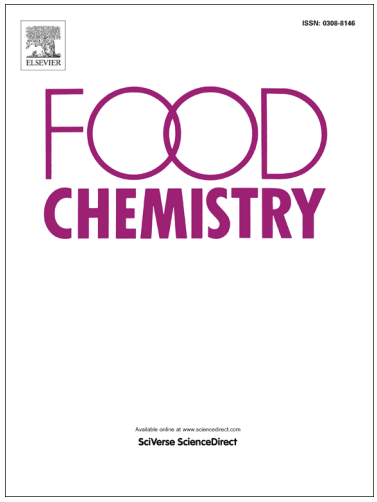


Enhancing the Hardness of Potato Slices after Boiling by Combined Treatment with Lactic Acid and Calcium Chloride: Mechanism and Optimization

Jia Liu, Chengrong Wen, Mei Wang, Sijie Wang, Nan Dong, Zunguo Lei, Songyi Lin, Beiwei Zhu

PII: S0308-8146(19)30906-9
DOI: https://doi.org/10.1016/j.foodchem.2019.05.106
Reference: FOCH 24832

To appear in: *Food Chemistry*

Received Date: 29 December 2018
Revised Date: 9 May 2019
Accepted Date: 14 May 2019

Please cite this article as: Liu, J., Wen, C., Wang, M., Wang, S., Dong, N., Lei, Z., Lin, S., Zhu, B., Enhancing the Hardness of Potato Slices after Boiling by Combined Treatment with Lactic Acid and Calcium Chloride: Mechanism and Optimization, *Food Chemistry* (2019), doi: https://doi.org/10.1016/j.foodchem.2019.05.106

Enhancing the Hardness of Potato Slices after Boiling by Combined Treatment with Lactic Acid and Calcium Chloride: Mechanism and Optimization

Jia Liu[a,b], Chengrong Wen[a], Mei Wang[b], Sijie Wang[b], Nan Dong[b], Zunguo Lei[b], Songyi Lin[a,*], and Beiwei Zhu[a,*]

[a] Dalian Polytechnic University, National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian 116034, PR China

[b] Guizhou Academy of Agricultural Science, Institute of Food Processing technology, Guiyang 550006, PR China

[*]**Corresponding author**

Dalian Polytechnic University, National Engineering Research Center of Seafood, School of Food Science and Technology, No. 1 Qinggongyuan, Ganjingzi District, Dalian 116034, PR China.

Tel.: +86-411 86323262

E-mail address: zhubeiwei@163.com;Songyi Lin: linsongyi730@163.com

E-mail for First author：Jia Liu: mcgrady456@163.com

E-mail for all co-authors: Chengrong Wen:948821706@qq.com ;Mei Wang: 1375898692@qq.com ; Sijie Wang: 394344127@qq.com ; Nan Dong: 460288583@qq.com ; Zunguo Lei 404427917@qq.com.

Abstract: Potatoes usually suffer from greatly decrease of hardness after boiling, which limits their processing potential in food industry. Moreover, methods for enhancing the hardness of potatoes after boiling are underexplored. In this study, the hardness of potato slices after boiling were increased from 288 *g* to 2342 *g* by the combined treatment of lactic acid (LA) and calcium chloride (CC). Through the analysis of the microstructure of the potato cells, the molecular weight distribution and natural sugar ratio of different soluble pectin fractions, and the enzymatic activities (polygalacturonase, PG and pectin methylesterase, PME), the possible mechanism behind the hardness enhancement by LA and CC pretreatment, namely the direct link between pectin and potato structure was revealed. The obtained results confirmed the target spot for enhancing the hardness of potatoes after boiling lay in PG activity and gelation of the pectin, which also could be used to help other plants resist the heat process if pectin existed in their cell wall.

Keywords: Potato; Pectin; Hardness enhancement; Lactic acid; Calcium chloride

1. Introduction

Potato (*Solanum tuberosum* L.) is a tuber widely consumed throughout the world typically in forms such as mashed potatoes, potato soups and salads, French fries and potato chips (Willard, 1993). Heat processing of potatoes in oil (frying) or water (boiling) before serving on the table is necessary to gelatinize the starch. The different evaporation rates of water at the surface and in the core of potato strips during frying make the outer shell hard and the inner core soft (Bouchon, 2009). While, boiling makes the bulk of the potato soft for preparing mashed potatoes. Thus, the processing of potato was limited because potato easily became soft after heat processing. In China, there is popular snack in Sichuan and Chongqing province called “leng chuan chuan”. This snack is prepared as follows. The vegetables and meat are first boiled and then immersed in a mixture of oil, flavouring and spices. After a few hours, the vegetables and meat absorb the taste compounds from the mixture, and the snack is ready to serve. However, it is strange that the potato slices are crisp after boiling (i.e., their soft mouth feel is lost). The secret of the recipe is soaking the potato slices in the pickle water (fermented juice in pickle) to obtain such a unique texture before boiling. The dominant microbe in pickles is lactic acid bacteria (LAB) (Liu, Han, & Zhou, 2011). Lactic acid (LA) is the main product of LAB metabolism and is responsible for preserving and improving the texture of vegetables (Leroy, & De Vuyst,

2004). Therefore, exploring whether LA is involved in improving the texture of potato slices after boiling is reasonable.

Normally, significant changes in potato texture occur a few days after slicing because the integrity of plant cells decreases, and the endogenous enzymes can act on the plant cell walls (decomposing the structural components) (Rocculi, et al. 2007). However, the texture changes quickly when potato slices are heated, and these changes can be attributed to two factors: starch and pectin. The gelatinization of starch granules during heating caused earlier researchers to believe that starch swelling was the reason that the potato texture changed more quickly. Jarvis and Shomer proved that the swelling of the cell wall and the starch in the potato cells impacted the texture of the potato (Jarvis, Mackenzie, & Duncan, 1992; Shomer, 1995; Shomer, Vasiliver, & Lindner, 1995). However, subsequent studies proved that there were other factors in addition to starch gelatinization that affected the texture of the potato tissue after boiling. Ng found that heat-induced softening of potato tissues resulted in the solubilization of pectic polysaccharides (Ng, & Waldron, 1997). Parker found that the edge-of-face structures could be strongly labelled with JIM5 but not with JIM7 (JIM5 and JIM7 were anti-homogalacturonan monoclonal antibodies, which linked with esterified and non-esterified pectin), indicating that the faces contained polygalacturonic acid with a low ester content (Parker, Parker, Smith, &

Waldron, 2001). In addition, the adhesion of the middle lamella to the face of the primary wall differed from the adhesion at the edge of each cell face. Ross found that when potato tubers contained higher levels of total pectin methylesterase (PME) activity, the degree of methylation of pectin in the cell wall was lower and consistently higher values of peak force and work done were observed during the fracture of cooked tuber samples (Ross, et al., 2010). Bordoloi found that the cell walls of parenchyma cells partially degraded upon boiling, resulting in loosening of the cell wall microfibrils (Bordoloi, Kaur, & Singh, 2012). Ormerod considered that the weakening of the potato tissue upon boiling was primarily controlled by thermal degradation of the middle lamella (Ormerod, Ralfs, Jobling, & Gidley, 2002).

Taken together, most researchers agree that the texture change in potatoes after boiling is caused by changes in the polysaccharides in the cell wall, such as pectin degradation. Namely, the change of potato structure was accompanied with the change of pectin during or after heat processing. However, why the change of pectin take place and how pectin influence the potato structure during or after heat processing is still a mystery. Through the tracking of the change of pectin chemical structure and relevant enzyme activity, hardness enhancement after boiling of potato slices pre-soaked with LA was elucidated in this study. Besides, calcium could affect the fruit quality and preservation based on the

knowledge that calcium in cell could interact with the uronic acid carboxyl functions in pectin polysaccharide chains to create the so-called pectin 'egg-box' to maintain the cell wall stabilization and integrity (Vicente, Saladié, Rose, & Labavitch, 2007). In order to maximumly enhance the hardness of potato after boiling, calcium chloride (CC) in combination with LA was used in the optimization experiment to achieve the greatest hardness of potato slices after boiling.

2. Materials and methods

2.1 Materials

Potatoes were obtained from a local supplier in Dalian, Liaoning, China (Cultivar: Favorita; Harvest time: 03-Aughst-2018; Test time: 08-Aughst-2017 to 13-November-2017; Potato storage condition: temperature of 4 °C; Humidity of 90%; Potato size: diameter range of 4-5 cm, and length range of 7-8 cm). The monosaccharide standards including arabinose (Ara), galactose (Gal), fucose (Fuc), xylose (Xyl), rhamnose (Rha) and galacturonic acid (GalA), were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). An enzyme-linked immunosorbent assay (ELISA) kit (MEIMIAN) was purchased from Huyu Biological Technology Co., Ltd. (Shanghai, China). Chromatography-grade acetonitrile and formic acid were also used. All chemicals without further purification were used as received.

