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Full Length Research Paper

Influence of growth stage and variety on the pigment levels in *Ipomoea batatas* (sweet potato) leaves

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The study of pigments and nutritional composition in *Ipomoea batatas* has been intensive, yet little studies have been conducted on their leaves. *I. batatas* (sweet potato) leaves which are discarded after the harvesting period can be used as a potential source of natural dye extraction due to their high level of carotenoids (particularly lutein). Selection of leaves material is dependable on factors such as leaves varieties and stages of growth developments. Variations in chlorophyll a, chlorophyll b, total carotenoids and lutein level were determined spectrophotometrically at different stages of leaves development in 6 different varieties of *I. batatas* leaves. Result showed that chlorophyll a, b, total carotenoids and lutein pigments increased rapidly when measured from day 1 to 7 and remained stagnant or decreased at older stages (day 15 to 18). Both the *I. batatas* var Oren and Biru Putih contained the highest amount of carotenoids and lutein from day 7 onwards compared to the other varieties and thus can be used as a suitable source of natural yellow dye.

Key words: *Ipomoea batatas*, pigments, developmental stage, spectrophotometric, lutein.

INTRODUCTION

Ipomoea batatas or sweet potato originated from the Northwest of South America and has been dispersed world-wide because of its high yield potential and wide adaptability. Sweet potato plants are easy to grow and are planted mainly for their storage roots. The leaves are usually made into animal feeds or discarded after the storage roots are harvested. The nutritional value of *I. batatas* leaves is gaining recognition, as the understanding between diet and health increases. *I. batatas* leaves with their high nutritive value and antioxidants may become an excellent leafy vegetable (Islam, 2006). Besides, this crop has higher tolerant to pest, diseases and moisture and can be grown several times a year, making it a potential leafy vegetable (Islam, 2006). Lately, *I. batatas* leaves have been studied as a potential source of cheap natural yellow dye that can be used to replace the current usage of synthetic yellow dye. *I. batatas* leaves have advantage over other sources due to their low economic value and their ability to provide long term supply.

Leaves contain pigments or biochromes that are mainly used in the absorption of light. Pigments such as chlorophylls are needed by plants to absorb sufficient amount of light through photosynthesis. During leaf development, the level of pigments in the leaves increases to provide energy through photosynthesis (Lefsrud et al., 2007). Lutein is a xanthophyll that has medical importance which can prevent loss of sight caused by age-related macular degeneration (Cardinault et al., 2003). A study by Ishiguro and Yoshimoto (2006) found that the *I. batatas* leaves contained high amount of lutein compared to other green leafy vegetables. Hence, *I. batatas* leaves are further studied to be a potential source of natural yellow dye (contributed by the presence of lutein). However, there are several factors that can contribute to the level of lutein pigment in plant such as plant variety and stages of leaves development. Hence, the main objective of the present study is to select the suitable starting material to be used as the source of natural yellow dye extraction by studying the lutein, total carotenoids, chlorophyll a and chlorophyll b

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contents in the different developmental stages and different *I. batatas* varieties.

MATERIALS AND METHODS

The six different varieties of *I. batatas* leaves used in this experiment are *I. batatas cv.* Batu Kelantan (BK), *I. batatas cv.* Batu Biasa (BB), *I. batatas cv.* Biru Putih (BP), *I. batatas cv.* Oren (Oren), *I. batatas cv.* Vitato (Vit) and *I. batatas cv.* Indon (Indon). They were harvested from a sweet potato farm in Tanjung Sepat, Kuala Langat, Selangor and located about 90 km from the Post Harvest Laboratory, University of Malaya. The leaves used were harvested at the different developmental stages (young (1 to 3 days), immature (5 to 7 days), mature (9 to12 days), fully developed (15 to 18 days). The formation of leaf bud at the petiole is calculated as day 1 of the leaf development. The leaves were labelled and annotated with the date of collection and deposited at the Post Harvest Laboratory, University of Malaya.

Sample preparation

Fifty leaves of each developmental stage were thoroughly washed to remove dirt and air-dried before pigments analyses were conducted. Stems and petioles of the leaves were removed before extraction. The leaves were firstly ground into fine powder using a mortar and pestle with liquid nitrogen. One gram of finely ground leaf powder was added to 50 ml of methanol (Merck, USA) and the sample mixture was vortexed to aid the extraction process. The mixture was then placed in an orbital shaker (Stuart Scientific S01, United Kingdom) for 120 min under dim light condition. The mixture was centrifuged at 12 000 rpm and 4°C for 10 min using a refrigerated centrifuge (Beckman J2-M1, California, USA) and the resulting pellet was discarded and the supernatant was retained for analysis.

