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Influence of Developmental Stage on Activities of Polyphenol Oxidase, Internal Characteristics and Colour of Lettuce cv. Grand Rapids

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ABSTRACT

An analyzing study to evaluate the relationship between Polyphenol Oxidase (PPO) activity, internal qualities and degree of browning was conducted on lettuce var. Grand Rapids in order to understand the basis changes relating to the appearance of leaf browning and some quality characteristics. For PPO activity, Factorial experiment in completely randomized design was arranged with two factors: developmental stage (Factor A) at four levels (28, 42, 59 and 73 days after planting, DAP) with three different parts of plant (Factor B) (stem, leaf and root), while Completely Randomized Design (CRD) was applied for studying the internal qualities and browning appearance in leaves at 28, 42, 59 and 73 DAP. The experiment was carried out during May-July, 2009, with four replications, ten plants per replication. The results showed that each developmental stage and the various sections of the plant affected PPO activity. The leaf section of the plant particularly affected PPO, especially at 73 DAP. In addition, at the more mature stages of the leaf, more phenolic substance, ascorbic acid content and pH value were apparent, while quinone content decreased at advancing leaf age. In addition, as the plants reached an older stage, the leaf colour in terms of L* and b* decreased. Thus, the appropriate harvest for lettuce var. Grand Rapids should be at 59 DAP in order to face fewer incidence of browning and maintain good quality.

Key words: PPO activity, lettuce, browning appearance, phenolic, leaf colour

INTRODUCTION

Lettuce (*Lactuca sataiva*), an important economic vegetable crop belonging to Compositae family (Asteraceae), is a popular vegetable and considered one of the most important all year round crops in Thailand. The lettuce planting area in all regions of the country is around 2, 119.2 ha with an estimated production of 15, 499.87 ton/year. Most of the lettuce is used for fresh consumption in fast foods and vegetable salads. Fresh lettuce is a significant source of dietary antioxidants. These include phenolics, ascorbic acid, carotenoids, tocopherols and glucosinolates, all of which have protective effects against various forms of cancer and cardiovascular and cerebrovascular diseases (Lister, 2003; Nicolle *et al*., 2004; Liorach *et al*., 2008). Moreover, lettuce is considered a low-acid food or health food (Dupont *et al*., 2000). Altunkaya and Gökmen (2008) reported that lettuce is highly susceptible to enzymatic browning. As such, leaf browning is a major problem that affects

product losses resulting from rejection by consumers, shortened storage life and economic loss (Zhang *et al*., 2001). The appearance of this physiological disorders can be observed visually on leaf surfaces (Franck *et al*., 2007) during the preharvest period (Kays, 1999). Generally, browning in plants, was induced by several factors attributing to the activity of the PPO enzyme(Mayer, 1987). A loss of leaf colour is caused by enzymatic browning which is associated with the developmental stages of the plant. In plants, PPOs are localised in plastids, while plant phenolic substrates are mainly located in the vacuole. Therefore, the majority of enzymatic browning occurs when this sub-cellular compartmentation is lost (Rigal *et al*., 2000). Degl'Innocenti *et al*. (2007) also cited that the patterns of PPO activity could be related to their sensitive to browning incidence. However, there are few studies on the comparative browning level of lettuce during these growth stages. A further understanding of the browning appearance during the development stage is needed. The purposes of this study were threefold: quantify the distribution of PPO activity in the various organs of the lettuce plant, such as the stem, leaf or root; clarify internal substances related to enzyme-catalysed browning of fresh lettuce; compare the differences in leaf colour of Grand Rapids lettuces during its different maturity stages in order to determine the best quality. In addition, the quantification of the level of PPO activity and an understanding of the changes of other internal qualities at certain stages of development would be helpful for relating the occurrence of leaf browning to certain events and ultimately could provide a basis for predicting the optimal harvesting time in order to reduce quality loss.

