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Preparation of starch phosphate carbamides and its application for improvement of noodle quality

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Abstract: Phosphorylated corn starch derivatives show improved pasting properties compared to native corn starch. In this study, starch phosphate carbamides (SPC) were prepared through a dry process reaction of starch with urea and phosphate salts. The effects of major factors on SPC pasting properties were studied by response surface methodology. Validation results showed that the polynomial quadratic models were adequate for predicting SPC pasting properties. Fourier transform infrared spectroscopy (FTIR) and a Rapid Visco Analyzer (RVA) were used to characterize SPC. RVA results showed SPC had higher peak viscosity, trough, breakdown, setback and final viscosities and lower gelatinization temperature compared to native starch. FTIR showed the characteristic absorption of the ester phosphate group in SPC at 1244/cm. SPC with high viscosity stability were applied to replace part of wheat flour used for making noodles. The best quality noodles were obtained with 12% SPC substitution.

Keywords: starch phosphate carbamides; response surface methodology; pasting properties; noodles

Starch modification such as phosphorylation enhances the positive attributes, eliminates the shortcomings of native starch, and makes the starch paste stable at high acidity and high shear (Kaur *et al.* 2012; Shukri & Shi 2017). Starch phosphate esters render various functionality and extend industrial applications of starch (Xu *et al.* 2017). Starch phosphate esters exhibit enhanced rheological and textural properties of starch paste, and have been used as thickening agents, emulsifiers and stabilizers in the food industry (Shukri & Shi 2017). Starch phosphate esters also show enhanced bioactivities, such as anti-inflammatory and antioxidant properties (Wang *et al.* 2018).

Native and modified starches are mostly heated to make starch paste when used in the food industry. Pasting properties are among the key rheological properties for starches. Pasting behaviour is determined by viscosity changes (Zaidul *et al.* 2007). High stability of paste viscosity is desired when cooking starch for food applications (Abraham 1993). Modified starch paste clarity also affects the appearance of starch based products. Improved paste viscosity and clarity were obtained for starch with varying amounts of phosphate ester derivatives (Singh *et al.* 2004). One of the most common types of phosphorylated starch is starch phosphate carbamides (SPC) developed by Heinze *et al.* (2003). Under solid state conditions, SPC are synthesized by reacting starch with urea and phosphoric acid salts (Solarek 1986). During the reaction, urea is incorporated into the helical structure of amylose and hydrophilic phosphate and carbamide groups are introduced to loosen the starch matrix and substitute the hydroxyl groups (Hebeish *et al.* 1991; Heinze *et al.* 2003; Landerito & Wang 2005). Incorporation of the phosphate group into the starch structure increases the hydration capacity of starch pastes after gelatinization, prevents crystallization and affects the viscosity of the final products (Sung *et al.* 2005). SPC impart improved appearance,

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hygroscopicity, solution transparency, swelling, and viscosity (Jane 2009; Sang *et al*. 2010).

For the preparation of SPC, reaction conditions such as reagent concentration, molar ratio of reagents, and reaction temperature are the most important factors (Passauer & Bender 2017). In this study, phosphate salts were used instead of phosphoric acid. Response surface methodology was used to evaluate the effect of these factors on pasting properties and clarity of SPC. Formation of SPC was confirmed by FTIR. SPC were used to partially replace wheat flour to improve noodle quality.

MATERIAL AND METHODS

Materials and reagents. In this study, as the most common starch, corn starch was purchased from Changzhi Jinze Biotech Co., China. Food grade sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), urea, hydrogen chloride and sodium hydroxide were obtained from Tanjin Kemiou Chemical Reagent Co., China.