Pigments analysis

The pigments in the leaf mixture were measured using a spectrophotometer (MRC UV-200RS, Israel). A quartz cuvette was used for spectrophotometric analysis. Absorbance readings were taken at 470, 652 and 665 nm. The amount of pigments in the leaves was calculated based on the equation provided by Lichtenthaler and Buschmann (2001). The formula used for calculation is as follow:

c_a (µg/ml) = 16.72 $A_{665.2}$ – 9.16 $A_{652.4}$
c_b(µg/ml) = 34.09 $A_{652.4}$– 15.28 $A_{665.2}$
$c_{(x+c)}$ (µg/ml) = (1000 A_{470}–1.63 c_a – 104.96 c_b)/221,

where c_a, c_b and $c_{(x+c)}$ is chlorophyll a, chlorophyll b and mixture of xanthophylls and carotenes respectively.

The lutein level in *I. batatas* leaves were determined using spectrophotometric method as described previously by Bulda et al. (2008) with some modifications. Liquid nitrogen frozen sample was ground with mortar and pestle with 4:1 mixture of petroleum ether (PE) (AJAX, Australia) and Tetrahydrofuran (THF) (Fisher, USA). The mixture was filtered through Whatman No. 1 filter paper. Saponification process was performed to remove chlorophylls and lipids from the extract by adding freshly prepared KOH (1g/ml) to the extract. The mixture was incubated for 5 min at 45°C, cooled on ice and the sample was left to settle. The uppermost coloured fraction was used for analysis. The lutein levels in leaves at different developmental stages were measured using leaves harvested at different times. The lutein level was calculated and plotted into a graph. The equation to calculate the lutein content is as follow:

C_{lut} = 11.51 A_{480} – 20.61 A_{495}

The analyses of pigments were completed in triplicates and the data were tabulated. Statistical analyses were performed using one-way ANOVA and Tukey's Test in SPSS (SPSS19, IBM).

RESULTS AND DISCUSSION

Material selection is an important process when selecting a suitable source to be used in subsequent extraction process. However, the amount of carotenoids in green leafy vegetables are subjected to natural variation, variety or cultivar, climate and stage of maturity and this may account for part of the divergence for the same foods (Kimura and Roddriguez-Amaya, 2002; Chen and Chen, 1992). Previous studies on pigments accumulation in plants includes in kale, clover and tomato leaves (Yoo et al., 2003). However, no study has been conducted for the pigments levels in the different varieties of the *I. batatas* leaves.

The levels of carotenoids and lutein are particularly important in search for a suitable source for the natural yellow dye extraction. Although the level of chlorophyll a and chlorophyll b in leaves does not influence the extraction of the natural yellow dye as they are removed during the saponification process, these pigments captured our interest because chlorophyll contents in these leaves allows the study of the physiological state of the plant during the different stages of development.

The maximum concentration of chlorophyll a, chlorophyll b, carotenoids and lutein occurred in the first week of the leaves development. The chlorophylls and carotenoids content were shown in Figure 1 and found to be varied among the different varieties (Tables 1, 2 and 3). The concentration of these secondary metabolites may change as plants grow and reaches maturity because of the physiological changes that occur in the plants during the growing cycle. Deterioration of chlorophyll usually represents the senescence process that might be taking place in the leaf which resulted in the appearance of yellow, red or orange leaves.

Figure 1 showed the changes in chlorophyll a, b and carotenoids level at different developmental stages of the leaves. As shown in Figure 1a, the Biru Putih variety showed a rapid increase in the chlorophyll a level from day 1 to 5, followed by a stagnant period when the leaves were fully expanded and a rapid decrease from day 12 onwards. The Batu Biasa (Figure 1e) and Batu Kelantan (Figure 1d) varieties showed a similar pattern in term of changes in the chlorophyll a level although the latter had a more dramatic decrease from day 7 onwards. On the other hand, maximum peak from day 9 to day 12 was observed in Vitato variety (Figure 1f). However, at day 9 when the leaves reaches full maturity, the differences in the chlorophyll a content was found to

