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Influences of modified atmosphere packaging using active breathable films on antioxidant activity and quality of minimally processed Teaw leaves (*Cratoxylum formosum* Dyer)

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Abstract

A study was conducted to investigate influences of modified atmosphere packaging using active breathable films (FF3) on postharvest qualities of minimally processed Teaw leaves (*Cratoxylum formosum* Dyer), a tropical leafy vegetable having high total phenolic content and antioxidant activity, kept at 10 and 20ºC. Teaw leaves stored at 20ºC became wilted, and having visible microbial appearance within 2-3 days, which were considered unmarketable in regardless of either FF3 or Low Density Polyethylene (LDPE, as control) films studied. High permeabilities of FF3 film to O_2 and CO_2 significantly affected levels of modified atmosphere conditions in package headspaces. At all temperatures studied, high O_2 concentrations (17-19% v/v) and relatively low CO_2 concentrations (< 0.1% v/v) accumulated in FF3 bags and such gaseous accumulations were in contrast to those accumulated in LDPE bags i.e. lower O_2 (9-15% v/v) and higher CO_2 (2-6% v/v). Low levels of CO_2 in FF3 bags apparently minimised rates of losses of ascorbic acid and antioxidant activity during 5 days of storage, although there were no significant differences in the parameters measured in leaves kept in LDPE bags. There were no significant effects of packaging films and storage temperatures on total phenolic contents, which slightly increased from the initial value and became stable throughout the storage period. Higher mass loss percentage of Teaw leaves kept in FF3 bags were noticed and were attributed to high permeabilities of FF3 to water vapour. However extents of mass losses were significantly reduced when leaves were kept at 10ºC. Whilst total viable counts of leaves packaged in FF3 bags tended to be lower than those in LDPE due to lower humidity levels accumulated, there was unclear effects of packaging films on yeast and mould. Consumer panel tested accepted overall quality of the products packaged in the FF3 kept at 10ºC of which the visual appearances of Teaw leaves appeared to be rated with higher scores, compared to those in LDPE bags.

Introduction

Teaw (*Cratoxylum formosum* Dyer) is a tropical leafy vegetable extensively grown and traditionally consumed as fresh leaves in Thailand. Teaw leaves reportedly had high antioxidant activity because of their high content of phenolic compounds which can reduce oxidative reaction in turn minimising carcinogenic development in human health (Maisuthisakul *et al*., 2007; Maisuthisakul *et al*., 2008). Furthermore Teaw is an important nutritional sources such as vitamins and energy (Maisuthisakul *et al*., 2008). Similar to other leafy vegetables (Lee and Kader, 2000), the life of harvested Teaw is limited by rapid weight loss which can result in quality deteriorations including physical (e.g. wilting) and antioxidant properties. To date, whilst report on packaging development to extend shelf life of Teaw leaves evidently is limited, modified atmosphere packaging (MAP) using plastic film such as LDPE has commercially been utilised to minimise quality changes of a range of fresh produce (Rojas-Grau *et al*., 2009).

However, there is much evidence to show that MAP alone is unlikely to be completely effective for controlling postharvest quality changes, particularly when these are in response to storage temperature fluctuations. Compared to the respiration rate, permeabilities to O_2 and CO_2 of films typically used in the horticultural industry are less sensitive to temperature changes (Utto *et al*., 2005). Such mismatch could lead to cause rapid accumulation of CO_2 and depletion of O_2 in the package and such

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gaseous concentration changes become pronounced under ambient or high storage temperature (Yam and Lee, 1995) to which horticultural products often encounter throughout supply chains in Thailand and other developing countries.

