Effect of dry heat, microwave and ultrasonic treatments on physicochemical properties of potato starch with or without pectin

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Abstract

Purpose: To investigate the effects of dry heat, microwave and ultrasonic treatments on the physicochemical properties of potato starch alone or blended with pectin.

Methods: The physicochemical properties of potato starch gels prepared using microwave, ultrasonic and dry heat treatments were assessed. Pasting properties, gel strength, thermal properties and crystal texture of the potato starch were determined using Rapid Visco analyzer, texture profile analyzer, differential scanning calorimeter and x-ray diffractometer.

Results: Dry heat and ultrasonic treatments significantly increased the peak viscosity of the potato starch, and significantly decreased its setback and pasting temperatures (p < 0.05). Dry heat treatment significantly increased the hardness, while dry heat and ultrasonic treatments significantly improved retrogradation of the potato starch (p < 0.05). Transparency of potato starch paste was significantly increased by the different treatments, except microwave treatment (p < 0.05). Potato starch gels blended with pectin and subjected to any of the treatments exhibited significantly increased hardness, when compared with raw potato starch (p < 0.05). The retrogradation of the potato starch was significantly improved by the different treatments. Dry heat and ultrasonic treatments significantly decreased the syneresis of potato starch with or without pectin (p < 0.05). The three treatments also significantly affected the gelatinization enthalpy of the potato starch with or without pectin, and exerted some effects on the crystallinity of the gels.

Conclusion: The results obtained in this study suggest that differences in physicochemical properties of potato starch gels are due mainly to the degree of damage to starch granules caused by different treatments. The addition of pectin to potato starch gel greatly improves its hardness and retrogradation.

Keywords: Potato starch, Pectin, Ultrasonic treatment, Gelatinization enthalpy, Retrogradation

INTRODUCTION

Potato starch, a tuber starch accounts for about 6% of global starch production, and it is ranked third in the world based on its usefulness. Potato starch is characterized by low gelatinization temperature, large particle diameter, high paste viscosity and high paste transparency. Amylose makes up 23.2% of potato starch, and it is characterized by high swelling force (19.0 g/g),
high solubility (17.5 %) and high paste viscosity [1].

Blends of starch and non-starch polysaccharide hydrocolloids are used as raw or auxiliary materials for food or drug production, because they have different effects on starch gelatinization, viscosity, flow performance and aging properties of binary gels [2,3]. The key factors that control the formation and properties of a binary gel include molecular weight of the components, polydispersity and proportion of amylose in the starch. The effects of molecular weight and polydispersity on thermodynamic compatibility of polysaccharide ligands interacting with each other can be seen from their effects on water activity in solution [4].

The effects of anionic gum solutions (sodium alginate, sodium carboxymethyl cellulose and sodium xanthate) on properties of potato starch under different pH conditions (6.0 and 8.0) before dry heat treatment have been reported [5]. In one of such studies, the viscosity of potato starch paste was significantly decreased. Xanthan gum has more pronounced influence on viscosity spectrum of binary gels than sodium alginate or carboxymethyl cellulose (CMC). It restrains particle expansion, and enhances shear stability of the paste. The viscosity and shear stability of potato starch is greatly improved using mixed gums of xanthan and alginate. The viscosity of potato starch is also greatly increased by using sodium alginate at pH 6 and xanthan at pH 8 before heat treatment.

In a previous study, the blending of potato starch with ionic gums such as sodium alginate, CMC, and xanthan (1 % based on starch solids) followed by dry heat treatment for 0, 2, or 4 h at 130 °C significantly reduced the pasting temperature of the starch. Dry heating with xanthan and CMC increases the paste viscosity of potato starch, while sodium alginate decreased it. Extrapolated onset temperature ($T_o$), conclusion temperature ($T_c$), and peak temperature ($T_p$) of potato starch blended with ionic gums are significantly decreased after dry heat treatment. The gel structure of potato starch blended with CMC appears compressed after dry heat treatment. Dry heat treatment improves functional properties of potato starch to some extent [6]. Guar gum increases the peak and final viscosity of potato starch [1]. Rheological properties of blends of potato starch with guar gum, xanthan gum, and k-carrageenan have been described [3]. However, less attention has been paid to potato starch systems blended with pectin. The aim of this study was to investigate the effects of dry heat, microwave and ultrasonic treatments on the physicochemical properties of potato starch alone or blended with pectin.