Preparation of starch phosphate carbamides. Starch phosphate carbamides (SPC) were prepared by a dry method according to Heinze *et al*. (2003) but phosphoric acid was replaced by phosphate salts since phosphate salts are easier to operate than phosphoric acid. Dry methods do not use any solvent and save the cost of facilities, compared to traditional industrial production that adds phosphorylating agents to the starch suspension or reaction in organic solvents (Tian *et al.* 2018). Corn starch, urea and phosphate salts were mixed homogeneously. The mixture was heated in a vacuum oven. After reacting for 3 hours, the product was washed three times with deionized water and ethanol, respectively. The product was dried at 50°C in a vacuum oven (DZF-6050; Hangzhou HuIer Instrument Inc, China). Peak viscosity was used as the response variable. Results of screening experiments (data not shown) showed that factors including phosphate salts (0.01–0.05 of native starch, w/w), urea concentrations (0.02–0.06 of native starch, w/w), ratio of NaH_2PO_4 to Na_2HPO_4 (0.5 : 1.5) and reaction temperature (130–150°C) exhibited the effect on pasting properties of SPC. These four factors were used as independent variables in the following response surface design.

Pasting property measurement. Pasting properties were measured by a Rapid Visco Analyzer (RVA-4; Newport Scientific, Australia). Two grams of SPC with a moisture content of 14% were added to 25 ml deionized water to form a uniform paste in RVA canisters. A heating and cooling cycle was used. The temperature profile consisted of equilibrating the starch slurry at 45°C for 1 min, raising the temperature to 95°C at a heating rate of 10°C/min, holding the temperature at 95°C for 2.5 min, lowering the temperature to 50°C at 10°C/min, and holding the temperature at 50°C for 2 min. Pasting profiles were determined in triplicate to confirm the reproducibility of the data and the evaluated parameters were averaged.

The pasting temperature (PT), peak viscosity (PV), trough (TR), final viscosity (FV) and their derivative parameters: breakdown (BD = PV – TR) and setback (SB = FV – TR) were recorded on a personal computer equipped with Thermocline software (Newport Scientific, USA). Two derived parameters, viscosity stability (VS = BD/PV) and gel forming ability (GFA = SB/TR), were calculated and used as the response variables. The third response variable is paste clarity. Starch paste clarity was measured by boiling 1% starch aqueous dispersion at 100°C for 15 min with constant stirring, and then cooling it down to 25°C. The transmittance was measured at 650 nm using a UV-1901 spectrophotometer (Puxi; China).

Response surface design. Response surface methodology (RSM) was applied to evaluate the interaction effect of four factors (phosphate salts and urea concentrations, ratio of NaH_2PO_4 to Na_2HPO_4 and reaction temperature) and to obtain the optimal reaction conditions. A four-factor three-level central composite face centred (CCF) design was employed. Levels of independent variables and the corresponding response values are shown in Table 1. The polynomial quadratic model in equation (1) was employed.

$$Y = b_0 + \sum_{n=1}^{4} b_n x_n + \sum_{n=1}^{4} b_{nn} x_n^2 + \\ + \sum_{n=m-1}^{3} \sum_{m=2}^{4} b_{nm} x_n x_m \quad (1)$$

where: Y – predicted response variable; b_0 – constant (intercept); b_n – linear coefficient; b_{nn} – quadratic coefficient; b_{nm} – cross product coefficient; X_n and X_m – independent variables.

The response surface regression was used to analyse the experimental data using Design Expert 7.1.3 (Stat-Ease Inc., USA).

Fourier transform infrared (FTIR) spectroscopy. FTIR was used to characterize the SPC prepared under the optimal conditions. The spectra of native

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Table 1. The central composite face centred (CCF) design and response variables