128 *2.2 Sample Treatment*

129 Potatoes were obtained from a local supplier in Dalian, Liaoning,
130 China. Potato tubers were firstly washed with tap water. Then, it was
131 peeled and cut into 3-mm-thick slices (potato with a diameter range of 4-
132 5 cm was used, and 3 potato slices separately cut from 3 different
133 potatoes were arranged in 1 group, then followed by different treatment
134 and analysis) for subsequent experiments.

135 *2.2.1. Soaking Treatment (Sila, Smout, Vu, T& Hendrickx, 2004)*

136 The potato slices were soaked in distilled water with 0.5% lactic acid
137 (*v*/*v*) and 0.5% calcium chloride (*w*/*v*) for 10 h. Then, the samples were
138 washed with distilled water to remove solvent remaining on the surface,
139 packaged in aluminium foil vacuum pouches (20×30 cm, only one slice
140 was put into a pouch and vacuum degree was -70 kPa, as showed in
141 supplementary 1) and stored at 25 °C until analysis (less than 10 min).

142 *2.2.2. Thermal Treatment (Kaur, Singh, Sodhi, & Gujral, 2002)*

143 After soaking treatment, the samples in their aluminium foil vacuum
144 pouches were immersed in boiling water for specified times (1, 3, 5, 7,
145 and 10 min) and then rapidly cooled to room temperature by placing them
146 under running tap water (10-15 °C) for 10 min.

147 *2.3. Texture Measurement*

148 The sample hardness was evaluated by texture profile analysis (TPA)
149 using a TA.XTplus texture analyzer (Stable Micro Systems Co.,

Ltd.,Godalming, UK) equipped with a P/5 probe (the center portion of potato slice was used for the test and 30%-40% area of the slice was covered by the probe). The test program was set as follows: samples were compressed to 50% (1.5 mm compression) of their original height (3 mm thickness); time between two compressions: 5 s; post-test speed: 1.00 mm/s; testing speed: 1.00 mm/s (García-Segovia, Andrés-Bello, & Martínez-Monzó, 2008). The sample hardness, the peak force of the first compression cycle, was automatically calculated from the TPA curves using the software supplied by Texture Technologies Corp.

2.4. Galacturonic Acid (GalA) Determination

The GalA levels in fresh and boiled potatoes were measured spectrophotometrically at 525 nm as reported in a previous study (Kang, Hua, Yang, Chen, & Yang, 2015). (1) Sample preparation: a 5-g sample of potato and 35 mL of anhydrous ethanol (75 °C) were added to a tube, shaken and heated at 85 °C for 10 min. After the treatment, the tube was cooled with tap water. The volume was brought up to 50 mL with 60% ethanol and centrifuged at 8000 rad/min for 15 min 3 times (the supernatant was discarded each time). (2) Sample measurement: the pellet obtained by centrifuging each sample was washed into a 100-mL cylinder using 95 mL of distilled water. NaOH (5 mL, 1 M) was added to the cylinder, and the mixture was incubated for 15 min. Then, the solution was filtered, and the filtrate was ready for testing. Then, 1.0 mL of the

filtrate, 0.25 mL of 0.1% carbazole ethanol solution and 5.0 mL of sulfuric acid were added into a 25-mL tube and shaken in a water bath at 85 °C for 20 min. Then, the mixture in the tube was cooled with tap water (10-15 °C) and used for spectrophotometric determination within 1.5 h.

2.5. Morphological Observation

The structure of potato slice was observed using scanning electron microscopy (SEM). The determination was performed following the method (Chan, & Tian, 2005). Potato slice subjected to different treatments was firstly lyophilized for 24 h and then broken off for SEM observation. The fractured surface of the potato sample was coated with gold powder to avoid charging under the electron beam. Then, samples were observed using a S-4800 scanning electron microscope (Hitachi, Tokyo, Japan).

2.6. Pectin Extraction and Characteristic Analysis

Sequential Extraction of Pectic Polysaccharides. The extraction process was performed following the protocol (Gwanpua, et al., 2014). Potato slices were homogenized by pureeing in a kitchen blender for 5 min and were then filtered (300 mesh gauze) to remove the starch granules. Then, the residue was suspended in ethanol (95%) in a potato to ethanol ratio of 1:10 (w/v). To remove the soluble sugars, the suspension was kept at 85 °C for 20 min and then filtered. Finally, the residue was homogenized in acetone (ratio of 1:5, w/v) and stirred for 2 h at 25 °C

194 before a final filtration, followed by freeze drying to obtain the alcohol-
195 insoluble residue (AIR). The water-soluble, chelator-soluble, and
196 Na_2CO_3-soluble pectin (WSP, CSP and NSP) fractions were then
197 extracted from the AIR. (1) The WSP was extracted by stirring 3 g of
198 AIR in 50 mL of distilled water for 20 h at 4 °C; (2) the CSP was
199 obtained by stirring the residue resulting from the WEP extraction in 50
200 mL of 0.05 M cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA,
201 pH 6.5) solution at 25 °C for 10 h; (3) the NSP fraction was obtained by
202 stirring the residue resulting from CSP extraction in 50 mL of 0.05 M
203 Na_2CO_3 solution with 0.02 M $NaBH_4$ at 4 °C for 20 h.

204 *Molecular Weight Distribution*. The molecular weight distributions
205 of the different pectin fractions were investigated by size exclusion
206 chromatography (Shpigelman, Kyomugasho, Christiaens, Van Loey, &
207 Hendrickx, 2014). A Shimadzu LC-20A series HPLC system (Shimadzu
208 Co., Kyoto, Japan) with a reflective index detector (RI -20) was used. The
209 separation was performed on a TSKgel G5000PWXL column (TOSOH,
210 Tokyo, exclusion limits of 4000-800000 Da) with a particle size of 10 µm
211 and a pore size of 100 nm. A 1.0-mL aliquot of the pectin fraction (WSP,
212 CSP, and NSP) was solubilized in 1 mL of eluent and filtered through a
213 0.45 µm filter before being injected into the column. The eluent contained
214 0.1 M 2-(N-morpholino) ethanesulfonic acid buffer (pH 6.5) and 0.1 M
215 NaCl to stabilize the pH and minimize the ionic interactions during the

analysis. The flow rate and column temperature were 1.0 mL/min and 40 °C, respectively.

2.7. Natural Sugar Ratio Determination

2.7.1. Pectin hydrolysis (Kurita, Fujiwara, & Yamazaki, 2008)

Pectin samples (2 mg) and 2 M Trifluoroacetic acid (TFA, 2 mL) were added to an ampoule and sealed with an alcohol torch. The pectin was hydrolysed at 110 °C in an oven for 3 h. Then, the digested sample was dried in an N_2 evaporator at 45 °C. The dried sample was dissolved in 200 µL of ultrapure water, and the sample was neutralized with 0.1 mol/L NaOH by pH test paper. The final volume of sample was brought up to 1 mL.

2.7.2. Derivatization procedure with PMP (1-phenyl-3-methyl-5-pyrazolone) (Stepan, & Staudacher, 2011)

A 400-µL aliquot of a pectin sample or a standard neutral sugar solution, 50 µL of 0.02 mol·L^{-1} lactose standard solution, 450 µL of 0.3 mol·L^{-1} NaOH and 450 µL of 0.5 mol·L^{-1} PMP were mixed in a vortex mixer. Then, the mixture was incubated at 70 °C for 30 min. After cooling to room temperature, the mixture was neutralized by adding 450 µL of 0.3 mol·L^{-1} HCl. The resulting solution was extracted 5 times by adding 1 mL of chloroform. Then, this mixture was vigorously mixed and centrifuged at 3000 *g* for 5 min (the organic layer was carefully removed). The final aqueous layer was concentrated to dryness and dissolved in 1

mL of water to obtain a solution with a standard final volume. The solution was filtered through a 0.45-mm membrane filter and evaluated by HPLC.

