CHANGES IN PHYSICO-CHEMICAL PROPERTIES, POLYPHENOL COMPOUNDS AND ANTIRADICAL ACTIVITY DURING DEVELOPMENT AND RIPENING OF MAOLUANG (*Antidesma bunius* L. Spreng) FRUITS

Luchai Butkhup[1] and Supachai Samappito[2]*

[1]Laboratory Equipment Center and [2]Department of Biotechnology
Faculty of Technology
Mahasarakham University, Maha Sarakham Province 44000, THAILAND
*Corresponding author: e-mail: Samappito@msu.ac.th

(Received October 20, 2009/Accepted March 16, 2010)

A B S T R A C T

Influence of fruit development and ripening on the changes in physico-chemical properties, antiradical activity and the accumulation of polyphenolic compounds were investigated in Maoluang fruits. Total phenolics content (TP) was assayed according to the Folin-Ciocalteu method, and accounted for 19.60-8.66 mg GAE/g f.w. The TP gradually decreased from the immature to the over ripe stages. However, the total anthocyanin content (TA) showed the highest content at the over ripe stage, with an average value of 141.94 mg/100 g f.w. The antiradical activity (AA) of methanolic extracts from Maoluang fruits during development and ripening were determined with DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging. The highest AA was observed at the immature stage accompanied by the highest content of gallic acid and TP. Polyphenols were quantified by HPLC. The level of procyanidin B2, procyanidin B1, (+)-catechin, (–)-epicatechin, rutin and *tran*-resveratrol as the main polyphenol compounds, increased during fruit development and ripening. Other phenolic acids such as gallic, caffeic, and ellagic acids significantly decreased ($p < 0.05$) during fruit development and ripening. At over ripe stage, Maoluang possess the highest antioxidants. Thus, the over ripe stage would be the appropriate time to harvest when taking nutrition into consideration. This existing published information provides a helpful daily diet guide and useful guidance for industrial utilization of Maoluang fruits.

Key words: maoluang, polyphenol, phenolic acids, antiradical activity, DPPH assay, HPLC

INTRODUCTION

Polyphenols are important secondary metabolites of plants. They are widespread in nature, with > 8000 phenolic structures currently known (Bravo, 1998). They are ubiquitous in all plant organs and are frequent constituents of the human diet. The colourless polyphenol compounds can act as co-pigments and provide protection against UV radiation and insect attack, but they also can be substrates in browning reactions (Amiot et al., 1995; Mathew and Parpia, 1971). In recent years, polyphenols have become an intense focus of research interest because of their potential health-beneficial effects with regard to their antioxidant capacity (Hertog et al., 1997; Stoclet et al., 2004), for example, ellagic acid has been described as an antimutagen and an anticarcinogen and polyphenols are reported to reduce coronary heart disease (Amiot et al., 1995; Hertog et al., 1997; Kahkonen et al., 1999; Maas et al., 1990). Besides the health-protective effects, polyphenols are partially responsible for many quality criteria in plant-derived foods and beverages. The colour, astringency, stability, bitterness, and aroma of foods and beverages can depend on the content of polyphenolic compounds (Atanasova et al., 2002; Vidal et al., 2004). In contrast to the positive aspects mentioned above, the oxidation of phenolic compounds is largely responsible for the browning of fruits during handling and storage. Polyphenols are also responsible for undesirable hazes and flavour that occur during the preparation of fruit juices and wine (Mathew and Parpia, 1971).

Maoluang (*Antidesma bunius* L. Spreng) is a wild plant belonging to the *Euphobiaceae* family. In Indonesia, the use of *A. bunius* as a medicinal plant has been practiced long before recorded history. The study by Eun-Mi and Jae-Kwan (2005), investigated several medicinal plant extracts traditionally and commonly used in Indonesia. The study focused on their inhibitory action against NO release from macrophages and antioxidant activity. These scientists found that leaf crude extract of *A. bunius* showed high inhibitory effect on NO release in RAW264.7 cells (110.4±2.1%), and high reducing power (393.5±12.7). In this study the leaf crude extracts had a high concentration (249.5±3.2 mg/g) of total phenolics. Maoluang can be cultivated successfully in many regions particularly in Northeast Thailand. The fruit is an economically important crop. They display an attractive bright red colour before full ripeness and are red-black when fully ripe. They have a special sweet-sour taste and a distinctive flavour, and are popular with local people. Maoluang fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, vitamins (Samappito and Butkhup, 2008b), anthocyanins, flavonoids and phenolic acids (Butkhup and Samappito, 2008a). Haripyaree, Guneshwor and Damayanti (2010) report that methanolic extract of *A. bunius* fruit which is

grown in Manipur, India, showed higher antioxidant activity, with an average IC_{50} value of 100.08 µg/ml, when compared to other fruits.

These days Maoluang fruits are also widely used to make excellent jam, jelly, juice, juice concentrate and red wine. Fruit maturity is important to the overall quality of fruit and their derived products, such as juice and red wine. Yet it is unclear, to what extent the polyphenol contents change during the fruit maturity period. Changes in polyphenol content and antioxidant capacity of fruits are often associated with ripening of fruits (Burda et al., 1990; Amiot et al., 1995; Kobayashi et al., 2008).