To overcome this problem, utilisations of films having high permeability to oxygen and cool temperature storage are considered a key approach. An example of the films is referred to so-called Active breathable films (FF3) manufactured and commercialised by Thantawan Industry Company (Thailand). FF3 film is LDPE based film with proprietary polymeric macromolecular modification, have higher oxygen permeability ($P^{O_2}_{FF3}$ 2.31 fmol.m.s^{-1}.m^{-2}.Pa^{-1}), compared to that of LDPE ($P^{O_2}_{LDPE}$ 0.34 fmol.m.s^{-1}.m^{-2}.Pa^{-1}) (Boonsiri, 2010). The FF3 film thus is considered a potential packaging film for extending shelf life of Teaw leaves because the film technically should allow O_2 to permeate into the package with faster rate and increase O_2 concentration in the headspace for maintaining aerobic respiration process of the leaves.

Although there are reports on applications of FF3 on, for example, rambutan, longon, mango and straw mushroom (Boonsiri, 2010), those on Teaw leaves are not available. The purpose of this study is to investigate influences of MAP using the active breathable (FF3) on key postharvest qualities and antioxidant activity of minimally processed Teaw leaves.

Materials and Methods

Materials

Fresh Teaw leaves were purchased from a wholesale market, Warinchamrab district, Ubon Ratchathani Province, Thailand. Mean hue angle value (±SD), h^o, were 102.97 ± 0.01. Leaves were delivered to the Postharvest Technology Laboratory, Faculty of Agriculture, Ubon Ratchathani University. Defect-free leaves of uniform size and colour were selected. The leaf rachises were cut down to length of ~10 cm. Old leaves (dark red colour leaves) on the rachises were taken out leaving most young leaves. The leaves were later equilibrated to storage temperatures studied for 10 hours prior being randomly assigned to different experimental treatments. Plastic film packages of which their sizes are 7" x 11" were employed. These were categorized into two groups including (1) clear LDPE bag (control; 30 µm thickness, film transmission rates to O2, CO_2 and water vapour are, 2,500 ml.m^{-2}.day^{-1}, 13,500 ml.m^{-2}.day^{-1}, and 8 g.m^{-2}.day^{-1}, respectively) and (2) FF3 film bags (Thantawan Industry Company, Thailand; 28 µm thickness, film transmission rates to O_2, CO_2 and water vapour are, 18,272 ml.m^{-2}.day^{-1}, >30,000 ml.m^{-2}.day^{-1}, and 25.1 g.m^{-2}.day^{-1}, respectively).

Teaw leaves, ~70g, were packaged into individual bags which were later kept at the storage temperatures tested. Storage trial period was 7 days. Storage temperatures were 10ºC and 20ºC. The former represents the optimal temperature commonly utilised for long term storage of horticultural products (Robinson *et al*., 1975), while the latter was selected as a challenging high temperature that the products would be encountered during the supply chain and is typical of the temperature during uncontrolled (ambient) storage (Bassal and El-Hamahmy, 2011). Although the 20ºC chosen in the present work was considered lower than the ambient temperature in Thailand (averagely 30ºC), utilisations of MAP for fresh vegetables at such high storage temperature would compromise their benefits to prolong shelf life. The high temperature effect can stimulate reduced O_2 and increased CO_2 of which the levels of both gases may become close to the fermentation threshold. Further decrease in O_2 is considered critically undesirable effect that can lower storage quality (Cameron *et al*., 1995; Yearsley *et al*., 1997). Also, the simulated 20ºC condition would provide some understandings on effects of FF3 on O_2 levels accumulated in the bag i.e. at which levels of O_2 and CO_2 would be maintained during 20ºC storage and how these could affect storage quality of Teaw leaves, thus this storage temperature was chosen in the present work.

Methods

Postharvest quality parameters were measured after the first day of treatment and every 2 days thereafter. The parameters include modified atmosphere condition (O_2 and CO_2 concentration) in package headspace, mass loss, ascorbic acid content, total phenolic content, antioxidant activity (i.e. free radical scavenging activity), and microbial quality. Sensory assessment of consumer toward Teaw leaves packaged in different plastic bags kept under storage temperatures was also conducted.

Modified atmosphere condition (O_2 and CO_2 concentrations)

Measurements of O_2 and CO_2 inside the packages were conducted with a packaging atmosphere analyzer (Model MAP test 3050, Hitech Instruments, Luton, UK). A 20 ml gas sample was taken through a septum with a hypodermic syringe for analysis.

