Changes in Chemical Compositions and Enzymatic Activities During Fruit Ripening in Hawthorn (*Crataegus Pinnatifida*)

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ABSTRACT—*In this study, changes in some chemical compositions associated with fruit quality and enzymatic activities were investigated during ripening of hawthorn fruits from the 88th to 148th day after full bloom day (from August 11 to October 10, 2013). Significant differences in these indices were found between different maturation stages as well as hawthorn cultivars. During ripening of hawthorn fruits, the content of total soluble solids (TSS) and the reducing sugar (RS) increased continuously and progressively. The pH values of hawthorns first decreased significantly and then increased. The activities of polyphenoloxidase (PPO) and peroxidase (POD) in hawthorns decreased significantly during ripening. The catalase (CAT) and phenylalanine ammonia lyase (PAL) activity in Mopan hawthorns decreased significantly, while they first decreased significantly and then increased during ripening of Dajinxing hawthorns. The outcomes of this study provide additional and useful information for fresh consumption and processing as well as utilization of dropped unripe hawthorn fruits.*

Keywords—Chemical compositions, Enzymatic activities, Hawthorn, Ripening

1. INTRODUCTION

Hawthorn is an important component of many processed food products because of its excellent flavour, attractive color and high content of many macro- and micro-nutrients (Cao *et al*., 1995; Özcan *et al*., 2005). In China, the hawthorn species (*Crataegus spp*.) is widely cultivated for its edible fruits (Cui *et al*., 2006), and have recently attracted increasing attention in the field of nutraceuticals and medicine because their leaves, flowers, and both green (unripe) and red (ripe) berries are widely reported health benefits besides rich nutrient contents (Wang *et al*., 2011; Kirakosyan *et al*., 2003), e.g., the reduction of the risk of cardiovascular diseases (Pittler *et al*., 2003; Chang *et al*., 2005) and offering antioxidant, anti-inflammatory, vasorelaxing, antityrosinase and hypolipidemic effects (Bahorun *et al*., 2003; Kao *et al*., 2005; Quettier-Deleu *et al*., 2003; Chai *et al*., 2014).

However, some factors affect the physico-chemical properties, nutritional value and active components in fruit and vegetables (Lee & Kader, 2000; Kirakosyan *et al*., 2003), and some studies showed that the nutritional compositions, physical and biochemical properties as well as enzymatic activities of fruits and vegetable were most affected by maturity and ripening stages (Opara *et al*., 2012; Zheng *et al*., 2012; Menz *et al*., 2010; Awad *et al*., 2011; López-Miranda *et al*., 2011), and hawthorn fruits were no exception (Liu *et al*., 2011)**.** Catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO), and the non-enzymatic antioxidant compounds such as phenols, ascorbate and glutathione have been viewed as a synergistic antioxidant defensive system, whose combined purpose is to protect cells from active oxygen damage (Agarwal & Pandey, 2004). Phenylalanine ammonia lyase (PAL) enzyme converts phenyl alanine to phenolics via the phenylpropanoid pathway (André *et al*., 2009). PPO and POD can catalyse the oxidation of phenols to produce brown pigments (Nokthai *et al*., 2010). CAT constitutes the most efficient and elaborate system available in both plants and animals to control H_2O_2 concentrations. It catalyzes the dismutation of H_2O_2 to water and oxygen. SOD dismutates superoxide radicals to hydrogen peroxide and O_2 in a reaction that is spontaneous and extremely rapid, thus protecting the cells from damage by superoxide radical reaction products. However, little information is available on changes in antioxidant and browning related enzyme activity changes in hawthorn during ripening even though Liu et al. (Liu *et al*., 2011) studied changes of phenolic compounds in hawthorn (*Crataegus grayana*) fruits during ripening. The objective of this study was to investigate the changes in some chemical compositions and enzymatic activities in hawthorns during ripening, which may be useful for optimal harvest timing, and the processing and utilization of hawthorns, especially unripe hawthorns.

