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INTERACTION BETWEEN CROPPING SYSTEMS AND STORAGE DURATION ON NITRATE, NITRITE, ANTIOXIDANT AND POSTHARVEST QUALITY OF *Lactuca sativa*

[1]Azimah, H; [1*]Ahmad, S. H.; [2]Rosenani, A. B.

[1]Department of Crop Science,

[2] Department of Land Management, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

*Corresponding Author

ABSTRACT

Leafy green vegetables are highly demanded by Malaysian consumers due to their rich source of essential nutrients needed for good health. The increase usage of nitrogen fertilizers and livestock manure by growers to produce dark green leafy vegetables could lead to abundant chlorophyll pigment and a higher content of nitrate. The conversion of nitrate to nitrite and eventually to carcinogenic nitrosamines during storage could affect human health. Thus, a study was conducted to determine the effects of organic and conventional cropping systems and storage duration on the quality of leafy vegetables stored under refrigerated temperatures. *Lactuca sativa* were obtained from organic and conventional farms. Damaged-free vegetables were selected, cleaned, packed in perforated polyethylene bags and stored in a refrigerator at 6±1 °C. Nitrate and nitrite contents, nitrate reductase (NR) activity, water loss, chlorophyll content, total phenolic content (TPC), total flavonoid content (TFC), 2, 2-diphenyl-2-picrylhydrazyl (DPPH), colour hue (h°), chromaticity (C*) and lightness (L*) were measured at 0, 3, 6 and nine days of storage. The experiment was conducted using a randomized complete block design, arranged in a factorial experiment, with five replications. The *L. Sativa* from the two cropping systems showed 4.5 to 8% weight loss and decreased firmness by 33 to 46% during storage. Both organic and conventional *L. sativa* showed a shift in colour from pale green to yellowish dull green due to the degradation of chlorophyll content. The organic and conventional *L. sativa* were 78% reduced in nitrate contents and 99% increase in nitrite content as storage days increased. The TPC and TFC both showed positive correlations with nitrate content while DPPH had the

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opposite. It can be suggested that *L. sativa,* should not be kept under refrigerated (6±1 °C) storage for more than 3 days.

Keywords: organic, conventional, nitrate reductase activity, chlorophyll content, weight loss

INTRODUCTION

The current demand for vegetables by consumers is high and consumption of green leafy vegetables is expected to be an upward trend due to their contents of beneficial compounds, including phytochemicals, minerals, and vitamins (Connor et al., 2005; Li and Kubota, 2009). There is also a preference by consumers for dark green leaves as they are considered fresh and healthier for consumption. In order to meet consumers demand, most growers tend to use high amounts of nitrogen fertilizer to produce high biomass yield and dark green leafy vegetables. The high nitrogen fertilizer rates result in higher nitrate uptake, and contribute to higher biomass yield, abundant pigment-protein complexes in the thylakoid of the chloroplast and also affect nitrate reductase activity in the cytosol. Thus, dark green leafy vegetables are indicative of excessive use of nitrogen fertilizers. Since nitrogen is a primary component of chlorophyll and other organic compounds (Chapman and Barreto, 1997), there is a positive correlation between nitrogen and chlorophyll (Tuncay, 2011).

Plants take up nitrogen in the form of nitrate ions and stored nitrates in vacuoles during planting before undergoing nitrate reduction in cytosol by nitrate reductase (NR) activity. Nitrate and NR exist in the cytosol, therefore, the amount of nitrite formed in cytosol is also dependent on the NR activity, the microbial population and availability of nitrate. There is a close relationship between NR activity and nitrate concentration in plants (Caba et al., 1995) as nitrate induces the expression of both the uptake and reduction systems (Bussi et al., 1997) in the photosynthetic process. Therefore, NR is assumed to be the rate-limiting step in nitrate assimilation and an inducible enzyme (Skrdleta et al., 1979). After harvest, the nitrate in vegetables is converted to nitrite by nitrate reductase (NR) activity by reaction with amines, amides and enzymes in the chloroplasts (Kross et al., 1992) during storage (Du et al., 2007; Bartsch et al., 1988) and eventually to carcinogenic nitrosamines that could cause cancer.

About 80-90% of the nitrogen from fertilizers or organic materials, absorbed in the form of nitrates by plants, are essential for protein synthesis (Chung et al., 2003; Santamaria et al., 1999). In Malaysia, leafy vegetable are under organic and conventional systems. Nitrogen sources of both cropping systems are either synthetically or organically produced. Synthetic nitrogenous fertilizers, such as urea, sodium nitrate, ammonium chloride and ammonium nitrate, are commonly used on conventional farms to increase yields of leafy vegetables (Wang and Li, 2004). In organic vegetable farms, sources of nitrogen are from chicken manure, compost, and

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animal or plant based commercial organic fertilizers. However, the quality of most organic fertilizers is not guaranteed in terms of C/N ratio and characterization of composted materials (Kala et al., 2011). Unregulated application of nitrogen fertilizers to produce green leafy vegetables result in luxury uptake of nitrate leading to excessive accumulation in vegetables regardless of the cropping systems. Nevertheless, the studies have shown that organic vegetables have a tendency to have lower nitrate content than conventional vegetables (Worthington, 2001).