Mass loss measurement

Leave mass (g) was determined using an electronic balance (Company, Japan) with an error range of 0.01 g. Mass loss was expressed as a percentage of the initial fresh mass.

Sample preparation for measurements of ascorbic acid, total phenolic content, and antioxidant activity

Juice extracted of leave samples was prepared following the methods of Swain and Hillis (1959) with some modifications. 20g of leaf samples was mixed with methanol (80% v/v) and homogenized for 1 minute and then transferred to polypropylene tubes. The samples were vortexed and allowed to stand for 1 h at room temperature to technically allow solvent extraction to be completed. Juice obtained was centrifuged at 2000g for 15 min at 20 °C. The supernatant was filtered through a Whatman no. 1 filter, after which the filtrate samples were kept in the volumetric flask wrapped with aluminium foil and stored in 4ºC chamber for further usages.

Ascorbic acid content

Ascorbic acid was determined according to AOAC (1990) with modifications. 1 ml of juice extracted was pipetted into the 250 ml flask and 9 ml of 5% Trichloroacetic acid was added into the flask which later was thoroughly shaken and titrated with indophenol solution (25% DCIP and 21% $NaHCO_3$ in water). The titration was conducted until a light but distinct rose pink colour appears and persists for more than 5 seconds. The indophenol solution was standardised daily with 0.02 mg/ml ascorbic acid solution. Ascorbic acid level was reported in mg/100g juice.

Total phenolic content (TPC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteau (F&C) and gallic acid assay described by Kim *et al*. (2003) with some modifications. 1 ml of the juice extracted was added to 25 ml volumetric flask containing 1 ml distilled water. 5 ml of Folin and Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 minutes, 4 ml of 7.5% sodium bicarbonate solution was added. The solution was mixed vigorously and left to stand in the dark for 2 h after which the absorbance was then determined at 765 nm. A calibration curve was prepared with gallic acid and the results were expressed as mg GAE/100 g fresh weight. A range of gallic acid concentrations from 100 to 500 µg/g was used to prepare the calibration curve.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay

The DPPH free radical scavenging activity was determined using methods utilized by Brand-Williams *et al*. (1995) with some modifications. 0.1 ml of the juice extracted was added to 3.9 ml DPPH solution. The mixture was mixed vigorously and left to stand in the dark for 30 min. The absorbance was then determined at 515 nm. The absorbance of the control was obtained by replacing the sample with methanol. A calibration curve was prepared using ascorbic acid (AA) and the results were expressed as mg AA/100 g fresh weight. A range of ascorbic concentrations from 20-100 µg/g was used to prepare the calibration curve.

Microbial evaluation

Total viable counts and yeast and mould counts were evaluated on plate count agar (PCA; Merck, Darmstadt, Germany) and potato dextrose agar (PDA; Merck, Darmstadt, Germany), respectively using the pour plate technique as described in Aneja (2009) with modifications. Twenty five grams of Teaw leaves was added with 225 ml 0.1% peptone water, pummeled in a stomacher for 2 min. A series of dilutions were prepared in 0.1% sterile peptone water (w/v) and parallel 1 ml samples of appropriate dilution were tested with PCA or PAD agars. PCA and PDA plates were incubated at 35ºC for 1-2 and 3-5 days, respectively.

Sensory assessment

Sensory evaluation of minimally processed leaves was performed using 40 untrained panelists. Overall acceptance was evaluated using a 9-point hedonic scale (9 = excellent, and 1 = poor) as describe by Samakradhamrongthai *et al*. (2009) with some modifications. Each panel will determine acceptance to visual appearance, colour, odour, taste and overall acceptance of Teaw leaves. The sensory evaluations for individual experimental treatments were conducted on day 3 of the storage period because of microbial loads of the leaves were in a safety range for consumption (referred to Microbial standards for food and food-package contact; Department of Medical Sciences, Ministry of Public Health, Thailand) of which levels of total plate count and yeast and mould should be less than 1×10^6 CFU/g and 1.0×10^4 CFU/g, respectively.