**EXPERIMENTAL**

**Materials**

Potato starch was purchased from Beijing Minsong Economic and Trade Co. Ltd. (China); pectin was obtained from Tang Ruisi Food Materials Inc. (USA), while magnetic stirrer was purchased from Tianjin Honor Instrument Co., Ltd. (China). Mechanical pulverizer was a product of Beijing Ever Bright Medical Treatment Instrument Co., Ltd. (China); Rapid Visco Analyzer (RVA-Series 4) was obtained from Newport Scientific Pty., Ltd. (Australia), while Texture Analyzer (SMS, model TA-XT2i) was purchased from Stable Micro System, (England).

**Treatments**

**Simple blend**

Pectin (3 g) was dissolved in 170 ml distilled water and stirred vigorously. This was followed by addition of 100 g of potato starch. The mixture was stirred on a magnetic stirrer for 2 h at room temperature, and then dried to constant weight in an oven at 40 °C. The blend of potato starch and pectin was crushed using a pulverizer and passed through a 150 μm sieve. The raw potato starch without pectin was subjected to the same procedure and used as the control.

**Dry heat treatment of potato starch**

The blend of potato starch and pectin was subjected to dry heating at 120 °C for 3 h in the oven, and then cooled at room temperature. The control starch without pectin was subjected to the same dry heat treatment and was used for comparison with the blend.

**Microwave treatment**

The blend of potato starch and pectin was microwaved with medium heat for 2 min, and then cooled at room temperature. The raw starch
without pectin was subjected to the same microwave treatment and was used for comparison with the blend.

**Ultrasonic treatment**

Pectin (3 g) was dissolved in 170 ml of distilled water and stirred vigorously. This was followed by addition of 100 g of potato starch. The mixture was stirred on a magnetic stirrer for 2 h at room temperature. The resultant solution was then treated with ultrasound. The ultrasonic conditions were set as follows: ultrasonic power (50 W), whole ultrasound duration (10 min) at intervals of 25 sec and ultrasound working time of 4 sec. The blend was dried to constant weight in an oven at 40 °C, crushed with a pulverizer, and passed through a 150 μm sieve. The raw starch without pectin was treated the same way and used for comparison with the blend.

**Assessment of pasting properties**

Moisture content of potato starch was adjusted to 14 % according to sample weight. Then, the pasting properties of the starch (2.0 g) was measured using Rapid Visco analyzer. The peak, trough, breakdown, final, setback and pasting temperatures, and peak time were recorded and compared [7].

**Measurement of gel strength**

Potato starch (2 g) dissolved in 25 ml of distilled water was placed in an aluminum canister, and completely gelatinized based on the RVA measurement. Then, the resultant paste was stored at 4 °C for 24 h to form gel. The texture of the gel was analyzed using texture analyzer fitted with a 36 mm probe (P36R). The test protocol was set up as follows: pre-test speed was 1 mm/sec, test speed was 0.5 mm/sec, post-test speed was 1 mm/sec, compression ratio was 25 % deformation while force was 5 g. The procedure was performed in triplicate and the mean taken.

**Evaluation of retrogradation**

Retrogradation was measured according to the method described by Wang et al [8]. Starch suspension (10 mg/ml) prepared with distilled water was put in boiling water bath and heated for 30 min. The solution was mechanically stirred during the heating process and then cooled at room temperature. The resultant paste was transferred at room temperature to 100 ml graduated cylinder and the precipitation volume was observed at intervals of 2 h for 24 h.

**Measurement of paste transparency**

The transparency of potato starch paste was measured according to the method described by Hu et al, with some modifications [9]. Starch suspension (1 %) was prepared in distilled water, heated in boiling water bath for 30 min, and cooled at room temperature. The starch suspension was constantly stirred to prevent precipitation, and distilled water was added intermittently to keep the total volume of the suspension constant. The transmittance of the starch paste was read at 620 nm and recorded as the starch transparency.

**Freeze-thaw stability studies**

The freeze-thaw stability of potato starch with or without pectin was determined based on the method of Wang et al., with some modifications [10]. Starch suspension (3 %, w/v) was prepared with distilled water and heated in boiling water bath for 30 min with moderate mechanical agitation, and then cooled at room temperature. Aliquot of the paste (30 ml) was transferred to 50 ml cold centrifuge tubes, and then thawed and equilibrated at room temperature for 1.5 h.