The nitrate and nitrite levels of vegetables after harvest can be affected by the storage and processing methods (WHO, 2003; EFSA, 2008). In addition, prolong or adverse storage condition of vegetables, particularly, nitrate rich vegetables, might lead to a reduction of nitrate to nitrite (Philips, 1968). The nitrite concentrations in fresh, undamaged vegetables are usually very low, but under improper postharvest storage conditions and poor sanitation (Chung et al., 2003), nitrite concentration could increase as a result of bacterial contamination or endogenous NR activity, reducing nitrate to nitrite (ESFA, 2008; WHO, 2012). Under proper refrigerated storage, reduction of nitrate to nitrite tends to be delayed, however, the reduction varied between samples. Even though the toxicity level of nitrites is small, but the accumulated of nitrosamine compounds in the organisms threaten human health. This occurrence has awakened public awareness about the nitrate and nitrite levels in local vegetables in Hong Kong (Anon, 2010). Postharvest storage duration is very crucial to ensure consumers are getting the safety and best benefits of leafy vegetables. Thus, the objective of this experiment was to determine the effects of organic and conventional cropping systems and storage duration (0, 3, 6 and 9 days) on nitrate and nitrite contents, nitrate reductase activity, weight loss, chlorophyll content, total phenolic content (TPC), total flavonoid content (TFC), 2, 2-diphenyl-2-picrylhydrazyl (DPPH) activity and leaf colour of the *Lactuca sativa*. *Lactuca sativa* was chosen in this study based on its popularity of consumption and as a model crop for the nitrate quality response (Cometti et al., 2011).

MATERIALS AND METHODS

Leaf lettuce were obtained from both organic and conventional farms in Selangor, Malaysia. The lettuce were transported in polyethylene-lined polystyrene iced-chest to the Postharvest Laboratory, Faculty of Agriculture, Universiti Putra Malaysia. Uniform-sized and damaged-free lettuce were selected, cleaned with water and air dried. About 200 g sample (equal to about 5-7 plants) were placed into a polyethylene (PE) bag (51 cm wide x 76 cm long x 0.045 mm thick). One bag represented one replication. The PE bags were labeled, packed in a plastic crates and stored in a refrigerator (NR-B53FE, National, Malaysia) for 9 days at 6 ± 1 °C with 60% relative humidity without light. Weight loss, Nitrate and nitrite contents, NR activity, TPC, TFC, DPPH

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radical-scavenging activity, weight loss, leaf colour and chlorophyll content were measured at 0, 3, 6 and 9 days of storage.

1. Nitrate and Nitrite Contents Determination

The extraction for nitrate and nitrite analysis was according to Muramoto (1999) with some modification, while determination of nitrate and nitrite content was by using a flow injection analysis system (Lachat Instruments, 1992). One gram sample of *L. sativa*, containing 0.5 g leaf petiole and 0.5 g leaf blade was chopped into fine pieces, and 200 ml deionized water was added. The sample was extracted by using Muramoto (1999) method with slight modification. The sample was homogenized with a blender (MX-799S, Panasonic, Malaysia) for 1 min. A 30 mL sample of the homogenate was placed in a centrifuge tube, and 0.5 mL of the discolouring agent was added successively. The tube was capped and shaken well by hand after each addition of the decolouring agent. The discolouring agent contained magnesium carbonate ($MgCO_3$) and calcium hydroxide ($Ca(OH)_2$) in a ratio of 1:2 (v/v) in order to remove the green chlorophyll colour and obtain a transparent solution. The sample was centrifuged (Hettich Zentrifugen EBA 270, United Sates) at 3,500 RPM for 10 min. The supernatant was then filtered through a filter paper (Whatman no. 1) to eliminate the turbidity and get a clear solution. 20 ml filtrate of each sample were put into test tube and samples were arranged in test tube rack, and put on the autosampler of autoanalyzer. Nitrate content was determined according to the modified method of Sechtig (2003) using the flow injection analyzer (Lachat QuikChem 8000). Nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column (Lachat Part No. 50327) attached to the analyzer. The nitrite (reduced nitrate plus original nitrite) in the sample was determined by diazotizing with sulfanilamide followed by coupling with N-1-naphthyl-ethylenediamine dihydrochloride. The resulting water soluble dye had a magenta colour and was read at 520 nm (interference filter). Nitrite was determined by removing the copperized cadmium column. Nitrate and nitrite contents were expressed as milligram nitrate per kilogram on a fresh weight basis (mg NO_3 /kg FW) unless otherwise stated.

2. Nitrate Reductase activity

For NR activity, each 0.3 g vegetable sample was chopped and placed in a test tube (16 mm x 150 mm) and added with 10 ml incubation medium containing 0.1 M potassium phosphate buffer (pH 7.5) of 0.1 M KNO_3 and 5% (v/v) isopropanol. The test tube was sealed with a rubber stopper and incubated in a water bath for 1 hour in the dark room at 30 °C. After incubation, the samples were placed in a boiling water bath to stop the NR activity. The samples were cooled to room temperature. Five millilitres of sulphanilamide and 5 ml of N-1-naphthyl-ethylenediamine dihydrochloride were added and mixed by a vortex mixer (Model SA7, Stuart, United Kingdom). After 10 min, the samples were measured by using a spectrophotometer (Model S1200,

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Spectrawave, England) with absorbance reading at 540 nm. The nitrite released to the medium was expressed as µmol NO_2^-.$h^{-1}g^{-1}$ FW (Snell and Snell, 1955).

3. Phytochemicals and antioxidant activity

The leaf samples were chopped into small pieces, and ground using liquid nitrogen in order to grind leaf samples with a mortar and pestle. The extraction and quantification of TPC (expressed as gallic acid equivalents (GAE) in mg per 100 g fresh weight) and TFC (expressed as mg g-1rutin fresh weight) were according to the methods of Jaafar et al., (2010). The DPPH free radical-scavenging activity (expressed as ascorbic acid equivalent of antioxidant capacity (AEAC) in mg AA/100 g fresh weight) used by Miliauskas, et al. (2004) was adopted with modifications. In a vial, 1 g of powdered vegetable sample was added to 50 mL methanol with continuous swirling for 1 hour at room temperature. After that, the mixture was filtered through filter paper (Whatman ™ No.1) and the methanol extract was ready for the analysis of TPC, TFC and DPPH.