Runs	Factors				Response		
	X_1	X_2	X_3	X_4	viscosity stability	gel forming ability	clarity (%)
1	0.01 (–1)	0.5 (–1)	130 (–1)	0.06 (1)	0.39	1.28	48.2
2	0.05 (1)	0.5 (–1)	130 (–1)	0.06 (1)	0.48	1.22	49.6
3	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.39	1.42	77.3
4	0.03 (0)	1.5 (1)	140 (0)	0.04 (0)	0.40	1.12	54.2
5	0.01 (–1)	1.5 (1)	150 (1)	0.06 (1)	0.01	0.08	27.7
6	0.03 (0)	0.5 (–1)	140 (0)	0.04 (0)	0.45	1.33	67.4
7	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.41	1.39	55.6
8	0.03 (0)	1 (0)	140 (0)	0.06 (1)	0.40	0.88	84.6
9	0.05 (1)	1.5 (1)	130 (–1)	0.02 (–1)	0.49	1.61	32.0
10	0.05 (1)	0.5 (–1)	150 (1)	0.06 (1)	0.41	0.66	85.7
11	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.43	1.35	60.2
12	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.43	1.36	64.5
13	0.05 (1)	1 (0)	140 (0)	0.04 (0)	0.50	1.06	79.7
14	0.01 (–1)	1.5 (1)	130 (–1)	0.06 (1)	0.37	1.30	38.3
15	0.05 (1)	1.5 (1)	150 (1)	0.06 (1)	0.41	0.37	80.4
16	0.01 (–1)	0.5 (–1)	150 (1)	0.02 (–1)	0.33	0.70	61.5
17	0.01 (–1)	1 (0)	140 (0)	0.04 (0)	0.31	1.01	38.6
18	0.03 (0)	1 (0)	140 (0)	0.02 (–1)	0.47	1.18	38.7
19	0.05 (1)	0.5 (–1)	130 (–1)	0.02 (–1)	0.50	1.47	22.8
20	0.05 (1)	1.5 (1)	150 (1)	0.02 (–1)	0.51	1.02	44.3
21	0.01 (–1)	0.5 (–1)	150 (1)	0.06 (1)	0.06	0.16	40.0
22	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.43	1.36	60.9
23	0.05 (1)	1.5 (1)	130 (–1)	0.06 (1)	0.48	1.13	42.4
24	0.05 (1)	0.5 (–1)	150 (1)	0.02 (–1)	0.54	1.06	72.1
25	0.01 (–1)	1.5 (1)	130 (–1)	0.02 (–1)	0.37	1.45	29.1
26	0.01 (–1)	0.5 (–1)	130 (–1)	0.02 (–1)	0.40	1.35	39.3
27	0.03 (0)	1 (0)	130 (–1)	0.04 (0)	0.41	1.32	49.9
28	0.01 (–1)	1.5 (1)	150 (1)	0.02 (–1)	0.30	0.78	35.8
29	0.03 (0)	1 (0)	140 (0)	0.04 (0)	0.42	1.15	59.3
30	0.03 (0)	1 (0)	150 (1)	0.04 (0)	0.33	0.68	69.2

X_1 – phosphate salt concentration, range: 0.01–0.05; X_2 – ratio of NaH_2PO_4 to Na_2HPO_4, range: 0.5–1.5; X_3 – temperature (°C); range: 130–150; X_4 – urea concentration, range: 0.02–0.06; numbers in the brackets mean the coded level in CCF design

starch and SPC were acquired on a Shimadzu FTIR instrument (Shimadzu Corporation, Japan) using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr. Before making the discs, both starch samples were dried at 105°C for 12 hours. Samples were scanned from 400/cm to 4000/cm with an interval of 2/cm.

Noodle preparation, cooking and sensory evaluation. SPC with high viscosity stability and gel forming ability can enhance the sensory properties of noodles. An optimal condition to obtain high viscosity stability and gel forming ability was used to prepare SPC. Noodles were made by substituting different proportions (4, 6, 8, 10, and 12%) of SPC for formulated wheat flour to evaluate the effect of SPC addition. Trial runs were conducted to choose the substitution levels ranging from 4 to 12%. Noodles were prepared based on the method described by Li *et al.* (2012). Cooking and sensory evaluation procedures are described here. All of the noodles were cooked for 5 min in boiling water. Cooked noodles were rinsed in tap water and drained for 3 min before sensory evaluation. Sensory evaluation was performed by 15 trained panellists in triplicate according to Li *et al.* (2012). All panellists consumed noodles as their staple food regularly every day. Each panellist was given five samples randomly

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at the same time. All panellists were asked to score the attributes using a scale of 0–9. Sensory attributes included colour, appearance, firmness, elasticity, slipperiness and flavour. The final values from the panellists for each attribute were averaged.