Mature Maoluang fruits are important for their beneficial effects and quality of fruit, or for industrial processing of foods and beverages. However, no information is available on the composition of polyphenols and antiradical activity of Maoluang fruit during development and ripening. Therefore, the objectives of this study were to determine the changes in physico-chemical properties, polyphenol compounds and antiradical activity in Maoluang fruit during development and ripening. The data obtained form a good basis for evaluating the nutritional importance of Maoluang fruit.

MATERIAL AND METHODS

Fruit material

Maolung fruit was randomly harvested in the early morning (6.00-8.00 a.m.). It had been cultivated on the hillsides of Phupan and the neighbouring lands that are 400-567 m over sea level, in Sakon Nakhon Province, Northeast Thailand. Maolung was collected in bunches and randomly sampled: one sample was taken from the top, one from the bottom, and one from the middle of the cluster. Special care was taken to obtain an even distribution among berries from the inside and the outside of the bunch. In this study, we designated the fruit as immature from the first to second month of development after full bloom. Fruit ripening started in the third month and ended in the fifth month. The sampled fruits were subdivided into immature, mature, mid-ripe, ripe and over ripe stages, on the basis of skin and state of maturity. In the immature stage, the entire Maoluang had a light green skin.. The next stage was called the mid-ripe stage, in which the skin was ca. 60% yellow-red and 40% pale green. For the ripe and over ripe stages, the skin colours of Maoluang fruits were completely reddish and red-black, respectively (Tab. 1). One kilogram of each sample was randomly selected, and then stored at −20 °C for further study.

Reagents and chemicals

Methanol, acetonitrile and phosphoric acid were of HPLC grade (Tedia Company, USA). Deionised water was prepared by a Milli–Q Water Purification system (Millipore, MA, USA). Gallic acid, (+)-catechin, (–)-epicatechin, rutin, procyanidin B1, caffeic acid, procyanidin B2, myricetin, ellagic acid, *trans*-resveratrol, ferulic acid, luteolin, quercetin, naringenin

Table 1. Collection data and the colour of Maoluang fruit and the state of fruit maturity

Harvest NO.	Harvest date	Maturity	Fruit colour and state of fruit maturity
1	15 June 2008	immature	unripe, skin light green, pulp white, fruit hard
2	15 July 2008	mature	unripe, greenish, pulp white, fruit hard
3	15 August 2008	mid ripe	half ripe, green-red, pulp white
4	15 September 2008	ripe	ripe, skin completely reddish, pulp red
5	15 October 2008	over ripe	post-ripe, skin completely red-black, pulp black-red

and kaempferol standards were purchased from the Sigma company (USA). Standard stock solutions of polyphenol compounds were prepared in methanol at a concentration of 0.50 g/l. All sample solutions were filtered through 0.45 μm membrane filter (Millipore) prior to injection on the chromatograph.

Extraction and hydrolysis

The collected berry samples (Tab. 1) were frozen immediately after arrival and stored at -20 °C until extraction time. Storage time was less than 2 months. The frozen berries (75-100 g) were thawed in a microwave oven and homogenized. Ground samples (5 g) were extracted and hydrolyzed with 50 ml of 60% aqueous methanol containing 1.2 M HCl and 8 mg/l ascorbic acid as antioxidant. The mixture was refluxed at 85 °C for 2 h to ensure complete extraction (Butkhup and Samappito, 2008). Then, the extracts were filtered through Whatman No. 1 paper under vacuum, and the residue was repeatedly extracted with the same solvent until it was colourless, and centrifuged (10 min, 5000 g). Methanol was evaporated from the supernatants on a rotary evaporator at 50 mm Hg pressure and 50 °C for determining polyphenol content and antiradical capacity.

General composition and colour analysis

The total soluble solids (TSS) content of samples were measured at 25 °C using Abbe refractometer (Japan). The pH was determined with a combination electrode at 25 °C. Titratable acidity (TAc) was determined by titration to pH 8.1 with 0.1 N NaOH solution and calculated as grams of citric acid per 100 g of sample (AOAC, 1984). Fruit colour was measured using a Minolta Chroma Meter CR-300 series (Japan). The colour determination was reported as L^* (brightness, 100 = white, 0 = black), a^* (+, red; -, green) and b^* (+, yellow; -, blue) colour values. A white tile (No: 13133060) was used to standardize the instrument. Dry matter was de-

termined by drying fruits at 70 °C under vacuum (AOAC, 1984).

Determination of total phenolic content (TP)

TP content of the methanolic extracts was determined using the Folin-Ciocalteu reagent (Kahkonen et al., 1999). The extract solution (200 µl) was transferred into a test tube and then mixed thoroughly with 1 ml of Folin–Ciocalteu reagent. After mixing for 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer, then allowed to stand for a further 30 min in the dark. Then, they were centrifuged at 3300 g for 5 min. The absorbance of extracts and a prepared blank were measured at 765 nm using a spectrophotometer (UV–Vis model 1601, Shimadzu, Kyoto, Japan). The measurement was compared to a standard curve prepared with gallic acid solutions and expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight.