2.7.3. High-performance liquid chromatography (Stepan, et al., 2011)

The derivatives were separated on a Thermo BDS C18 column (250 × 4.6 mm i.d., 5 μm). The mobile phase consisted of A and B eluents. Eluent A: a mixture of 15% acetonitrile with 0.05 M phosphate buffer solution (KH_2PO_4-NaOH, pH 7.1). Eluent B: a mixture of 40% acetonitrile with 0.05 M phosphate buffer solution (KH_2PO_4-NaOH, pH 7.1). Both eluents were filtered through 0.2-mm-pore hydrophilic propylene membrane filters for degassing and sterilization. The gradient conditions were as follows: 0–10 min, 0–10% B; 10–40 min, 10–40% B; 40–50 min, 40–0% B; 50–60 min, 0% B. The eluate was monitored at 245 nm, the flow rate was 0.7 $mL \cdot min^{-1}$, the injection volume was 10 μL, and the column was maintained at 35 °C. Through plotting the ratio of the peak areas of the sugar to the internal standard against the sugar concentration, the linearity of each calibration curve (fucose, rhamnose, galactose, galacturonic acid, xylose and arabinose) was confirmed. The concentrations of the unknown samples were calculated by the aid of the standard curves. Standard curves were also prepared for the pectic samples as described in the previous section.

2.8. Cell Wall Degradation Enzyme Extraction and Analysis

Polygalacturonase (PG) or pectin methylesterase (PME) were extracted as the former method (Xie, Yuan, Pan, Wang, Cao, & Jiang, 2017) with a slight modification. The sample in the reference method was tomato, thus, an ice cold 80% (v/v) ethanol was used in order to remove the effect of the red colour. However, this pretreatment was removed in this study because the extraction was clean. 5.0 g samples of potato tissue (Reference method: 10 g) that has been subjected to different treatments were homogenized using a cold mortar and pestle in 10 mL (Reference method: 5 mL) of 50 mmol L^{-1} sodium acetate buffer (pH 5.5, containing 1.8 mol L^{-1} NaCl) and then centrifuged at 7000×*g* for 30 min (Reference method: 12000×g for 20 min) at 4 ℃. The supernatant was collected as the crude enzyme extract and stored in a refrigerator at 4 °C until analysis. The PG and PME activities were measured using an enzyme-linked immunosorbent assay (ELISA, MEIMIAN, Huyu Biological Technology Co., Ltd., Shanghai, China) as follows:

(1) A 40-μL aliquot of diluted sample (diluted with phosphate-buffered saline (PBS) containing 5% bovine serum albumin) was added to coat the microtiter plate, and then 10 μL of enzyme extract was added. The plate was incubated for 30 min at 37 °C after being sealed with a closure plate membrane.

(2) After a certain period (30 min), the closure plate membrane was removed from the plate. The liquid inside the plate was discarded, and the

plate was air dried and then washed with buffer (PBS containing 20 ppm Tween 20) (holding for 30 s, then draining). This washing procedure was repeated five times, and after the final wash, the plate was patted dry.

(3) HRP-conjugate reagent (50 μL) was added to each well (except the blank well), and then the plates were incubated for 30 min at 37 ℃. After incubation the plates were washed five times with PBS containing 20 ppm Twain 20 (holding for 30 s, then draining), and after the final wash, the plates were patted dry.

(4) Chromogen solution A (50 μL, containing sodium acetate, citric acid and 30% hydrogen peroxide) and chromogen solution B (50 μL, containing EDTA-Na, citric acid, glycerol and tetramethyl benzidine) were added to each well in turn, and the colour was allowed to develop for 10 min at 37 ℃ (protected from light). The reaction was stopped by adding 50 μL of stop solution (1.0 mol L^{-1} sulfuric acid) at which point the colour immediately changed from blue to yellow.

(5) The absorbance of each sample was recorded at 450 nm within 15 min, and the enzyme activity is reported as U g^{-1}. A calibration curve was prepared by plotting the absorbance against the concentration of a PG or PME standard.

2.9. Optimization of the Pretreatment Procedure

Response surface methodology (RSM, three-level, three-variable) based on the Box-Behnken design (Bezerra, Santelli, Oliveira, Villar, &

304 Escaleira, 2008) was used to observe the influence of the concentrations
305 of LA and CC and the soaking time on the hardness of the potato slices.
306 The hardness of the potato slices was chosen as the dependent variable
307 response, and the three independent variables were the concentration of
308 LA (X_1), concentration of CC (X_2), and soaking time (X_3). The factorial
309 design consisted of 5 central and 12 factorial points. The coded and
310 uncoded independent variables was listed in Table 1, and all the trials in
311 this series were performed at least in triplicate. Software named Design-
312 Expert (STAT-EASE Inc., Minneapolis, MN, USA version 7.0) was
313 applied to evaluate the experimental design and for statistical analysis.
314 The following second-order polynomial equation was applied to show the
315 predicted responses (hardness of the potato slices) as a function of the
316 independent variables.

317 $$Y_i = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1^2 + a_{22}X_2^2$$
318 $$+ a_{33}X_3^2$$

319 where X_i represents the independent variables, a_0 is a constant, and a_i,
320 a_{ii}, and a_{ij} are the linear, quadratic, and interactive coefficients,
321 respectively.

322 *2.10. Statistical Analysis*

323 All measurements in this study were performed in triplicate. Tukey's
324 HSD test was used to compare the means. The data were subjected to
325 analysis of variance (ANOVA), and differences among the tested samples

were considered significant at $p<0.05$. All computations and analyses were using SPSS19.0.

3. Results and discussion

3.1. Texture Characteristics and Galacturonic Acid (GalA) Contents of Potato Slices Following Different Treatments

The potato samples had the highest hardness (2828 *g*) when freshly sliced, and the hardness decreased upon soaking in distilled water (DW) for 10 h, as shown in Fig. 1. As the boiling time increased from 3 to 10 min, the hardness of the potato slices decreased from 1999 *g* to 288 *g*. Potato slices soaked in LA (725 *g*) and CC (1042 *g*) for 10 h showed a higher hardness values after heating for 10 min. Negatively charged galacturonic acid residues in pectin could interact through ionic bonds in the presence of Ca^{2+} and form a calcium-pectate structure to improve the strength of the cell walls. However, why the hardness of the potato slices was improved after soaking in LA remains unclear. The variation in the GalA contents of the potato slices was opposite with that of the hardness values during the same treatment procedures. Freshly sliced potato and slices soaked in DW for 10 h had lower GalA contents than samples subjected to other treatments. With increasing heating time, the GalA content in the potato slices increased from 21.67 to 36.33 g/kg. This change may be due to the increase in the solubility and diffusivity of pectin after thermal treatment (Rico, Martin-Diana, Frias, Barat,

JHenehan, & Barry-Ryan, 2007). Potato slices soaked in LA and CC and then boiled for 10 min had GalA contents similar to those of potato slices soaked in DW for 10 h and then boiled for 3 min. The primary wall contributes to the wall structural integrity and cell adhesion in plants (Somerville, et al., 2004). Thus, the loss of pectin from the plant primary cell walls and the middle lamellae caused a decrease in the potato hardness. However, the LA and CC were likely to serve as protectors, preventing the loss of pectin from the cell wall.

3.2. Scanning Electron Microscopy (SEM) Analysis of Potato Slices Following Different Treatments

In the fresh and unheated potato slices, the cell walls were linked together, and the starch granules were scattered throughout the interior of the cells (Fig. 2 a and b). The starch granules in the potato samples deformed and aggregated into one mass filling the whole interior of the cell after heating for 3-10 min (Fig. 2 c, d, and e). An obvious intercellular separation was observed in potato slices after heating for 10 min due to the thermal breakdown of the pectin in the middle lamella and the cell wall, which decreased cell integrity (Parker, et al., 2001). The pectin is responsible for joining the face of the middle lamella to the face of the primary walls (Fuentes, Vázquez-Gutiérrez, Pérez-Gago, Vonasek, Nitin, & Barrett, 2014). Interestingly, this phenomenon (intercellular separation) did not occur in the potato slices that were soaked in LA (Fig.

2 f) and CC (Fig. 2 h) and then heated for 10 min. However, there was a subtle difference between the two treatments upon close inspection of the surface of the cells. The surfaces of the potato cells (Fig. 2 f) appeared to be coated with a layer of a colloidal substance that bound these cells together. The same phenomenon was observed in potato slices pre-soaked in 0.8% acetic acid and then heated for 30 min (Zhao, Shehzad, Yan, Li, & Wang, 2017). Pectin with a high degree of methoxylation can form a gel under acidic conditions. Thus, the acidic conditions during pre-soaking in LA may promote gelation of the pectin, and the gel could then act as an adhesive to firmly bind the potato cells together. However, the cell surfaces shown in Fig. 2 h appeared to be strongly fixed. From the picture (Fig. 2 f and h), it seemed that the mechanisms by which pre-soaking in LA and CC improved potato hardness were different. However, we did find some more interesting result (200 X) from the SEM image from potato treated with LA after 10 min boiling, which was recorded in Fig. 2 (g). Firstly, the cell structure was very complete as we can see from the image; Secondly, the gelatinized starch (the cell wall was taken off and leaving porous substance in the cell) was trapped in the potato cell, which again proved that the potato cell was complete after 10 min boiling.