The thawed tubes were centrifuged at 3000 g for 15 min, and the volume of water released from the gel after 1, 3, 5, and 7 freeze-thaw cycles was measured using a graduated cylinder and expressed as percent of water separated or syneresis.

**Differential scanning calorimetry (DSC)**

Thermal properties of the potato starch were determined using a differential scanning calorimeter fitted with a thermal analysis data station. The starch (0.1 g) was dispersed in 0.2 ml distilled water and evenly mixed. The mixed sample (8 - 12 mg) was put on a steel pan and sealed. The scanning temperature ranged from 30 - 100 °C at a ramp rate of 10 °C/min. The curve peaks were analyzed with TA Universal Analysis instrument.

**X-ray diffraction (XRD)**

X-ray diffraction analysis of the starch was performed using an X-ray diffractometer [11]. The samples were first ground and sieved using 125 μm screen. The operating conditions were: diffraction angle (2θ) of 5 - 80 °, emission slit of 1°, anti-scattering slit of 1°, receiving slit of 0.3 mm, step width of 0.02 °, and preset time of 0.1 sec.
Statistical analysis

Data are expressed as mean ± SD, and the statistical analysis was performed using Microsoft Excel (2007). The different treatment groups were compared using Duncan multiple test range. Values of p < 0.05 were considered statistically significant.

RESULTS

Pasting properties

As shown in Table 1, in the absence of pectin, the peak viscosity of potato starch was significantly increased by dry heat and supersonic treatments (p < 0.05). Dry heat and supersonic treatments significantly decreased the setback and pasting temperatures of potato starch, and the peak viscosity was reached at a shorter time (p < 0.05). However, microwave treatment had little effect on the gelatinization of potato starch. On addition of 3 % pectin, the pasting properties of potato starch and pectin blend without any treatments were barely changed, relative to raw potato starch (p > 0.05).

| Treatments | Peak /cP | Trough /cP | Breakdown /cP | Final /cP | Setback /cP | Pasting temp /℃ | Peak time /min |
|------------|----------|------------|---------------|-----------|-------------|-----------------|---------------|
| ck         | 2706±11  | 2166±12    | 540±10        | 2886±11   | 720±8       | 71.8±0.2        | 4.53±0.12     |
| A          | 6672±12  | 2652±15    | 4020±14       | 3114±15   | 462±12      | 65.6±0.1        | 3.60±0.10     |
| B          | 2821±14  | 2288±12    | 533±16        | 3027±14   | 739±13      | 72.75±0.2       | 4.67±0.14     |
| C          | 6166±13  | 2479±10    | 3687±15       | 2891±17   | 412±12      | 67.9±0.1        | 3.73±0.13     |
| D          | 2688±10  | 1923±8     | 765±12        | 2666±12   | 743±8       | 71.2±0.1        | 4.67±0.10     |
| E          | 860±9    | 495±11     | 365±16        | 617±10    | 122±15      | 67.9±0.1        | 3.73±0.09     |
| F          | 1672±12  | 1306±12    | 366±10        | 1969±8    | 663±10      | 68.65±0.2       | 4.27±0.08     |
| G          | 2604±12  | 1916±10    | 688±12        | 2524±16   | 608±10      | 71.15±0.2       | 4.87±0.09     |

ck: Raw potato starch; A: potato starch subjected to dry heat treatment without pectin; B: potato starch subjected to microwave treatment without pectin; C: potato starch subjected to supersonic treatment without pectin; D: blend of potato starch and pectin without any treatment; E: blend of potato starch and pectin subjected to dry heat treatment; F: blend of potato starch and pectin subjected to microwave treatment; and G: blend of potato starch and pectin subjected to supersonic treatment.