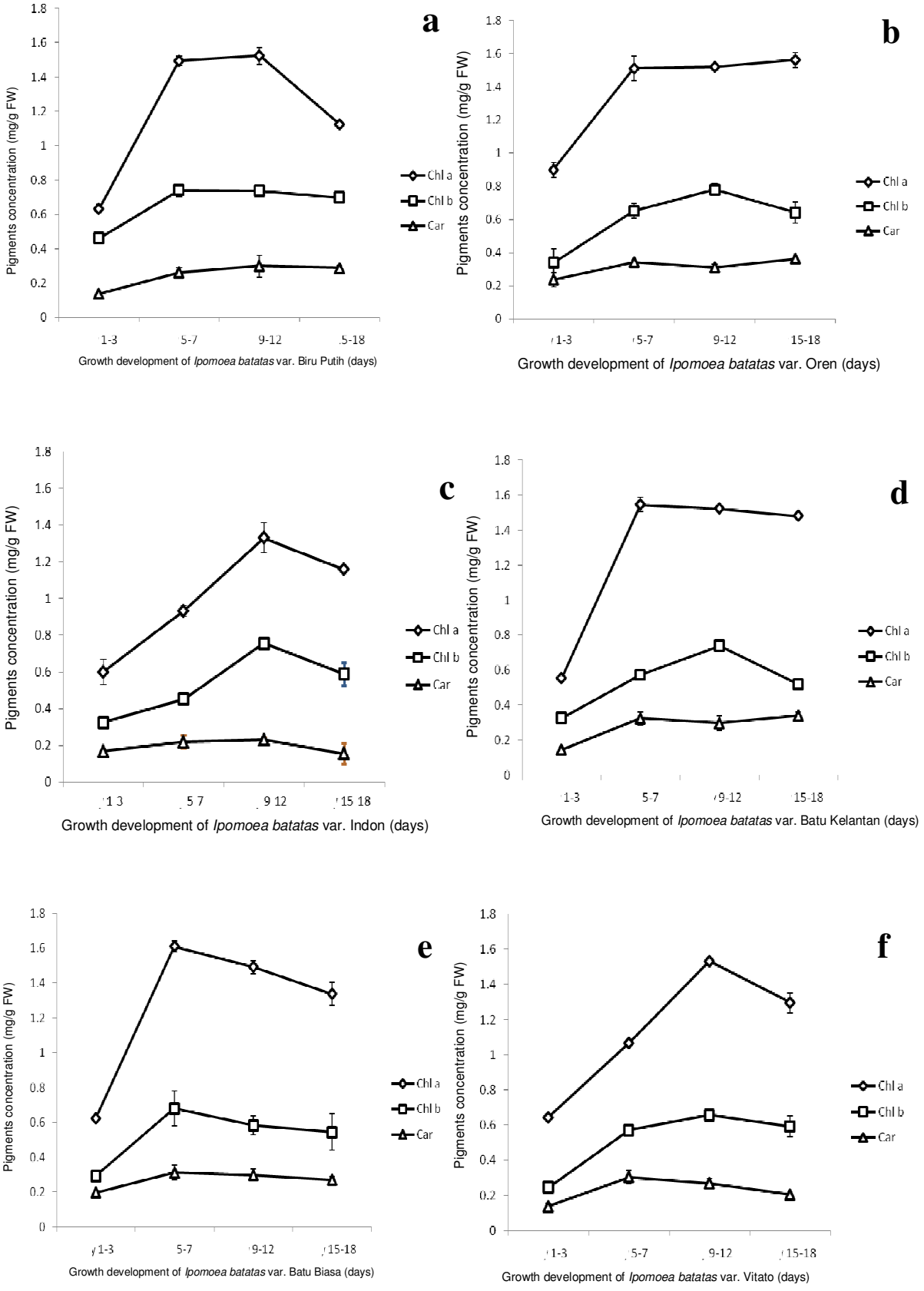

Figure 1. The Chlorophyll a (Chl a), Chlorophyll b (Chl b) and carotenoids (Car) levels in the different development stages of the different varieties of *Ipomoea batatas* L. leaves.

Table 1. Chlorophyll a concentration in the different varieties of *I. batatas* leaves at different stages of leaves development.