3.3. Molecular Weight (MW) Profiles of Soluble Pectin Polysaccharides from Potato Slices Following Different Treatments

391 SEM images showed an obvious intercellular separation in the
392 potato slices after boiling for 10 min, and this observation directly proved
393 that the binding of the middle lamella to adjacent cells was interrupted
394 (experimental condition: potato slice soaked in distilled water for 10 h
395 with 10 min heat treatment). The pectin matrix is the main component of
396 the middle lamella (Knox, 1992), and it prevents the aggregation and
397 collapse of the internal structure of the cell wall (experimental condition:
398 potato slice soaked in 5 g/L lactic acid for 10 h with 10 min heat
399 treatment). Thus, changes in the pectin polysaccharides during heating
400 were determined by exclusion chromatography. The pectin fractions,
401 including (1) the water-soluble (water-soluble pectin, WSP), (2)
402 Cyclohexane-diamine-tetra acetic acid (CDTA)-soluble (CDTA-soluble
403 pectin, CSP) and (3) Na_2CO_3-soluble (Na_2CO_3-soluble pectin, NSP)
404 fractions, extracted from the cell walls of the potato slices were likely to
405 be loosely, ionically and covalently linked to hemicellulose and cellulose,
406 respectively, in the cell wall structure. The changes in these pectin
407 fractions in the potato slices during the heat treatments are shown in Fig.
408 3. For the WSP fraction of the fresh potato slices (WSP, A), a major peak
409 was observed at the beginning of the profile at 7.5 min followed by two
410 peaks for lower M_W pectins between 10-11 min. The main peak gradually
411 separated into two peaks (at 7.5 min and 9.5 min) after boiling for 3-10
412 min (WSP, B-E). The profile of the WSP fraction of potato slices soaked

in 0.5% LA for 10 h was significantly different, and it contained a new peak (7.1 min) after 10 min of boiling. The M_W of the WSP fraction of potato slices (G) treated with 0.5% CC for 10 h seemed to be intermediate between that of the WSP fraction of potato slices (B) boiled for 3 min and that of the WSP fraction of potato slices (C) boiled for 5 min. The intensity of the molecular weight profiles of the CSP fraction was much higher than those of the WSP and NSP fractions. Thus, no changes were observed among these samples (CSP-1, A-G). However, the changes in the M_W of the CSP fraction after different treatments were observed when the signal intensities were adjusted to levels similar to those of WSP and NSP (after the adjustment of signal intensities, the CSP fraction was summarized as CSP-2, A-G). The M_W of CSP-2 in potato slices decreased (the peak shifted from 8.9 min to 10 min) after boiling. The CSP-2 fraction of potato slices treated with 0.5% LA for 10 h showed only one main peak at 7.1 min. The M_W of CSP-2 (G) of potato slices treated with 0.5% CC for 10 h resembled that of CSP-2 (C) of potato slices boiled for 3 min. The profile of the NSP fraction of fresh potato slices had two peaks at 6.9 min and 10.8 min. As the treatment time increased, a broad peak at approximately 8.7 min appeared. The profile of the NSP fraction of potato slices soaked in LA for 10 min showed only one peak at 7.1 min, and the tail peak at 10.8 min had disappeared. The M_W of the NSP (G) fraction of potato slices treated with 0.5% CC for 10 h resembled that of

the NSP (D) fraction of potato slices boiled for 5 min. The WSP and CSP-2 fractions of potato slices treated with LA showed the same M_W peak at 7.1 min, which was completely different from the peaks of other samples. This result was probably due to the gelation of the pectin, which formed an aggregate with a larger M_W. Unlike the profile of potato slices boiled for 10 min, a broad peak at approximately 8.7 min did not appear in the profile of the NSP fraction of potato slices treated with LA. It seemed that these pectin fractions were also involved in gelation. The M_W distribution of the potato slices soaked in CC (and heated for 10 min) was quite similar to the M_W distribution of the potato slices thermally treated for 3-5 min, which was likely a result of a delay in the changes caused by heat treatment. Upon thermal treatment, the pectin in CC-treated common bean consistently showed a higher M_W than that in beans treated with demineralized water (Njoroge, et al., 2016). The high M_W pectins in both the water-soluble and chelating agent-soluble fractions were solubilized and extensively depolymerized into pectins with lower M_W distributions (Broxterman, Picouet, & Schols, 2017).

3.4. Sugar Profile of Soluble Pectin Polysaccharides from Potato Slice Following Different Treatments.

The extractable pectic polysaccharides in fresh and cooked potatoes are composed mainly of GalA (701.42 and 1077.68 mg/g) with moderate amounts of Gal (41.2 and 57.75 mg/g), Fuc (25.02 and 26.34 mg/g) and

457 Rha (17.48 and 19.27 mg/g) and minor amounts of Ara (5.57 and 12.6
458 mg/g) and Xyl (6.82 and 9.05 mg/g). Heating processes were associated
459 with decreases in the textural characteristics of potato slices as well as
460 homogalacturonan depolymerization (the contents of GalA in WSP were
461 significantly increased by heating) and loss of neutral sugar side-chains in
462 rhamnogalacturonan I and rhamnogalacturonan II (the contents of Ara,
463 Gal, Fuc, Xyl, and Rha in WSP were significantly increased by heating).
464 The increase in the contents of GalA and other neutral sugars in the CSP
465 fraction of potato slices after heat treatment followed the same trend that
466 was seen in the sugar profiles in WSP. However, heat treatment
467 decreased the sugar content in the NSP fraction. The CDTA- and
468 Na_2CO_3-extractable fractions were the ionically and covalently bound
469 soluble fractions of pectin, respectively. The CDTA fraction comes from
470 the middle lamella (with a lower neutral sugar content than the Na_2CO_3
471 fraction from fresh potato slices), while the Na_2CO_3 fraction is released
472 from the primary cell wall. Table 2 shows that 82% of the Ara and 93%
473 of the Rha were in the NSP fraction, which implied that
474 rhamnogalacturonan (RG) played an important role in the construction of
475 the cell wall structure. GalA was the main component in the CSP fraction,
476 which implied that homogalacturonan (HG) was responsible for
477 connecting adjacent cells. The increase in GalA and neutral sugar
478 contents in the WSP and CSP fractions after heat treatment may be due to

479 the NSP fraction, which became less tightly bound to the extracellular
480 matrix during the heating process. Compared with potato slices boiled for
481 10 min without treatment, LA and CC treatment decreased the dissolution
482 of GalA and other neutral sugars (lower sugar contents in the WSP and
483 CSP fractions and a higher sugar content in the NSP fraction were
484 observed). This result indicated that the improved textural characteristics
485 of the boiled potato slices after LA and CC treatment were accompanied
486 by maintenance of the GalA and other neutral sugar contents in both the
487 middle lamella and the primary cell wall. The impact of heating on the
488 textural characteristics of carrots has been studied (Sila, Doungla, Smout,
489 Van Loey, & Hendrickx, 2006; Greve, McArdle, Gohlke, & Labavitch,
490 1994), and the concentration of pectin-related neutral sugars (rhamnose,
491 arabinose, and galactose) increased with increasing thermal processing
492 time in accordance with pectin solubilization (Sila, 2006). The CSP and
493 NSP fractions were characterized by lower amounts of neutral sugars than
494 the WSP fraction (Sila, 2006). Nevertheless, the amount of neutral sugars
495 in the NSP fraction also declined with increasing thermal processing time
496 (Sila, 2006). The GalA content in ginseng pectin increased 1.62-fold after
497 steaming at 100 °C and 2.06-fold after steaming at 120 °C (Jiao, Zhang,
498 Wang, Li, Liu, & Liu, 2014).

499 *3.5. Polygalacturonase (PG) and Pectin Methylesterase (PME) Activity*
500 *of Potato Slices Following Different Treatments*

The degradation of pectic polymers in the native plant cell wall is mostly due to the activity of pectinases, including PG and PME (Caffall, & Mohnen, 2009). PG can directly hydrolyse HG, while PMEs can hydrolyse the constituent methyl esters, yielding HG with a low degree of methylation that could then be cleaved by PG (Wang, Yeats, Uluisik, Rose, & Seymour, 2018). Thus, the activities of these two kinds of enzymes were determined in the test samples. The specific activities of PG and PME when potato was harvested, were 0.118 ± 0.004 U/g and 0.472 ± 0.025 U/g. As shown in Fig. 4, the specific activities of PG (from 0.124 to 0.200 U/g) and PME (from 0.498 to 0.707 U/g) significantly increased with increasing thermal treatment time (from 0 to 10 min). Increasing the temperature of the thermal treatment caused a gradual decrease in the enzyme activity in carrot (Jolie, Duvetter, Verlinde, Buggenhout, Loey, & Hendrickx, 2009) and an increase in the inactivation rate constant in banana (Ly-Nguyen, Van Loey, Smout, Verlent, Duvetter, & Hendrickx, 2003). The differences between the results in this study and previous studies on the influence of thermal treatment on enzyme activities in plants may be due to the different experimental systems (packed and unpacked). The aluminium foil vacuum pouches lowered the heat transfer rate from the boiling water to the potato slices. Thus, we could observe slower heating processes than can be achieved in the absence of packaging. With the same thermal

treatment time, potato slices soaked in LA for 10 h showed the same PME activity and significantly lower PG activity than untreated potato slices. CC treatment significantly inhibited both PME and PG activity in potato slices. In both orange and A. *aculeatus*, the specific activity of PME also decreased in the presence of CC (Videcoq, Garnier, Robert, & Bonnin, 2011).