Experimental design and statistical analysis

The experimental design and statistical analysis were conducted in accordance to Complete Randomized Design. Quality parameters and gaseous

data were measured with 3 replicates for individual days investigated. All data were subjected to analysis of variance and the Duncan Multiple Range Test (p = 0.05) to determine significant differences among Teaw leaf samples as affected by packaging materials and storage temperature. Specific to the quality attributes measured on Day 5, data were analysed using t-Test (p = 0.05) because products kept at 20ºC were discarded due their marketing unsuitability. The statistical analyses were performed using R Statistical Package. Experiments were repeated twice during May-August 2011.

Results and Discussions

Modified atmosphere condition

For all packaging bags and storage temperatures tested, atmospheric conditions in package headspaces were modified throughout the storage period i.e. decreased in O_2 and increased in CO_2 concentrations (Figure 1). It can be seen that there were no data after day 3 of bags stored at 20°C due to unmarketable quality of leaves i.e. wilted and visual microbial appearance. Rates of gaseous concentration changes in headspace of packages kept at 20°C were faster than those kept at 10°C because high temperature increased respiration rates of leaves and film permeabilities to both O_2 and CO_2 which subsequently increased rates of gaseous permeation through packaging materials (Cameron *et al*., 1995; Yam and Lee, 1995). Research findings on effects of storage temperatures on gaseous changes in MAP of Teaw leaves were similar to those of other horticultural products such as chillies (Wall and Berghage, 1996), blueberries (Rosenfeld *et al*., 1999) and carrots (Alasalvar *et al*., 2005). Considered at the same temperature, atmospheric concentrations in LDPE bags were highly modified concentration levels, compared to those in FF3 bags (Figure 1).

At 10°C, the quasi-steady state concentrations of both O_2 and CO_2 in LDPE bags which apparently were established after day 3 of storage, were ~14-15% and 0.5-2 % (v/v), respectively, whilst those in FF3 bags were ~17-18% and < 0.1% (v/v), respectively. The higher O_2 and lower CO_2 concentrations in FF3 bags compared to those in LDPE bags importantly are attributed to higher gas permeability of FF3. Similar results in gaseous levels in ABF bags were reported in longan packages (Boonsiri, 2010) and cabbages (Penpattanakul and Taprap, 2011). High variations were observed in gaseous concentrations on Day 3 and 5. These may be attributed to senescence and/ or deterioration of the leaves stored particularly at 20°C in which gaseous concentrations could rapidly change due to high respiration rates i.e. giving off high CO_2 and rapid depleting O_2 concentrations.

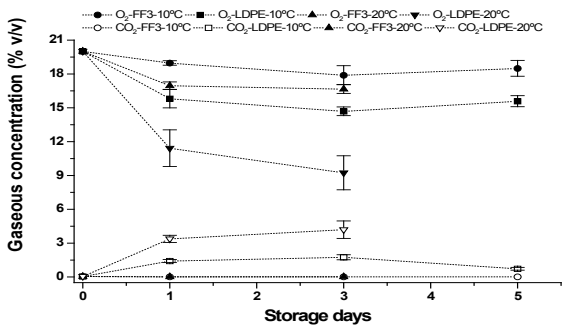

Figure 1. Changes of O_2 and CO_2 concentrations in headspaces of LDPE and FF3 bags for Teaw leaves, at 10ºC and 20ºC (Data and bars represented mean ± standard error (SE), 6 replicates)

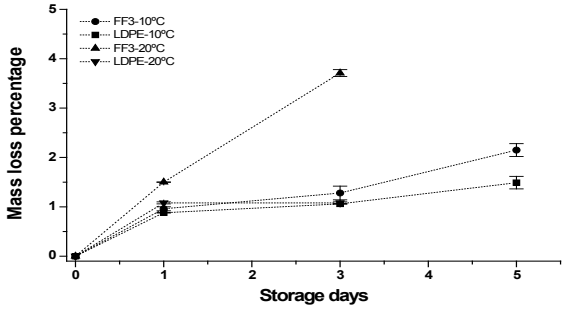

Figure 2. Mass loss percentage of Teaw leaves packaged in LDPE and FF3 bags kept at 10ºC and 20ºC (Data and bars represented mean ± standard error of mean (SE), 6 replicates)