Stages of leaves development (days)	Chlorophyll a concentration (mg/g FW)					
	BP	Oren	Indon	BK	BB	Vit
1-3	0.632±0.003 ^{a}	0.900±0.046 ^{abcdef}	0.600±0.067 ^{c}	0.555±0.011 ^{d}	0.624±0.024 ^{e}	0.645±0.010 ^{f}
5-7	1.495±0.027 ^{adef}	1.511±0.074 ^{bef}	0.933±0.030 ^{ef}	1.546±0.041 ^{cef}	1.610±0.034 ^{def}	1.067±0.004 ^{f}
9-12	1.526±0.049 ^{ae}	1.522±0.013 ^{be}	1.330±0.083 ^{e}	1.523±0.005 ^{ce}	1.492±0.037 ^{de}	1.532±0.008 ^{fe}
15-18	1.125±0.007 ^{abcdf}	1.563±0.045 ^{bdef}	1.158±0.008 ^{cdef}	1.482±0.012 ^{de}	1.338±0.064 ^{e}	1.297±0.059 ^{f}

The different letters beside the values indicates the statistically significant differences among the different varieties at each stages of leaves development at the P<0.05 level in Tukey's HSD test. Values are means calculated in triplicates ± SD.

Table 2. Chlorophyll b concentration in the different varieties of *I. batatas* leaves at different stages of leaves development.

Stages of leaves development (days)	Chlorophyll b concentration (mg/g FW)					
	BP	Oren	Indon	BK	BB	Vit
1-3	0.461±0.008^{abcdef}	0.337±0.085^{bdef}	0.324±0.004^{e}	0.328±0.005^{cdf}	0.293±0.029^{d}	0.246±0.012^{f}
5-7	0.743±0.036^{abef}	0.652±0.045^{be}	0.452±0.006^{e}	0.573±0.006^{c}	0.681±0.101^{e}	0.571±0.003^{f}
9-12	0.738±0.004^{ad}	0.780±0.036^{bdf}	0.755±0.027^{ef}	0.741±0.005^{cd}	0.585±0.054^{de}	0.658±0.037^{f}
15-18	0.698±0.022^{ac}	0.642±0.065^{b}	0.589±0.063^{e}	0.519±0.019^{c}	0.545±0.104^{d}	0.593±0.060^{f}

The different letters beside the values indicates the statistically significant differences among the different varieties at each stages of leaves development at the P<0.05 level in Tukey's HSD test. Values are means calculated in triplicates ± SD.

Table 3. Carotenoids concentration in the different varieties of *I. batatas* leaves at different stages of leaves development.

Stages of leaves development (Days)	Carotenoids concentration (mg/g FW)					
	BP	Oren	Indon	BK	BB	Vit
1-3	0.139±0.003^{abd}	0.235±0.044^{bcef}	0.167±0.005^{e}	0.148±0.009^{c}	0.200±0.014^{df}	0.138±0.016^{f}
5-7	0.263±0.028^{a}	0.341±0.014^{be}	0.218±0.035^{e}	0.326±0.037^{ce}	0.313±0.042^{de}	0.305±0.036^{f}
9-12	0.300±0.064^{a}	0.310±0.020^{b}	0.231±0.007^{e}	0.298±0.041^{c}	0.299±0.034^{d}	0.269±0.028^{f}
15-18	0.287±0.015^{a}	0.362±0.107^{bef}	0.154±0.056^{e}	0.342±0.005^{ce}	0.272±0.019^{d}	0.204±0.009^{f}

The different letters beside the values indicates the statistically significant differences among the different varieties at each stages of leaves development at the P<0.05 level in Tukey's HSD test. Values are means calculated in triplicates ± SD.

be not significant between the varieties. The sudden spike up in the chlorophyll a level from day 1 to 7 leaves showed that adaptation took place in the plant to perform photosynthetic activity to produce sufficient energy to accommodate rapid plant growth. The chlorophyll a pigment is the major pigment in plants that absorb light of all wavelengths except green and therefore gives leaf its green colour. Other