Determination of total anthocyanin content (TA)

TA content of berries was measured using a spectrophotometric pH differential protocol (Boyles and Wrolstad, 1993). Powdered berry extract (75 mg) was accurately weighed and put into a 100 ml volumetric flask. About 80 ml of distilled water were added to the flask and sonicated for 15 minutes or until dissolved. The solution was allowed to cool to room temperature for 4 hours and then diluted to volume with water and mixed. If the solution has turbidity, a portion of it must be centrifuged to help remove particulates. A 1.0 ml aliquot of the berry solution was removed and placed into a 25 ml volumetric flask. The sample was diluted 5:1 in 0.025 M potassium chloride buffer (pH 1.0) and mixed. A second 1.0 ml aliquot of the berry solution was removed and placed into a 25 ml volumetric flask. The sample was diluted 5:1 in 0.4 M sodium acetate buffer (pH 4.5) and mixed. The absorbance of the mixture was measured at 515 and 700 nm using distilled water as a blank sample. The absorbance of the diluted sample (A) was as follows: $A = (A_{515} - A_{700})$ pH $1.0 - (A_{515} - A_{700})$ pH 4.5. TA content was calculated as the total of monomeric anthocyanin pigment (mg/100 gram f.w.) = (A x MW x DF x 1000)/(ε x 1), where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ε in this formula correspond to the predominant anthocyanin in the sample. Since the sample composition was unknown, pigment content was calculated as cyanidin-3-glucoside, where MW = 449.2 and ε = 26,900.

Determination of antiradical activity (AA)

A 0.1 ml aliquot of the methanol extract prepared above, was mixed with 3.9 ml of an 80% ethanolic 0.6 mM DPPH solution. The tubes were vortexed for 15 s and allowed to stand for 180 min, as described by Cai et al. (2003). After this, the absorbance of the mixture was meas-

ured at 515 nm using the Hewlett Packard UV–Vis spectrophotometer (UV–Vis model 1601, Shimadzu, Kyoto, Japan). Most tested compounds react completely within 180 min in this condition. Reaction time for vitamin C is less than 1 min due to its fast oxidation. Ethanol (80%) was used as a blank solution, and DPPH solution without test samples (3.9 ml of DPPH + 0.1 ml of 80% ethanol) served as the control. All tests were performed in triplicate. The antiradical activity of the test samples was expressed as the median effective concentration for radical-scavenging activity (EC_{50}): TP (mg) of antioxidant (test sample) required for a 50% decrease in absorbance of DPPH radicals, and inhibition (%) of DPPH absorbance = $(A_{control} - A_{test})$ x $100/A_{control}$. A plot of absorbance of DPPH vs. concentration of antioxidant was made to establish the standard curves (dose-response curves) and to calculate that EC_{50}. $A_{control}$ is the absorbance of the control (DPPH solution without the test sample), and A_{test} is the absorbance of the test sample (DPPH solution plus 0.1 ml of 5 µM test compound). Ascorbic acid served as a standard. The results of the assay were expressed relative to an ascorbic acid equivalent.

RP-HPLC-DAD analyses

Determination of polyphenol compounds was carried out by reversed-phase HPLC as described in details by Butkhup and Samappito (2008). Clear samples in the amount of 20 µl were injected on HPLC apparatus consisting of Shimadzu (Shimadzu Cooperation Analytical & Measuring Instruments Division Kyoto, Japan) LC–20AD Series pumping system, SIL–10AD Series Auto injector system and SPD–M20A Series Diode array detector. The data were collected and analyzed with the use of a Shimadzu computing system. The column used was an Apollo C_{18} (Alltech Associates, Deerfield, IL, USA) (ø 4.6 mm x 250 mm, 5 µm) protected with guard column Inertsil ODS–3 (ø 4.0 mm x 10 mm, 5 µm; GL Science Inc., Tokyo, Japan). The mobile phase for polyphenol determination was acetonitrile/deionised water (2/97.8, v/v) containing 0.2% phosphoric acid (solvent A), and acetonitrile-/deionised water (97.8/2, v/v) containing 0.2% phosphoric acid (solvent B) at a flow rate of 0.6 ml/min and a column temperature which was 40 °C. The UV–Vis spectra were recorded from 190 to 400 nm, with detection at 254 nm. The linear gradient started with 20% solvent B, 50% solvent B at 30 min, 60% solvent B at 35 min, 20% solvent B at 40 min at isocratic elution until 55 min. Identification was carried out by comparing the retention times and the spectra with those of authentic standards. Polyphenol compounds were purchased from Sigma and used for calibration which were accurately weighted and dissolved in methanol to prepare a stock solution standard of 500 mg/l. The concentrations of polyphenol compounds were calculated with the help of a corresponding external standard. The external standard procedure was implemented

preparing standards of polyphenols with concentrations in the range of 0.01-100 mg/l for (+)-catechin, rutin, myricetin, quercetin, naringenin, kaempferol, 0.05-100 mg/l for, *trans*-resveratrol, 0.50-100 mg/l for gallic acid, caffeic acid, (–)-epicatechin, ellagic acid, ferulic acid, and 1.00-100 mg/l for luteolin, procyanidin B1, procyanidin B2.