Table 2: Gel properties of potato starches

| Treatment | Hardness /g | Springer | Cohesiveness | Gumminess /g | Chewiness /g |
|-----------|-------------|----------|--------------|--------------|--------------|
| ck        | 59.50±2.18  | 0.99±0.03 | 0.86±0.02    | 51.07±1.99   | 51.01±2.30   |
| A         | 191.51±2.09 | 0.98±0.02 | 0.94±0.05    | 179.56±2.04  | 175.91±2.14  |
| B         | 139.36±2.80 | 0.97±0.05 | 0.91±0.06    | 127.72±1.86  | 124.13±2.54  |
| C         | 165.85±2.22 | 0.95±0.05 | 0.92±0.02    | 153.13±2.01  | 146.13±2.58  |
| D         | 208.63±2.32 | 0.95±0.08 | 0.93±0.04    | 194.39±1.78  | 183.84±2.05  |
| E         | 433.38±3.02 | 0.92±0.04 | 0.92±0.05    | 400.16±1.96  | 365.96±2.62  |
| F         | 219.86±2.88 | 0.96±0.02 | 0.95±0.05    | 209.16±2.08  | 201.35±2.03  |
| G         | 299.26±2.56 | 0.98±0.04 | 0.95±0.07    | 283.42±2.24  | 278.98±2.14  |

Note: ck, Raw potato starch; A, Potato starch by dry heating treatment without pectin; B, Potato starch by microwave treatment without pectin; C, Potato starch by supersonic treatment without pectin; D, Blends of potato starch and pectin, without any treatment; E, Blends of potato starch and pectin by dry heating treatment; F, Blends of potato starch and pectin by microwave treatment; G, Blends of potato starch and pectin by supersonic treatment.
**Retrogradation**

As shown in Figure 1, in the absence of pectin, the precipitation volume of raw potato starch was significantly reduced and showed obvious retrogradation ($p < 0.05$). The precipitation volume of potato starch subjected to microwave treatment was also significantly reduced ($p < 0.05$). However, dry heat and ultrasonic treatments significantly improved potato starch retrogradation ($p < 0.05$). The precipitation volume of potato starch subjected to dry heat and ultrasonic treatments after 24 h of storage were significantly increased ($p < 0.05$). After the addition of pectin, the retrogradation of potato starch subjected to the different treatments were further improved.

**Paste transparency and freeze-thaw stability**

The raw potato starch exhibited low paste transparency (28 ± 2 °C). The paste transparency of potato starch subjected to the different treatments were significantly increased, except microwave treatment without pectin, when compared with that of raw potato starch ($p < 0.05$). The blend of potato starch and pectin subjected to dry heat treatment showed highest transmittance (65 ± 3.5 °C). After subjecting the starch gels to different treatments, the photorefractive index and reflectance of the pastes were significantly reduced, and the transparency was significantly improved ($p < 0.05$). Dry heat and ultrasonic treatments significantly reduced the syneresis of potato starch with or without pectin, while microwave treatment significantly increased the water volume separated from the starch, relative to raw potato starch ($p < 0.05$). Thus, freeze-thaw stability of potato starch subjected to dry heating and ultrasonic treatments was significantly increased and exhibited a weak retrogradation trend. These results are shown in Figure 2.

**Thermal characteristics**

Gelatinization temperature for the endothermic transition of the raw potato starch ranged from 62.76 to 83.08 °C, while the enthalpy change ($\Delta H$) was 4.66 J/g. The different treatments significantly altered the gelatinization enthalpy of the potato starch with or without pectin ($p < 0.05$). The wider gelatinization temperature (61.47 ± 0.20 to 85.35 ± 0.29 °C) and the lowest gelatinization enthalpy (2.50 ± 0.09 J/g) were found in the blend of potato starch and pectin subjected to dry heating treatment (Table 3).

**Crystalline properties**

As shown in Figure 3, the raw potato starch powders showed the strongest diffraction peaks at 29 angles of 15.143 °, 17.021 ° and 22.956 °. However, the first peak (15 °) was conspicuously missing from the diffraction patterns of potato starch subjected to different treatments with or without pectin, and the potato starch subjected to microwave treatment without pectin was predominantly associated with B type crystallinity. Although the starch presented a bimodal diffraction distribution, the second peak was weakened and its strength significantly reduced. However, the positions of the two peaks in the derivation patterns were maintained close to 17 and 22 °, respectively.
Table 3: Gelatinization parameters of potato starch subjected to different treatments with or without pectin