The high gaseous permeable properties of films including FF3 film contribute high rates of mass transfer across packaging materials of both O_2 and CO_2 (Yam and Lee, 1995). Given higher rates of O_2 transfer across FF3 (from outside to in-bag), rates of decrease in O_2 levels attributed to respiration rate are lower, compared to rates of O_2 concentration reductions in LDPE bags (Figure 1). Although Teaw leaves packaged in FF3 bags can have high respiration rates (i.e. giving off CO_2) because of high oxygen levels (Mannapperuma and Singh, 1994), lower CO_2 levels (~0.05 % v/v) accumulated in FF3 bag are noticeable, compared to those in LDPE bags (Figure1). Such results on CO_2 accumulations were observed in both storage temperatures and these can be articulated through the higher CO_2 permeability of FF3 contributing higher rate of CO_2 permeation from in-bag to outside environment. Higher O_2 and lower CO_2 levels accumulated in FF3 bags can provide benefits on minimising development of anaerobic respiration of products packaged which can cause off-flavours (Kader *et al*., 1989) and other undesirable effects such as sunken texture and discoloration attributed to CO_2 injury (Fawbush *et al*., 2008).

Mass loss

Table 1. Changes of vitamin C, total phenolic content and antioxidant activity of minimally processed Teaw leaves stored in different packaging systems and temperatures

Total Vitamin C (mg/100g)					
Day		FF3-10°C	LDPE-10°C	FF3-20°C	LDPE-20°C
0[1/]	14.32±0.01				
1		13.70 ± 2.31[a][2/]	11.8 5 ± 1.70[a]	12.59 ± 1.70[a]	8.89 ±2.22[b]
3		9.63 ± 0.64 [a]	9.26 ± 1.70 [a]	9.26 ± 1.70 [a]	11.11 ± 2.94 [a]
5		9.26 ± 2.31[ns][3/]	9.26 ± 0.64[ns]		
Total Phenolic Content (mgGAE/100gFW)					
Day		FF3-10°C	LDPE-10°C	FF3-20°C	LDPE-20°C
0	68.72 ± 2.50				
1		71.81 ± 2.52[a]	72.74 ± 3.90[a]	71.14 ± 1.60[a]	71.81 ± 2.52[a]
3		71.58 ± 7.92[a]	72.32 ± 6.94[a]	71.27 ± 8.01[a]	70.98 ± 7.08[a]
5		72.43 ± 4.34[ns]	72.52 ± 4.24[ns]		
Antioxidant activity (mgAA/100gFW)					
Day		FF3-10°C	LDPE-10°C	FF3-20°C	LDPE-20°C
0	36.70 ± 0.14				
1		24.00 ± 3.85[a]	19.41 ± 1.17[bc]	21.52 ± 3.74[ab]	16.52 ± 2.99[c]
3		19.79 ± 2.50[a]	17.95 ± 2.80[a]	11.23 ± 1.53[a]	13.03 ± 1.82[b]
5		20.32 ± 5.98[ns]	22.61 ± 2.03[ns]		

[1/] Data on Day 0 represent average ± standard deviation (9 replicates) and they were not used in analysis of variance (ANOVA)
[2/] Mean (± SD) values (6 replicates) followed by the same letter within rows are not significantly different at P = 0.05 according to ANOVA and the Duncan Multiple Range Test
[3/] Non-significance difference (p=0.05) according to t-Test

Table 2. Sensory assessment of Teaw leaves packaged in FF3 and LDPE film bags, kept at 10°C and 20°C