2. MATERIALS AND METHODS

2.1 Hawthorn Materials

Hawthorn cultivars (*Crataegus pinnatifida*) Dajinxing and Mopan, are two main cultivars with wide adaptability across different hawthorn-growing regions of China and used in the study. Hawthorn fruits were picked by hand in the production base of hawthorn (Linfen, Shanxi, China) from the 88th to 148th day after full bloom day (namely, from August 11 to October 10, 2013) in seven stages (R1−R7) of maturity, at roughly 10-day intervals. Each sample was picked from three trees at five randomly selected collection points from different sides of each tree. Immediately after harvesting, the samples were used for the chemical analyses and determination of enzymatic activities.

2.2 Chemical Analyses

Deseeded hawthorns flesh homogenate was prepared by blending and filtered using a cheese cloth and then subjected to chemical analysis. The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter. The total soluble solids (TSS) were determined by using an Atago digital refractometer dbx-30 at 25 °C. The reducing sugar (RS) contents were quantified in according to Jemai *et al*. (1986).

2.3 Enzymes extraction

Enzymes extraction was carried out by homogenizing 10 g of frozen material in 20 mL of extraction buffer at 4 °C using a homogenizer. Extraction buffer contained 50 mM potassium phosphate (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM cysteine, 1% (w/v) soluble polyvinylpyrrolidone (PVP), 0.1 mM phenylmethanesulfonyl fluoride (PMSF), and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at 12000g for 20 min at 4 °C. The supernatant was designated as the enzymatic extract and stored at −20 °C for further analysis.

2.4 Peroxidase assay

Peroxidase activity was carried out according to Miranda *et al*. (1995). The reaction mixture contained in 1 mL: 8 mM H_2O_2, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5 and least amount of enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of POD activity was defined as the amount of enzyme which increases the OD 1.0 per min under standard assay conditions.

2.5 Catalase assay

Catalase activity was determined according to Bergmeyer (1974). Two and half mL of substrate solution was made up of 25 mM H_2O_2 in a 75 mM sodium phosphate buffer pH 7.0 and enzyme solution. The decrease in absorbance at 240 nm and 25 °C was recorded for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min under standard assay conditions.

2.6 Polyphenoloxidase assay

Polyphenoloxidase activity assay was carried out with catechol as a substrate according to the spectrophotometric procedure of Jiang *et al.* (2002). The enzyme solution (0.2 mL) was rapidly added to 2.8 mL of 20 mM catechol solution prepared in 0.01 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 400 nm and 25 °C was recorded for 3 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min.

2.7 Phenylalanine ammonia lyase (PAL) assay

PAL activity was determined by incubating a 0.1 mL mixture of enzyme extract and 2.9 mL of a 0.1 M sodium borate buffer (pH 8.0) solution containing 3mM L-phenylanine for 1 h at 37 °C. An increase in the PAL activity at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hour.

2.8 Statistical Analysis.

All experiments were conducted three times independently and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were performed with significant level being considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Changes in chemical compositions of hawthorn during ripening

The chemical compositions affecting the quality of hawthorns were investigated the results are shown in Table 1. There was a significant difference in the levels of the TSS among ripening stages and hawthorn cultivars, as well as in the RS and pH values of hawthorns. During the fruit growth, the content of TSS increased continuously by 217% and

230% ($p < 0.05$), from 4.26 to 13.52 °Brix for Dajinxing hawthorn and from 3.75 to 12.38 °Brix for Mopan hawthorn by the end of maturity, respectively. Different from the TSS of Dajinxing hawthorns did not change much between stages R6 and R7. Similar to TSS, the RS content increased continuously and significantly ($p < 0.05$) by approximately 2.57-fold and 2.61-fold, from 1.97% to 7.03% for Dajinxing and from 1.53% to 5.52% for Mopan by the end of maturity, respectively. The increases in TSS and RS may be attributed to the hydrolysis of the starch component in the hawthorn with maturity of fruits (Zheng *et al*., 2012), which is desirable for hawthorn taste. In addition, the increase in TSS can bring the hawthorns colors because there was a marked positive correlation between the content of anthocyanin and total sugars of hawthorn fruits (Qi *et al.*, 2005). A similar change in the total sugar content has also been reported in pomegranates (Kulkarni & Aradhya, 2005) and apples (Zheng *et al*., 2012). In this study, the levels of TSS and RS of Dajinxing was higher than that of Mopan hawthorns at the same stage, which may come from differences in hawthorn cultivars.

