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Electrochemical Fingerprinting of Potatoes and Their Compositional Changes During Storage

Xubin Hua[1,2], *Qiao Lin*[1,2,*], *Junhua Liu*[1,2], *Bibo Shi*[1,2], *Li Cai*[1,2], *Fayong Gong*[1,2], *Zhengtao Li*[1,2]

[1] Xichang University, Xichang, Sichuan, China
[2] Panxi Crops Research and Utilization Key Laboratory of Sichuan Province, Xichang, Sichuan, China
[*]E-mail: qianlinqql@163.com

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Potatoes undergo significant changes in storage and these changes are critical for processing quality. In this study, the effect of six varieties of potatoes on processing quality during 180 days of storage was studied. Changes in the main substances, starch structure and characteristic indicators were monitored. At the same time, electrochemical fingerprints of potatoes during storage were collected to clarify the relationship between storage time and processing quality of potatoes. Potatoes showed an overall decrease in starch content during storage. The reducing sugar content showed a pattern of increase followed by decrease. There was a highly significant positive correlation between current values of electrochemical fingerprints and starch. There was no significant correlation between current values and reducing sugars. There was no significant correlation between current value and starch phosphorylase activity. There was a positive correlation between current value and amylase activity. There was also a positive correlation between current value and polyphenol oxidase.

Keywords: Electrochemical fingerprints; Potato; Polyphenol oxidase; Phosphorylase activity; Amylase activity

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual herb of the genus Solanum in the family Solanaceae, originating from the Peruvian-Polivian region in the western foothills of the central Andes in South America, bordering the Pacific Ocean. Potato is the fourth largest food crop in the world after wheat, rice and corn. Potatoes contain about 10-30% starch, which is more easily absorbed than the starch of cereal crops. The average protein content in potatoes is 2% in fresh potatoes [1,2]. However, its protein has a biomass value of 90-100, which is very similar to that of whole egg protein (100). The dietary fiber content of potato is 0.6%-0.8%, which is 2-12 times higher than that of rice, millet and wheat flour [3–5]. Potatoes contain about 0.2% fat and 2/3 of unsaturated fatty acids, which are low-fat foods. 100 g of

fresh potatoes contain about 342 mg of potassium, 8 mg of calcium, 0.8 mg of iron and 40 mg of phosphorus. Potatoes are also rich in 18 amino acids, including all essential amino acids [6–9].

The potato is still a living organism after harvesting. It is still undergoing various metabolic processes during storage, transportation, marketing and processing, which is the main factor affecting the quality status of potatoes during storage [10–12]. After harvesting, potatoes enter a dormant period, and the dormancy and germination process can be divided into three stages: the first stage is the potato block maturity period, which shows that the potato block epidermis has not been completely corked [13–15]. The water inside the potato evaporates rapidly to the outside, and the weight of the potato decreases sharply due to vigorous respiration and large water loss. If the storage temperature is higher at this time, it is more likely to accumulate water vapor, thus causing the rotting of potatoes [16]. After about 20-35 days of maturation, the potato skin is fully corked. The evaporative and respiratory intensity gradually decreases into a dormant state. The second stage is called the deep dormancy period. During this period the respiration of the potato slows down and nutrient consumption is reduced to a minimum [17–19]. The dormancy period of potato can be maintained for a longer period of time, usually around 2 months and up to more than 4 months. The third stage is called the late dormancy. At this time, the dormancy of potatoes ended, respiration again turned vigorous. The degree of potato weight reduction is directly proportional to the degree of sprouting [20,21]. Germination will make the potato tissue contains a lot of starch conversion and cause the appearance of wilting, while the potato germination parts produce toxic substances lobotropin [22–24]. This can result in loss of sales, processing, or even complete loss of food value. In this study, the effect of six varieties of potatoes on processing quality during 180 days of storage was studied. Changes in the main substances, starch structure and characteristic indicators were monitored. At the same time, electrochemical fingerprints of potatoes during storage were collected to clarify the relationship between storage time and processing quality of potatoes.

2. EXPERIMENTAL

2.1. Sample and storage conditions

The test varieties Sweet Potato (No. 6), Green Potato (No. 9), Atlantic, Mira, Sichuan Sweet Potato (No. 5), Sweet Potato 97 were harvested on October 7, 2020 and stored in semi-open brick cellars after 10 d of healing treatment. The date of storage in the cellar was 0 d and measurements were taken every 30 d. Seven measurements were taken. Enzyme activity was measured from 0 to 120 d, every 30 d. Varieties with the same name but different numbers are generally developed from the same research institute. However, varieties with different numbers differ in their characteristics. Therefore, some varieties will be widely planted. The varieties we choose are all widely grown varieties.

