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# Influence of ripening stages on physicochemical characteristics and antioxidant properties of bitter gourd (*Momordica charantia*)

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**Abstract:** *Momordica charantia* is known to contain with antioxidant properties and bioactive compounds to lower of diabetic diseases. Objective this study was investigate the influence of ripening stages on the phenolic bioactive substances and the corresponding antioxidant activity of bitter melon (*Momordica charantia*). The ripening of bitter melon fruit divided to four stages (RS1, RS2, RS3 and RS4). The results of this study were more ripened the fruit, lightness ($L^*$), yellowish ($b^*$) and chroma increased. Other ways, more ripened the fruit, the pH value and titratable acidity decreased. Total phenolic content and FRAF of RS 4 was highest compared other samples but DPPH of RS 4 was lowest among all the samples. However DPPH and FRAP value of bitter gourd on ripening stages showed no significant difference ($p>0.05$) among samples.

**Keywords: Maturity, bitter melon, phenolics, DPPH, FRAP**

## Introduction

Prevalence of diabetes mellitus in Malaysia is gradually increasing 6% according to First Malaysian Nation Health and Morbidity Survey (Malaysia, Ministry of Health 1986-1987) and increased to 8.2% after 10 years (Malaysia, Ministry of Health 1996-1997). By year 2010, the global incidence of type 2 diabetes is estimated to increased 50% and will double by 2025 (WHO, 2000). The increasing trend is largely due to population growth, obesity, ageing, unhealthy dietary intake and lack of exercise (Noor Hashimah *et al*., 2010). Antioxidants have been detected in a larger number of food and agricultural products, including cereal, grains, vegetables, fruits, plant extracts (Burits and Bucar, 2000; Yu *et al*., 2002a, b) and medical plants (Faridah *et al*., 2006; Wong *et al*., 2006 and Chan *et al*., 2007). May be an important property of plants medicines associated with the treatment of several ill fated diseases including diabetes. Thus herbal plants are considered useful means to prevent and ameliorate certain disorder such as diabetes, arthelosclerosis and other complications (Scartezzini *et al*., 2000), as a hypoglycemic agent for diabetis patient (Jayasooriya *et al*., 2000; Khattak *et al*., 2005).

Bitter gourd (*Momordica charantia*) is one of the popular vegetables in Asia. It is used for the preparation of several dishes. It can be fried, deep-fried, boiled, pickled, juices and dried to drink as tea. It is a good source of phenolic compounds which posses potent antioxidant activity (Budrat and Shotipruk, 2008; Myojin, *et al*., 2008). All parts of bitter gourd, including the fruit, taste bitter. The fruit is oblong and resembles as small cucumber, young fruit is emerald green that turn to orange-yellow when ripe (Grover and Yadaf, 2004). Consumers eat bitter melon when the fruit is particularly immature or unripe and therefore when discussing bitter melon maturity refers to maturity for eating quality. Maturity is very hard to judge from the outward appearance of the fruit but external fruit color of the whole fruit can be used. The seed coat must be creamy or pale green – brown with over maturity indicated by any shade of pink seed coat (Morgan and Midmore, 2002).

Kalt (2005) has been reported the changes of phenolic composition and antioxidant activity during postharvest storage and handling and refered Skrede *et al*. (2000) during processing. The other hand. Research groups have also analyzed the growing performance and fruit quality attributes of specific cultivars, as well as differences in growing locations in respect to phenolic compound content and antioxidant activity (Kalt *et al*., 2001). In general, fruit becomes sweeter, less green and softer as it ripens. However the acidity as well as sweetness rises during ripening, for a part of kind fruit. Bitter gourd is harvested as a physiologically ripening stage, before the true onset of ripening. It is important, if the fruit is to be transported overland (taking from 1-5 days from origin to market place), that fruit be selected that harvest started the physiologically process of ripening, for they will produce ethylene that hastens improving at store fruit (Morgan and Midmore, 2002).

There have been many studies conducted on fresh vegetables and fruits, mainly to quantify the antioxidant activity of the various cultivars of fruit and the influence of maturity on their bioactive compounds (Howard *et al*., 2000; Marin *et al*.,

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2004). Therefore, the purpose of this study was to investigate the influence of ripening stages on the physicochemical characteristics and antioxidant properties of bitter melon (*Momordica charantia*).