For TFC, 1 mL of ethanol extract was mixed with NaNO3 (0.3 mL) in a test tube covered with an aluminium foil and allowed to stand for 5 minutes. Secondly, an amount of 10% AlCl3 (0.3 mL) was added followed by addition of 1 M NaOH (2 mL). Lastly, the results were measured at 510 nm using a spectrophotometer (UV-3101P, Labomed Inc, USA) with rutin as a standard (results expressed as mg g-1rutin fresh sample). The mean value of five representative plants was used to represent each experimental unit.

TPC of the vegetable extracts was determined using the Folin–Ciocalteu assay reported by Kähkönen et al. (1999). Folin–Ciocalteu reagent (1.5 ml; diluted 10 times) and sodium carbonate (1.2 ml; 7.5% w/v) were added to the extracts (300 µl; triplicate). After 30 min, absorbance was measured at 765 nm using a spectrophotometer (Model S1200, Spectrawave, England). Total phenolic content was expressed as gallic acid equivalents (GAE) in mg per 100g.

The DPPH free radical-scavenging activity used by Miliauskas, et al. (2004) was adopted with modifications. Different dilutions of the extract (1 ml; triplicate) were added to 2 ml of DPPH (5.9 mg/100 ml methanol). Absorbance was measured at 517 nm after 30 min by using a spectrophotometer (Model S1200, Spectrawave, England). Free radical scavenging activity was calculated as IC_{50} (inhibitory concentration) and expressed as ascorbic acid equivalent of antioxidant capacity (AEAC) in mg AA/100 g (Leong and Shui, 2002) as follows:

$$\text{AEAC (mg AA/100g)} = IC_{50\ (\text{ascorbate})} / IC_{50\ (\text{extract})} \times 100\ 000$$

The IC50 of ascorbic acid used for calculation of AEAC was 0.00387 mg/ml.

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4. Weight loss

After storage, the vegetables were weighed after removal from each PE bag using an electronic weighing scale. The weights were recorded and weight loss were calculated and expressed in percentage.

5. Leaf colour and chlorophyll contents

Measurements of the vegetable leaf colour were made at three equidistant points on the leaf surface of each leaf blade with a chromameter (Minolta CR-300, Minolta Corp., Japan). The results were expressed as lightness (L*), chromaticity (C*) and hue (h^o).

For chlorophyll content, three leaf discs of 0.59 cm^2 were randomly sampled from the same leaf where colour was measured, placed in a vial wrapped in aluminium foil and added to 20 ml of 80% acetone. The samples were kept in a dark place for 3 days until all the chlorophyll were extracted from the discs. The chlorophyll a and b contents were determined by using a spectrophotometer (Model S1200, Spectral Wave, England) with absorbance readings at 663 and 645 nm. Total chlorophyll content was calculated using the equation described by Coombs et al. (1985) as follows:

Chlorophyll a (mg/cm^2) = 12.7 (A_{663}) – 2.35 (A_{645})
Chlorophyll b (mg/cm^2) = 18.61 (A_{645}) – 4.68 (A_{663})

$$\text{total chlorophyll} = \frac{3.5 \times (\text{chlorophyll a} + \text{chlorophyll b})}{0.59}$$

= Total chlorophyll extracted leaf area in vial (ml)

0.59 = Surface area leaf for the chlorophyll extract (cm^2)

6. Experimental design and statistical analysis

The experiment was conducted using a randomized complete block design, arranged in a factorial experiment, with five replications. Each replication consisted of five samples of a leafy vegetable. The factors comprised two cropping systems (organic and conventional) x four storage days (0, 3, 6 and 9) in *L. sativa.* Data were analysed using the analysis of variance, and significant treatment means were separated by Duncan's multiple range test (DMRT) at $P \leq 0.05$ (SAS, version 9.3). When there were significant interaction effects between cropping system and storage duration on the measured variables, the results indicated that cropping system (organic and conventional) differences were not the same at different storage duration (0, 3, 6 and 9 days). Further partitioning of the interaction sum of square was conducted to understand the nature of the interaction between cropping system and storage duration on the measured variables. Regression analysis was conducted to determine the relationship between the dependent

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variables and storage duration by each cropping system. When there was no significant regression responses, the effects of storage duration on the dependent variables evaluated in each cropping system was compared using the pooled least significant difference (LSD) test at $P \leq 0.05$. The correlations between the dependent variables were determined using the CORR procedure of SAS. Unless otherwise noted, only results significantly different at $P \leq 0.05$ and significant quadratic responses are discussed.

RESULTS AND DISCUSSION

1. Contents of nitrate and nitrite, and NR activity

There were significant interactions between cropping system x storage duration on contents of nitrate and nitrite, and NR activity of *L. sativa* (Table 1).The nitrate content of organic and conventional *L. sativa* leaves showed a significant quadratic relationship with storage duration (Figure 1A). The trends of decrease in nitrate contents of *L. sativa* between the organic and conventional cropping systems at storage days 3 and 9 were similar, with reductions of 37.6% and 45.7%, respectively, compared to storage day 0. There were significant and positive quadratic relationships between nitrite contents and storage durations of organic and conventional *L. sativa* (Figure 1B).The nitrite contents of both organic and conventional *L. sativa* increased by 97% and 98%, respectively during storage days 3 and 9 compared to storage day 0.

For NR activity, only the conventional *L. sativa* leaves showed a significant quadratic relationship with storage duration while NR activity of organic *L. sativa* was not related to storage duration (Figure 1C). The NR activity of conventional *L. sativa* leaves increased slightly until day 6, followed by a 23% increase until the end of storage. At initial storage day, the intercept value in the quadratic equation ($Y = 0.097 + 0.013x - 0.002x^2$) of NR activity of organic *L. sativa* leaves (Figure 1C) was 63% higher than those of conventional *L. sativa*.