3.6. Optimization of the Pretreatment of Potato Slices with LA and CC

The data from 17 experiments (Table 1) related to the hardness of the potato slices were fitted to the following second-order polynomial: Y_i=954.4+107.8 X_1 +91.4 X_2 +262.9 X_3 +1.4 X_1X_2 -0.9 X_1X_3 +0.8 X_2X_3 - 6.0-X_1^2 -7.5 X_2^2-30.5X_3^2.

The ANOVA results are shown in Supplementary 2. The model coefficient proved the validity of using this method to describe the effect of pretreatment on the hardness of potato slices after boiling. The coefficient of determination R^2 was 0.9234, showing that the obtained model could explain 92.34% of the variability in the response. The model also gave a *p* value for the hardness (0.0642) of the potato slices (higher than 0.05), indicating that the lack-of-fit was not significant relative. The maximum potato slice hardness (2342 ± 202 *g*) was achieved using the optimal conditions (soaking time = 9.45 h, LA concentration = 7.21 g/L, and CC concentration = 4.27 g/L). The difference ($p > 0.05$) between the experimental and predicted values (2355 *g*) was not significant.

As shown in Supplementary 3, the LA concentration increased to 8 g/L, the contact probability between pectin and LA (which promoted the gelation of pectin) and the inhibitory effect on PG (which maintained the cell structure) increased, causing an increase in the hardness of the potato slices. However, a higher LA concentration decreased the hardness of the potato slices by increasing the permeability of the plant cell membrane, which enhanced the mobility of the endogenous enzymes (causing damage to the cell wall). The hardness of the potato slices increased as the CC concentration increased from 1 to 4 g/L. As this concentration increased, the inhibitory effect of CC on PG and PME might also increase. Further increases in the CC concentration decreased the hardness of the potato slices. The combination of LA and CC has a substantial effect on the hardness of the potato slices after boiling. Increasing the pre-soaking time also increases the contact probability between the pectin and the reagents (LA and CC). However, long LA and CC soaking times are detrimental to the hardness of the potato slices.

4. Conclusion

Based on cell wall chemical and enzymatic analyses, softening of the potato tissue upon heat treatment may be due to the solubilization of pectin, which is accompanied by an increase in GalA, a decrease in pectin Mw and an increase in PG activity. The mechanism of enhancing the post-boiling hardness of potato slices by pretreatment with LA and CC

lies in the following factors. (1) Pre-soaking of the potato slice in LA inhibited PG activity, which decreased degradation of the structure of the cell wall. Nevertheless, the acidic environment during pre-soaking in LA may promote gelation of the pectin, and the gel could act as an adhesive agent to firmly bind the potato cells together. (2) Pre-soaking the potato slices in CC significantly increased potato hardness by strongly inhibiting PG activity. Through the combined use of LA and CC, the hardness of boiled potato slices was significantly increased to 2342 *g* (boiled potato slice without treatment, 288 *g*). LA and CC were the permitted additives in Food Chemicals Codex, and had been extensively applied in food processing, such as acidifying (LA, concentration of 1%-20%, *w*/*w*) of whey (Saffari, & Langrish, 2014), extending the shelf-life (LA, concentration of 1.5%, w/w) of common carp (Noori, Khanzadi, Fazlara, Najafzadehvarzi, & Azizzadeh, 2018), improving antibacterial activity (LA, concentration of 0.08%-0.75%, *w*/*w*) of gelatin (Moreira, et al., 2019), retaining freshness (CC, concentration of 1%-4%, *w*/*v*) of strawberry (Chen, et al., 2011), improving the structure (CC, concentration of 0.2%-0.5%, *w*/*v*) of cheese curd (Moudrá, Pachlová, Černíková, Šopík, & Buňka, 2017). Thus, the technology developed in the current study is safe for human consumption and can be applied in the development of novel potato-based foods, such as non-fried or pickled potato slice (the crispy mouthfeel of potato slice can be retained after

589 gelatinization of starch, while less worry about the excessive intake of
590 lipid, please see supplementary 4).

591 **CONFLICTS OF INTEREST**

592 There are no conflicts to declare.

593 **ACKNOWLEDGMENTS**

594 This research was conducted by financial supported from the
595 National Natural Science Foundation of China (31760463), Construction
596 Project of Innovative Talents Base of Guizhou Provence ([2016] 22) and
597 National Key R&D Program of China (2016YFD0401304).

Reference

Bouchon, P. (2009). Understanding oil absorption during deep-fat frying. *Advances in Food and Nutrition Research*, *57*(9), 209-234.

Bordoloi, A., Kaur, L., & Singh, J. (2012). Parenchyma cell microstructure and textural characteristics of raw and cooked potatoes. *Food Chemistry*, *133*(4), 1092-1100.

Broxterman, S. E., Picouet, P., & Schols, H. A. (2017). Acetylated pectins in raw and heat processed carrots. *Carbohydrate Polymers*, *177*, 58-66.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965-977.

Caffall, K. H., & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, *344*(14), 1879-1900.

Chan, Z., & Tian, S. (2005). Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. *Postharvest Biology and Technology*, *36*(2), 215-223.

Chen, F., et al. (2011). Quality attributes and cell wall properties of strawberries (*Fragaria annanassa* Duch.) under calcium chloride treatment. *Food Chemistry*, *126*(2), 450-459.

Fuentes, A., Vázquez-Gutiérrez, J. L., Pérez-Gago, M. B., Vonasek, E.,

620 Nitin, N., & Barrett, D. M. (2014). Application of nondestructive
621 impedance spectroscopy to determination of the effect of temperature on
622 potato microstructure and texture. *Journal of Food Engineering*, *133*, 16-
623 22.
624 García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2008).
625 Textural properties of potatoes *(Solanum tuberosum* L., cv. *Monalisa*) as
626 affected by different boiling processes. *Journal of Food Engineering*,
627 *88*(1), 28-35.
628 Gwanpua, S. G., et al. (2014). Pectin modifications and the role of pectin-
629 degrading enzymes during postharvest softening of Jonagold apples.
630 *Food Chemistry*, *158*, 283-291.
631 Greve, L. C., McArdle, R. N., Gohlke, J. R., & Labavitch, J. M. (1994).
632 Impact of heating on carrot firmness: changes in cell wall components.
633 *Journal of Agricultural and Food Chemistry*, *42*(12), 2900-2906.
634 Jiao, L., Zhang, X., Wang, M., Li, B., Liu, Z., & Liu, S. (2014). Chemical
635 and antihyperglycemic activity changes of ginseng pectin induced by heat
636 processing. *Carbohydrate Polymers*, *114*, 567-573.
637 Jolie, R. P., Duvetter, T., Verlinde, P. H., Van Buggenhout, S., Van Loey,
638 A. M., & Hendrickx, M. E. (2009). Size exclusion chromatography to
639 gain insight into the complex formation of carrot pectin methylesterase
640 and its inhibitor from kiwi fruit as influenced by thermal and high-
641 pressure processing. *Journal of Agricultural and Food Chemistry*, *57*(23),

642 11218-11225.

643 Jarvis, M. C., Mackenzie, E., & Duncan, H. J. (1992). The textural
644 analysis of cooked potato. 2. Swelling pressure of starch during
645 gelatinisation. *Potato Research*, *35*(1), 93-102.

646 Knox, J. P. (1992). Cell adhesion, cell separation and plant
647 morphogenesis. *The Plant Journal*, *2*(2), 137-141.

648 Kurita, O., Fujiwara, T., & Yamazaki, E. (2008). Characterization of the
649 pectin extracted from citrus peel in the presence of citric acid.
650 *Carbohydrate Polymers*, *74*(3), 725-730.