Attributes	10°C		20°C	
	FF3	LDPE	FF3	LDPE
Appearances	7.56 ± 1.17[ab]	7.03 ±2.23[b]	6.31±3.21[bc]	5.98±0.12[c]
Colour	4.32±1.87[a]	3.65±1.18[a]	3.78±1.51[a]	4.26±2.37[a]
Odour	6.53±0.71[a]	6.12±3.13[a]	6.73±1.59[a]	5.93±2.13[a]
Taste	5.49±1.32[a]	5.13±2.15[a]	5.31±0.15[a]	5.20±1.21[a]
Overall acceptance	7.48±0.56[a]	6.79±1.31[b]	5.18±0.37[c]	5.03±1.56[c]

Means within each attribute (row) followed by the same letter are not significantly different according to Duncan Multiple Range Test (DMRT) ($p<0.05$).

Mass losses of Teaw leaves packaged in both LDPE and FF3 continuously occurred throughout the storage period. Higher mass loss rates apparently were found in the leaves packaged in FF3 bags and kept at 20°C, compared to those in LDPE bags and kept at 10°C (Figure 2), although there were no significance differences among treatments such as results observed on day 1 and 3. It can be noticed that there was no clear difference in mass loss percentages of leaves kept in LDPE at 10°C (LDPE-10°C) and those kept in LDPE at 20°C (LDPE-20°C). The result may be attributed to high humidity accumulated in headspaces of LDPE bags. Although the relative humidity (RH) levels were not measured, it could be estimated that the levels would be higher than 90% RH due to some presences of condensates on inner surfaces of the LDPE bags kept at both temperatures. Because LDPE film is a good barrier to water vapour (Robertson, 1993), high RH levels in LDPE bags would minimise water loss. The highest mass loss rates of Teaw leaves were noticed in the treatment which combines with FF3 and 20°C (Figure 2). On Day 3 of the storage period, mass loss percentage of such treatment was ~3.70 which was considered approximately 2-fold higher than the percentages identified of other treatments.

At the same temperature, higher mass loss rates of Teaw leaves packaged in FF3 bags compared to those packaged in LDPE are attributed to film permeability to water vapour, of which the permeabilities of FF3 and LDPE are ~4.50 and ~1.54 $fmol.m.s^{-1}.m^{-2}.Pa^{-1}$, respectively (Boonsiri, 2010). Based these data, higher rate of water vapour transferring across FF3 film as well as higher mass loss rate of Teaw leaves packaged in FF3 bag (mainly contributed by water vapour loss) can be expected, given assumptions on water loss rate and effective skin permeance to water vapour of Teaw packaged in both bags are similar. In study of lime, rambutan and logan at 5-12°C, Boonsiri (2010) reported that there were no significances in effects of FF3 and LDPE film bags on mass loss percentages of which were in a range of 0.5-1.0%. Differences in experimental data reported by Boonsiri (2010) and research findings observed in the present work may be attributed to morphological characteristics of the products i.e. fruit and leafy vegetable. The vegetable have generally higher surface area to volume ratio (SA:V ratio) than the fruit have. For example SA:V ratio of edible leaves and citrus fruit are 5-10 and 0.005-0.015, respectively (Ben-Yehoshua, 1987).

Ascorbic acid

Initial ascorbic acid content of minimally processed Teaw leaves was 14.32 ± 0.01 mg/100g (9 replicates) (Table 1). Ascorbic acid contents of Teaw leaves determined in the present work is comparable to those reported for other vegetables such as red lettuce (18 mg/100g), onion (25 mg/100g) (Klein and Perry, 1982), red cabbage (20 mg/100g) and cauliflower (20 mg/100g) (Padayatty *et al*., 2003), importantly suggesting contributions of Teaw leaves to the diet. Ascorbic acid contents of Teaw leaves continuously declined during the storage period, compared to the content level measured on Day 0 (Table 1), regardless of treatments studied. Degradation of ascorbic acid would be attributed to wounds e.g. taking old leaves out from the rachises occurred during minimally processed Teaw leaves. The wounds subsequently could cause deteriorations of cellular integrity and molecular compartmentations by enzymes resulting in releasing of oxidative enzymes, especially ascorbic acid oxidase (EC 1.10.3.3) which catalyses the oxidation reaction of ascorbic acid to dehydroascorbic