## Materials and Methods

*Determination of ripening stage*

For sample collection of bitter gourd, four ripening stages have been choice. The description of the selected maturity stages for sample showed at Table 1. refered to Fama, Malaysia. Ripening stages were sorted according to the progressive fruit development by the following parameters: color changes of fruits, hardness of fruits and color changes of seeds on fruits.

**Table 1.** Description of the selected maturity stages for sample collection of bitter melon (*Momordica charantia*)

| Abbreviation | Maturity stages | Color of fruits | Hardness of fruit | Color of seed |
|---|---|---|---|---|
| RS1 | Unripe | Dark green | Hard | White-yellowish |
| RS2 | Ripe | Light green | Hard | White-yellow-redness |
| RS3 | Ripe | Green-yellowish | Tender | White-yellow-redness |
| RS4 | Overripe | Yellow-orange | Most tender | Red |

*Color, pH and Titratable Acidity*

Color juice of bitter melon were determined using Minolta CR-300 Chroma Meter (*Minolta Corp*., *Osaka, Japan*) and results were expressed as lightness and darkness ($L^*$), redness ($+a^*$) and greenness ($-a^*$) and yellowness ($+b^*$) and blueness ($-b^*$). The chromameter was calibrated with a white standard. The pH was determined by a glass-electrode pH meter (Metrohm Herisam, Switzerland) used buffers of pH 4.0 and 7.0 for calibration and total acidity as (%) of mallic acid (A.O.A.C. 2000).

*Extraction of sample*

Extraction was conducted following the method described by Conner *et al*. (2005). 0.5 g grinded sample was mixed vigorously with 3 mL of methanol 80% and centrifuged for 15 min at 3000 rpm. Supernatant was collected in a 10 mL volumetric flask. The residue was treated again twice with 3 mL methanol 80% and 15 min centrifugation. Supernatants were collected and standardised to a final volume of 10 mL.

*Determination of the total phenolic content*

Total phenolic contents (TPC) of the fruit extract were determined using Folin-Ciocalteu assay which was described by Singleton and Rossi (1965). 40 µl of properly diluted fruit extract solution were mixed with 1.8 ml of Folin-Ciocalteu reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing 5 min at room temperature, 1.2 ml of (7.5% w/v) sodium carbonate solution were added. The solutions were mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured at 765 nm, using a UV-visible spectrophotometer (Tunable versamax microplate reader absorbance). A calibration curve was prepared, using standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l, $r^2$= 0.997). Result expressed on dry weight (dw) as mg GAE/100 g of sample.

*DPPH radical-scavenging activity*

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by Akuwuoh *et al*. (2005). The method described by 200 µL. The aqueous extract sample (0.05 mg/mL) was added to 2 ml of a 0.001 M DPPH in methanol. Laser UV-Vis spectrophotometer (*Tunable versamax microplate reader*), absorbance at 517 nm was determined after 1 h. The percent inhibition of activity was calculated as $[(A_o - A_e)/A_o] \times 100$ ($A_o$ = absorbance without extract; $A_e$ = absorbance with extract).

*Ferric reducing/ antioxidant power (FRAP) assay*

FRAP assay was performed as previously describe by Benzie and Strain (1999). Briefly, a 40 µl aliquot of properly diluted fruit extract was mixed with 3 ml of FRAP reagent. Then, the reaction mixture was incubated at 37°C for 4 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 h instead of 4 min. FRAP reagent should be pre-warmed at 37°C and should always be freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-trazine (TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM $FeCl_3$. $6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6. A calibration curve was prepared, using an aqueous solution of ferrous sulphate $FeSO_4$. $7H_2O$ (200, 400, 600, 800 and 1000 ppm). FRAP values were expressed on a fresh weight basis as micromoles of ferrous equivalent Fe(II) per g of sample.

*Statistical Analysis*

Analyses were repeated at least 3 times for each sample. All data were analyzed using ANOVA by means of the GLM proc-SAS software (6.12 version, 1997). Significant differences between ripening of stages were detected using LSD (Least Significant Differences) at the 5% level of significance.

## Results and Discussions

*Physicochemical characteristics*

The possible of explanation for the color change in fruit peel was associated with the enzymatic degradation of chlorophyll as fruit ripeness increased (Ding *et al*., 2007). However, fruits lightness ($L^*$) increase as ripening day processed (Lewallen and Marini, 2003). Mwithiga *et al*. (2007) reported that changes in the fruit flesh color of tamarillo fruit were well pronounced with 44% of decreased in $L^*$ values as fruit ripeness progressed.