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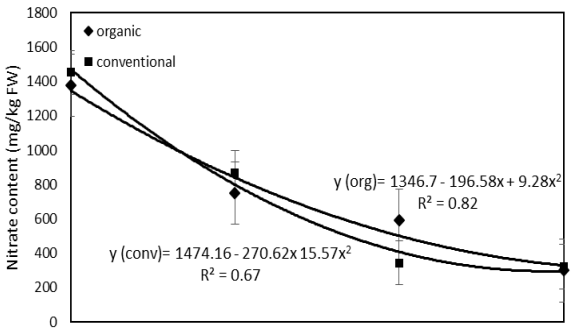
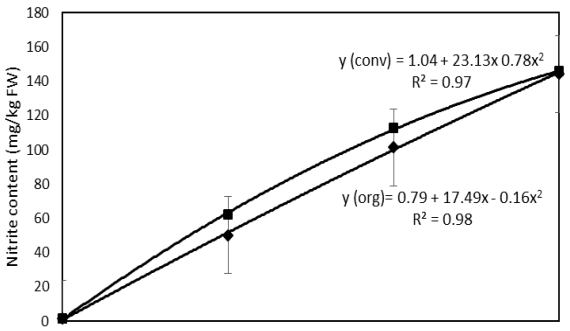
Table 1. Main and interaction effects of organic and conventional cropping systems (CS) and storage duration (SD) at 0, 3, 6 and 9 days on nitrate and nitrite contents, nitrate reductase (NR) activity, weight loss, chlorophyll content, L*, C* and hº colour values, total phenolic content (TPC), total flavonoid content (TFC) and DPPH free radical-scavenging activity of *Lactuca sativa 'Leaf lettuce'*.

Measured variable	CS	SD	CS x SD
Nitrate	ns	*	*
Nitrite	*	*	*
NR activity	*	*	*
Weight loss	*	*	*
Chlorophyll content	*	*	*
L*	*	*	ns
C*	*	*	*
h°	ns	*	*
TFC	*	*	*
TPC	*	*	*
DPPH	*	*	*

ns,* = non-significant or significant at P≤ 0.05, respectively. L* = lightness, C* = chromaticity, hº = hue. (n = 25).





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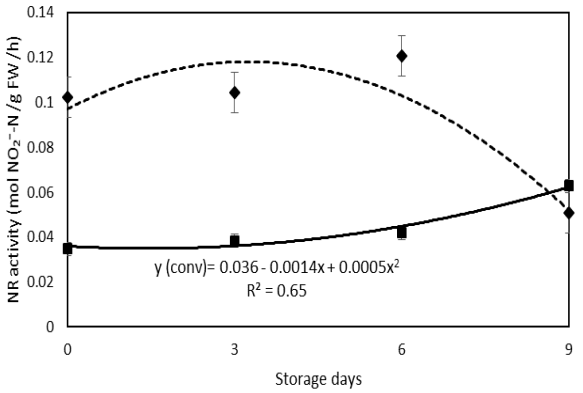

Figure 1. Nitrate content (A), nitrite content (B) nitrate reductase (NR) activity (C) of organic (org) and conventional (conv) *Lactuca sativa* 'Leaf lettuce' stored for 3, 6 and 9 days at 6 ± 1 °C. Solid line indicates a significant quadratic response at $P \leq 0.05$. Vertical bars = SE of five replicates for each treatment. Nitrate content for *Lactuca sativa* = 1300 mg/kg fresh weight in Hong Kong (Chung et al., 2011). n = 25

The study also showed a significant negative correlation between nitrate and nitrite contents of *L. sativa* (Table 2), as well as between nitrate and NR activity, indicating that the nitrate content decreased while nitrite content and NR activity increased during storage. Nitrite contents are usually very low in fresh vegetables, but under unsanitised postharvest storage conditions, nitrite levels tend to increase in vegetables (Ayaz et al., 2007). There are close relationships between NR activity and nitrate contents in leafy vegetables as NR activity was expected to be the rate inhibitor for nitrate metabolic conversion or reduction (Caba et al., 1995; Bussi et al., 1997), and NR appeared to be an inducible enzyme (Skrdleta et al., 1979). Also, water loss during storage could be a factor in nitrate reduction and nitrite increase during storage. Water loss had a negative relationship with nitrate content as opposed to a positive correlation with nitrite content and NR activity of *L. sativa* (Table 2). Thus, as fresh weights of vegetables are reduced during storage, their nitrate contents decreased while nitrite contents and NR activities increased. The nitrate and water contents of rape, Chinese cabbage, and spinach were positively correlated (Qiu et al., 2014), since leafy vegetables with higher levels of nitrate accumulation had greater volumes of water in the tissues (Burns et al., 2011).

In the present study, nitrate contents of both organic and conventional L. sativa were reduced, while nitrite content increased as storage days increased. The nitrate contents of both cropping systems were slightly higher on initial day of storage, but decreased afterwards. However, the nitrate content of organic and conventional L. s

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ativa from the study were similar with the nitrate content of L. sativa in Hong Kong (Nitrate content = 1300 mg/kg fw) as reported by Chung et al. (2011). Under certain storage conditions, nitrate can be converted into nitrite in vegetables. The nitrate and nitrite levels of vegetables after harvest could be affected by microbial activity, temperature and NR activity (WHO, 2012; EFSA, 2008).

Table 2. Correlation coefficients (r) between nitrate and nitrite contents, nitrate reductase (NR) activity, chlorophyll content, weight loss, colour values [lightness (L*), chromaticity (C*) and hue (hº)], total phenolic content (TPC), total flavanoid content (TFC) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay of *Lactuca sativa* 'Leaf lettuce' obtained from organic and conventional farms.