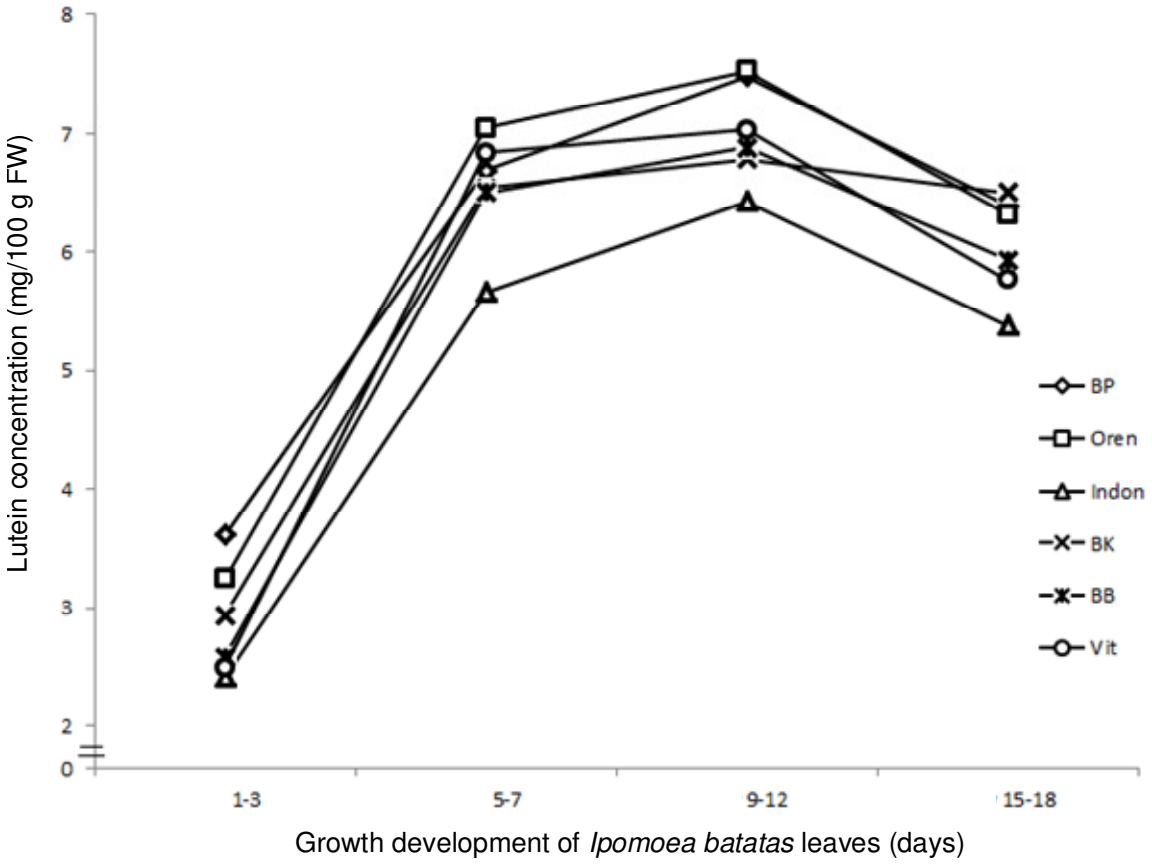

Figure 2. Lutein (mg/100 g FW) concentration in different Varieties of *Ipomoea batatas* (Sweet potato) leaves at different developmental stage.

accessory pigments in leaves such as chlorophyll b, carotenoids and xanthophylls (lutein), absorb energy at different wavelength which is not absorbed by chlorophyll a.

The chlorophyll b concentration in the leaf extracts ranked between the chlorophyll a and carotenoids pigments. The increase of the chlorophyll b level occurred most rapidly during the first week of the leaf development (Table 2). After the first week of leaves development, the chlorophyll b level in the Biru Putih (Figure 1a) and Batu Biasa (Figure 1e) varieties decreased whereas the level in Indon (Figure 1c) variety increased and peaked at day 9 to 12 before it dropped rapidly. All varieties had a maximum level of chlorophyll b from day 9 to day 12 except for the Batu Biasa variety which had the highest chlorophyll b level from day 5 to day 7 and decreased after it reaches maturity. The drop in the chlorophyll b level signifies the beginning of senescence in the *I. batatas* leaves. Senescence refers to the sequential degradation process and decline in the level of RUBISCO and chlrorophyll in leaves which resulted in the desiccation and abscission of the leaf (Barry et al., 1992).

Carotenoids function as photosynthetic accessory pigments and essential structural components of the photosynthetic antenna and reaction center complexes in leaves (Bartley and Scolnik, 1995). Carotenoids pigments are produced in higher plants to protect them from the damaging effects by their own endogenous photo-sensitizer, the chlorophyll (Tee, 1995). The changes in the carotenoids level observed are less drastic for all the varieties compared to chlorophyll a and b pigments. The increment of carotenoids level was observed from day 1 to 7 of the leaves development for all the leaves varieties.