MATERIALS AND METHODS

The research was carried out at the experimental field, Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the Northeast of Thailand in the period between May to July, 2009. Lettuce cv. Grand Rapids was planted from seed. The seedlings were transplanted 25 DAP and grown singly in 2 L pot filled with a sandy loam soil : rice husk : manure ratio 1: 1 : 1 and placed under field conditions. A Factorial in Completely Randomized Design (CRD) was arranged and composed of two factors: developmental stage (Factor A) at four levels (28, 42, 59 and 73 DAP) with different parts of the plant (Factor B) at three levels (stem, leaf and root) for evaluation of PPO activities. Measurements for internal qualities and colour were conducted specifically on leaves at the same before mentioned developmental stages by using a CRD. Each treatment was carried out in four replicates, ten plants per replication. Plants were cut at each developmental stage and taken to the laboratory within a few minutes of cutting. The following were evaluated: (1) PPO activity was carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per 60 sec. (2) Phenolic content was performed as described by Ribeiro *et al*. (2008). The content of phenolic was expressed as g/100 g fresh weight (FW) of leaf. (3) Quinone content was extracted as described by Pirie and Mullins (1976). Quinone content was expressed as absorbance at 437 nm per g fresh weight. (4) Ascorbic acid content was measured using juices squeezed from the flesh of the leaf with the use of distilled water at a ratio of 1:3. The measurements followed the method of AOAC (1990) and were expressed as mg ascorbic acid /100 mL juice (5) The measurement of pH values was carried out with the use of juices described earlier in number 4 and a pH meter ID 100D, from Singapore was used. (6) Leaf colour was

measured on the leaf surface with a Hunter Lab Model No. 45/0-L, Serial No. 7092, USA. The CIE colour values L^* (black = -100 and white = +100), a^* (red) (- = green and + = yellow) and b^* (yellowness) (- = blue and + = yellow) were measured to describe the colour of lettuce's leaf. The collected data were statistically analyzed using the SPSS Version 6 (SPSS, 1999).

RESULTS

The recorded data received from measuring PPO activity, internal qualities and colour of 'Grand Rapids' lettuce at different ages (28, 42, 59 and 73 DAP) demonstrated the following results:

PPO activity: The results showed that PPO activity could be detected in various sections of the plant during the developmental stages. PPO activity increased sequentially when the plants entered their older stage. PPO activity increased quickly during plant growth and reached the maximal content at late harvesting of 73 DAP. In addition, changes in PPO activity were also influenced by different tissues. The results from Table 1 showed that PPO extracted from leaves had the highest activity, followed by the root and then the stem. For interaction of the developmental stage and various analyzed tissues, the results revealed that the highest PPO activity was observed in the leaf cut at late harvesting of 73 DAP (Table 1).

Phenolic: The phenolic content showed a surge of content during plant growth, suggesting the browning appearances of lettuce was affected by plant age. Significant changes in total phenolic content were found in lettuce leaves at different plant ages, although phenolic content in lettuce's leaves featured the maximal content (2439.72 g/100 g FW) at the early developmental time (28 DAP). Afterwards, the levels of phenolics decreased dramatically to a value of 964.49 and 287.09 g /100 g FW at the ages of 42 and 59 DAP, respectively, then increasing markedly to a high level of 2425.54 g/100 g FW at the late-harvesting of 73 DAP (Table 2).

Quinone: With respect to quinone content, the results revealed a leaf from the early stage of 28 DAP showed the maximal quinone content of 0.1299 per g FW. Afterwards, quinone content from leaves of 42, 59 and 73 DAP showed insignificant quinone content of 0.0852, 0.0866 and 0.0935/g FW, respectively (Table 2).

Ascorbic acid content: Content of ascorbic acid showed an increasing trend with plant age. At 28 DAP, the lettuce leaf showed a significantly lower ascorbic acid level of 12.72 mg ascorbic acid/100 mL juice. Afterwards, there was a similarly high amount of ascorbic acid of 14.00-14.05 mg ascorbic acid/100 mL juice as growth progressed from day 42 to 73 DAP (Table 3).

pH: In a comparison of the pH value of lettuce's leaves during plant growth, the data indicated that plant age had a highly significant effect on the pH value of leaves. The mean values of pH of leaf age at the early stage of 28 DAP showed the highest pH value of 7.20. Afterwards, as plant age reached 42 DAP and 59 DAP, the samples decreased the pH value remarkably to 5.52 and 5.98, respectively, then increased again to 6.81 as growth progressed to the late-harvest time at 73 DAP (Table 3).

Leaf colour: The results showed that leaf colour, measured by monitoring in terms of L*, a* and b* values, changed significantly during the different plant developmental stages. From Table 4,