2.2. Reagents

Trichloroacetic acid was purchased from Chengdu Kelong Chemical Reagent Factory. Anhydrous ethanol was purchased from Chengdu Kolon Chemical Co. Ethylenediaminetetraacetic acid

(EDTA) was purchased from Tianjin Jindong Tianzheng Fine Chemical Reagent Factory. Ammonium molybdate, acetic acid, sodium hydroxide, magnesium chloride, glucose, hydroquinone, potassium iodide, disodium hydrogen phosphate, citric acid, potassium dihydrogen phosphate, sodium sulfite, 2-mercaptoethanol and propanetriol were purchased from Tianjin Damao Chemical Reagent Factory. Phosphoenolpyruvate, Tricine, Hepes, 3,5-dinitrosalicylic acid (DNS), adenosine diphosphate glucose (ADPG), DL-Dithiothreitol (DTT), Nicotinamide adenine dinucleotide phosphate (NADP), Army-6-phosphate dehydrogenase (G-6-PD), α -D-army-1-phosphate disodium salt tetrahydrate (G-1-pNA2), pyruvate kinase, hexokinase was purchased from Shanghai Yuanye Biotechnology Co., LTD.

2.3. Starch determination

Fresh potatoes were washed, dried and weighed (A). The tubers were then completely submerged in water at 17.5°C and weighed (B). The specific gravity of the tubers was calculated and compared to the Mepkep table to obtain the starch content.

2.4. Determination of reducing sugar content

Accurately weigh 7 g of chopped potato sample, add 5-10 mL of 85% ethanol to grind. The mixture was extracted in a constant temperature water bath at 50°C for 20 min to leach out reducing sugars and then centrifuged (4000 r/min, 15min). The supernatant was collected and the residue was washed with 20 mL of distilled water and centrifuged again. The supernatant from the 2 centrifugations was put into a 100 mL volumetric flask, and the volume was fixed to the scale as the reducing sugar solution to be measured. Add 1.5 mL of DNS reagent into a test tube, boil for 5 min, transfer to a 25 mL volumetric flask, shake well, and measure the absorbance at 540 nm.

2.5. Enzyme activity determination

Polyphenol oxidase (PPO): 5 g of potato sample was accurately weighed and placed in a mortar and pestle with 5 mL of 0.2 M disodium hydrogen phosphate-0.1 M citric acid buffer at pH=7. Add 0.5 g of PVP and a small amount of quartz sand, grind the homogenate and transfer to a centrifuge tube. Then rinse the mortar with 5 mL of 0.2 M disodium hydrogen phosphate-0.1 M citric acid buffer, pH=7. Combine the buffers and freeze centrifuge at 7500 r/min for 30 min at 4°C to obtain the crude enzyme extract. 2 mL of 0.1 M disodium hydrogen phosphate - citric acid buffer was added to 0.5 mL crude enzyme solution, and the temperature was kept warm in a water bath at 30°C for 4 min. Add 1 mL of 0.12 M catechol to the solution and immediately measure the absorbance value at 410 nm at 10 s intervals. Blanks were replaced with 0.1 M disodium hydrogen phosphate-citrate buffer for catechol, and the change in absorbance of 0.001 per second was used as 1 unit of polyphenol oxidase activity.

Starch phosphorylase: Weigh 5 g of potato sample, grind to homogenate in ice bath and fix 100 mL. The samples were extracted by immersion at 4°C for 2 h, then frozen and centrifuged for 15 min (3000×g), and the supernatant was the enzyme extract. Add 0.2 mL citrate buffer (0.5 M, pH=6.0), 0.2

mL soluble starch (0.5%), 0.1 mL enzyme extract, 0.5 mL distilled water, and 0.1 mL disodium glucose-1-phosphate (0.1 M) to the tube to be tested in order. Add 0.5 mL trichloroacetic acid (5%), 0.1 mL enzyme extract, 0.2 mL citric acid buffer (0.5 M, pH=6.0), 0.2 mL soluble starch (0.5%), 0.5 mL distilled water, and 0.1 mL disodium glucose-1-phosphate (0.1 M) to the blank test tube in order. The above solution was rapidly placed in a water bath at 30°C for 10 min, cooled, and the reaction was terminated by adding 0.5 mL of trichloroacetic acid (5%) to the tube to be tested. Then add 2 mL of ammonium molybdate solution, 1 mL of sodium sulfite solution and 1 mL of hydroquinone solution to the above test tubes and blank test tubes respectively. Then, add distilled water to fix the volume to 20 mL, mix well and let stand for 30 min. The absorbance was measured at 730 nm and the phosphorus content of the enzyme extract was calculated from the measured absorbance value on the standard curve. The phosphorus content was used to express the enzymatic activity of starch phosphorylase.

Amylase: Weigh 5 g of potato, add 5 mL of distilled water and a small amount of quartz sand to a mortar, grind into a homogenate and transfer to a centrifuge tube. The residue was washed into a centrifuge tube with 10 mL of distilled water, and the extract was left to extract for 15-20 min at room temperature, stirring once every few minutes to make it fully extracted. The solution was centrifuged at 3000r/min for 10min, and the supernatant was poured into a 50mL volumetric flask with distilled water and fixed to the scale, which was the amylase extract. Take 1 mL of amylase extract in the test tube and blank tube respectively. Add 2 mL of DNS reagent to the blank tube, then put the tube to be tested and the blank tube in a water bath at 40℃ for 10 min, and then add 1 mL of 10 g/L starch solution respectively. Hold the reaction at 40°C for 5 min, then add 2 mL of DNS reagent to the tube to be tested. Boil each tube in a water bath for 5 min, then remove and cool with running water, and add distilled water to fix the volume to 25 mL. The absorbance values were measured at 540 nm wavelength after shaking well, and the maltose content was calculated from the maltose standard curve to express the enzyme activity.