	Nitrate	Nitrite	NR activity	TPC	TFC	DPPH	Weight loss	Chlorophyll content	L*	C*
Nitrite	-0.90**	--								
NR activity	-0.53**	0.61**	--							
TPC	0.61**	-0.74**	-0.40*	--						
TFC	0.67**	-0.69**	-0.78**	ns	--					
DPPH	-0.54**	0.65**	0.59**	ns	-0.57**	--				
Weight loss	-0.74**	0.87**	0.78**	-0.62**	-0.71**	0.70**	--			
Chlorophyll content	0.44**	-0.50**	ns	0.51**	ns	ns	-0.46*	--		
L*	ns	ns	0.59**	ns	-0.50**	ns	ns	ns	--	
C*	ns	ns	ns	ns	ns	ns	ns	0.52**	0.71**	--
h°	0.74**	-0.80**	-0.68**	0.53**	0.63**	-0.72**	-0.80**	0.48**	ns	ns

ns,*, ** = non-significant or significant at P≤0.01. L* = lightness, C* = chromaticity, hº = hue. (n = 200).

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Microbial reduction of nitrate in leafy vegetables during postharvest storage could result in accumulation of high nitrite contents (Chung et al., 2004; Domanska-Blicharz et al. 2004; Prasad and Chetty, 2008; Tamme et al., 2009). Nitrite tends to increase dramatically via microbiological reduction of nitrate in vegetables, and nitrate content decreases during a period of storage at ambient temperature (Phillips, 1968, Ezeagu, 1996). This implies that the activity of endogenous NR in vegetables tends to be inactivated under the cold storage condition. Yaneva et al. (1996) reported that cold temperature could strongly reduce the activity of NR in leaves of green vegetables by disturbing the internal electron transport of nitrate reductase. Nitrate reduction occurs at shoots which were catalyzed by NR activity. Specifically, the reaction of nitrate reduction to nitrite occurs in the cytosol outside any organelle (Salisbury and Ross, 1992). Abundant levels of nitrate in cytosol clearly increased NR activity, largely because of rapid synthesis of the enzymes. In leaves and stems, light also increases NR activity when nitrate is available. Light activates one or both photosystems in photosynthesis, which transports stored nitrate from vacuoles to cytosol where the NR activity occurs (Granstedt and Huffaker, 1982). In the current study, the NR enzyme in the vegetables was considerably activated during the 9 days of storage. Thus, it might contribute to the significant microbial reduction of nitrate, which led to the accumulation of high nitrite levels. Microorganisms may have been present on the surface of the vegetables and within the roots. Improper handling and storage of vegetables can also lead to bacteria contamination. Such bacteria could multiply resulting in much higher levels within the tissue when conditions, such as temperature and moisture, are particularly favourable for bacterial growth. It has been proven that nitrite concentrations in fresh, unscathed, well-stored vegetable tissues are particularly low (Chung et al., 2004; Ezeagu, 1996). It seems that the activity rate of NR maintains an equilibrium with one of the NR enzymes under suitable storage conditions. The variations may be dependent on the differences between species-specific nitrate reductase activities and the influence of levels of bacterial contamination. This might imply that the dramatic reaction of microbiological reduction (the effect of nitrate reductase) during storage at room temperature could probably cause a significant elevation in nitrite levels in vegetables. Thus, fresh vegetables purchased from retail market should be stored immediately in a refrigerator and consumed within 3 days of storage. Improper storage conditions at ambient temperature present a risk in terms of increased accumulation of nitrite. Proper storage of nitrate-containing vegetables at refrigerated temperatures is suggested as the appropriate way to prevent bacterial nitrite formation and thus improved safety during vegetable consumption. Under proper storage, nitrite and nitrate reductase activity rates are stable, thus, resulting in lower nitrate content of the stored vegetables (Chung et al., 2004). Therefore, it has shown that *L. sativa* should not be kept under refrigerated (5 °C) storage for more than 3 days, as the nitrite content could increase tremendously.

2. Phytochemicals and antioxidant activity

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The interaction effects between cropping system x storage duration on contents of total flavonoid, total phenolic, and ascorbic acid as well DPPH free radicle scavanging activity of *L. sativa* were significant (Table 1). The regression response between total flavonoid content and storage duration of organic and conventional *L. sativa* showed significant, negative and quadratic relationships (Figure 2A). Total flavonoid content of conventional leaves was higher than organic on initial storage day and dropped steeply during storage with a 62% decrease compared to a 56% decrease in organic leaves. Similarly, the relationship between total phenolic content and storage duration of conventional leaves was significant, negative and quadratic (Figure 2B). The storage duration of conventional leaves affected 83% ($R^2 = 0.83$) of the changes in the total phenolic content. Particularly, total phenolic content of conventional *L. sativa* leaves deteriorated by 12.6% in the first 3 days of storage. However, there was no significant relationship between the total phenolic content of organic *L. sativa* leaves and storage duration. Also, there was no significant relationship between ascorbic acid and storage duration of both organic and conventional *L. sativa* (Figure 2C). For conventional *L. sativa* leaves, there was no significant decrease in ascorbic acid until 3 days of storage as there was no significant difference between ascorbic acid on 0 and 3 days of storage in organic *L. sativa* leaves. The conventional *L. sativa* leaves had higher ascorbic acid after 6 days of storage than those of organic leaves. For DPPH assay of organic and conventional *L. sativa* leaves, there was a significant, positive and a quadratic relationship between DPPH and storage duration (Figure 2D). There were no differences in DPPH assay between organic and conventional *L. sativa* leaves up to. However, after 3 days of storage, there followed quadratic increases in DPPH of organic and conventional *L. sativa* leaves by 78% and 59%. This indicated that the DPPH of organic leaves was increased dramatically after day 3 compared to those of conventional.