Statistical analyses

All the compounds and parameters reported below were evaluated in triplicate, in each of the samples. The statistical analysis of the data was carried out by analysis of variance (ANOVA) and the LSD (least significant difference) test, to show measurements which can be considered statistically different. A significance level of $p = 0.05$ was used.

RESULTS AND DISCUSSION

Fruit ripening is associated with important biochemical changes that modify colour, texture, taste and other quality traits. The changes of some physico-chemical characteristics of Maoluang fruit used in this study are presented in Table 2. The values reported are means of tree assays of each group performed in triplicate. The colour change of skin was reflected by the variation of the L^*, a* and b^* values determined in Maoluang fruit. The L^* value decreased with fruit ripening as colour became deep/dark. The Maoluang fruit's skin colour is green at the beginning of ripening and becomes red in conjunction with chlorophyll degradation and anthocyanin accumulation. The a^* value variation was used as an index of redness and greenness. The a^* value increased in the early fruit ripening stages and then decreased in the ripe mature stage, as the violet colour developed Similar results were reported by Tosun et al. (2008) and Bureau et al. (2009) for blackberry and red apricot fruits, respectively. The b^* value variation was used to express yellowness and blueness. The b^* value was observed as the Maoluang fruit decreased from the immature to the over ripe stage ($p < 0.05$). It is known that the fruit weight evolution during fruit maturation is directly related to the fruit size. During fruit development and ripening, fruit weight increased from about 0.32 g (immature stage) to 0.68 g (over ripe stage). Dry matter changes in the fruit samples decreased ($p < 0.05$) as the fruit ripened.

During ripening, a slight and insignificant increase in the total soluble solids (TSS) content occurred at the immature and mature stages. But at the ripe stage, the change in TSS was significant ($p < 0.05$) and the final TSS content was 18.40% (Tab. 2). The pH is becoming increasingly recognized for its important contribution to product quality. This is especially true about red wine because it plays a key role in prevention of microbial spoilage, malolactic fermentation occurrence and colour stability of wines. The pH slightly increased from about 2.20 to 3.95 during fruit development and ripening. In contrast, the titratable acidity (TAc) showed a gradual decrease from about 1.40 to 0.75%.

Table 2. Some physico-chemical properties and antioxidant activity of the Maoluang fruit in different maturation stages

Properties	Harvest NO.				
	1	2	3	4	5
*L**	39.50±2.41a[2]	32.27±2.53b	21.14±1.62c	9.20±1.35d	2.34±1.41e
*a**	-1.46±0.15d	4.86±1.24c	31.68±1.30a	27.36±1.57b	26.40±1.22b
*b**	21.06±1.94a	16.20±1.35b	10.17±1.43c	2.12±0.62 d	1.87±0.46d
Fruit diameter [cm]	0.50±0.02d	0.60±0.03c	0.90±0.05b	1.10±0.06ab	1.20±0.08a
Fruit weight [g]	0.32±0.03c	0.38±0.04c	0.49±0.02b	0.64±0.04a	0.68±0.03a
Dry matter [%]	35.88±2.42a	33.08±2.10a	27.43±2.20c	19.36±2.18d	25.75±2.47c
TSS [% at 25 °C]	3.50±1.50d	4.42±1.24d	8.20±1.22c	14.50±1.13b	18.40±1.20a
pH	2.20±0.11e	2.76±0.23d	3.18±0.12c	3.52±0.24b	3.95±0.15a
TAc [% citric acid]	1.40±0.13a	1.29±0.08a	0.96±0.04b	0.72±0.05c	0.57±0.04d
Maturation index [%][1]	2.50±0.10e	3.43±0.82d	8.54±1.31c	20.14±1.10b	32.28±1.14a
TA [mg/100 g f.w.]	ND	ND	4.51±1.14c	86.42±6.50b	141.94±4.32a
TP [mg GAE/ g f.w.]	19.60±1.37a	13.51±1.46b	10.70±1.52c	10.67±1.04c	8.66±1.14d
AA [%]	85.37±1.46a	80.04±1.18b	74.30±1.61c	73.26±1.25c	69.18±1.42d
EC_{50} [μg/μg DPPH]	0.06±0.02e	0.12±0.03d	0.32±0.04c	0.58±0.06b	0.96±0.04a

[1]Maturation index: total soluble solids content/titratable acidity (TSS/TAc)
[2]Different letters in same row denote significant differences according to the LSD test ($p < 0.05$)
Values are the means ± standard deviation ($n = 3$)
f.w. – fresh weight
ND – not detected

Acidity was inversely correlated to pH. The ripe fruit sample which had a low acid content, had a correspondingly high pH. Evolution of TAc and the pH of Maoluang recorded above, agree with published data on strawberries (Voća et al., 2008). Organic acids usually decline during ripening as they are respired or converted to sugars. The maturation index is regarded as the most reliable measure of fruit quality such as fruit flavour. The maturation index relates TAc (sourness) and sugar level (sweetness) and should be 30 to 32 (Gallander, 1983) for wine production. The maturation index is an important quality factor. One can predict from the finding of this study that the mid ripe stage is of less quality than those of the ripe and over ripe stages, respectively. In the over ripe stage, the high ratio of 32.28% may be regarded as low acid and would have a sweet taste.