2.6. Electrochemical fingerprint determination

The electrochemical fingerprint was recorded by CHI1200C electrochemical workstation. A three-electrode system has been used for electrochemical fingerprint recording, where a commercial glassy carbon electrode (GCE, 3 mm), an Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively. A small amount of potato (0.01 g) was carefully mixed with 2 mL of solvent. Then, the slurry was sonicated for 5 min for extraction. Then, 2 μL of potato tissue dispersion was drop coated on the working electrode surface and dried naturally. The voltammetric profile (fingerprints) of plant leaf were recorded using differential pulse voltammetry (DPV) in either PBS (pH 7.0, 0.1 M) or ABS (pH 4.5, 0.1 M).

3. RESULTS AND DISCUSSION

The changes in starch content during storage was shown in Figure 1. At 0 d, the highest starch content was found in all varieties of potatoes with 23.32%, 21.41%, 15.23%, 21.52%, 18.64% and

18.97% for Sweet Potato (No. 6), Green Potato (No. 9), Atlantic, Mira, Sichuan Sweet Potato (No. 5), Sweet Potato 97, respectively. The percentage of Sweet Potato 97 was 23.32%, 21.41%, 15.23%, 21.52%, 18.64% and 18.97%, respectively. With the extension of storage time, the overall starch content showed a decreasing trend, but showed a wave-like change characteristic of drop-lift-drop-lift. The starch content dropped to the lowest point at 150 d with 13.22%, 5.21%, 5.60%, 4.46%, 6.29% and 5.23%, respectively. However, at the end of storage (180 d) the starch content increased [25]. After harvesting, potatoes go through three physiological stages: post-ripening, dormancy and sprouting. The first stage of post-ripening period, the potato block skin of corking is not yet complete. Combined with the higher storage temperature at this time the water in the tuber evaporates rapidly outward [26,27]. The weight of the potato block is significantly reduced, as shown by a sharp decline in dry matter content [28]. We noted that We noticed that at the end of storage, the dry matter content showed a "V" shaped trend, which may be due to the change in temperature. This may be caused by the change in temperature.

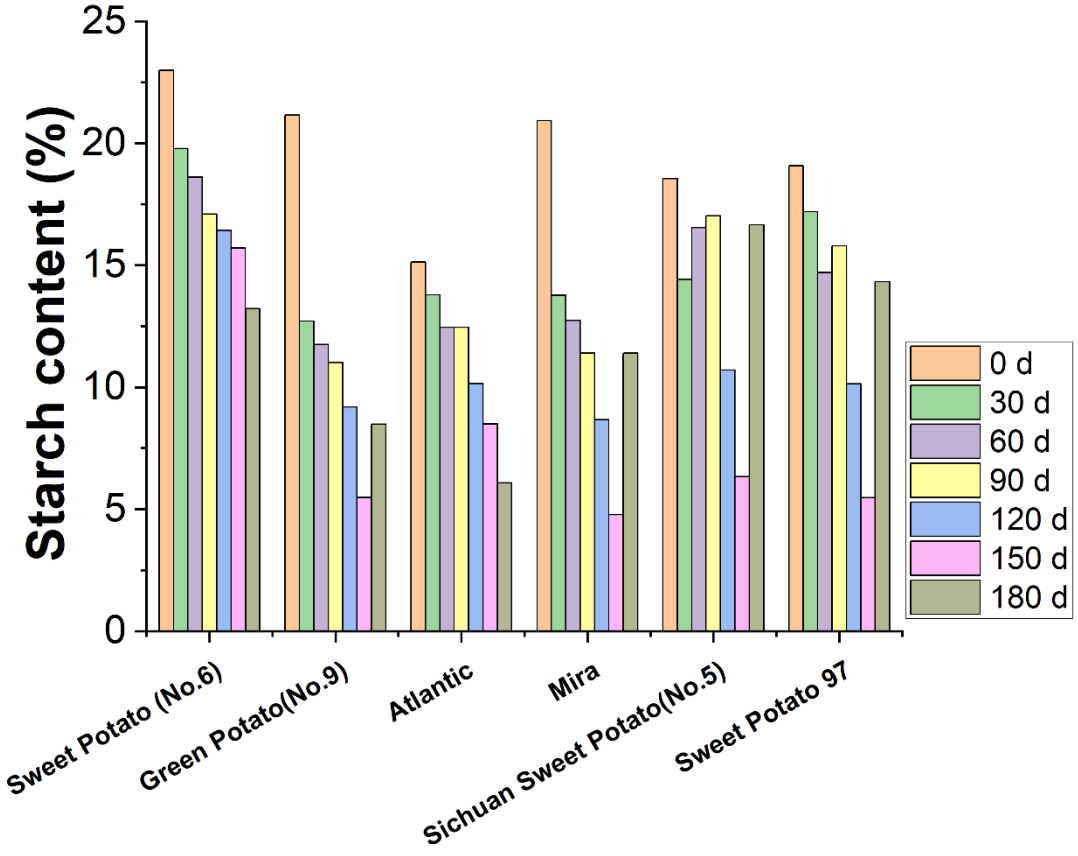

Figure 1. Changes in starch content of Sweet Potato (No.6), Green Potato(No.9), Atlantic, Mira, Sichuan Sweet Potato(No.5), Sweet Potato 97 during storage.