Statistical analysis. Statistical analyses were performed using Design Expert 7.1.3 (Stat-Ease Inc., USA). Evaluation of a single factor effect was subjected to analysis of variance (ANOVA) and Tukey's HSD test to examine if differences between multiple means were significant at $P < 0.05$. Experimental data from the response surface design were fitted to a second-order polynomial model and regression coefficients were obtained.

RESULTS AND DISCUSSION

Effect of interaction effects. Second-order polynomial response surface models were applied to fit the experimental values (Table 1). Parameter estimates and fitness of the second-order polynomial equations are shown in Table 2. An R^2 value of at least 0.80 means a good fit of a model and the R^2 values for three equations were 0.97, 0.94, and 0.92, respectively, indicating only 3, 6 and 8% of the variations were not explained by the models. The P for lack of fit were greater than 0.05 for all three models, indicating that all of them fitted well. These observations implied that the three models were suitable for adequate representation of the actual relationship among the selected factors. All three models are highly significant ($P < 0.01$). For viscosity stability and gel forming ability, it is worthwhile to note that not all terms associated with the ratio of NaH_2PO_4 to Na_2HPO_4 (X_2) were significant. These results indicated that the ratio of NaH_2PO_4 to Na_2HPO_4 (X_2) within the range of 0.5–1.5 had no significant effects on viscosity stability and gel forming ability. Therefore, phosphate salts, urea concentrations and reaction temperature were the major contributing factors to improvement of viscosity stability and gel forming ability. Similarly, the effects of phosphoric acid and urea were found to be significant in Heinze *et al*. (2003). For paste clarity, all linear terms had significant effects. The effect of the ratio of NaH_2PO_4 to Na_2HPO_4 was also found significant in Passauer *et al*. (2010).

Table 2. Parameter estimates and fitness of the models for three response variables

R^2	Viscosity stability 0.97		Gel forming ability 0.94		Clarity (%) 0.92	
ANOVA	Parameter estimates	Prob > F	Parameter estimates	Prob > F	Parameter estimates	Prob > F
Model	−8.7273	0.002	−22.6500	< 0.0001	−1046.4384	0.0021
X_1	−0.2918	< 0.0001	−0.3390	0.0173	−50.5774	0.0044
X_2	−0.0368	0.0553	0.4232	0.5060	100.8018	0.0378
X_3	0.1353	< 0.0001	0.3621	< 0.0001	14.6703	0.0023
X_4	0.2542	< 0.0001	0.7844	< 0.0001	18.5799	0.0165
X_1X_2	5.1000×10^{-3}	0.4639	−0.0255	0.4505	1.6850	0.5341
X_1X_3	2.4094×10^{-3}	< 0.0001	4.1780×10^{-3}	0.0227	0.3923	0.0097
X_1X_4	4.6750×10^{-3}	0.0147	-5.0352×10^{-3}	0.5497	1.5368	0.0347
X_2X_3	-4.7250×10^{-4}	0.7326	-6.1964×10^{-3}	0.3614	−0.6620	0.2304
X_2X_4	1.9250×10^{-3}	0.7805	−0.0446	0.1950	1.2400	0.6463
X_3X_4	-2.3269×10^{-3}	< 0.0001	-4.2752×10^{-3}	0.0202	−0.1098	0.4196
X_1^2	-3.3570×10^{-3}	0.4383	−0.0265	0.2152	−1.3347	0.4299
X_2^2	0.0267	0.6979	0.3285	0.3312	−14.7551	0.5834
X_3^2	-4.9378×10^{-4}	0.0104	-1.3860×10^{-3}	0.1108	−0.0494	0.4645
X_4^2	-3.8430×10^{-3}	0.3764	−0.0280	0.1909	−0.7097	0.6723
Lack of fit	NA	0.2302	NA	0.1893	NA	0.1699

X_1 – phosphate salt concentration, range: 0.01–0.05; X_2 – ratio of NaH_2PO_4 to Na_2HPO_4, range: 0.5–1.5; X_3 – temperature (°C), range: 130–150; X_4 – urea concentration, range: 0.02–0.06; equations were derived by the second-order polynomial response surface model

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Table 3. Experimental and predicted values of responses under optimal conditions