In the present study, total flavonoid and total phenolic contents in *L. sativa* of conventional and organic leaves decreased while DPPH increased throughout 9 days of storage. This finding is in agreement with Siti Fairuz et al. (2012), where the total phenolic content of 'Butterhead' lettuce showed a 28% reduction while DPPH was increased by 10.9% during 8 days of storage under refrigerated condition.

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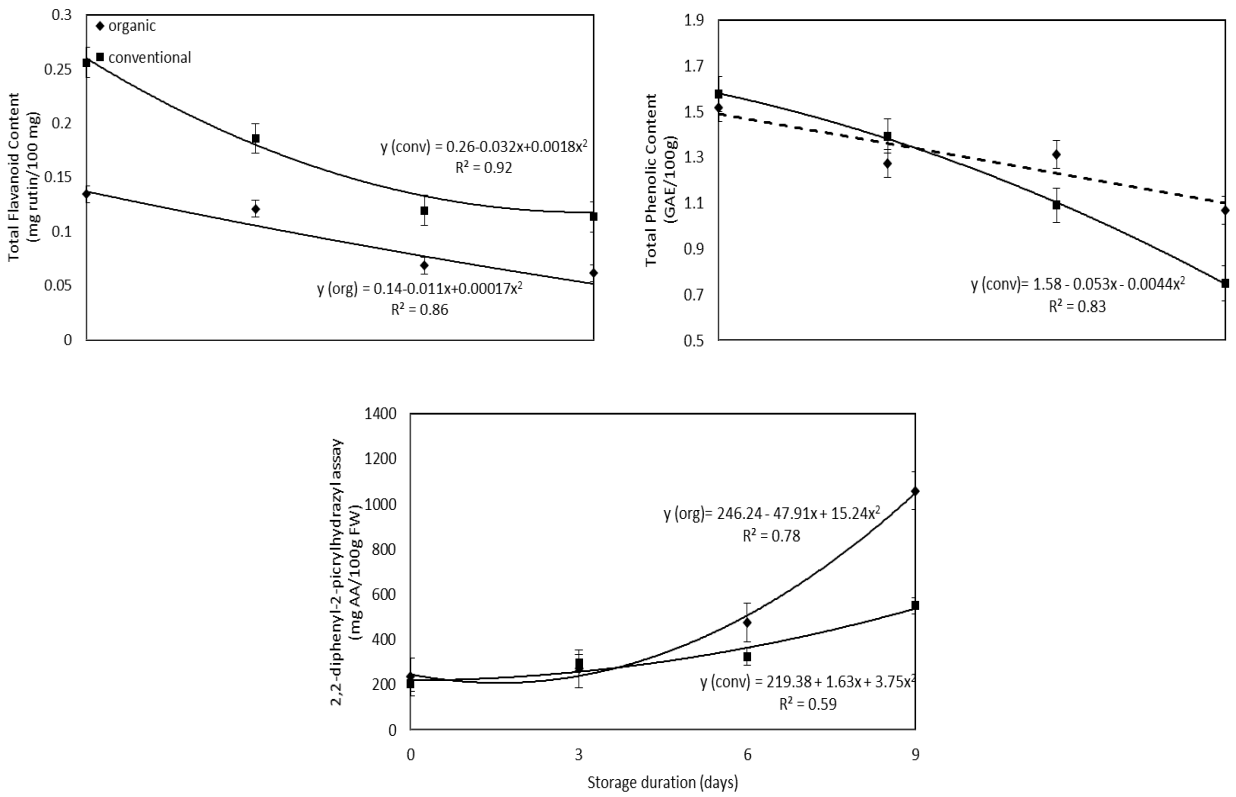

Figure 2. Total flavanoid content (A), total phenolic content (B) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay of organic (org) and conventional (conv) *Lactuca sativa* 'Leaf lettuce' stored for 3, 6 and 9 days at 6 ± 1°C. Solid line indicates a significant quadratic response at $P \leq 0.05$.Vertical bars = SE of five replicates for each treatment. n = 25.

The negative correlation between TPC and DPPH in Butterhead lettuce, is similar to the result in this study where there were significant negative correlations between DPPH and TPC as well as with TFC (Table 2). There were positive significant correlation between nitrate and TFC, as well as with TPC. On the contrary, nitrate content had a negative significant correlation with DPPH. A significant and positive correlation between nitrogen and total phenolic contents, as well as total flavonoid content in *Labisa pumila* has also been reported (Ibrahim et al., 2011). Since the increase in nitrogen could result in intensification of nitrate content in leaves, this showed that higher nitrogen had a significant impact on the production of total phenolic and flavonoid production. The breakdown of cellular structure during postharvest storage could be the factor causing a decrease in the phenolic and flavonoid contents (Toor and Savage, 2006). According to Yap (2016), low nitrogen rates increased the DPPH antioxidant capacity, indicating the negative relationship between nitrogen and DPPH, irrespective of the fertilizer types applied on

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Orthosiphon stamineus. The ascorbic acid of conventional *L. sativa* was significantly reduced by 3 days of storage while ascorbic acid of organic *L. sativa* showed no changes during 9 days of storage. The study was in agreement with that of Chung (2012) whereby the ascorbic acid content in conventional *Brassica rapa* cul. Choy sum, decreased after four days of storage compared to those that are organic-grown. The excessive application of nitrogen fertilizer could lead to the lower ascorbic acid of a commodity.