At the beginning of storage, the six varieties of potatoes had the lowest reducing sugar content. The reducing sugar content increased gradually with storage time, especially in the middle of storage (Figure 2). Sweet Potato (No. 6), Mira and Sweet Potato 97 reached the highest reducing sugar content at 60 d with 0.93%, 0.72% and 0.81%, respectively. Green Potato (No. 9), Atlantic and Sichuan Sweet Potato (No. 5) reached the highest reducing sugar content at 90 d, with 0.92%, 1.04% and 0.88%,

respectively. Sweet Potato (No. 6), Green Potato (No. 9), Atlantic, Mira, Sichuan Sweet Potato (No. 5), and Sweet Potato 97 all showed a 3- to 5-fold increase in reducing sugar content at the highest point compared to the lowest point at 0 d. After this period, the reducing sugar content started to decrease, but all increased significantly compared to the beginning of storage. High temperatures and high respiratory consumption reduce the dry matter content more. However, high temperature increases the water loss from tubers, which can relatively increase the dry matter content of tubers [29,30]. The varieties with high dry matter content are suitable for frying and baking processing, with high product yield and low oil content of fried products [31].

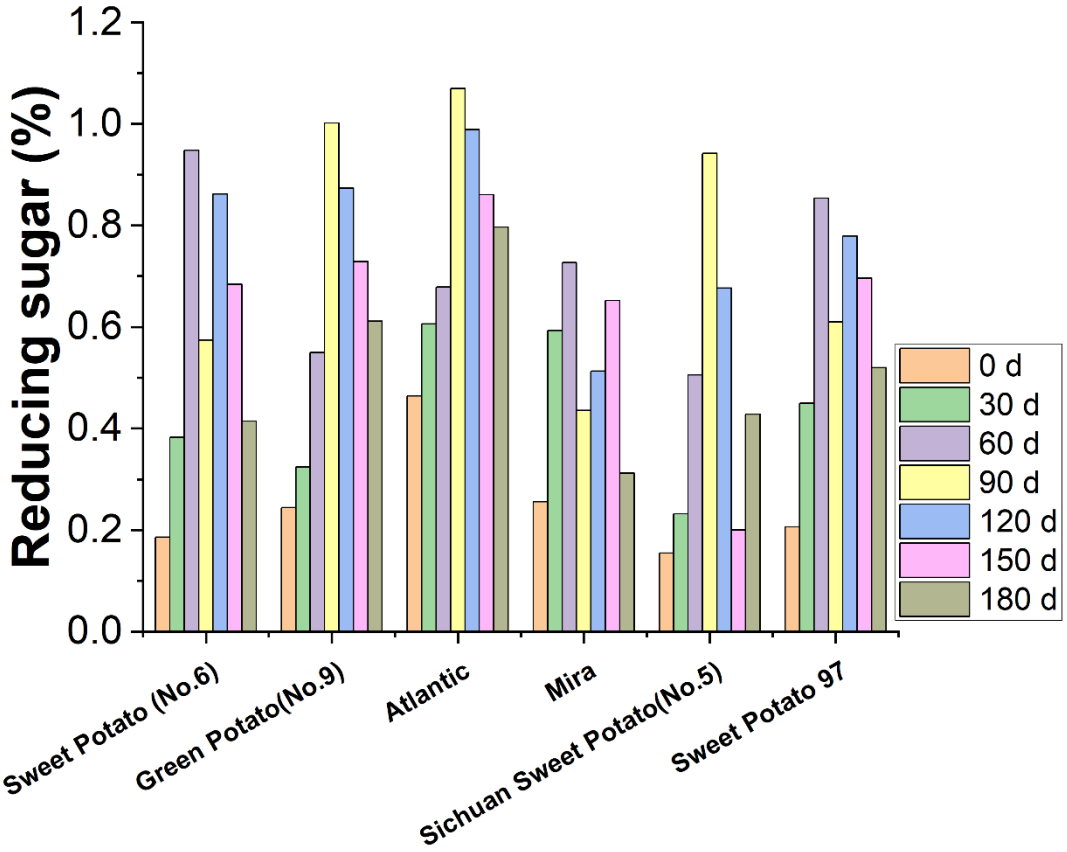

Figure 2. Changes in reducing sugar of Sweet Potato (No.6), Green Potato(No.9), Atlantic, Mira, Sichuan Sweet Potato(No.5), Sweet Potato 97 during storage.

The crystalline and amorphous structures of starch show different characteristics on the XRD spectra, with the crystalline region showing spikes and the amorphous region showing diffuse characteristics. Figure 3 shows the crystallization characteristics of the starch of six potato varieties during storage. As can be seen from the figure, the strong spike diffraction at 2θ of 5.6° and 17.0° was observed for all varieties of potato starch, indicating that the potato starch granules have a typical B-type crystalline structure [32]. The changes in the crystallization characteristics of potato starch with storage time were consistent for different varieties.

