4 ± 0.6[B]	19.2 ± 1.2[B]
NMS	39.6 ± 0.0[B]	53.1 ± 1.0[A]	7.3 ± 1.0[E]	72.1 ± 1.9[BCE]	6.9 ± 0.1[A]	21.1 ± 1.8[B]

Note: [a] Values are means ± SD. Values with different superscript uppercase letters in the same column are significantly different at $P < 0.05$. [b] RDS, rapidly digestible starch; SDS, slowly digestible starch, RS, resistant starch.

rapid digestion within the first 20 mins in the small intestine, resulting in a high postprandial glycemic response. In contrast, SDS is digested slowly but completely from 20 to 120 mins of digestion, helping to maintain plasma glucose levels over time. RS, on the other hand, resists digestion in the upper gastrointestinal tract but undergoes fermentation in the colon, producing short-chain fatty acids that are beneficial for colonic health [30,45]. The results exhibited an increase in AC in both potato (comparing WPS and NPS) and maize (comparing WMS and NMS) system contributed to higher RS content but lower RDS content for native granular starches. Lower RDS translates to higher SDS + RS, which is related to increased AC, and an important indicator of starch digestion benefitting both postprandial glycemic and colonic health. The effect of AC on increasing SDS or RS is well-documented in previous reports [42,46,47]. However, higher SPC (comparing LPPS and HPPS) is associated with lower RS and higher contents of RDS and SDS, leading to lower SDS + RS content. This is likely due to less ordered structures of HPPS compared to LPPS caused by the C3P, which increases the accessibility of digestive enzymes [48,49]. In addition, all potato starches showed higher RS contents than those of maize starches due to their densely packed outer shell providing a physical barrier for the access/binding of digestive enzymes [50].

After 1 day of retrogradation at 4 °C, a decreased RDS and a slightly increased SDS and RS (also increased SDS + RS content) were observed in the maize system with increasing AC (comparing WMS and NMS). NMS with a higher AC had a faster retrogradation rate, as supported by its higher SBV value (Fig. 3B), turbidity (Fig. 1), and storage and loss modulus (Table 1). These retrograded starches belong to RS3 [51]. However, higher AC in the potato system (comparing WPS and NPS) resulted in opposite results, *e.g.,* significantly increased RDS and decreased RS and SDS (also decreased SDS + RS content), although the NPS also had a higher SBV, storage and loss modulus than WPS. Elevating SPC (comparing LPPS and HPPS) led to significantly higher RS and RS + SDS but lower RDS. These results indicate the complex effects of SPC on retrograded starch digestibility in the presence of AC.

3.6. Pearson correlation analysis between multi-structures and properties of potato and maize starch samples

3.6.1. Pearson correlation between the multi-structures and turbidity

The Pearson correlation was used to analyze the relationships between the multi-structures reported in a previous study [3] and the properties of potato and maize starch samples (Fig. 4).

The turbidity stored at 4 °C for 5 days was significantly correlated with the relative content of single helices, and a less significant correlation with apparent amylose starch (AAC) or relative content of AM (RC$_{AM}$) was also found for turbidity. These results indicated that the turbidity is mainly attributed to AM reorganization, and more AM can form more single helices with endogenous lipids. Higher turbidity of starches with higher AC was also reported in previous studies [27,33]. However, no significant correlations were found between the turbidity and SPC/C3P/C6P, indicating that phosphate monoesters had little effect on the turbidity.

3.6.2. Pearson correlation between the multi-structures and swelling power and solubility

A significant positive correlation was found between swelling power and C3P, and less significantly positive relationships were also observed with SPC and C6P. Hence, our present data indicated that swelling power is largely affected by the SPC, mainly in the form of C3P, further supporting our hypothesis on the effects of C3P [52]. Both the repulsion effects of phosphate monoesters and the less ordered and loose crystalline lamellae caused by C3P led to easier hydration and more water uptake [3,5]. The weak negative correlation between the swelling power and total crystallinity and the significant positive relationship with the thickness of crystalline lamellae (d_c) further supported this statement. The negative correlation between the swelling power and relative content of fa (RC$_{fa}$, DP 6–12) or fb1 (RC$_{fb1}$, DP 12–24) AP chains indicated that starches with fewer AP chains with DP 6–24 had a higher swelling power. Our previous study suggested that AP chains with DP 6–18 mainly contribute to crystallites [53]. In this case, starch with fewer AP chains with DP 6–24 has lower crystallinity, and water can quickly enter the less ordered crystalline regions during heating, thereby improving the water uptake.