651 Kang, J., Hua, X., Yang, R., Chen, Y., & Yang, H. (2015).
652 Characterization of natural low-methoxyl pectin from sunflower head
653 extracted by sodium citrate and purified by ultrafiltration. *Food*
654 *Chemistry*, *180*, 98-105.

655 Kaur, L., Singh, N., Sodhi, N. S., & Gujral, H. S. (2002). Some properties
656 of potatoes and their starches I. Cooking, textural and rheological
657 properties of potatoes. *Food Chemistry*, *79*(2), 177-181.

658 Liu, S. N., Han, Y., & Zhou, Z. J. (2011). Lactic acid bacteria in
659 traditional fermented Chinese foods. *Food Research International*, *44*(3),
660 643-651.

661 Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional
662 starter cultures for the food fermentation industry. *Trends in Food*
663 *Science & Technology*, *15*(2), 67-78.

Ly-Nguyen, B., Van Loey, A. N., Smout, C., Verlent, I., Duvetter, T., & Hendrickx, M. E. (2003). Effect of mild-heat and high-pressure processing on banana pectin methylesterase: a kinetic study. *Journal of Agricultural and Food Chemistry*, *51*(27), 7974-7979.

Moreira, T. F. M., et al. (2019). Hydrogels based on gelatin: Effect of lactic and acetic acids on microstructural modifications, water absorption mechanisms and antibacterial activity. *LWT- Food Science and Technology*, *103*, 69-77.

Moudrá, K., Pachlová, V., Černíková, M., Šopík, T., & Buňka, F. (2017). The combined effects of fat content, calcium chloride, and coagulant concentration on the development of cheese curd structure. *International Dairy Journal*, *73*, 92-97.

Ng, A., & Waldron, K. W. (1997). Effect of steaming on cell wall chemistry of potatoes (*Solanum tuberosum* cv. *Bintje*) in relation to firmness. *Journal of Agricultural and Food Chemistry*, *45*(9), 3411-3418.

Njoroge, D. M., et al. (2016). Mechanistic insight into common bean pectic polysaccharide changes during storage, soaking and thermal treatment in relation to the hard-to-cook defect. *Food Research International*, *81*, 39-49.

Ormerod, A., Ralfs, J., Jobling, S., & Gidley, M. (2002). The influence of starch swelling on the material properties of cooked potatoes. *Journal of Materials Science*, *37*(8), 1667-1673.

686 Parker, C. C., Parker, M. L., Smith, A. C., & Waldron, K. W. (2001).
687 Pectin distribution at the surface of potato parenchyma cells in relation to
688 cell− cell adhesion. *Journal of Agricultural and Food Chemistry*, *49*(9),
689 4364-4371.
690 Rocculi, P., et al. (2007) Effects of the application of anti-browning
691 substances on the metabolic activity and sugar composition of fresh-cut
692 potatoes. *Postharvest Biology and Technology*, *43*(1): 151-157.
693 Ross, H. A., et al. (2010). Potato tuber pectin structure is influenced by
694 pectin methyl esterase activity and impacts on cooked potato texture.
695 *Journal of Experimental Botany*, *62*(1), 371-381.
696 Rico, D., Martin-Diana, A. B., Frias, J. M., Barat, J. M., Henehan, G. T.
697 M., & Barry-Ryan, C. (2007). Improvement in texture using calcium
698 lactate and heat-shock treatments for stored ready-to-eat carrots. *Journal*
699 *of Food Engineering*, *79*(4), 1196-1206.
700 Shomer, I. (1995). Swelling behaviour of cell wall and starch in potato
701 (*Solanum tuberosum* L.) tuber cells-I. Starch leakage and structure of
702 single cells. *Carbohydrate Polymers*, *26*(1), 47-54.
703 Shomer, I., Vasiliver, R., & Lindner, P. (1995). Swelling behaviour of
704 cell wall and starch in potato (*Solanum tuberosum* L.) tuber cells-II.
705 Permeability and swelling in macerates. *Carbohydrate Polymers*, *26*(1),
706 55-59.
707 Shpigelman, A., Kyomugasho, C., Christiaens, S., Van Loey, A. M., &

708 Hendrickx, M. E. (2014). Thermal and high pressure high temperature
709 processes result in distinctly different pectin non-enzymatic conversions.
710 *Food Hydrocolloids*, *39*, 251-263.
711 Stepan, H., & Staudacher, E. (2011). Optimization of monosaccharide
712 determination using anthranilic acid and 1-phenyl-3-methyl-5-pyrazolone
713 for gastropod analysis. *Analytical Biochemistry*, *418*(1), 24-29.
714 Somerville, et al. (2004). Toward a systems approach to understanding
715 plant cell walls. *Science*, *306*(5705), 2206-2211.
716 Sila, D. N., Doungla, E., Smout, C., Van Loey, A., & Hendrickx, M.
717 (2006). Pectin fraction interconversions: insight into understanding
718 texture evolution of thermally processed carrots. *Journal of Agricultural*
719 *and Food Chemistry*, *54*(22), 8471-8479.
720 Sila, D. N., Smout, C., Vu, T. S., & Hendrickx, M. E. (2004). Effects of
721 high-pressure pretreatment and calcium soaking on the texture
722 degradation kinetics of carrots during thermal processing. *Journal of*
723 *Food Science*, *69*(5), E205-E211.
724 Videcoq, P., Garnier, C., Robert, P., & Bonnin, E. (2011). Influence of
725 calcium on pectin methylesterase behaviour in the presence of medium
726 methylated pectins. *Carbohydrate Polymers*, *86*(4), 1657-1664.
727 Vicente, A. R., Saladié, M., Rose, J. K. C., & Labavitch, J. M. (2007).
728 The linkage between cell wall metabolism and fruit softening: Looking to
729 the future. *Journal of the Science of Food and Agriculture*, *87*(8), 1435-

730 1448.

731 Wang, D., Yeats, T. H., Uluisik, S., Rose, J. K., & Seymour, G. B. (2018).
732 Fruit softening: Revisiting the role of pectin. *Trends in Plant Science*,
733 *23*(4), 302-310.

734 Willard, M. (1993). Potato processing: past, present and future. American
735 *Potato Journal*, *70*(5), 405-418.

736 Xie, F., Yuan, S., Pan, H., Wang, R., Cao, J., & Jiang, W. (2017). Effect
737 of yeast mannan treatments on ripening progress and modification of cell
738 wall polysaccharides in tomato fruit. *Food Chemistry*, *218*, 509-517.

739 Zhao, W., Shehzad, H., Yan, S., Li, J., & Wang, Q. (2017). Acetic acid
740 pretreatment improves the hardness of cooked potato slices. *Food*
741 *Chemistry*, *228*, 204-210.

742 **Figure caption**

743 Fig.1 Hardness and galacturonic acid (GalA) content of potato slice with
744 different treatment. (Fresh sample; soaked in distilled water for 10 h
745 without heat treatment; soaked in distilled water for 10 h with 3 min heat
746 treatment; soaked in distilled water for 10 h with 5 min heat treatment;
747 soaked in distilled water for 10 h with 10 min heat treatment; soaked in 5
748 g/L lactic acid for 10 h with 10 min heat treatment; soaked in 5 g/L
749 calcium chloride for 10 h with 10 min heat treatment; data columns
750 representing hardness and points representing GlaA content bearing
751 different capital and lowercase letters are significantly different by
752 Tukey's HSD test, $P < 0.05$; 3 samples in each treatment was tested; the
753 determination of hardness and galacturonic acid (GalA) content were
754 performed in triplicate)

755

756 Fig.2 SEM images (a-f, and h with a magnification of x100; g with a
757 magnification of x200) of potato slice with or without boiling and
758 treatment. (Fresh sample, a; soaked in distilled water for 10 h without
759 heat treatment, b; soaked in distilled water for 10 h with 3 min heat
760 treatment, c; soaked in distilled water for 10 h with 5 min heat treatment,
761 d; soaked in distilled water for 10 h with 10 min heat treatment, e; soaked
762 in 5 g/L lactic acid for 10 h with 10 min heat treatment, f; soaked in 5 g/L
763 lactic acid for 10 h with 10 min heat treatment, g; soaked in 5 g/L calcium

764 chloride for 10 h with 10 min heat treatment, h)

765 Fig.3 Molecular weight (M_W) profiles of soluble pectin polysaccharides
766 (including water-soluble pectin, WSP; CDTA-soluble pectin, CSP;
767 Na_2CO_3-soluble pectin, NSP) of potato slice with or without boiling and
768 treatment. (Fresh sample, A; soaked in distilled water for 10 h without
769 heat treatment, B; soaked in distilled water for 10 h with 3 min heat
770 treatment, C; soaked in distilled water for 10 h with 5 min heat treatment,
771 D; soaked in distilled water for 10 h with 10 min heat treatment, E;
772 soaked in 5 g/L lactic acid for 10 h with 10 min heat treatment, F; soaked
773 in 5 g/L calcium chloride for 10 h with 10 min heat treatment, G; 3
774 samples in each treatment was tested; the determination of M_W was
775 performed in triplicate)

776

777 Fig.4 Polygalacturonase (PG) and pectin methylesterase (PME) activity
778 of potato slice with different treatment. (Fresh sample; soaked in distilled
779 water for 10 h without heat treatment; soaked in distilled water for 10 h
780 with 3 min heat treatment; soaked in distilled water for 10 h with 5 min
781 heat treatment; soaked in distilled water for 10 h with 10 min heat
782 treatment; soaked in 5 g/L lactic acid for 10 h with 10 min heat treatment;
783 soaked in 5 g/L calcium chloride for 10 h with 10 min heat treatment;
784 data columns representing PG activity and points representing PME
785 activity content bearing different capital and lowercase letters are

786 significantly different by Tukey's HSD test, $P < 0.05$; 3 samples in each
787 treatment was tested; the determination of PG and PME activity was
788 performed in triplicate)

Supplementary 1 Package style of potato slice for thermal treatment.