Response	Optimal condition levels				Response values	
	phosphate salt concentration	ratio of NaH_2PO_4 to Na_2HPO_4	temperature (°C)	urea concentration	experimental (mean ± s.d.)	predicted
VS	0.0489	0.5	143	0.02	0.5708 ± 0.0003	0.5721
GFA	0.045	0.5	140	0.05	1.7573 ± 0.0008	1.7582
Clarity (%)	0.0476	0.6	147	0.0585	87.64 ± 0.07	87.18

VS – viscosity stability; GFA – gel forming ability

Optimal conditions and validation. Optimal conditions for three response variables are shown in Table 3. Under each optimal condition, additional independent experiments were conducted. The results showed that the experimental and predicted values were not significantly different.

Pasting properties. Based on the optimal conditions, SPC were prepared under the condition of the ratio of NaH_2PO_4 to Na_2HPO_4 equalling 0.5, phosphate salt concentration of 0.048, urea concentration of 0.04 and reaction temperature of 143°C. Such a reaction condition was designed to obtain SPC with high viscosity stability and gel forming ability as the priority. RVA was used for the characterization of SPC. Pasting parameters (peak viscosity, trough, breakdown, final viscosity, setback, pasting temperature) were acquired. In the viscosity profile during pasting, SPC exhibited increased peak viscosity (4516 cP) and decreased pasting temperature (62.65°C) compared to native starch (1398 cP for peak viscosity and 73.45°C). A similar pattern was found for hydroxypropylated corn starch (Chung *et al.* 2008). Pasting temperature is the temperature indicating an initial increase in viscosity. Decreased pasting temperature may be caused by the formation of phosphate ester and carbamate bonds in SPC that can rapidly absorb water causing its starch granules to quickly swell resulting in easy gelatinization of starch at a lower temperature (Asmeda *et al.* 2016). Compared to native starch, increased peak viscosity (1398 cP to 4516 cP) and trough (737 cP to 2260 cP) of SPC were due to the cross-linking by phosphate ester bonds (Jane 2009). An increase in breakdown viscosity (661 cP to 2256 cP) indicates that SPC have higher paste stability and can withstand greater heating and mechanical shear stress during industrial processing compared to native starch. This is the result of strengthened internal cross-linking within SPC granules (Otegbayo 2014). Final viscosity of SPC reached as high as 4368 cP while that of native starch was only 1122 cP, which indicates the formation of a rigid gel structure during cooling (Asmeda *et al.* 2016). Setback during pasting involves re-association, retrogradation or reordering of starch molecules (Otegbayo 2014). High setback viscosity implies a high degree of recrystallization of the gelatinized starch and lower resistance to retrogradation during cooling (Abdel-Aal *et al.* 2002). Compared to the setback value of native starch (385 cP), the higher setback value (2280 cP) of SPC means SPC are more prone to retrogradation. Further work should be performed to lower the setback value of SPC.

FTIR. SPC prepared under optimal conditions were subjected to FTIR analysis. FTIR spectra (Figure 1)

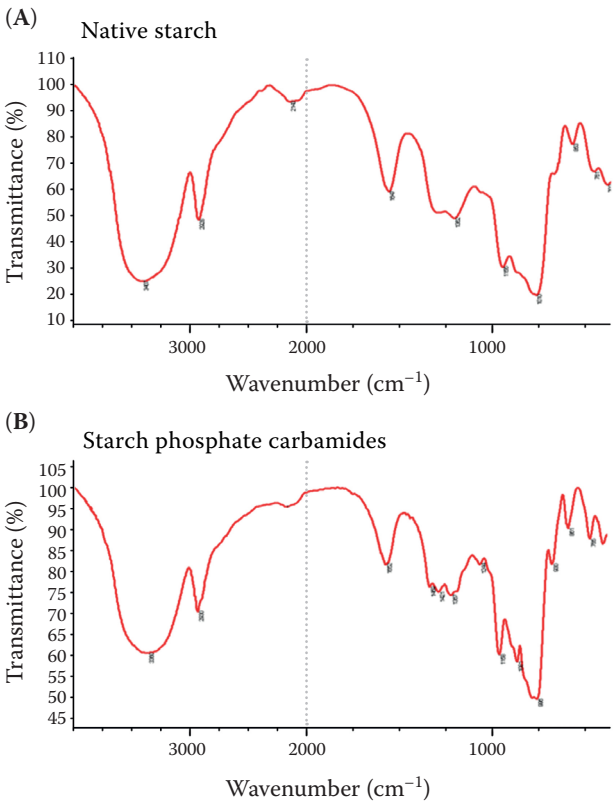

Figure 1. FTIR spectra of native starch (**A**) and starch phosphate carbamides (**B**)