Anthocyanins are members of the group of polyphenolics that contribute to the red, blue and purple colours of plant tissues. They largely contribute to the visual quality of fruits. The concentrations of anthocyanins increase with ripening. The total anthocyanin content (TA) of the Maoluang fruits during development and ripening are presented in Table 2. Being responsible for the colour of Maoluang fruits, TA presented a gradual accumulation in the course of ripening. The changes in TA concentrations agree with Alarcao-E-Silva et al. (2001) for arbutus berry. Interestingly, TA has been detected at the mid ripe stage. The highest content of TA ($p < 0.05$) was at the over ripe stage. At this stage TA had an average value of 141.94 mg/100 g f.w. that was higher than those reported in red grape (cultivar Cabernet Sauvignon) with 99.08 mg/100 g f.w. (Hulya Orak, 2007). However, Maoluang fruit had a lower TA content than those reported in red grape (cultivar Alfonse Lavalle) which had 189 mg/100 g f.w. (Carreno et al., 1997), and blackberry with 792.67 mg/100 g f.w. (Tosun et al., 2008).

The total phenolic content (TP) and antiradical activity (AA) tended to decrease continuously during fruit development and ripening. According to Atanasova et al. (2002) and Vidal et al. (2004), the decrease in polyphenol content of fruits causes a loss in astringency and bitterness during ripening. The highest TP and AA were found at the immature stage with 19.60 mg GAE/g f.w. and 85.37%, respectively. Whereas, the over ripe stage contained the lowest TP and AA (8.66 mg GAE/g f.w. and 69.18%, respectively), about a 55.82 and 18.96% decrease, respectively. These results suggest that phenolic components of Maoluang fruit have a major effect on AA, as reported for other fruits (Kahkonen et al., 1999; Kobayashi et al., 2008). In our study, the quantity of TP was similar to those values reported by Tosun et al. (2008) and Alarcao-E-Silva et al. (2001) for blackberry and arbutus berry, respectively, ranging from 15.50 to 9.37 mg GAE/g f.w. The EC_{50} value is defined as the concentration of extract required for 50% scavenging of DPPH or hydroxyl

radicals under experimental conditions. The EC_{50} value is employed as a parameter widely used to measure the AA; a smaller EC_{50} value corresponds to a higher antioxidant activity. It was observed that the EC_{50} slightly increased from about 0.06 to 0.96 μg/μg DPPH during fruit development and ripening. EC_{50} was inversely correlated to AA. The over ripe fruit sample which had a low AA, had a correspondingly high EC_{50} value. The lowest EC_{50} values for Maoluang fruit were found at the immature stage which were also the richest in TP.

Besides sugars and organic acids, polyphenols as secondary metabolites, to a certain extent can also contribute to sweet, bitter or astringent flavours of fruit , while they can also contribute to aroma (Tomas-Barberan and Espin, 2001). A number of studies have shown that the presence of polyphenols in food and especially in fruit, can be particularly important for consumers, because of their beneficial health properties (Maas et al., 1990; Amiot et al., 1995; Hertog et al., 1997; Kahkonen et al., 1999; Stoclet et al., 2004). The polyphenols analysed in our experiment are presented in Table 3. Comparing the different stages of development and ripening, analysis of variance revealed significant differences for results based on a fresh weight basis. The sum of polyphenols based on a fresh weight basis showed significant increase from unripe to ripe stages. Over ripe fruits showed the highest amount of polyphenols (3550.39 mg/100 g f.w.). In the course of ripening, the main polyphenol compounds increased slightly from the unripe to the ripe stages. Procyanidin B2 (2.35-2006.39 mg/100 g f.w.), procyanidin B1 (4.80-1332.91 mg/100 g f.w.) and (+)-catechin (8.23-175 mg/100 g f.w.) were the main polyphenol compounds. Procyanidin B2, procyanidin B1 and (+)-catechin, rutin (0.10-16.60 mg/100 g f.w.) were also present in relatively high quantities. All of the other polyphenols were present in lesser quantities. (–)-Epicatechin showed a slight but not significant increase of from 1.31 to 1.27 mg/100 g f.w. between immature and mid ripe stages and then it significantly decreased ($p < 0.05$) to reach 6.35 mg/100 g f.w. at the last stage (over ripe fruits). Both (–)-epicatechin and (+)-catechin belong to the group of catechins. Auger et al. (2004) report that this is a very important group of compounds in the Mediterranean diet. Maoluang fruits analysed in our study contained more (+)-catechin than (–)-epicatechin. The highest value of (+)-catechin was achieved in the over ripe stage. The results for myricetin showed a slight but not significant increase from 0.25 to 0.36 mg/100 g f.w. from the unripe to the ripe stages. Many authors have already examined changes in polyphenol compounds during fruit development and ripening. For American cranberry (Irina and Nicholi, 2004) and red raspberries (Shiow et al., 2009), polyphenol compounds were lowest in young fruit but increased during fruit development and ripening. Investigations

of Veberic et al. (2008) on fig fruits also revealed that (–)-epicatechin, (+)-catechin and rutin, the main polyphenols of this fruit, remained relatively high in quantity during fruit development and ripening.