During ripening of hawthorn fruits, changes in the pH values of Dajinxing and Mopan had a similar trend, but the variation range of the pH value was different for two hawthorn cultivars (Table 1). The pH values of Dajinxing first decreased significantly ($p < 0.05$) from 5.25 to 3.65 and reached the minimum at stage R6, and then it increased ($p < 0.05$) to 4.42 at R7 stage, while the pH values of Mopan first decreased significantly ($p < 0.05$) from 4.98 to 3.08 and reached the minimum at stage R6, and then it increased ($p < 0.05$) to 3.93 at R7 stage. Liu *et al.* (2010) reported that the malic acid, citric acid, quinic acid were the major organic acids and the total acid content of the full mature hawthorn fruits varied from 3.1 to 11.8 g/100 g DM because of differences in the cultivars or species. Similar changes were also found in most edible fruits (Scheerens, 2006; Johnson *et al*., 2011; Zheng et al., 2012; Distefano *et al*., 2009).

Table 1: Content of chemical compositions in hawthorn fruits at different ripening stages [a]

	Dajinxing			Mopan		
stages	TSS (°Brix)	RS (%)	pH	TSS (°Brix)	RS (%)	pH
R1[b]	4.26±0.13 e	1.97±0.05 g	5.25±0.08 a	3.75±0.08 g	1.53±0.05 g	4.98±0.09 a
R2	6.15±0.15 d	2.25±0.03 f	5.18±0.12 a	4.91±0.12 f	1.96±0.04 f	4.88±0.06 a
R3	8.38±0.24 c	3.04±0.04 e	4.69±0.07 b	6.55±0.22 e	2.53±0.06 e	4.39±0.08 b
R4	9.66±0.11 b	4.13±0.11 d	4.22±0.10 c	7.96±0.25 d	2.90±0.03 d	3.62±0.05 d
R5	10.14±0.24 b	5.42±0.12 c	3.83±0.05 d	9.04±0.24 c	3.72±0.12 c	3.30±0.08 e
R6	13.12±0.36 a	6.35±0.08 b	3.64±0.06 d	10.82±0.44 b	4.45±0.15 b	3.08±0.04 f
R7	13.52±0.35 a	7.03±0.06 a	4.42±0.11 c	12.38±0.24 a	5.52±0.08 a	3.93±0.09 c

[a] Numbers represent mean values of three independent replicates ± SD. [b] R1-R7 refer to the different maturation stages. Different letters indicate statistically significant differences between the means (P < 0.05) for each nutrient.