The level of carotenoids in the Indon (Figure 1c), Vitato (Figure 1f) and Batu Biasa (Figure 1e) varieties declined as the leaves aged whereas in the Biru Putih variety, the carotenoids level remained stagnant from day 7 onwards. The Oren variety showed a high level of carotenoids although a slight decline was spotted from day 9 to day 12. However, the differences in the carotenoids level in mature leaves are not significant (Table 3). As observed in this study, the carotenoids contents were found to be highest in the *I. batatas* var. Oren (Figure1b) which coincidently has orange coloured storage roots compared to the low level of carotenoids in the Indon variety which has purple flesh. However, so far no study has been conducted to determine the relationship between the storage roots colour and the

Table 4. Lutein concentration in the different varieties of *I. batatas* leaves at different stages of leaves development.

Stages of leaves development (days)	Lutein concentration (mg/100 g FW)					
	BP	Oren	Indon	BK	BB	Vit
1-3	3.614±0.066^{acdef}	3.244±0.055^{bdef}	2.417±0.043^{e}	2.938±0.066^{ce}	2.586±0.287^{d}	2.493±0.327^{f}
5-7	6.689±0.241^{ae}	7.047±0.250^{be}	5.660±0.051^{e}	6.544±0.320^{ce}	6.507±0.197^{de}	6.836±0.131^{fe}
9-12	7.486±0.078^{acdef}	7.529±0.131^{bcdef}	6.431±0.250^{ef}	6.790±0.052^{c}	6.884±0.167^{de}	7.033±0.182^{f}
15-18	6.393±0.228^{adef}	6.319±0.075^{bef}	5.379±0.132^{e}	6.503±0.368^{cdef}	5.934±0.045^{de}	5.762±0.066^{f}

The different letters beside the value indicates the statistically significant differences among the different varieties at each stages of leaves development at the $P<0.05$ level in Tukey's HSD test. Values are means calculated in triplicates ± SD.

pigment levels in their leaves.

Figure 2 showed the lutein content in the different *I. batatas* leaf varieties. Lutein is an important nutritional source under the carotenoids group and is only available through dietary intake for human. Besides, lutein is known to contribute the highest portion of carotenoids in higher plants photosynthetic apparatus which accounts up to 40% of total carotenoids in leaves (Osto et al., 2006). For this reason, green leaves have the potential to be used as a suitable source of lutein. Lutein in leaf have gained recognition in improving eye sight as well possess health benefits. This was previously thought to be the function of beta carotene that was also found in green leafy vegetables (Landrum and Bone, 2001).

The lutein level in the leaves for all varieties increased drastically during the first week of development in which the leaves undergone rapid development and increment in size. However, this is followed by a significant drop after the 12th day of development which probably contributed by senescence. Lutein level was observed to be at the lowest during the first 3 days of the leaf development. At this stage, the young leaves might be provided with food generated from bigger leaves to complement the low number of pigments production. This is further confirmed by the lower chlorophyll level in young leaves when compared to the other developmental stages. However, as leaf expansion occurred, pigmentation in leaves also increased to boost photosynthesis which resulted in the increased chlorophyll content in leaves (Taiz and Zeiger, 1998). From day 9 to 18, the level of lutein decreases which implies the senescing process that is occurring in the leaves.

As shown in (Table 4), *I. batatas* var. Oren leaf shown the highest level of lutein which is approximately 7.529 ± 0.131/ 100 g FW and followed closely by the BP variety. However, the lutein levels between the two varieties do not differ significantly. The study on kale showed that the highest level of lutein was recorded at 15.1 mg/100 g in 1 to 2 weeks old of kale leaves (Lefsrud et al., 2007). Besides, previous research conducted by Ishiguro and Yoshimoto (2006), shown that the amount of lutein detected in spinach is between 4.4 to 15.9 mg/100 g FW. Both spinach and kale are currently ranked the highest spot for green leafy vegetables with the highest lutein content. From this study, we can conclude that the concentration of lutein in the *I. batatas* var. Oren and Biru Putih is comparable to that of spinach.

In conclusion, development stages and variety can influence the level of chlorophyll a, chlorophyll b, carotenoids and lutein content in the *I. batatas* leaves. Chlorophyll a and carotenoid levels were found to increase drastically during the first week of leaf development. Chlorophyll b on the other hand increased in amount up to the 12th day. Lutein level in the leaf increased during the first week and decreased after the leaves reaches maturity. From this study, we can conclude that harvesting the leaves of *I. batatas* var. Oren and BP from day 7 onwards will ensure maximum carotenoids and lutein contents thus can be used as suitable starting materials for the natural yellow dye extraction.

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