3. Weight loss and chlorophyll content

For *L. sativa*, there were significant interaction effects between cropping system and storage duration on fresh weight loss and chlorophyll content (Table 1). Organic and conventional *L. sativa* both showed significant quadratic relationships between weight loss and storage duration (Figure 3A). Even though weight loss of both organic and conventional *L. sativa* leaves were increased in a similar trend, weight loss of the organic *L. sativa* had increased sharply than conventional leaves. The weight loss was rapidly increased after 3 days of storage in both organic and conventional leaves. However, the weight loss of organic *L. sativa* leaves was significantly higher than conventional leaves after 6 days of storage. Kader et al. (1973) reported that about 20-25% water loss occurred in lettuce after 8 days of storage at ambient temperature. The higher water loss in organic *L. sativa* after day 6 could be due to the lack of pre-cooled practices after harvest. Pre-cooling is the most important process of extending the shelf life of commodity since pre-cooling could remove the field heat (Tao et al., 2007; Thompson, 2004), thus, could lower the respiration rate and delay the metabolic and physiological activities (Xiangyou et al., 2014). Even if the vegetables were stored at the same temperature and condition, differences in water loss could be because of differences in form, structure and physiological behavior (Nunes and Emond, 2007). There was a significant, negative and quadratic relationship between chlorophyll content and storage duration of conventional grown *L. sativa* (Figure 2D). However, there was no relationship between chlorophyll content and storage days for organic *L. sativa*. The chlorophyll content of conventional *L. sativa* leaves showed no changes during the first 3 days, slightly decreased from day 3 to day 6, and rapidly decreased until day 9 (Figure 3B). The changes in the colour of the green leaves could be attributed to the degradation and breakdown of chlorophyll pigment (Ferrante et al., 2004), a symptom of postharvest senescence in green vegetables.

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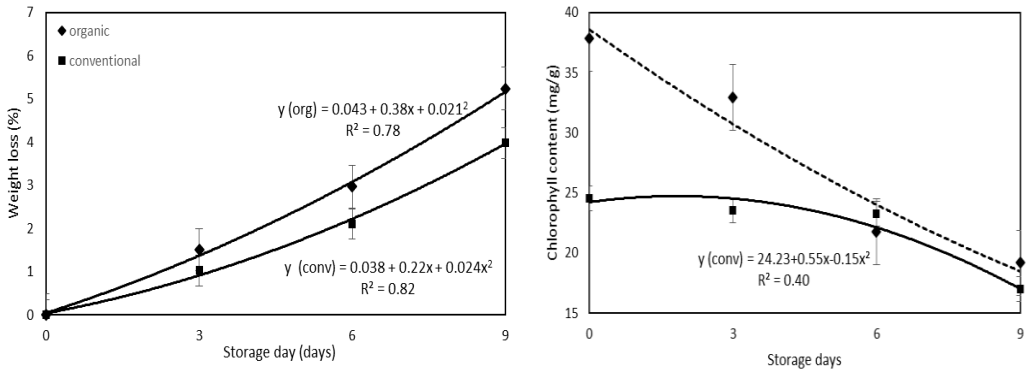

Figure 3. Weight loss (A) and chlorophyll content (B) of organic (org) and conventional (conv) *Lactuca sativa* 'Leaf lettuce' stored for 3, 6 and 9 days at 6 ± 1 ºC. Solid line indicates a significant quadratic response at $P \leq 0.05$.Vertical bars = SE of five replicates for each treatment. n = 25.

Yellow carotenoids co-exist with green chlorophylls in green vegetables (Yamauchi and Watada, 1991). Thus, chlorophyll degradation lead to unmasking of yellow pigments, subsequently, resulting in the yellowing of green vegetables (Gross, 1991) during storage.

From the study, *L. sativa* showed a significant and negative correlation between chlorophyll content and weight loss during 9 days of storage (Table 2). The finding is in agreement with that of Nunes and Emond (2007) who found close correlations between weight loss and colour and other physical quality attributes. There were also positive relationships between chlorophyll content and TPC, as well as with DPPH. However, the result is in contrast with Meyer et al. (2006) and Havaux and Kloppstech (2001) where they showed that there were negative relationships between the secondary metabolites and chlorophyll content. It was said it is a sign of gradual switch of investment from protein to polyphenols production, which suggested that the production of secondary metabolites was competing with light harvesting protein and there were competition between protein and secondary metabolites biosynthesis pathway and its metabolites regulation. However, during storage condition, the reduction or breakdown of chlorophyll content also caused the reduction in TPC and TFC as well. The result also showed significant negative correlation between weight loss and TPC, as well as with TFC. This indicated that as more water is transpired during storage, TPC and TFC would be decreased. Therefore, *L. sativa* should not be stored in a home refrigerator (5 ºC) for more than 3 days to maintain its quality.

4. *Colour values of L*, C* and h°*

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L. sativa leaf colour from both cropping systems were measured and expressed in values of hue (h°), chromaticity (C*) and lightness (L*). There were significant interaction effects between cropping system x storage duration on h° and C* values except but not on L* values of *L. sativa* (Table 1). There was a significant, negative and a quadratic relationship between the h° values of organic *L. sativa* leaves, with 67% variation in h° value due to the storage duration (Figure 4A). There was no relationship between the h° value and storage duration of conventional *L. sativa* leaves. The h° values were similar between the organic and conventional leaves, during 0 and 3 days of storage. There was also no relationship between C* value and storage duration of organic and conventional (Figure 4B).