The variation of PPO activity in potato tubers with storage time is shown in Figure 4A. Except for Sichuan Sweet Potato (No.5), which showed a continuous increasing trend during storage, all other

varieties showed an increasing and then decreasing pattern. Sweet Potato (No.6) and Mira had maximum PPO activity at 60 d storage time with 42.2 and 61.4 U/g-min, respectively. Green Potato (No. 9), Atlantic and Sweet Potato 97 reached the highest PPO activity at 90 d of storage time with 71.3, 75.1 and 55.3 U/g-min, respectively. Sichuan Sweet Potato (No.5) showed a maximum PPO activity of 56.2 U/g-min at 120 d. The lowest PPO activity values for Sweet Potato (No. 6), Green Potato (No. 9), Atlantic, Mira, Sichuan Sweet Potato (No. 5), and Sweet Potato 97 occurred at 0 d of storage time, with the lowest values of 22.4, 35.2, 34.9 The lowest values of PPO activity were 22.4, 35.2, 34.9, 31.2, 29.2 and 37.3 U/g-min at 0 d storage time.

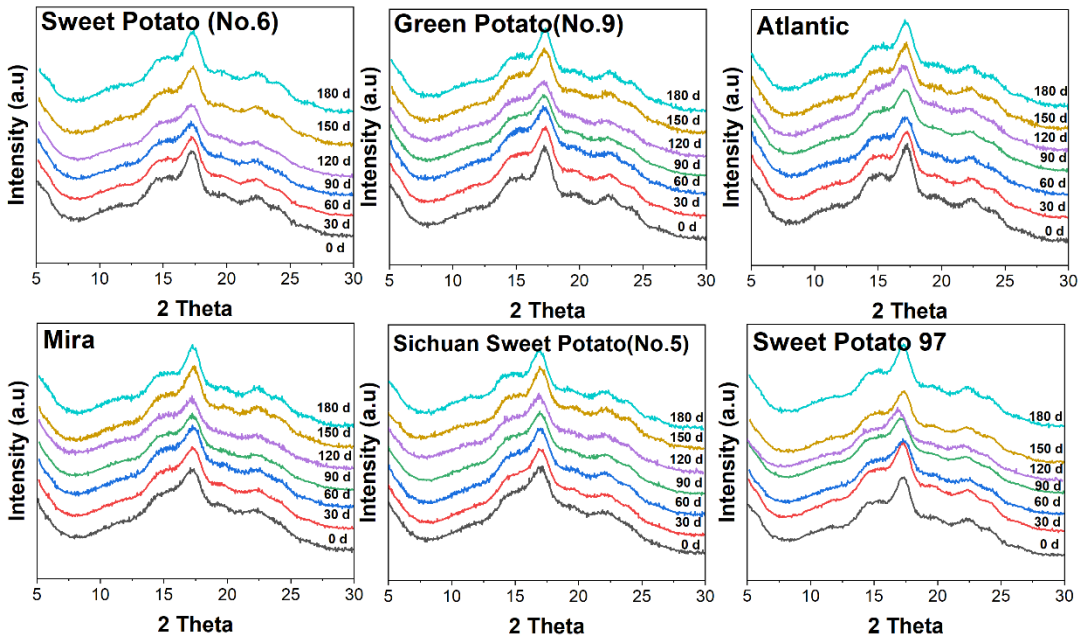

Figure 3. XRD patterns of Sweet Potato (No.6), Green Potato(No.9), Atlantic, Mira, Sichuan Sweet Potato(No.5), Sweet Potato 97 during storage.

From Figure 4B, the starch phosphorylase activities of Sweet Potato (No. 6), Green Potato (No. 9) and Atlantic all had maximum values at 60 d of storage time, with maximum values of 1.71, 1.80 and 1.92 mg/g-min, respectively. The maximum values of starch phosphorylase activity in Mira, Sichuan Sweet Potato (No.5) and Sweet Potato 97 were 2.34, 2.49 and 2.05 mg/g-min at 90 d of storage time, respectively. The lowest value of starch phosphorylase activity in Sweet Potato (No.6) was 1.12 mg/g-min at 30 d storage time. Green Potato (No.9) and Atlantic had the lowest values of starch phosphorylase activity at 120 d of storage time with the lowest values of 1.24 mg/g-min and 1.14 mg/g-min, respectively. The starch phosphorylase activity of Mira, Sichuan Sweet Potato (No. 5) and Sweet Potato 97 decreased to the lowest values at 60 d of storage time with the lowest values of 1.64, 1.20 and 0.99 mg/g-min, respectively. The overall starch phosphorylase activity of Mira was higher among the six varieties.

The amylase activity in potatoes was all low in the early stages of storage and there was a large increase in activity in the later stages of storage (Figure 4C). The amylase activity of Sweet Potato (No.

6), Atlantic, Sweet Potato (No. 5) and Sweet Potato 97 all had maximum values at 60 d of storage time with maximum values of 1.24, 1.05, 1.39 and 1.12 mg/g-min, respectively. The maximum amylase activity of Green Potato (No.9) and Atlantic occurred at 90 d of storage time with 1.11 and 1.23 mg/g-min, respectively. The minimum values of amylase activity for Sweet Potato (No. 6), Green Potato (No. 9), Atlantic and Mira were 0.23, 0.71, 0.34 and 0.25 mg/g-min at 30 d of storage time, respectively. Sichuan Sweet Potato (No. 5) and Sweet Potato 97 had the lowest amylase activity at 0 d storage time, with minimum values of 0.36 and 0.81 mg/g-min, respectively.