Significantly negative correlations were observed between the water solubility and RC$_{AM}$ or average chain length (ACL) of AM (ACL$_{AM}$), indicating that starch with less AC and shorter AM chains has higher water solubility. In addition, the significantly positive correlation between the water solubility and relative content (RC) of AP1 (RC$_{AP1}$, short amylopectin chains (DP 6–36) and degree of branching (DB) suggested that AP chains with DP 6–36 are responsible for starch solubility, and more branches resulted in higher solubility in our study. These results are inconsistent with the well-known responsibility of AM on starch leaching. During heating, at low temperatures, mainly AM leaching occurs, whereas AP leaching increases with increasing heating temperature; especially when the temperature is above 70 °C, starch is fully gelatinized, and its granular structure is disrupted, making AP being the dominant leaching component for normal potato starches [52]. The testing temperature was 95 °C in our study, and thus, AM leached first, but finally, AP was the main component for leaching, especially for WPS and WMS samples with only AP. Higher solubility for starch with lower AC was also reported by comparing waxy and normal wheat starches under the same conditions during heating from 50 to 90 °C [54]. Moreover, it is reasonable that short AP chains (DP 6–36) with a smaller molecular weight mainly contributed to the leaching process. In addition, higher DB indicates more AP molecules and, thus, more AP chains with DP 6–36, thereby resulting in more AP leaching. However, AM is regarded as a swelling inhibitor and granular structure maintainer, and these leached AM molecules can form gel networks to inhibit swelling. Shorter AM chains mean lower molecular weight, making them easier to leach out from those swollen starch granules than longer chains [55]. However, no correlations were found between the water solubility and SPC/C3P/C6P, indicating little effect of phosphate monoesters on the starch leaching. This is reasonable, as phosphate groups are preferably located on the longer AP chains with DP > 24 [3,56].
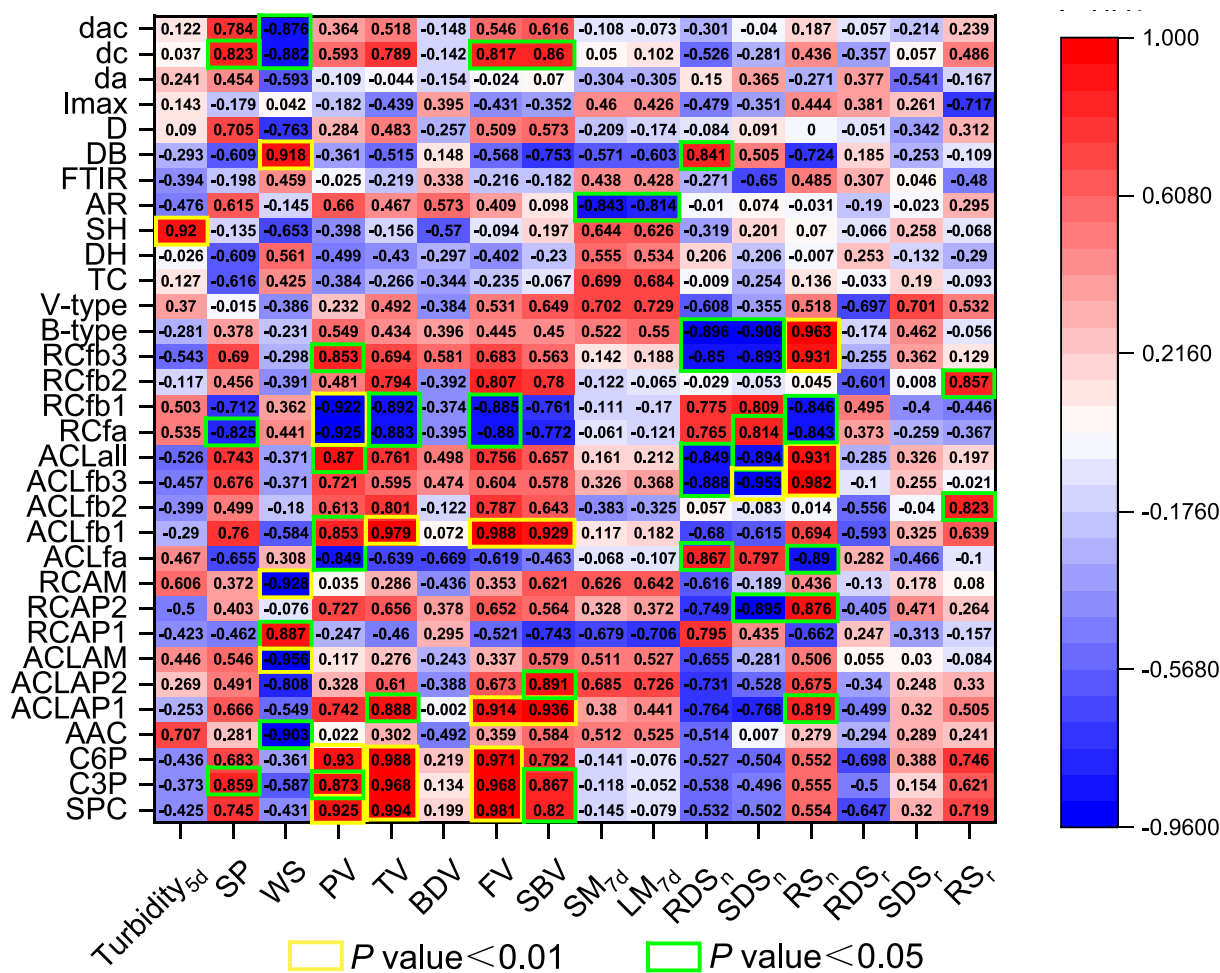
Fig. 4. Pearson correlation analysis between multi-structures and properties of potato and maize starch samples.
Note: SPC, starch phosphate content; C3P, C-3 phosphate monoester content; C6P, C-6 phosphate monoester content; AAC, apparent amylose content; ACL_X, average chain lengths (DP) of fraction X; RC_X, relative content of fraction X; AP1, short amylopectin chains (DP 6–36); AP2, long amylopectin chains (DP 37–100); fa, amylopectin chains (DP 6–12); fb1, amylopectin chains (DP 13–24); fb2, amylopectin chains (DP 25–36); fb3, amylopectin chains (DP >36); TC, total crystallinity; B-type, B-type crystallinity; V-type, V-type crystallinity; SH, relative contents of single helices; DH, relative contents of double helices; AR, relative contents of amorphous region; FTIR, ratio at absorbance 1047/1016; DB, degree of branching; D, Bragg lamellar repeat distance; I_{max}, peak intensity; d_a, thickness of amorphous lamellae; d_c, thickness of crystalline lamellae; d_{ac}, long period distance. SP, swelling power; WS, water solubility; PV, peak viscosity; TV, trough viscosity; BDV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; SM, storage modulus; LM, loss modulus; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; n, Native granular starch; r, retrograded starch.