The sum of phenolic acids based on fresh weight showed a significant decrease ($p < 0.05$) from the unripe to the ripe stages. Over ripe fruits showed the lowest amount of phenolic acids (21.02 mg/100 g f.w.) (Tab. 3). With regard to the evolution of each compound, gallic acid (106.04-20.33 mg/100 g f.w.) was the main phenolic acid, followed by caffeic and ellagic acids, with trace amounts of ferrulic acid. Gallic acid is extremely well absorbed into the human body, compared with other polyphenols (Manach et al., 2005). In the review by Tomas-Barberan and Clifford (2000), gallic acid was shown to have a positive effect under *in vitro* conditions against cancer cells. The highest value of gallic acid was achieved in the immature stage. Ellagic acid is a naturally occurring polyphenol constituent of many plant species and has shown significant inhibition of colon, esophageal, liver, lung, tongue, and skin cancers in rats and mice by *in vitro* and *in vivo* antimutagenic and anticarcinogenic activity against chemical-induced cancers (Daniel et al., 1989). We found that ellagic acid achieved highest values (1.10 mg/100 g f.w.) in the immature stage and then significantly decreased ($p < 0.05$) to reach 0.15 mg/100 g f.w. at the last stage (over ripe fruits). Similar results were reported by Shiow et al. (2009) for red raspberries.

CONCLUSIONS

The changes in physico-chemical properties and concentrations of polyphenol compounds within the Maoluang fruits are important for their beneficial effects and quality. There are significant differences in the levels of TSS, TA, TP and polyphenol compounds of Maoluang fruit during development and ripening. L^* and b^* values, dry matter, TAc, TP and AA decreased, TSS, pH, maturation index and TA significantly increased with fruit development and ripening. Maoluang contains several polyphenol compounds with potent antioxidant properties. The amount of the polyphenol compounds significantly changed during the development and ripening process. Procyanidin B2, procyanidin B1, (+)-catechin, (–)-epicatechin, rutin and *tran*-resveratrol as the main polyphenol compounds increased, but other phenolic acids such as gallic, caffeic, and ellagic acids significantly decreased during fruit development and ripening. However, some climatic and agronomic changes can occur and these need to be examined. Based on our results, we can recommend that a harvest date for Maoluang fruit, in the middle of October at the over ripe stage, will give appropriate levels of pH, TSS, TA, TP, polyphenol compounds and AA for people who consume the fruit in their diets or for industrial processing of foods and beverages.

Table 3. Changes in polyphenol contents (mg/100 g f.w.) during Maoluang fruit maturation

Polyphenolic compound	Harvest No.				
	1	2	3	4	5
Polyhenols					
(+)-Catechin	8.23±5.20e*	68.89±2.14d	84.55±3.05c	136.80±2.52b	175.40±2.12a
(–)-Epicatechin	1.31±0.24c	1.53±0.22c	1.27±0.25c	4.21±1.01b	6.35±0.40a
Rutin	0.10±0.02d	0.12±0.01d	3.08±0.01c	10.13±0.11b	16.60±1.05a
Procyanidin B1	4.80±1.23e	130.54±4.04d	322.75±4.64c	594.85±6.50b	1332.91±9.28a
Procyanidin B2	2.35±1.24e	44.56±3.60d	218.17±5.01c	876.19±8.67b	2006.39±6.49a
Myricetin	0.25±0.14	0.29±0.12	0.34±0.12	0.32±0.10	0.36±0.11
trans- Resveratrol	0.16±0.08c	1.14±0.53b	4.59±1.10a	5.17±1.12a	5.98±1.16a
Luteolin	ND	ND	ND	0.05±0.02b	0.13±0.01a
Quercetin	ND	ND	1.85±0.28b	2.07±0.10b	4.11±0.14a
Naringenin	0.06±0.01d	1.87±0.18c	6.07±0.13b	3.14±0.10b	1.86±0.12c
Kaempferol	ND	ND	ND	0.12±0.06b	0.30±0.10a
∑ polyphenols	17.26±3.22e	252.39±4.40d	643.25±5.03c	1632.92±6.18b	3550.39±4.34a
Phenolic acids					
Gallic acid	106.04±3.16b	119.21±2.80a	37.19±1.50c	30.14±0.21d	20.33±0.32e
Caffeic acid	1.47±0.20a	1.21±0.26a	0.54±0.12c	0.50±0.12c	0.46±0.13c
Ellagic acid	1.10±0.14a	0.55±0.11b	0.24±0.04c	0.12±0.03d	0.15±0.04d
Ferulic acid	ND	ND	0.11±0.01a	0.08±0.04a	ND
∑ phenolic acids	108.61±2.03b	120.97±1.42a	38.04±1.40c	30.80±0.15d	21.02±0.08e

Values are the means ± standard deviation (n = 3)
ND – not detected
*Different letters in the same row indicate significant differences according to the LSD test ($p < 0.05$)

Acknowledgments: The authors would like to thank The Division of Research Development and Financial Assistance, Mahasarakham University, Thailand, for the financial support of our research. We would like to gratefully acknowledge the staff of the Microbiology Laboratory, Department of Biotechnology, Technology Faculty, Mahasarakham University, Thailand, for their collaboration and for providing laboratory facilities.