Table 1: Activities of PPO in different parts of lettuce during developing stage

	PPO activities at different times (sec)					
Factors	0	60	120	180	240	300
Factor A: Developmental stage (DAP)						
28	0.1496b	0.1916b	0.2276b	0.2589b	0.2868b	0.3111b
42	0.1734b	0.2293b	0.2746b	0.3148b	0.3487b	0.3791b
59	0.1357b	0.1730b	0.2080b	0.2413b	0.2702b	0.2974b
73	0.2741a	0.3681a	0.4323a	0.4823a	0.5200a	0.5508a
F-test	**	**	**	**	**	**
CV (%)	5.13	7.77	7.43	5.10	5.48	5.29
LSD	0.0184	0.0254	0.0299	0.0332	0.0355	0.0374
Factor B: Different parts of plant						
Stem	0.1060c	0.1352c	0.1585c	0.1798c	0.1987c	0.2157c
Leaf	0.2462a	0.3369a	0.4067a	0.4625a	0.5055a	0.5418a
Root	0.1973b	0.2495b	0.2917b	0.3307b	0.3652b	0.3964b
F-test	**	**	**	**	**	**
CV (%)	3.48	5.94	4.36	5.97	4.64	4.80
LSD	0.0155	0.0213	0.0245	0.0266	0.0280	0.0291
Interactions (AxB)						
Stem28DAP	0.0923ef	0.1210ef	0.1453fg	0.1685fg	0.1904ef	0.2102efg
Leaf28DAP	0.2154bc	0.2692bcd	0.3140bcd	0.3483bcde	0.3762bc	0.3978cd
Root28DAP	0.1411de	0.1847de	0.2236def	0.2599df	0.2938cde	0.3254cde
Stem42DAP	0.0583f	0.0765f	0.0921g	0.1069g	0.1217f	0.1344g
Leaf42DAP	0.2242bc	0.3150bc	0.3922b	0.4552b	0.5035b	0.5482b
Root42DAP	0.2376bc	0.2964bc	0.3394bc	0.3823bc	0.4208bc	0.4546bc
Stem59DAP	0.0840ef	0.1038ef	0.1210fg	0.1374g	0.1523f	0.1676fg
Leaf59DAP	0.1828cd	0.2415cd	0.3016bcde	0.3603bcd	0.4109bc	0.4559bc
Root59DAP	0.1404de	0.1738de	0.2013efg	0.2261efg	0.2473def	0.2687def
Stem73DAP	0.1895cd	0.2395cd	0.2756cde	0.3063cde	0.3302cd	0.3505cd
Leaf73DAP	0.3625a	0.5218a	0.6191a	0.6863a	0.7312a	0.7651a
Root73DAP	0.2702b	0.3430b	0.4023b	0.4543b	0.4987b	0.5367b
F-test	**	**	**	**	**	**
CV (%)	4.01	5.17	3.87	4.17	4.47	3.18
LSD	0.0255	0.0343	0.0396	0.0432	0.0456	0.0477

Letters within columns indicate Least Significant Differences (LSD) at **p = 0.01

Table 2: Content of phenolic and quinone in leaf of lettuce during developing stage

Age (DAP)	Phenolic (g/100 g FW)	Quinone (per g FW)
28	2439.72a	0.1299a
42	964.49b	0.0852b
59	287.09c	0.0866b
73	2425.54a	0.0935b
F-test	**	**
CV (%)	12.36	11.99
LSD	130.5929	0.0052

Letters within columns indicate Least Significant Differences (LSD) at **p = 0.01

Table 3: Ascorbic acid content and pH value of lettuce's leaf during developing stage

Age (DAP)	Ascorbic acid (mg ascorbic acid/100 mL juice)	pH
28	12.72b	7.20a
42	14.00a	5.52d
59	14.05a	5.98c
73	14.03a	6.81b
F-test	**	**
CV (%)	1.16	1.94
LSD	0.0669	0.0480

Letters within columns indicate least significant differences (LSD) at **p = 0.01

Table 4: Colour of lettuce during developing stage

Age (DAP)	L*	a*	b*
28	54.09a	-9.11	31.64a
42	50.62b	-9.01	29.34b
59	50.28b	-9.46	30.78ab
73	51.81ab	-9.43	31.17ab
F-test	**	ns	**
C.V. (%)	7.51	11.86	8.17
LSD	1.0192	0.2839	0.6523

Letters within columns indicate Least Significant Differences (LSD) at **p = 0.01, NS: Non significant

there were highly significant differences in the leaf colour in parameters of L* and b* between the four plant ages. The results showed that at the early stage of 28 DAP, lettuce leaves showed the significantly highest L* and b* of 54.09 and 31.64, respectively. As the plant advanced through its developing stages, less colouring of the leaf in terms of L* and b* became apparent, while a* value among treatments showed no significant difference.

DISCUSSION

This study can provide an important basis for understanding leaf browning in lettuce. In this research, fresh 'Grand Rapids' lettuce, harvested at the different growth stages, was compared and analyzed for PPO activity, chemical characteristics and leaf color.