The result of physicochemical of bitter melon based on ripening stages showed at Table 2. The RS 5 was the highest value $L^*$ (lightness) and $b^*$ (yellowness), 60.10 and 34.50, respectively and showed significant difference ($p<0.05$) compared other samples. The same results for measurement of chroma. The RS 4 was highest, 34.64. Value of $a^*$ (redness) of fruit showed no significant difference among all the samples at ($p>0.05$).

**Table 2.** Measurement of colors of bitter melon on ripening stages

| Sample | Measurements | | | |
|---|---|---|---|---|
| | L* | a* | b* | Chroma |
| RS 1 | $42.00^{b} \pm 3.00$ | $-1.86^{a} \pm 1.78$ | $8.25^{b} \pm 6.30$ | $14.22^{b} \pm 0.52$ |
| RS 2 | $43.68^{b} \pm 2.10$ | $-7.25^{a} \pm 1.00$ | $10.72^{b} \pm 1.40$ | $12.80^{b} \pm 1.37$ |
| RS 3 | $44.40^{b} \pm 2.46$ | $-6.75^{a} \pm 1.00$ | $13.40^{b} \pm 3.00$ | $15.12^{b} \pm 2.41$ |
| RS 5 | $60.10^{a} \pm 1.37$ | $-3.00^{a} \pm 0.06$ | $34.50^{a} \pm 1.58$ | $34.64^{a} \pm 1.60$ |

Data are expressed as mean ± SD (for each fruit n=3). The same letter in the same column indicates no significant differences ($p>0.05$).

The measurements of bitter melon for pH and titratable acidity showed at Figure 1. The ranges of pH on stage bitter melon were 4.86-5.20. All the samples showed no significant difference at ($p>0.05$). Titratable acidity on RS 1 and RS 2 were higher than other samples. Both the samples showed no significant difference ($p>0.05$). More ripening the bitter gourd showed decreased of mean on titratable acidity value. Postharvest developmental changes resulted in fruit splitting and ripening in bitter melon, thinning the edible flesh (Zong *et al*., 1993). The formation of pigment which caused color changes in the peel as well as flesh of the fruit is one of the important changes as the fruit goes through the last stage of development or ripening.

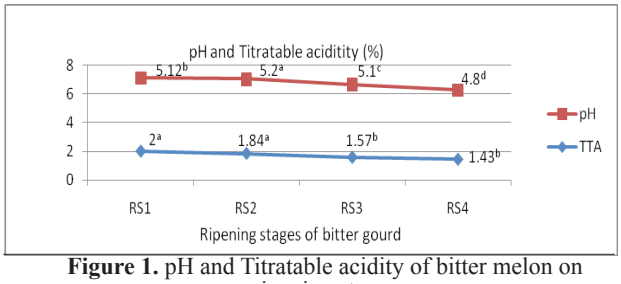


**Figure 1.** pH and Titratable acidity of bitter melon on ripening stages

The titratable acidity of samples decreased based on ripeness of maturity of fruit. RS 1 and RS 2 were similar, 2.00 and 1.84%, respectively and showed no significant difference at ($p>0.05$). This results similar to Illeperuma and Jayasooriya (2002) and Fisk *et al*. (2006) reported a decreased in titratable acidity with refrigerated storage of mangoes, attributed to the initiation of ripening in the presence of ethylene that is automatically stimulated by low temperatures in climacteric fruits such as mangoes and kiwifruit. However, this result was higher than reported Kulkarni *et al*. (2005), ranges of titratable acidity on fresh bitter gourd of fruits were 0.08 – 0.21%.

Antioxidant properties of bitter melon based on stages of maturity showed at Figure 2. Total phenolic content of bitter gourd were between Total phenolic content of bitter gourd on ripening stages were 2.8 – 4.2 mg GAE/100 fw or 224 – 336 mg GAE/100 g dw of samples (moisture content was 92%). RS1. 224 mg GAE/100 g dry weight was lowest of total phenolic content and RS4 was highest. However, RS 2 and RS 3 were the moderate of total phenolic content and showed no significant different at ($p>0.05$). However, these results showed lower total phenolics content compared last study. Kubola and Siriamornpun (2008) reported total phenolics content of green fruit and ripe fruit of bitter melon were 32.4 mg GAE/100 g fw and 22.4 mg GAE/100 fw, respectively. However, Wu and Ng (2007) reported that total phenolic content of dried wild bitter melon, using ethanol extraction was 6880 mg GAE/100 g dw and distilled water extraction was 5160 mg GAE/100 g dw.