3.6.3. Pearson correlation between the multi-structures and pasting properties

For pasting properties, PV, TV, FV, and SBV had similar correlations with structural parameters, while BDV had no significant correlation. PV, TV, FV had significantly positive correlations with SPC/C3P/C6P, indicating the dominant effects of SPC on starch pasting properties, compared to AC with no significant correlations. These results are attributed to the fact that the repulsion effect of phosphate monoesters and less ordered structures increased the ability to hydrate and swell, and those monoesters on adjacent AP chains also helped maintain the granular structures, but the increased swelling promoted more leached AM and/or short AP chains with DP 6–36 to form stronger networks [25,57]. Notably, SPC and C3P had a significant correlation with SBV, while C6P only had a weak correlation, indicating that starch with higher SPC, mainly C3P, had higher BDV. In addition, these pasting properties are also positively correlated with the average chain length of AP1 (ACL_{AP1}) and average chain length of fb1 (ACL_{fb1}) but negatively correlated with RC_{fa} and RC_{fb1}, indicating that longer but fewer AP chains with DP 6–36, especially those fa and fb1 chains with DP 6–24, are related to higher PV, TV, FV and SBV. These results are consistent with the negative correlations between the RC_{fa}/RC_{fb1} and swelling

power. PV is negatively correlated with average chain length of fa (ACL_{fa}) but positively correlated with the average chain length of all AP chains (ACL_{all}) and the relative content of fb3 (RC_{fb3}, DP > 36), indicating that starch with shorter fa chains with DP 6–12 and more and longer fb3 chains with DP > 36 had a higher PV. However, these correlations are likely attributed to the effects of SPC on the PV, TV, FV, and SBV, as potato starches with higher SPC had more and longer AP chains with DP > 24 but fewer short chains [3]. SBV and FV had positive correlations with d_c and the average chain length of AP2 (ACL_{AP2}, long amylopectin chains (DP 37–100)), consistent with our previous report on rice starches [6].