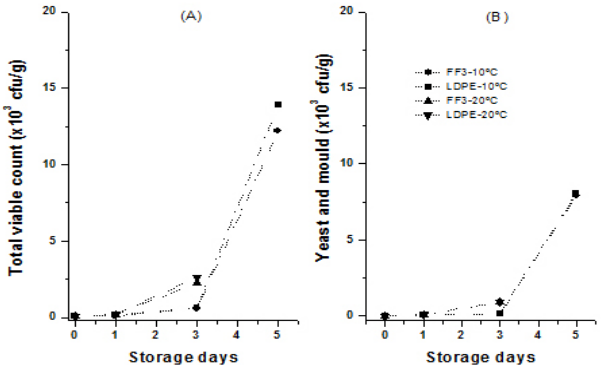

Figure 3. Changes in total viable counts (A) and yeast and mould (B) (Data represented mean with 6 replicates) of Teaw leaves packaged in both LDPE and FF3 bags kept at 10°C and 20°C.

acid (Nakamura *et al*., 1968; Yahia *et al*., 2001).

In spite of the decreasing trend, it can be observed that the ascorbic acid content of leaves in LDPE-20°C apparently increased after 3 days, although the content drastically decreased from Day 0 over 24 hour period (Figure3). Whilst increases in ascorbic acid content of Teaw leaves during storage have not been reported, ascorbic acid contents of intact tomatoes (Kline, 1987), fresh-cut bell pepper (Gonzalez-Aguilar *et al*., 2004) and strawberries (Agar *et al*., 1997) were reported increased during ripening period. Based on such information, maturity of Teaw leaves, particularly a set of LDPE-20°C leaves, could be considered an important factor among others causing an odd trend of changes in ascorbic acid content. The maturity is considered a key variation in horticultural products that can affect chemical and physiological properties of the products during storage periods (Hertog *et al*., 2004).

Whilst the ascorbic contents of Teaw leaves packaged in both types of packaging films were not significantly different, rates of changes in the contents of Teaw leaves packaged in FF3 apparently were lower than those of Teaw leaves packaged in LDPE bags, considered at the same temperature during 3 day storage. FF3 film apparently minimize loss of ascorbic acid content of Teaw leaves (Table 1), although O_2 concentrations in package headspaces of FF3 bags were high under which effects of O_2 on losses of ascorbic acid contents were expected to be pronounced. The lower rates of changes in ascorbic acid contents of Teaw leaves packaged in FF3 would be attributed to lower CO_2 concentrations accumulated in FF3 bags. In contrast, relatively high CO_2 concentrations (~2-6% CO_2) accumulated in LDPE headspace, considered at both storage temperatures. High CO_2 concentrations, for example i.e. 10% v/v, were reportedly stimulating decreases in ascorbic acid levels in fruits including berry fruits, strawberries, black currents and blackberries (Agar *et al*., 1997). Because CO_2 concentrations accumulated in LDPE bag were close to the level reported by Agar *et al*. (1997) (i.e. 10% v/v), effects of CO_2 on ascorbic acid content in Teaw leaves may be presumably similar to those on the fruit noted above.

Key mechanisms underlined such CO_2 effects on ascorbic acid however has not been elucidated. One possible mechanism is through which CO_2 stimulate ethylene generation (possibly through stimulating ACC-oxidase), subsequently increasing ascorbate peroxidase activity that reduces ascorbic acid content (Mehlhorn, 1990; Agar *et al*., 1997). Another involves with increasing pH of cellular cytoplasm due to high CO_2 which could change the compartmentation of ascorbic acid and its interconverting enzyme (Asada, 1992). The study on enzymological and compartmentation aspects involve with ascorbic acid is considered beyond the scope of the present work, yet is required further works to elucidate mechanism of high CO_2 on changes in ascorbic acid content of Teaw leaves.