Supplementary 2 Analysis of Variance of the Regression Coefficients of the Fitted Quadratic Equations for the Hardness of potato slice after pretreatment.

Supplementary 3 Response surface plots showing the interactive effects of degree of concentration of LA (X_1), concentration of CC (X_2), and soaking time (X_3) on the hardness of boiled potato slice.

Supplementary 4 Non-fried potato slice (left is the flavor of spicy, and right is the flavor of pickled pepper)

803 **Table 1. Box-Behnken Design Matrix, Experimental Values for Three-Level,**

804 **Three-Factor Response Surface Analysis**

no.[a]	decoded values			experiment values (g)[b,c]
	X_1 soking time (h)	X_2 lactic acid concentration (g/L)	X_3 cacium chloride concentration (g/L)	
1	5	4	4	2260.1±118.6
2	15	4	4	2049.1±161.9
3	5	12	4	2049.4±140.2
4	15	12	4	1947.6±174.0
5	5	8	1	1865.6±187.4
6	15	8	1	1939.7±84.3
7	5	8	7	1928.8±153.1
8	15	8	7	1952.0±228.6
9	10	4	1	1891.8±294.8
10	10	12	1	1850.3±284.9
11	10	4	7	2036.6±121.5
12	10	12	7	2033.3±145.9
13	10	8	4	2397.9±266.8
14	10	8	4	2398.9±245.8
15	10	8	4	2308.4±268.4
16	10	8	4	2321.5±244.7
17	10	8	4	2307.5±130.0

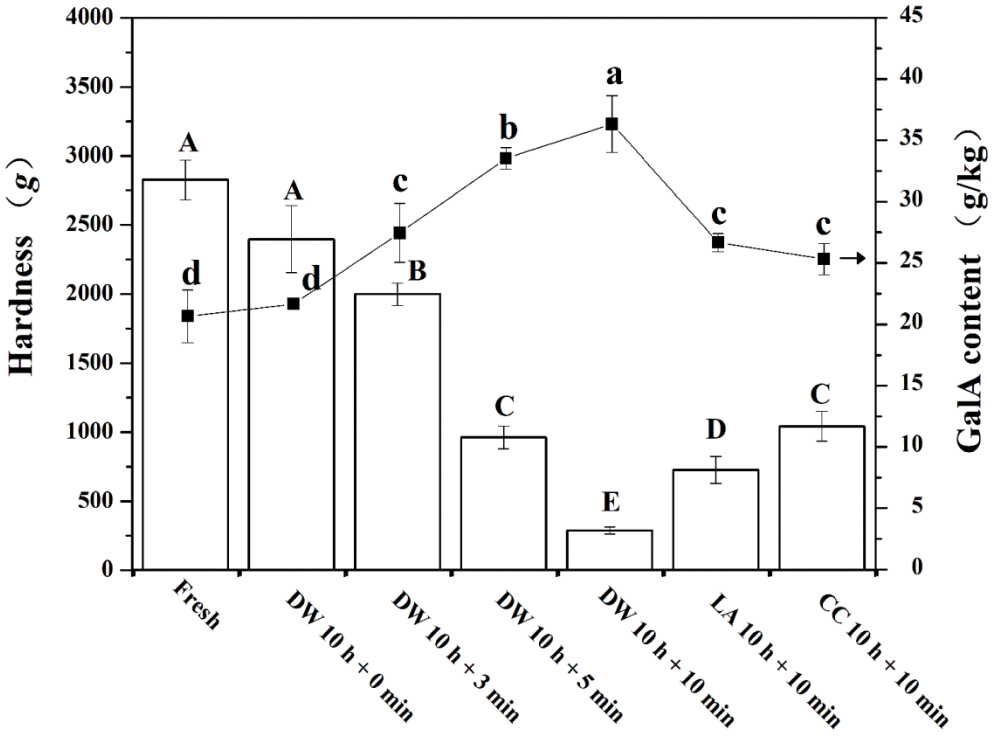
805 [a]Experiments no. were standard order in the Box-Behnken Design. [b]Hardness of
806 potato slice (g).[c]3 samples in each group was tested, and the whole design was
807 performed in triplicate

808

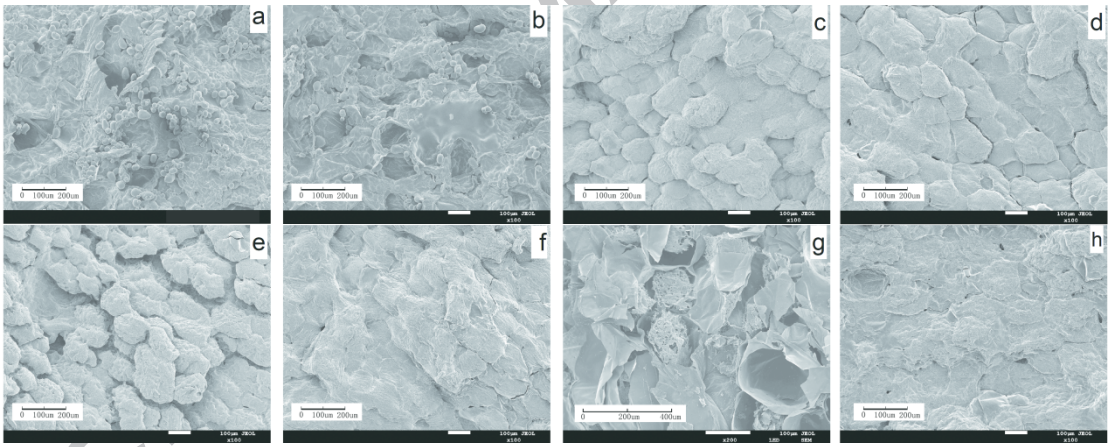
809 **Table 2. Sugar profile [arabinose (Ara), galactose (Gal), fucose(Fuc), xylose (Xyl),**
810 **rhamnose (Rha) and galacturonicacid(GalA), mg/g] of WSP,CSP, and NSP for**
811 **potato slice freshly prepared or soaked in distilled water (DW), lactic acid (LA)**
812 **and calcium chloride (CC), and thermally treated with 0-10 min.[a]**