3.1 Changes in CAT and POD Activity

The changes in CAT and POD activity of hawthorns during ripening are shown in Figure 1. The CAT activity of Dajinxing first decreased significantly ($p < 0.05$) by 93% from 4.26 to 0.29 units/g and reached the minimum at stage R5, and then it increased ($p < 0.05$) to 0.89 at R7 stage, while the CAT activity of Mopan first decreased continuously and significantly ($p < 0.05$) by 96% from 3.78 to 0.15 units/g by the end of R7 stage, but the CAT activity of Mopan hawthorns did not change much between stages R6 and R7 (Figure 1A). The POD activity of two hawthorn cultivars decreased significantly ($p < 0.05$) during ripening. The POD activity of Dajinxing decreased by 96% from 2.11 to 0.09 units/g at stage R3, after that no change was found with the processing of ripening; while the POD activity of Mopan decreased significantly ($p < 0.05$) by 92% from 3.25 to 0.26 units/g R5 stage, thereafter the CAT activity did not obvious change (Figure 1B). Free radical-induced oxidative stress plays a role in fruit maturation, ripening, and senescence (Rogiers *et al.*, 1998). H_2O_2 is a strong mediator of oxidative stress responses (Levine *et al*., 1994). Increased levels of H_2O_2 and lipid peroxides have been detected in ripening fruits (Shiow *et al*., 2001). While both CAT and POD activities reduce H_2O_2 to water, enzymatic oxidation of phenols is specifically coupled to peroxidatic reaction cycles, favoring subsequent polymerization processes and affecting the redox status of the bean. Besides the typical peroxidatic cycle, POD exhibits oxidase and hydroxylating activities involved in polymerization and depolymerization reactions (Chen *et al*., 1999). POD activities rely on the presence of H_2O_2. Consequently, CAT must exert a very tight control on POD activities in general, and in particular, during oxidative stress, which was supported by our studies. Similar results are found in blackberry (Shiow *et al*., 2001), tomato (Rabinowitch *et al.*, 1982). However, Awad *et al*. (2011) reported that CAT and POD activity first increased and then decreased in five date cultivars during development and ripening. López-Miranda *et al.* (2011) reported that the POD activity increased in Crimson Seedless table grape during ripening. These differences may be concerned in the sampling time, cultivars, and species of test materials.

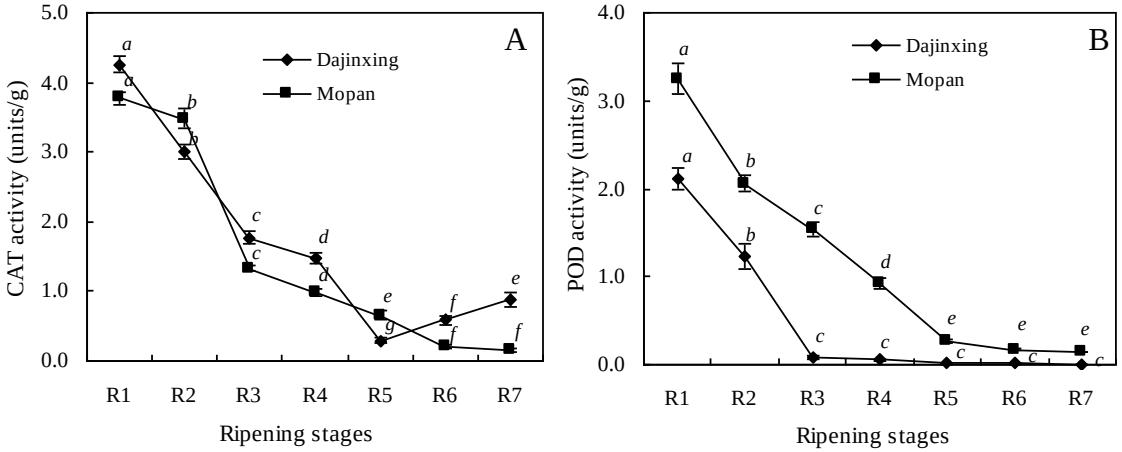

Figure 1: Changes of CAT (A) and POD (B) activity in hawthorns during ripening

3.2 Changes in PPO and PAL Activity

The changes in PPO and PAL activity of hawthorns during ripening are shown in Figure 2. Similar to changes of POD activity, the PPO activity of Dajinxing decreased by 94.7% from 3.03 to 0.16 units/g at stage R3, after that no change was found with the processing of ripening; while the PPO activity of Mopan decreased significantly ($p < 0.05$) by 92% from 3.62 to 0.29 units/g R5 stage, thereafter the PPO activity did not obvious change (Figure 2A). The change of PAL activity was very similar to that of CAT activity. The PAL activity of Dajinxing first decreased significantly ($p < 0.05$) by 80% from 14.53 to 2.87 units/g and reached the minimum at stage R3, and then it increased ($p < 0.05$) to 7.15 at R7 stage, while the PAL activity of Mopan first decreased continuously and significantly ($p < 0.05$) by 88% from 12.87 to 1.54 units/g by the end of R7 stage, but the PAL activity of Mopan hawthorns did not change among stages R5 toR7 (Figure 2B).