REFERENCES

Alarcao-E-Silva M.L.C.M.M., Leitao A.E.B., Azinheira H.G., Leitao, M.C.A. 2001. The arbutus berry: studies on its color and chemical characteristics at two mature stages. J. FOOD COMP. ANAL. 14: 27-35.

Amiot M.J., Tacchini M., Aubert S.Y., Oleszek W. 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. J. AGRIC. FOOD CHEM. 43: 1132-1137.

Atanasova V., Fulcrand H., Cheynier V., Moutounet M. 2002. Effect of oxygenation on polyphenol changes occurring in the course of wine-making. ANAL. CHIMICA ACTA. 458: 15-27.

Association of official analytical chemists – AOAC. 1984. Official methods of analysis of the Association of Official Analytical Chemists. 14 ed. Washington, DC: AOAC, 22.013, 22.059.

Auger C., Al-Awwadi N., Bornet A., Rouanet J.-M., Gasc F., Cros G. 2004. Catechins and procyanidins in Mediterranean diets. FOOD RES. INT. 37: 233-245.

Boyles M.J., Wrolstad R.E. 1993. Anthrocyanin composition of red raspberry juice: influence of cultivar, processing, and environmental factors. J. FOOD SCI. 58: 1135-1141.

Bravo L. 1998. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. NUTR. REV. 56: 317-333.

Burda S., Oleszek W., Lee C.Y. 1990. Phenolic compounds and their changes in apples during maturation and cold storage. J. AGRIC. FOOD CHEM. 38: 945-948.

Bureau S., Renard C.M.G.C., Reich M., Ginies C., Jean-Marc A. 2009. Change in anthocyanin concentrations in red apricot fruits during ripening. LWT. 42: 372-377.

Butkhup L., Samappito S. 2008a. Analysis of anthocyanin, flavonoids, and phenolic acids in tropical bignay berries. INT. J. FRUIT SCI. http://www.informaworld.com/smpp/title~db=all~content=t792306963~tab=issueslist~branches=8 - v88: 15-34.

Butkhup L., Samappito S. 2008b. An analysis on flavonoids contents in Mao Luang fruits of fifteen cultivars (*Antidesma bunius*), grown in Northeast Thailand. PAKIS. J. BIOL. SCI. 11: 996-1002.

Cai Y., Sun M., Corke H. 2003. Antioxidant activity of betalains from plants of the *Amaranthaceae.* J. AGRIC. FOOD CHEM. 51: 2288-2294.

Carreno J., Almela L., Martinez A., Fernandez-Lopez J.A. 1997. Chemotaxonomical classification of red table grapes based on anthocyanin profile and external colour. LEB. WISS TECHNOL. 30: 259-265.

Daniel E.M., Krupnick A.S., Heur Y.H., Blinzler J.A., Nims R.W., Stoner G.D. 1989. Extraction, stability, and quantification of ellagic acid in various fruits and nuts. J. FOOD. COMP. ANAL. 2: 338-349.

Dewanto V., Wu X., Adom K.K., Liu R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. AGRIC. FOOD CHEM. 50: 3010-3014.

Eun-Mi C., Jae-Kwan H. 2005. Screening of Indonesian medicinal plants for inhibitor activity on nitric oxide production of RAW264.7 cells and antioxidant activity FITOTERAPIA. 76: 194-203.

Gallander J.F. 1983. Effect of grape maturity on the composition and quality of Ohio Vidal blanc wines. AMER. J. ENOL. VITIC. 34: 139-141.

Haripyaree A., Guneshwor K., Damayanti M. 2010. Evaluation of antioxidant properties of some wild edible fruit extracts by cell free assays. EJEAFCHE 9: 345-350.

Hertog P.C., Feskens E.J.M., Kromhou D. 1997. Antioxidant flavonols and coronary heart disease risk. LANCET. 349: 699-703.

Hulya Orak H. 2007. Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. SCIENTIA HORT. 111: 235-241.

Irina O.V., Nicholi V. 2004. Flavonoid composition over fruit development and maturation in American cranberry, *Vaccinium macrocarpon* Ait. PLANT SCI. 167: 1043-1054.

Kahkonen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S. 1999. Antioxidant activity of plant extract containing phenolic compounds. J. AGRIC. FOOD CHEM. 47: 3954-3962.