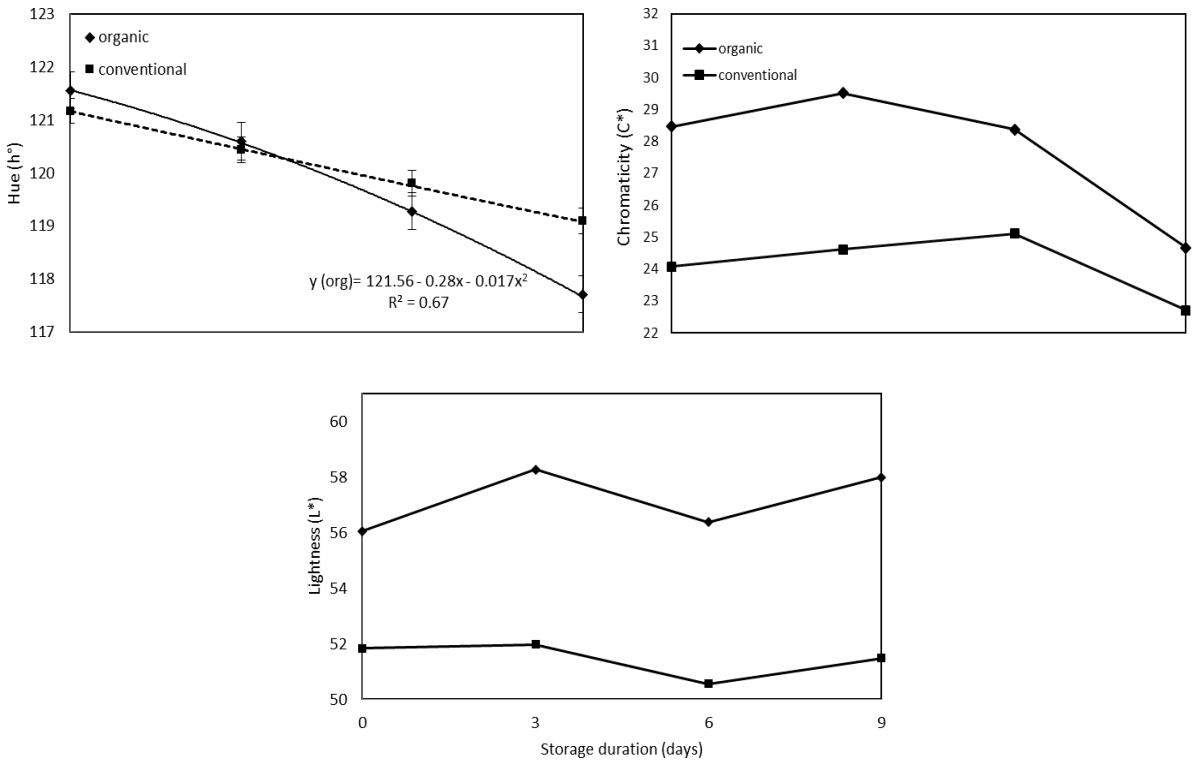

Figure 4. (A) Hue (h°), (B) chromaticity (C*) and (C) lightness (L*) colour values of organic (org) and conventional (conv) *Lactuca sativa* 'Leaf lettuce'stored for 3, 6 and 9 days at 6 ± 1 ºC. Solid line indicates a significant quadratic response at $P \leq 0.05$.Vertical bars = SE of five replicates for each treatment. n = 25.

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The C* value of organic *L. sativa* leaves showed a slight change upto 6 days of storage. Similarly, there was also a slight change in C* value of conventional leaves by 6 days of storage. Throughout the 9 days of storage, the C* value of the organic leaves was higher than those of conventional leaves, indicating that organic leaves were more intense in colour than those of conventional-grown. There was no significant interaction and regression responses between L* value and storage duration of organic and conventional *L. sativa* leaves (Figure 4C). The L* value of organic leaves was higher than those of conventional leaves as evidenced by the brighter green appearance compared to dark green leaves of conventional *L. sativa* although the brightness of organic leaves fluctuated during 3 to 9 days of storage as compared to conventional leaves. During storage, h° value of both organic and conventional *L. sativa* leaves changed from green to a light yellowish green. Based on h° and C* attributes, the colour of organic and conventional leaves changed from pale green to yellowish dull green by 9 days of storage. This might be due to 49.4% and 30.8% reduction of chlorophyll contents in organic and conventional *L. sativa*, respectively (Figure 3B).

Generally, consumers percieved leafy vegetables as being green in colour, however, the colours could be differentiated into either pale, dark, deep or grayish green by the C* values. Such differences in colour could only be differentiated by the use of a chromameter and not by a human eye. The h° value was negatively correlated with nitrite content, while nitrate content of *L. sativa* was positively correlated with h° value (Table 2). There was a strong and negative correlation between chlorophyll content and nitrite content, as nitrogen is an essential component of chlorophyll which is involved in the photosynthetic process (Cabrera, 1998; Barker and Bryson, 2007) and nitrite is an unstable reduction form of nitrogen. According to Ferrante and Maggiore (2007), degradation of the leaf pigments, such as chlorophyll and carotenoids, causes lost of leaf colour of vegetables. The colour is critical because it defines the appearance of the vegetables and influences consumers' choice (Ferrante et al., 2004).

CONCLUSIONS

The *L. sativa* from the two cropping systems showed 4.5 to 8% weight loss or shrinkage during storage. The organic and conventional vegetables showed that the colour changes from dark grayish, deep and pale green to bright grayish, and yellowish dull green during storage. The colour changes were influenced by the degradation of chlorophyll content in *L. sativa* during storage. The antioxidant and antioxidant activity such as TFC and TPC were decreased during storage, while DPPH was increased. The two organic and conventional leafy vegetables were 78 to 95.4% reduced in nitrate contents and 99% to increased in nitrite content as storage days increased. It is recommended that *L. sativa* in a refrigerated (6 ± 1 ^{o}C) storage should be consumed within 3 days to prevent the conversion of nitrate to nitrite. Meanwhile, quality of the

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vegetable could be maintained or even improved with the increase in total flavonoid and total phenolic contents and DPPH free radicle scavenging activity, besides preventing excessive leaf shrinkage and chlorophyll content degradation.

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