| Sample | Onset (°C)       | Maximum (°C)     | Stop (°C)    | Area (J/g) |
|--------|------------------|------------------|--------------|------------|
| ck     | 62.76±0.42       | 68.37±0.33       | 83.08±0.31   | 4.659±0.15 |
| A      | 63.25±0.18       | 69.91±0.19       | 85.62±0.20   | 2.761±0.10 |
| B      | 67.26±0.25       | 73.89±0.21       | 86.57±0.19   | 2.723±0.13 |
| C      | 64.22±0.22       | 71.72±0.18       | 86.25±0.25   | 2.866±0.12 |
| D      | 64.76±0.26       | 72.17±0.28       | 84.78±0.24   | 2.847±0.11 |
| E      | 61.47±0.20       | 68.49±0.14       | 85.35±0.29   | 2.504±0.09 |
| F      | 63.28±0.21       | 71.99±0.26       | 87.05±0.20   | 2.726±0.12 |
| G      | 65.07±0.16       | 72.35±0.20       | 85.88±0.22   | 2.574±0.11 |

Note: ck, Raw potato starch; A, Potato starch by dry heating treatment without pectin; B, Potato starch by microwave treatment without pectin; C, Potato starch by supersonic treatment without pectin; D, Blends of potato starch and pectin, without any treatment; E, Blends of potato starch and pectin by dry heating treatment; F, Blends of potato starch and pectin by microwave treatment; G, Blends of potato starch and pectin by supersonic treatment.

DISCUSSION

Starch gelatinization is the irreversible destruction of the ordered structure of starch molecules in aqueous solution when the temperature is elevated above the gelatinization temperature under heat treatment [12-14]. In the present study, in the absence of pectin, dry heat and ultrasonic treatments promoted the expansion of starch particles, thereby increasing the peak viscosity. On the other hand, when 3 % pectin was added, the peak viscosities of the starch subjected to dry heat and microwave treatments were significantly decreased, an indication that pectin may hinder the expansion of starch granules. It is likely that the different treatments induced changes in the surface of starch granules, which prevented or delayed the absorption of water molecules. In addition, particle degradation or polymer arrangements may cause starch particles to become more susceptible to shear, resulting in lower peak viscosity [14].

Microwave radiation is a non-ionizing physical modification technique which changes the structural characteristics of potato starch to form microemulsions [15]. Microwave heating rearranges the intramolecular structure of starch, thereby altering its water absorption capacity, solubility, expansion capacity, pasting properties, dehydration, shrinkage and rheological behavior [16-18]. It has been reported that microwave treatment promotes rapid rearrangement of starch molecules at low temperatures [15]. It changes the structure and properties of starch at a lower range of gelatinization temperature. The results obtained in this study suggest that changes in crystalline structures of potato starch with or without pectin when subjected to different treatments may have led to a significant reduction in the amount of energy required for disrupting the H-bonds within junction zones. The structure of the starch molecule may have been damaged during these treatments, or the starch granules may have been encased in a layer of pectin or some reactions occurred with pectin, so that gelatinization of the starch became more difficult.

A highly concentrated starch suspension gelatinizes and cools to form an elastic gel, and gelation depends on time and temperature. Starch gel is a metastable disequilibrium system formed during the aging process of starch and its...
Structure changes during storage [18,19]. Storage of starch at 4 °C causes it to age. During storage, a three-dimensional network structure is formed because of the rearrangement of amylose and recrystallization of amyllopectin side chains, thereby greatly increasing gel strength [19]. During aging of amylose, 40 - 70 glucose units aggregate to form a double helical structure, while amyllopectin is crystallized by rearrangement of its outermost short side chains (DP = 15) [20,21]. The aggregation and crystallization of amylose is accomplished in the first few hours of storage, while the aggregation and crystallization of amyllopectin is completed in the later stage of storage [22].

In this study, the different treatments significantly altered the hardness, gumminess and chewiness of potato starch. The addition of pectin significantly increased the hardness of the starch gels. The results obtained in the present study suggest that hardness, brittleness, elasticity, adhesiveness, degree of conglutination, chewiness and recoverability of starch may be reduced with increased frequency of ultrasound, and are in agreement with those previously reported [9]. The frequency and duration of ultrasound may determine the outcome of ultrasonic treatment. When the water content is sufficient and the heating temperature is elevated above the gelatinization temperature, the starch particles undergo irreversible expansion, leading to leaching of amylose into the solution.