Kobayashi H., Wang C., Pomper K.W. 2008. Phenolic content and antioxidant capacity of pawpaw fruit (*Asimina triloba* L.) at different ripening stages. HORTSCIENCE 43: 268-270.

Maas J.L., Galetta G.J., Stoner G.D. 1990. Ellagic acid, anticarcinogen in fruit, especially in strawberries: A review. HORTSCIENCE 26: 10-14.

Manach C., Williamson G., Morand C., Scalbert A., Rémésy C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. AMER. J. CLINIC. NUTR. 81: 230-242.

Mathew A.G., Parpia H.A.B. 1971. Food browning as a polyphenol reaction. ADD. FOOD RES. 19: 75-145.

Samappito S., Butkhup L. 2008. An analysis on organic acids contents in ripe fruits of fifteen Mao Luang (*Antidesma bunius*) cultivars, harvested from dipterocarp forest of Phupan valley in northeast Thailand. PAKIS. J. BIOL. SCI. 11: 974-981.

Shiow Y.W., Chi-Tsun C., Chien Y.W. 2009. The influence of light and maturity on fruit quality and flavonoid content of red raspberries. FOOD CHEM. 112: 676-684.

Stoclet J.C., Chataigneau T., Ndiaye M., Oak, M.H., El Bedoui J., Chataigneau M., Schini-Kerth V.B. 2004. Vascular protection by dietary polyphenols. EUR. J. PHARM. 500: 299-313.

Tomas-Barberan F.A., Clifford M.N. 2000. Dietary hydroxybenzoic acid derivatives – nature, occurrence and dietary burden. J. SCI. FOOD AGRIC. 80: 1024-1032.

Tomas-Barberan F.A., Espin J.C. 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. SCI. FOOD AGRIC. 81: 853-876.

Tosun I., Sule Ustun N., Tekguler B. 2008. Physical and chemical changes during ripening of blackberry fruits. SCI. AGRIC. 65: 87-90.

Veberic R., Colaric M., Stampar F. 2008. Phenolic acids and flavonoids of fig

fruit (*Ficus carica* L.) in the northern Mediterranean region. FOOD CHEM. 106: 153-157.

Vidal S., Francis L., Noble A., Kwiatkowski M., Cheynier V., Waters E. 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. ANALYTICA CHIMICA ACTA 513: 57-65.

Voća S., Dobričević N., Dragović-Uzelac V., Duralija B., Družić J., Čmelik Z., Babojelić M.S. 2008. Fruit quality of new early ripening strawberry cultivars in Croatia. FOOD TECHNOL. BIOTECHNOL. 46: 292-298.

ZMIANY WŁAŚCIWOŚCI FIZYKO-CHEMICZNYCH, ZAWARTOŚCI POLIFENOLI ORAZ AKTYWNOŚCI PRZECIWRODNIKOWEJ W OWOCACH ANTYDESMY WAWRZYNOLISTNEJ (*Antidesma bunius* L. Spreng) PODCZAS ICH ROZWOJU I DOJRZEWANIA

Luchai Butkhup i Supachai Samappito

S T R E S Z C Z E N I E

Badania miały na celu poznanie zmian właściwości fizyko-chemicznych, zawartości polifenoli i aktywności przeciwrodnikowej podczas rozwoju i dojrzewania owoców antydesmy wawrzynolistnej. Zawartość fenoli ogółem (TP), oznaczana metodą Folina-Ciocalteu, stopniowo zmniejszała się od 19,60 do 8,66 mg GAE/g f.w. wraz z przechodzeniem od stadiów niedojrzałości do przejrzałości. Natomiast zawartość antocyjanów ogółem (TA) była najwyższa u przejrzałych owoców, gdzie średnia jej wartość wyniosła 141,94 mg/100 g f.w. Aktywność przeciwrodnikową (AA) ekstraktów metanolowych z owoców antydesmy wawrzynolistnej oznaczano przez redukcję rodników DPPH (2,2-difenylo-1-pikrylohydrozylu). Najwyższą aktywność przeciwrodnikową, której towarzyszył wysoki poziom TP, w tym kwasu galusowego, zaobserwowano u roślin niedojrzałych. Polifenole były oznaczane za pomocą HPLC. W trakcie rozwoju i dojrzewania owoców poziom procyjanidyny B2, procyjanidyny B1, (+)-katecholu, (-)-epikatecholu, rutyny oraz trans resweratrolu jako głównych polifenoli zwiększył się. Natomiast poziom pozostałych hydroksykwasów aromatycznych, takich jak kwas galusowy, kawowy i elagowy znacząco spadł ($p < 0,05$). Owoce zawierały najwięcej przeciwutleniaczy, gdy były przejrzałe. Dlatego też okres, w którym owoce te są przejrzałe jest najlepszy do zbioru, biorąc pod uwagę ich właściwości odżywcze. Wyniki te dostarczają informacji dotyczących zastosowania owoców antydesmy wawrzynolistnej w codziennej diecie oraz przemysłowego ich wykorzystania

Słowa kluczowe: antydesma wawrzynolistna, polifenol, kwasy fenolowe, aktywność przeciwrodnikowa, oznaczenie DPPH, HLPC