The main step in enzymatic browning is the oxidation of phenolic compounds to corresponding quinines by PPO in the presence of oxygen. PAL is the first enzyme in the phenylpropanoid pathway and plays an important role in the synthesis of phenolic compounds in plants (Pina & Errea, 2008; André *et al*., 2009). Phenolic compounds are a group of chemical substances, which can be responsible for colour; they are present in most fruits and vegetables and also act as the key substrate for enzymatic browning (Alasalvar *et al*., 2001; Nokthai *et al*., 2010). The enzymatic browning of fruit and vegetables is mainly attributed to the oxidation of phenolics by PPO and/or POD (Jiang *et al*., 2004). The PPO, POD and PAL activity of hawthorn decreased significantly during the ripening of the fruit, which indicates that enzymatic browning does not happen easily in riper hawthorn than in unripe hawthorn. Huang *et al.* (2007) reported that PPO activity in yam decreased during growth, which was agreement with the present results. However, some studies reported that PPO activity in other fruits increased during ripening (López-Miranda *et al*., 2011; Venkatachalam & Meenune, 2012). Venkatachalam and Meenune (2012) reported that POD and PAL activity first increased and then decreased in longkong fruit during four different weeks of on-tree maturation. These differences may also result from test materials.

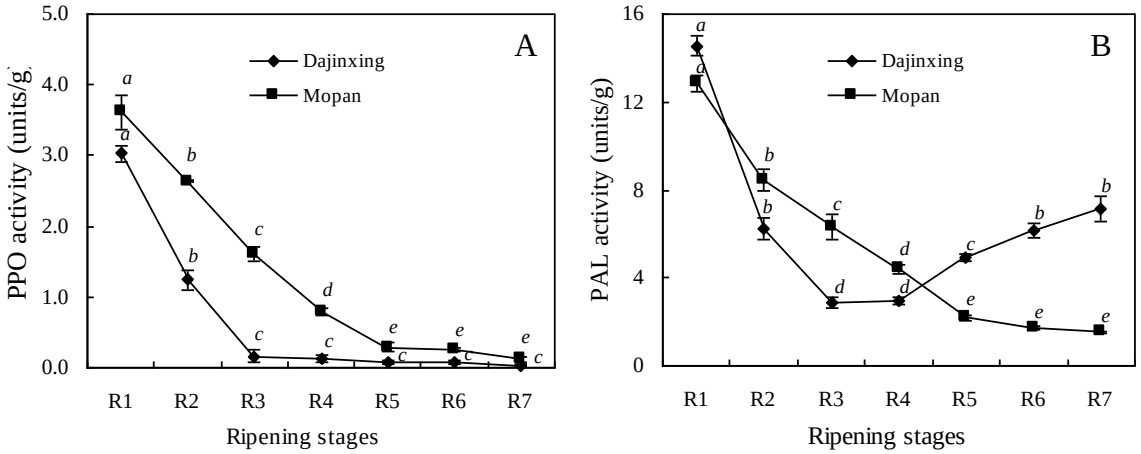

Figure 2: Changes of PPO (A) and PAL (B) activity in hawthorns during ripening

4. CONCLUSION

In conclusion, profiles of changes in some chemical compositions and enzymatic activities were investigated during ripening of hawthorn fruits. Differences in these indices were found among different maturation stages as well as hawthorn cultivars. During ripening of hawthorn fruits, the content of TSS and RS increased continuously and progressively. The pH values of hawthorns first decreased significantly and then increased. The activities of PPO and POD in hawthorns decreased significantly during ripening. The CAT and PAL activity in Mopan hawthorns decreased significantly, while they first decreased significantly and then increased during ripening of Dajinxing hawthorns. The outcomes of this study provide additional and useful information for fresh consumption and processing as well as utilization of dropped unripe hawthorn fruits.

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