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confirmed the formation of phosphate and carbamate groups in SPC by comparing with native starch. The band at 1728/cm indicates the formation of carbamate group. Similarly, in other studies, carbonyl group (C=O) was identified by the bands at 1707 (Heinze *et al.* 2003), 1710 (Kaputskii *et al.* 2007; Menzel *et al.* 2017) and 1717/cm (Lewandowicz *et al.* 2000). SPC exhibited stronger bands at 766 and 930/cm than native starch. These two bands indicate the out-of-plane bending band of the P-O-H groups and the stretching vibration of the P-O bonds, respectively (Kaputskii *et al.* 2007). The P=O vibrations were found to be in the range of 1200–1250/cm (Kaputskii *et al.* 2007; Khalil *et al.* 2002; Lewandowicz *et al.* 2000). In this study, the strong 1244/cm band present in SPC while absent in native starch indicates the formation of phosphate group.

Sensory evaluation. Sensory evaluation remains the most reliable method to evaluate the overall characteristics of cooked noodles. The result of sensory evaluation is shown in Figure 2. Noodles with 10% of SPC that replaced wheat flour showed the highest score. Scores of all attributes increased with substitution of SPC but started to decrease with PC substitutions above 8% or 10%. SPC substitutions of 10–12% increased the slipperiness greatly compared to the other proportions of substitution. These findings indicate that up to 10% of SPC substitution could give better sensory quality compared to regular noodles.

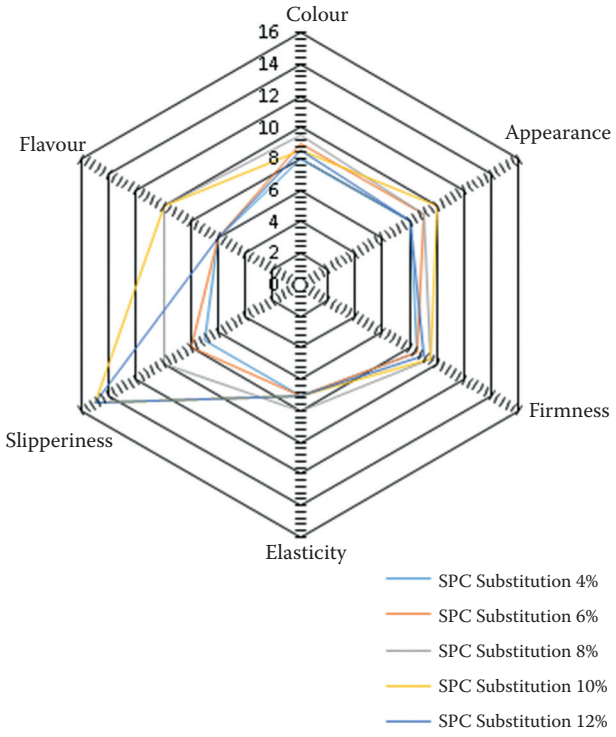

Figure 2. Sensory evaluation of noodles with SPC substitution ranging from 4% to 12%

CONCLUSIONS

In this study, a simple SPC preparation method was optimized to obtain high viscosity stability, gel forming ability and clarity with the aid of response surface methodology. FTIR spectra confirmed the formation of phosphate ester and carbamate group in SPC. RVA profiles showed improved viscosity and decreased pasting temperature. SPC substitution of 10% delivered the highest scores of sensory attributes of noodles. These findings provide guidance for the industrial production of SPC for enhancing noodle sensory properties. In the future, the nutritional effect of modified starch on noodles and other products will be evaluated comprehensively.

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