3.6.4. Pearson correlation between the multi-structures and rheology properties

Storage and loss modulus after storage at 4 °C for 7 days had the same correlations. They were significantly negatively correlated with the content of the amorphous region and had a weak positive relationship with total crystallinity. These results indicated that starches with higher levels of ordered structure likely had higher storage and loss modulus. Swollen starch granules with integrity are essential for forming a strong gel, and more ordered structures can limit the swelling and

help maintain the integrity, retaining more intact swollen granules during heating [5,7]. Furthermore, they also had weak positive correlations with V-type crystallinity and RC_{AM}, and a negative correlation with RC_{AP1}, suggesting that more AM, especially those formed V-type complexes with lipid, and less short AP chains with DP 6–36, are related to higher storage and loss modulus after 7 days of storage. Higher AC contributed to faster gelation and retrogradation, resulting in stronger gels [6]. Moreover, more AM tends to form more complexes with lipids, and these complexes lead to controlled swelling of the starch granules and maintain the integrity, thus promoting the formation of stronger gel [7]. However, short AP chains with DP 6–36 were responsible for the starch leaching, as discussed in section 3.6.2, and fewer short AP chains likely reflected less leaching and thus maintained the integrity. No correlations were found between the rheology properties and SPC/C3P/C6P, suggesting that phosphate monoesters are not a critical factor in determining the gel strength. A previous study also reported no significant correlation between the storage modulus and phosphate content [17].

3.6.5. Pearson correlation between the multi-structures and in vitro digestibility

The content of RDS and SDS for native starches had a similar correlation, while a reverse correlation was found between the RS and RDS/SDS. ACL_{AP1}, the average chain length of fb3 (ACL_{fb3}), ACL_{all}, RC_{AP2}, and RC_{fb3} had negative correlations with RDS and SDS but positive correlations with RS, while ACL_{fa} and RC_{fa} are both positively correlated with RDS and SDS but negatively correlated with RS. These results indicated that starches with more and longer AP chains, especially those with DP > 36, but less and shorter AP chains with DP 6–12 had higher RS but lower RDS and SDS in their native state. Fa chains with DP 6–12 and fb3 chains with DP > 36 have been suggested to be oriented in the crystalline and amorphous nano-lamellae, respectively [58]. AP chains with DP < 10 do not readily form double-helices with themselves [59] and can be defects in the crystalline regions, while long AP chains can stabilize them [60]. In addition, B-type crystallinity is positively correlated with RS but negatively correlated with RDS and SDS. The effects of ordered structures on increasing RS were well-documented in previous reports [36,61,62]. RDS alone is positively correlated with the DB, indicating that starch with more branches had a higher content of RDS. More branches mean higher AP content but less AM, and waxy starches are considered to be more easily digestible compared to normal starches due to their AP-only feature [63]. RS alone is significantly and negatively correlated with RC_{fb1}, indicating that less short AP chains with DP 12–24 are related to higher RS.

Only RS had a significant positive correlation with ACL_{fb2} and RC_{fb2} for retrograded starches, indicating that more and longer AP chains with DP 24–36 led to higher RS after retrogradation. As discussed in section 3.6.2, short AP chains with DP 6–36 are responsible for the starch leaching when heating, and these leached AP chains, especially those with DP 10–36, can link with each other to form ordered double helices during storage [59]. Notably, the SPC and C6P had a weak positive correlation with RS but a negative correlation with RDS of retrograded starches. This is likely attributed to the higher content of longer fb2 chains in starches with higher SPC/C6P [3].

4. Conclusion

Four types of potato starches with varied SPC/C3P/C6P were selected as models to investigate how starch phosphate monoesters, namely C3P and C6P, affect the multi-properties of potato starches, and whether C3P and C6P have different roles in those properties. HPPS, having higher SPC/C3P/C6P, showed lower turbidity, water solubility, storage and loss modulus after 7 days of storage at 4 °C, higher swelling power and pasting viscosities than LPPS. In addition, HPPS showed less RS in its native granular state (76% *vs.* 81%) but more RS after retrogradation (27% *vs.* 17%) compared to LPPS. Pearson correlation

suggested that SPC/C3P/C6P, combinedly, are significantly correlated with swelling power and pasting properties rather than water solubility, turbidity, and rheological properties, with the latter being more related to AC. In addition, and as hypothesized, C3P and C6P indeed displayed differential roles in affecting potato starch properties. Specifically, C3P played a notable role in enhancing the swelling power and SBV, while C6P likely affected the formation of RS during storage. These findings indicate that, while increasing the C3P can provide an optimal material for making puffed foods, elevating the C6P can be favorable for making RS3, *i.e.* retrograded RS.

CRediT authorship contribution statement

Li Ding: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Blennow:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Yuyue Zhong:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization.

Ethical statement

There are no human or animal subjects in this article.

Declaration of competing interest

The authors declare no conflict of interest in this work.

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