Changes in starch content during storage are influenced by two main factors: on the one hand, the ambient temperature during storage decreases with the weather temperature, and potato tubers undergo "low temperature saccharification" [31,33,34]. The interconversion between starch and reducing sugars causes the starch content to change continuously. At lower temperatures, the amylolytic enzyme activity in potato tubers is higher than the starch synthase activity, which converts starch into reducing sugars. Later, as the temperature gradually rose, the amylase activity was gradually higher than the amylolytic enzyme activity, and the reducing sugars were reversed to starch [35]. On the other hand, it is influenced by several metabolic pathways such as starch synthesis and catabolism related enzymes (e.g. starch phosphorylase and amylase). Therefore, the conversion between starch and reducing sugars is not equivalent. The time to reach the maximum value of reducing sugars during storage is not the same for different potato varieties [36]. This indicates that different varieties of potatoes have different physiological functions under the same storage conditions [37,38].

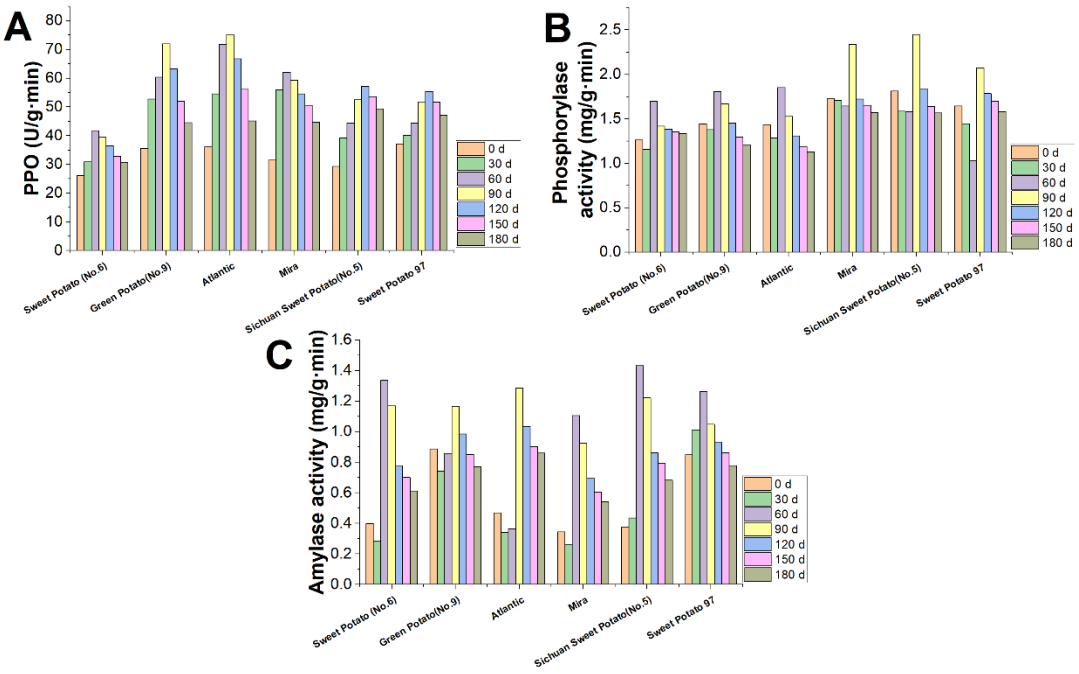

Figure 4. Changes in (A) PPO activity, (B) phosphorylase activity and (C) amylase activity of potato during storage