Pectin	Treatment	Ara	Gal	Fuc	Xyl	Rha	GalA
WSP	Fresh	0.75±0.134b	4.50+0.40d	7.42±0.06a	0.75±0.03ab	0.50±0.03a	200.89±2.52c
	DW 10 h + 0 min	2.60±0.13fg	2.34±0.93b	8.29+0.05d	1.12±0.61c	3.04±0.08g	280.14±6.50i
	DW 10 h + 3 min	6.33±0.43j	3.41±0.11c	8.35±0.08d	3.35±0.18h	6.18±1.32i	383.42±7.20l
	DW 10 h + 5 min	6.72±0.02k	19.04±0.52f	9.27±0.07e	5.31±0.26j	10.33±0.24n	521.39±5.07n
	DW 10 h + 10 min	8.43±0.04l	28.86±0.86j	9.25±0.08e	5.68±0.12j	16.58±0.98p	596.56±2.04o
	LA 10 h + 10 min	2.12±0.02e	7.41±0.18e	7.88±0.13bc	4.23±0.20i	1.84±0.02e	440.59±3.61m
	CC 10 h + 10 min	4.35±0.13i	7.50±0.05e	8.07±0.03c	3.46±0.07h	7.24±0.59j	160.71±5.52a
CSP	Fresh	0.23±0.01a	0.26±0.05a	7.29±0.25a	0.68±0.09a	0.66±0.11ab	180.51±8.01b
	DW 10 h + 0 min	0.27±0.01a	0.28±0.03a	7.23±0.21a	0.64±0.06a	1.02±0.02c	210.44±6.06cd
	DW 10 h + 3 min	0.39±0.09a	0.37±0.06a	8.23±0.06d	1.55±0.19b	1.27±0.02d	278.18±7.32hi
	DW 10 h + 5 min	0.34±0.01a	2.44±0.18b	9.47±0.25e	1.95±0.23d	1.95±0.27e	244.54±2.25f
	DW 10 h + 10 min	1.72±0.01d	7.43±0.19e	9.61±0.39e	2.56±0.05f	2.24±0.01f	290.61±1.19j
	LA 10 h + 10 min	1.15±0.01c	2.32±0.19b	7.25±0.18a	1.53±0.16b	0.77±0.11b	259.16±2.46g
	CC 10 h + 10 min	0.95±0.06bc	3.23±0.03c	8.22±0.03d	1.70±0.05d	1.93±0.06e	212.06±6.06cd
NSP	Fresh	4.59±0.03i	36.44±0.06l	10.31±0.51f	5.39±0.11j	16.32±2.16p	320.02±12.8k
	DW 10 h + 0 min	4.02±0.38h	39.06±1.97m	10.46±0.81f	4.99±0.06i	13.38±0.42o	261.06±5.12g
	DW 10 h + 3 min	2.83±0.12g	24.14±1.04h	7.47±0.14a	1.63±0.06d	8.82±0.10l	255.48±3.00g
	DW 10 h + 5 min	2.88±0.14g	27.01±2.28hi	7.96±0.04bc	1.13±0.72c	8.36±0.22k	238.87±8.19e
	DW 10 h + 10 min	2.45±0.27f	21.46±1.13g	7.48±0.01a	0.81±0.02d	3.65±0.70h	190.15±1.86b
	LA 10 h + 10 min	3.92±0.16h	33.62±2.22k	8.31±0.02d	2.73±0.32g	10.1±1.65m	222.52±7.26de
	CC 10 h + 10 min	4.43±0.35i	29.97±1.00j	7.49±0.05a	2.17±0.11e	10.85±0.31n	271.72±1.50h

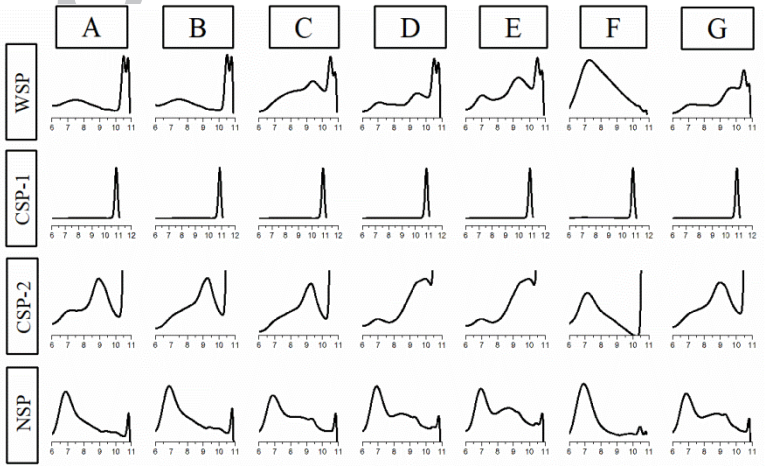
813 [a]3 samples in each treatment was tested, and the determination of sugar profile was performed in
814 triplicate.



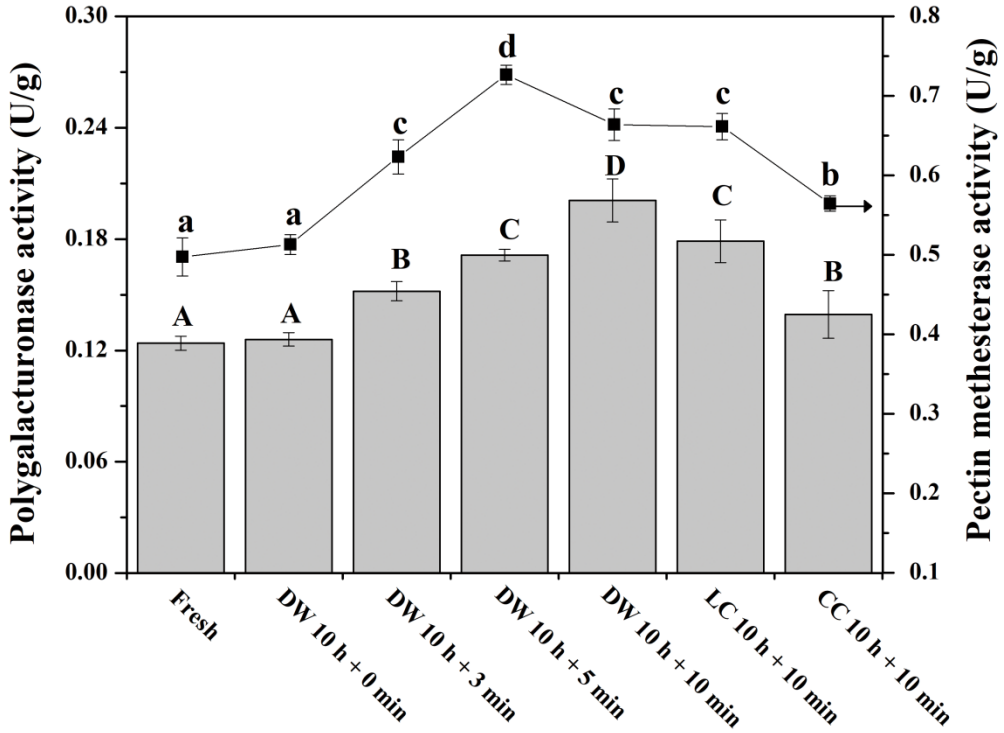
815



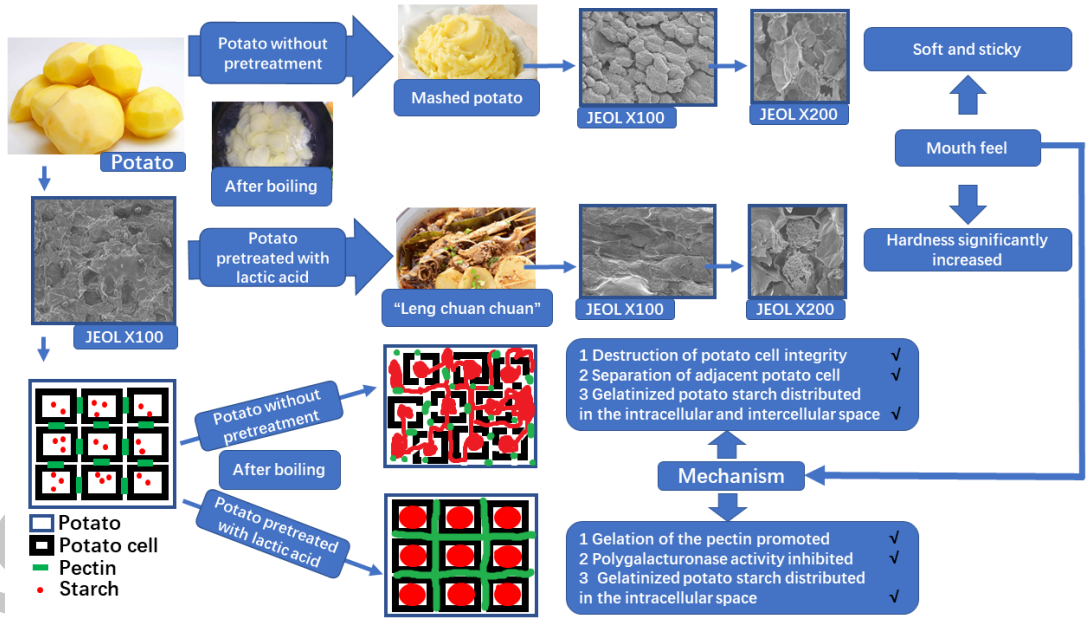
816



817



818



819

820 **Declaration of interests**

821

822 ☒ The authors declare that they have no known competing financial interests or

823 personal relationships that could have appeared to influence the work reported in this

824 paper.

825

826 ☐The authors declare the following financial interests/personal relationships which

827 may be considered as potential competing interests:

828

829

830

831

832

833

834 ■The hardness of boiled potato was significantly increased through

835 the combined use of LA and CC

836 ■Pre-soaking of the potato slice in LA promoted gelation of the

837 pectin

838 ■Pre-soaking of the potato slice in LA and CC inhibited PG activity

839

840