The PPO extracted from the lettuce plant at different stages of growth exhibited different levels of activity as shown in Table 1. It was obvious that PPO activity was present throughout the developmental stages. After 28 to 59 DAP, PPO enzymes maintained low activity. Activity then increased and reached maximum as the plants entered their advancing maturity stage, especially the later maturity stages. These results imply that lettuce has a potential for browning during the preharvest stage and especially at late-harvesting of 73 DAP. Generally, enzymatic browning of plant product was characterized by PPO activities (Peiser *et al*., 1998). Therefore, browning incidence in lettuce may be a development-associated activity of PPO based on the sensitivity of enzyme activity. This is in agreement with the results of Thipyapong *et al*. (1997) and Núñez-Delicado *et al*. (2007) who reported that PPO activity was considerably influenced by stages of development, such as tomato leaves and grapes. While Jiang *et al*. (2004) indicated that young tissues had significantly lower PPO activity than older tissues, this may be due to membrane integrity being maintained at the young stage. In addition, Lichanporn *et al*. (2009) cited that as the plant reached senescence and it led to a breakdown of the compartmentalization of enzymes, finally causing a rigorous symptom of enzymatic browning, especially harvest at a delayed maturity

stage (Benjawan *et al.*, 2008). Furthermore, these results were in agreement with Segovia-Bravo *et al.* (2009) who reported that in older plants the sub-cellular compartmentation was lost leading to intense leakage of PPO from the cells which in turn caused the plant to become sensitive to the browning appearance.

For the activity of the PPO analyzed from various tissues of lettuce, the results showed that the extent of PPO activity in 'Grand Rapids' lettuce was ubiquitous in various tissues. The total PPO activity of the leaf extracts was superior to that of the root and stem extracts. These results imply that the lettuce leaves were the most susceptible to browning compared with the root and stem, respectively. Yingsanga *et al.* (2008) cited that the higher activities of PPO in the leaf could be explained by higher rates of oxygen transmission into tissues due to their higher surface area and stomata density compared to the root and stem (Yingsanga *et al.*, 2006). While, Collier and Wurr (1981); Collier and Huntington (1983) indicated the reason leaves of lettuce plants were the most rigorous in developing browning incidence because the leaves are often larger and more succulent due to their rapid expansion and higher water content than the other sections.

A compared interaction of different plant ages and various plant organs also affected different enzymatic activities of PPO in Grand Rapids lettuce. The comparison revealed that leaves harvested from a plant aged 73 DAP had the maximum PPO activity. The delayed harvested lettuce tended to exhibit a progressive increase in the susceptibility of enzymatic browning due to a loss of cellular compartmentation and membrane disintegration (Franck *et al.*, 2007). Thus, the appropriate harvesting stage of lettuce should be done at 59 DAP in order to decrease the problem of leaf browning occurrence. Unfortunately, the identity of the different locations of PPO activity causing browning incidence in Grand Rapids lettuce is still unclear.

For the results on phenolic content, the results obtained from this study indicate that significant levels of phenolic components have been detected in lettuce and it was fluctuated according to plant age. Lettuce leaves at the early age of 28 DAP and the late stage of 73 DAP showed the highest phenolic content, while leaves from plant age of 59 DAP composed of only traces of phenolic substance. Thus, the concentrations of phenolic acids in lettuce were sensitive to the maturity stage. These results corresponded with King and Young (1999), who cited that the maturity stage was an important factor affecting the content of phenolic compounds. These results are also supported by the findings of Spanos and Wrolstad (1990), who claim that the phenolic content of pear depends primarily on level of maturity. Nevertheless, in lettuce leaves, as development progressed, the increase in PPO activity paralleled the increase in total phenol content. Thus, lettuces were susceptible to browning incidence during late-harvest due to an increase of both PPO activity and phenolic content and these facilitated the appearance of the browning disorder. This may be due to PPO, normally bound to membranes or walls, becoming active when released under altering plant metabolism and increasing phenolic synthesis when the plants reaches the advancing mature stage (Ruiz *et al.*, 1999). This is consistent with the observation of Muñoz-Muñoz *et al.* (2009), who found that some phenolic compounds have been shown to be good substrates involved in the oxidation of polyphenols by PPO enzymes (Ke and Saltveit, 1989; Nicoli *et al.*, 1991). These results were in agreement with the previous findings of Leoni *et al.* (1990) who reported that the phenolic compounds were the best substrate for artichoke PPO, while Vela *et al.* (2002) found the highest levels of PPO and phenolics occurred together in Algerie loquat at harvest. This coincidence of high PPO and phenolics enabled the loquat fruits to become more susceptible to enzymatic browning. Thus, both phenolic contents and PPO activity were found to be closely correlated to the degree of browning (Lee *et al.*, 1990) because phenolic compounds were the major browning substrates

present in plant tissues during late-harvesting (Sun *et al*., 2006). Furthermore, it may be possible that phenolic content might be largely regulated by PPO activity (Sun *et al*., 2009). While the opposite results were reported by Caldwell (2003) and Liorach *et al*. (2004) who cited that phenolic compounds acted as the main antioxidants in leafy vegetables. A general decrease of phenolic content was noticed in correspondence of maximum values of PPO activity (Chisari *et al*., 2010). Nevertheless, the major role of PPO in catalyzing the oxidation of phenolic substances and its involvement in the browning of lettuce leaves, was not fully understood and requires further investigation.