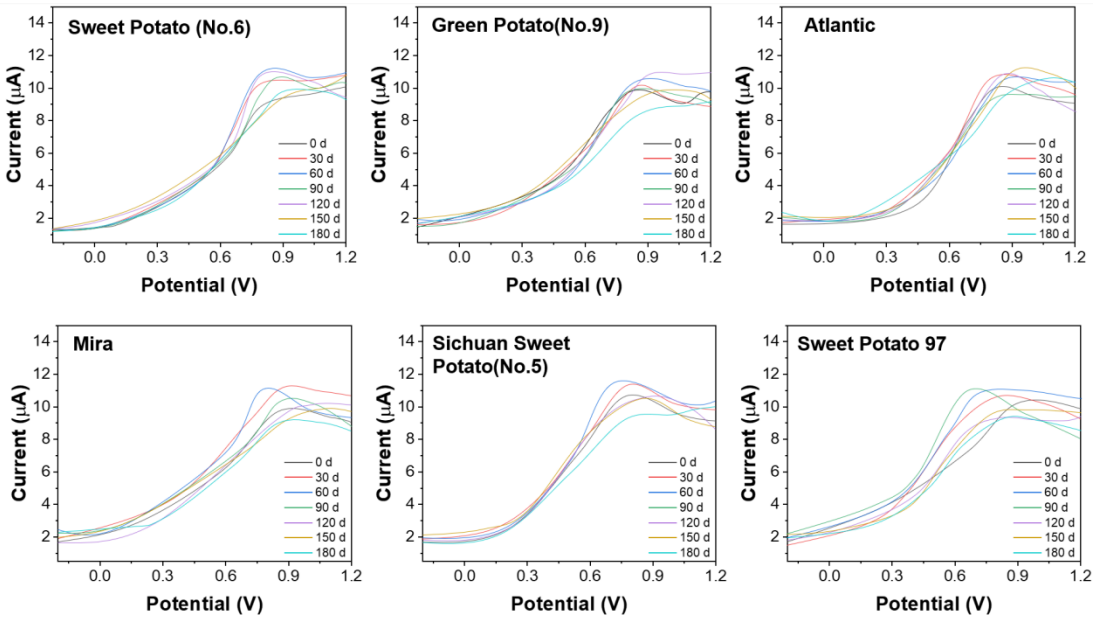

Figure 5. Electrochemical fingerprints of Sweet Potato (No.6), Green Potato(No.9), Atlantic, Mira, Sichuan Sweet Potato(No.5), Sweet Potato 97 during storage recorded under 0.1 M PBS (pH 7.0).

Figure 5 shows the electrochemical fingerprints of six potatoes over time. The electrochemical fingerprint in this figure was collected under PBS conditions. As can be seen from the figure, all potatoes showed electrochemical activity in the potential window. Some molecules underwent electrochemical oxidation during the scanning process [39–41]. These may be polyphenols, ascorbic acid and ketones [42–44]. The amount and type of these molecules vary according to the variety of potatoes. As a result, electrochemical fingerprints show different behavior. After a period of maturation (20-35 d), the potato epidermis has been fully corked and the evaporative and respiratory intensities begin to gradually diminish and turn into a dormant state. We noticed that at the end of storage, the electrochemical fingerprints showed a "V" shaped trend, which might be caused by the change of temperature [45]. High temperature and high respiratory consumption can cause a decrease in dry matter content [46]. But high temperature in turn increases water dissipation from tubers, which can change the behavior of electrochemical fingerprinting.

In order to further understand the feasibility of electrochemical fingerprinting technology, DPV curves of six potatoes under ABS were also collected. As shown in Figure 6, the four tobacco leaves also showed electrochemical activity under the condition of acidic buffer solution. Moreover, the electrochemical behavior under such conditions is different from that under PBS. The changes in starch content during storage were mainly influenced by two aspects. On the one hand, the ambient temperature during storage decreases with the weather temperature and the potato tubers show "low temperature glycation". The interconversion between starch and reducing sugars causes the starch content to change continuously [47]. At lower temperatures, the amylolytic enzyme activity in potato tubers is higher than the starch synthase activity, which converts starch to reducing sugars. Later, with the gradual warming of the temperature, the starch synthase activity is gradually higher than the amylolytic enzyme activity,

and the reducing sugars are reversed to starch. On the other hand, it is influenced by several metabolic pathways such as starch synthesis and catabolism related enzymes (e.g. starch phosphorylase and amylase) [48]. Therefore, the conversion between starch and reducing sugars is not equal. The time to reach the maximum value of reducing sugars during storage is not the same for different potato varieties, with a difference of about 30 d. This indicates that different varieties of potatoes have different physiological functions under the same storage conditions [49]. Newly harvested potato tubers throughout the storage period, from dormancy to dormancy lifted, the top buds sprout growth, to go through a series of material decomposition and synthesis, during all the various changes caused by the action of the corresponding enzymes. The electrochemical fingerprinting was only active when the tubers were dormant, and the electrochemical activity decreased rapidly after the sprouting of the terminal buds [50]. Even if the forced dormancy activity continues further, it is a terminal oxidase system for respiration of meristematic tissues during tuber dormancy.