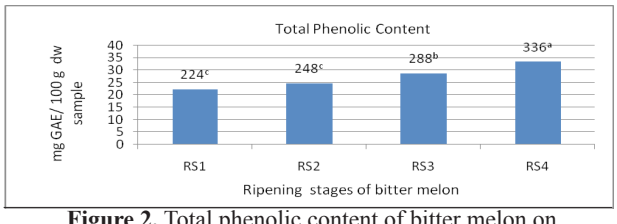


**Figure 2.** Total phenolic content of bitter melon on ripening stages

The other hand, Horax *et al*. (2005) reported total phenolic content for some varieties of flesh bitter melons were 539 - 775 mg/100 g GAE dw using oven-dried and 640 -802 mg/100 g GAE dw using freeze-dried. Budrat and Shotipruk (2008) reported total phenolic compound on bitter melon using subcritical water extraction for two times extraction were 769 and 5263 mg/100 g GAE dw, using soxhlet extraction (water as solvent) was 668 mg/100 g GAE dw and using solvent extraction (methanol as solvent) was 600 mg GAE/100g dw of sample.

The scavenging action of plant constituents has been found to relate to polyphenolic compounds (Hatono *et al*., 1989). Although the constituents of bitter melon, which show free radical scavenging action is still unclear, it is possible that the antioxidative properties of bitter melon are caused, at least in part, by the presence of polyphenols and other yet to be discovered antioxidant compounds.

The result of scavenging activity this study were 37% to 64.48%, there were ranges of DPPH on wild of bitter melon were 36.6% to 75.8% (Wu and Ng 2007). This result showed more ripened of bitter melon had a lower of DPPH. RS 4 was lowest and RS 3 was highest of DPPH value. However, all the samples showed no significant difference ($p>0.05$). Last reported, that radical scavenging activity for green fruit was 11.0% and ripe fruit 27.6% (Kubola and Siriamornpun, 2008). Generally, the results of this study higher than last result like reported.

**Table 3.** DPPH and FRAP of bitter melon based on stages of maturity

| Sample | DPPH (%) | FRAP (µg Fe (II)/ml fw) |
|---|---|---|
| RS 1 | $53.89^{a} \pm 11.16$ | $43.00^{a} \pm 1.15$ |
| RS 2 | $52.40^{ab} \pm 3.60$ | $45.00^{a} \pm 3.00$ |
| RS 3 | $64.48^{a} \pm 5.68$ | $47.00^{a} \pm 2.60$ |
| RS 4 | $37.00^{b} \pm 12.80$ | $49.50^{a} \pm 4.00$ |

Data are expressed as mean ±SD (for each fruit n=3). The same letter in the same column indicates no significant differences ($p>0.05$).

It has been reported that FRAP is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al*. 2003; Zhao *et al*., 2006). Antioxidant potential of ripeness of maturity stages of bitter melon was estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. RS4. 49.50 µg Fe (II)/ml fresh weight was highest and RS1. 43 µg Fe (II)/ml fresh weight was lowest of FRAP value. However, ferric reducing power of this results showed no significant difference ($p>0.05$) with ranges 43 – 49.50 µg Fe (II)/ml fresh weight. Antioxidant capacity of green fruit and ripe fruit of bitter melon were 79.9 and 59 µg Fe (II)/mg dry weight, respectively (Kubola and Siriamornpun 2008). Other researcher (Tosun *et al*., 2009) reported also there was relationship between total phenolic content and FRAP but results of this study did not similar results compared last reported. Maturity of stages on bitter melon showed no effect to ferric reducing power of bitter melon.

## Conclusions

Lightness ($L^{*}$), yellowness ($b^{*}$) and chroma of RS1 was lowest and RS 5 was highest. More ripened of bitter melon showed higher of lightness, yellowness and chroma. pH value and titratable acidity of R4 was lowest compared other samples and showed significant difference ($p<0.05$). Total pheolic of RS4 was highest compared other samples and showed significant difference ($p<0.05$). DPPH value of RS1 was highest and RS5 was lowest. However, DPPH and FRAP value all samples showed no significant difference ($p>0.05$). Generally, according to the results, more ripened stages of bitter melon fruit resulted higher of physicochemical characteristics, higher of total phenolic content and FRAP value but lower of DPPH value.

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