For quinone content, the results showed that the peak level for quinone content of lettuce leaf was observed only at early plant growth (28 DAP). Afterwards, quinone content decreased significantly and remained at stable levels as growth progressed during 42 to 73 DAP. Generally, the oxidation of phenolic compounds by the enzyme PPO produces *o*-diphenols. Then the o-diphenols mix together to produce o-quinones. These o-quinones were quite unstable and spontaneous polymerized to form brown, red or black pigments (Gawlik-Dziki *et al*., 2007; Ayaz *et al*., 2008). A gradual decrease in quinone content was responsible for browning in lettuce (Ke and Saltveit, 1986, 1989). Nevertheless, it is not clear whether the changed level of quinone was beneficial or detrimental to the browning incidence.

With ascorbic acid, the results showed that at the early stages of growth (28 DAP), the ascorbic acid content of lettuce's leaf appeared to be at the lowest level of 12.72 mg ascorbic acid /100 mL juice. The level then increased at 42 DAP and maintained a similar level throughout the late growth period (73 DAP). Jeffery *et al*. (2003) found that a variation in the ascorbic acid contents of Brassica vegetables was caused by many factors including maturity at harvest. These results corresponded to Deepa *et al*. (2007) who found that ascorbic acid in fresh sweet pepper showed an increasing trend with advancing maturity. In addition, the increase of ascorbic acid in lettuce could have been attributed to synergism of this compound with a phenolic substance (Altunkaya and Gökmen, 2008). Núñez-Delicado *et al*. (2007) reported that ascorbic acid had a great capability for the prevention of the degradation of phenolic compounds in fresh-cut lettuce (Altunkaya and Gökmen, 2009). Therefore, the results from this study showed that PPO activity in lettuce has been shown to be positively associated with phenolic content and ascorbic acid content. In contrast, Sapers (1993) cited that ascorbic acid may act as an antioxidant agent to inhibit the catalytic action of PPO. However, the exact mechanism of ascorbic acid on the mechanism of browning occurrence in lettuce is not yet clear. Further work is required to better understand the biochemical mechanism of ascorbic acid related to the browning appearance in Grand Rapids lettuces.

For pH value, the results showed that different developmental stages exhibited different pH values. The pH value of lettuce's leaves had the highest value of 7.20 at an early developmental stage (28 DAP). Afterwards, the pH levels were markedly reduced at 42 and 59 DAP and then they significantly increased again to the neutral level of 6.81 at late harvesting (73 DAP). Thus, the developmental stages of the plant affected pH alteration in the lettuce leaves. These results were in agreement with previous findings (Fang *et al*., 2007). In addition, Sun *et al*. (2009) found that the severity of PPO activity was pH-dependent. Generally the maximum PPO activity in most plants is at or near neutral pH values of 7.0 (Gawlik-Dziki *et al*., 2008). Thus, the activity of PPO was found to promote rapidly under the condition of higher pH of 6.81 at 73 DAP, while a lower pH at 42 and 59 DAP effected a decrease of PPO activity as a result of inappropriate pH (Altunkaya and Gökmen, 2008).

For the results on leaf colour, it was found that leaf colour in terms of L* (lightness) and b* (blue-yellow) tended to decrease their values with advancing developmental time, while the a* value (green-red) showed no significant difference irrespective o f plant ages. At early plant growth of 28 DAP, both L* and b* exhibited their highest level, then these values tended to decrease at 42 DAP through the 73 DAP. These results implied that as plant age advances, leaf colour changed toward a less bright green colour. This could be explained by, with the extension of development, increased PPO activity was found to be correlated with the susceptibility to browning in fresh lettuce measured by leaf colour (Zhang *et al*., 2000; De Castro *et al*., 2008).

In conclusion, PPO were found in various tissues of lettuce plants, especially in the leaf of plants aged at 73 DAP when the highest PPO activity was measured. As the plants reached an older stage, the contents of total phenol, ascorbic acid and pH increased, while quinone content and leaf colour in terms of L* and b* decreased. These findings show that as the lettuce plant reached an older stage, the plants were more sensitive to browning appearance and lower quality.

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