Retrogradation is a reaction that takes place when the amylose and amyllopectin chains in cooked, gelatinized starch realign themselves due to hydrogen bond interaction as the cooked starch cools [8,23]. Starch retrogradation involves the formation of gel structures, association of amylose chains to form double helix, and the aggregation of amyllopectin chains into double helical structures. Generally, amylose retrogrades much faster than amyllopectin, and the higher the amylose content, the more rapidly the starch retrogrades [23]. Amylopectin and its intermediates play important roles in retrogradation during refrigeration or storage of starch [24].

The results of this study showed that in the absence of pectin, dry heating and ultrasonic treatments significantly improved retrogradation of potato starch, while microwave treatment had no significant effect on starch retrogradation. However, the addition of pectin significantly improved retrogradation of potato starch regardless of the treatment. The two main factors that affect the transparency of starch paste are size distribution of starch granules and the ratio of amylose to amyllopectin [25]. In general, the larger the particle size or the higher the content of amyllopectin, the higher the transparency [9]. The paste transparency of potato starch subjected to different treatments were significantly increased, except microwave treatment without pectin, when compared with that of raw potato starch. The highest transmittance was obtained in the blend of potato starch and pectin subjected to dry heat treatment.

"Freeze-thaw" stability is an index of starch retrogradation. The volume of water separated from starch is directly related to the retrogradation trend of the starch [26]. The “freeze-thaw” stability of the whole system becomes worsened when starch retrogradation occurs rapidly [27]. Ice crystals are formed when phase separation occurs due to freezing of starch paste or gels, and during thawing, the paste or gel retains the aqueous phase rich in starch. Increasing the freeze-thaw cycles, increases the degree of phase separation, which is caused by retrogradation of amyllopectin. This facilitates the separation of water from dense networks, thereby leading to deterioration of starch quality [24]. Studies have shown that dual-frequency ultrasound exerts some effect on the freeze-thaw stability of corn starch [9].

In this study, dry heat and ultrasonic treatments significantly decreased the syneresis of potato starch with or without pectin, when compared with raw potato starch. These results suggest that dry heat and ultrasonic treatments may improve the freeze-thaw stability of potato starch and lead to a weak retrogradation trend. Thermal enthalpy is a measure of the crystallinity of amyllopectin which reflects the quality and quantity of starch crystals, as well as the energy required to disrupt the hydrogen bonds in the junction zones [28,29]. It has been reported that dual frequency ultrasonic treatment could reduce the enthalpy of corn starch, relative to single frequency ultrasound [9]. Dry heat, microwave and ultrasonic treatments significantly affected the gelatinization enthalpy of the potato starch with or without pectin. Wider gelatinization temperature range and the lowest gelatinization enthalpy were found in the blend of potato starch and pectin subjected to dry heat treatment. The raw potato starch powders exhibited the characteristic B-type of crystallinity [30]. It has been reported that ultrasound may not damage the crystal structure of corn starch, and that the peak intensity of the characteristic diffraction peaks of starch is increased by short-time ultrasound treatment.
may be significantly decreased [9]. Studies have also shown that diffused diffraction characteristic is enhanced and the crystal lattice arrangement of corn starch significantly decreased after ultrasound treatment [9].

The results obtained from crystallinity study are not in agreement with those previously reported. This may be due to the differences in raw materials or ultrasonic conditions applied. The first peak (15 °) was missing from the diffraction patterns of potato starch subjected to microwave treatment without pectin retained the characteristic B type crystallinity. The first peak of potato starch diffraction pattern was not observed in the presence or absence of pectin, an indication that the treatments may have exerted some effects on the crystal structure of the starch.

CONCLUSION

The results obtained in this study suggest that differences in the physicochemical properties of potato starch gels are due mainly to the degree of damage to starch granules caused by different treatments. The addition of pectin to potato starch gel greatly improves its hardness and retrogradation, while dry heating treatment enhances the transparency of the starch paste. Moreover, the crystal structure of potato starch and pectin mixture changed following the various treatments.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the all of author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Haiyan Gao and Jie Zeng conceived and designed the experiments; Kexin Meng, Tongchao Su, Meng Cao, Meng di Song, and Jikai Jiang conducted the research performed the experiments; Kexin Meng analyzed the data; Hanyan Gao contributed materials and analysis tools; Haiyan Gao and Jie Zeng wrote the paper.” Jie Zeng had primary responsibility for final content. All authors read and approved the final manuscript.

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