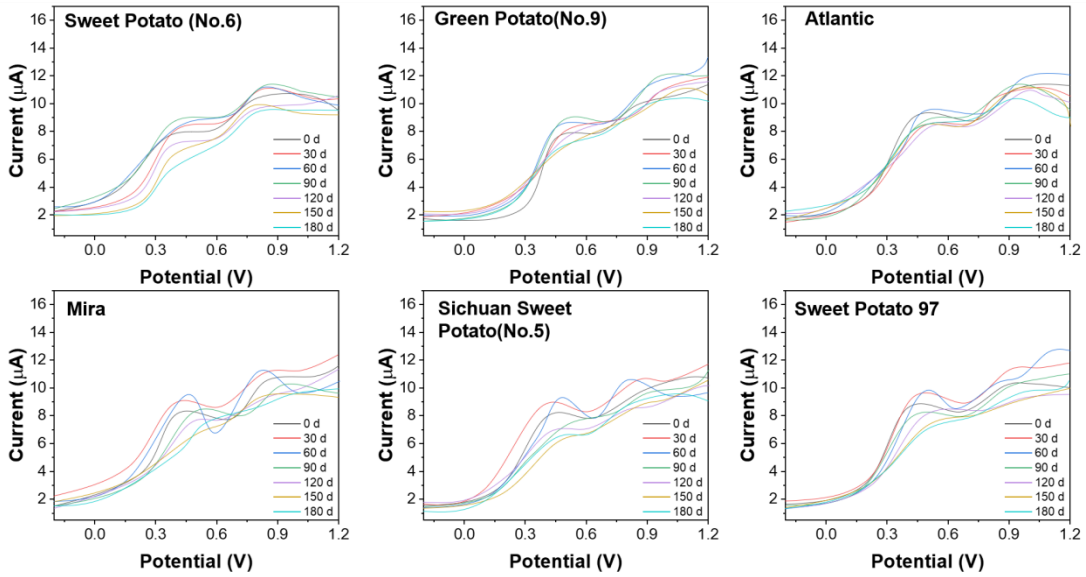

Figure 6. Electrochemical fingerprints of Sweet Potato (No.6), Green Potato(No.9), Atlantic, Mira, Sichuan Sweet Potato(No.5), Sweet Potato 97 during storage recorded under 0.1 M ABS (pH 4.5).

We correlated the peak electrochemical oxidation, starch characteristics and related enzyme activities of potatoes during storage. The results are shown in Figure 6. There was a highly significant positive correlation between current value and starch, r = 0.9814. There was no significant correlation between current value and reducing sugars. There was no significant correlation between current value and starch phosphorylase activity. There was a positive correlation between current value and amylase activity. There was also a positive correlation between current value and polyphenol oxidase.

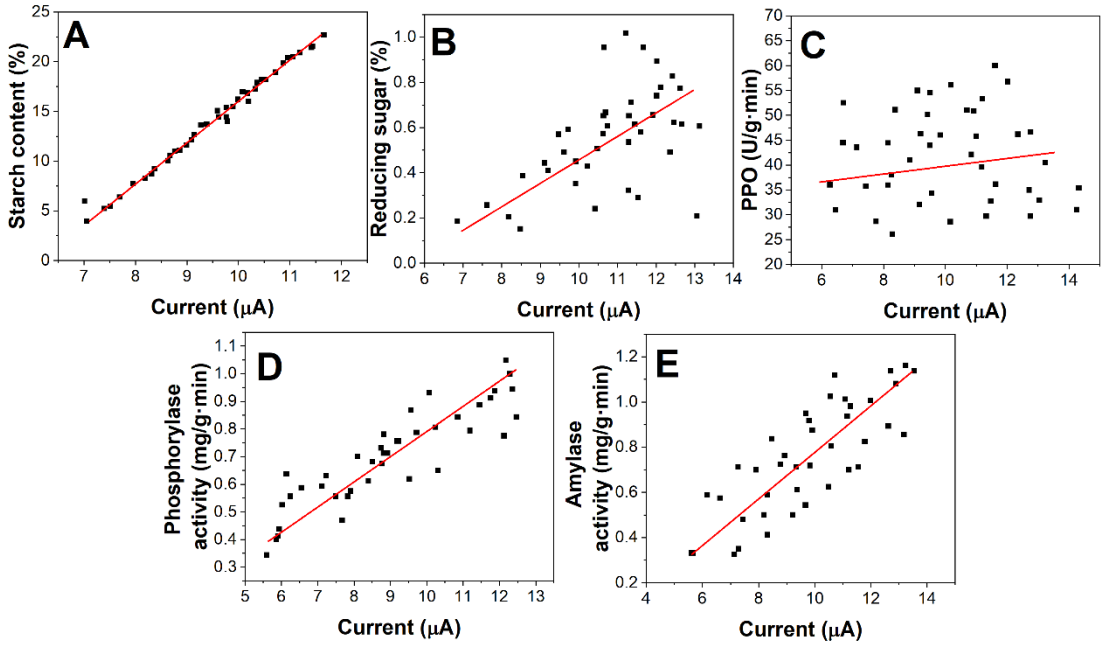

Figure 6. (A) Peak current vs. starch content; (B) Peak current vs. reducing sugar; (C) Peak current vs. PPO activity; (D) Peak current vs. phosphorylase activity; (E) Peak current vs. amylase activity;

4. CONCLUSION

In this work, electrochemical fingerprints, amylose, reducing sugars, PPO activity, phosphorylase activity and amylase activity were determined for six potatoes of different storage periods. The starch content of potatoes showed a trend of overall decrease and local increase - decrease - increase - decrease during storage. The reducing sugar content showed a change pattern of increasing and then decreasing. The crystal structure of the starch granules shows a typical B-type crystallization with crystalline diffraction peaks at diffraction angles 2θ(°) of 5.59°, 17.2°, 22.2° and 24°. Moreover, none of the crystal structures changed with storage time. Mira showed a continuous increase in PPO activity during the storage time of 0-120 d, while all other potato varieties showed an increasing and then decreasing trend in PPO activity during storage. Starch phosphorylase and amylase activity was low during the dormant period of potato. The activity was higher at the beginning of potato sprouting, and then with the growth of shoots, the activity of starch phosphorylase and amylase started to decrease again. Correlation analysis showed a highly significant positive correlation between current value and starch. There was no significant correlation between current value and reducing sugars. There was no significant correlation between current value and starch phosphorylase activity. There was a positive correlation between current value and amylase activity. There was also a positive correlation between current value and polyphenol oxidase.

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