Total phenolic content (TPC)

Total phenolic content of minimally processed Teaw leaves measured on day 0 was 68.72 ± 2.50 mg GAE/100g FW. The value reported in the present work is considered higher than that reported by Maisuthisakul *et al*. (2007), 38.65 ± 0.50 mg GAE/100g FW. The difference may be attributed to a range of factors including cultivar, maturity and environment conditions such as temperature, water and nutrient availability, and production techniques (Leonardi *et al*., 2000; Dumas *et al*., 2003). The research findings suggests that Teaw leaves appear to have higher TPC in comparisons to some common vegetables and fruits for example lettuce (13.40 mg GAE/100g FW), white cabbage (15.30 mg GAE/100g FW), chilli pepper (41.20 mg GAE/100g FW) (Bahorun *et al*., 2004), mangosteen (54.0 mg GAE/100g FW), and dragon fruit (21.0 mg GAE/100g FW) (Lim *et al*., 2007).

After 24h, TPC measured in all treatments (Table 1) slightly increased from the value reported on Day 0 and there were no significant differences in TPC among treatments. Values of TPC thereafter were apparently stable throughout the storage period, ranged from 70.98-72.74 mg GAE/100g FW. Utto *et al*. (2012) reported a similar trend in changes of TPC values of fresh-cut ripen papaya kept at 10°C. Wang and Stretch (2001) reported increasing TPC of several cultivars of cranberry kept at 0-20°C during 3 months storage. Increasing TPC may be attributed to metabolism of phenolic compounds which can be affected by postharvest storage conditions (Leonardi

et al., 2000; Dumas *et al*., 2003). Environmental or abiotic stresses for examples storage temperature and atmospheric condition can affect phenolic metabolism (Cano *et al*., 2003). Low temperature storage can lead to higher phenolic metabolism (Howard *et al*., 2002; Cisneros-Zevallos, 2003). Increasing TPC among Teaw leaves kept at 10°C (Table 1) accordingly may be induced due to the low temperature.

In the study on cranberry cultivar (Wang and Stretch, 2001), TPC values kept at higher temperatures (15 and 20°C) somewhat higher than those kept at 10°C. Similar trends were reported in anthocyanin contents of cranberry (Wang and Stretch, 2001), strawberries (Kalt *et al*., 1993), and lowbush blueberries (Kalt and McDonald, 1996). Increasing anthocyanin contents reported in those berry fruits were attributed to synthesis of phenolics; both anthocyanin and non-anthocyanin phenolic (Kalt *et al*., 1993). Whilst there are significant effects of storage temperatures on TPC of berry fruits aforementioned, the temperature effects on TPC of Teaw leaves apparently are not clear. Further study requires to investigate relevant biochemical processes including metabolism of phenolics to clarify responds of Teaw leaves to storage temperatures. In addition to storage temperature, the research findings suggest changes of TPC of Teaw leaves may not be dependent on atmospheric conditions developed in package headspaces (Table 1). The findings appear to be consistent to results reported by Aguayo *et al*. (2010) that TPC of fresh-cut Braburn apples were reported reasonably stable (no increasing trend observed) and no differences between TPC of products packaged in either ambient air or modified atmosphere condition (1-5% O_2 and 16-35% CO_2), kept at 4°C for 28 days.

Antioxidant activity

The DPPH assay of antioxidant activity of Teaw leaves on Day 0 was 36.73 ± 0.14 mg AA/100g FW (9 replicates). Antioxidant activities of Teaw leaves continuously decrease during storage period regardless of packaging films and storage temperatures (Table 1). It can be noticed that the pattern of decrease in the antioxidant activity with storage time and temperature followed the same trends as those observed in ascorbic acid (Table 1), indicating that these changes were the result of loss of ascorbic acid content. The finding suggests that ascorbic acid could be the main cause of antioxidant power in fresh Teaw leaves, in agreement with findings in many leafy vegetable such as African basil (*Ocimum gratissimum*) (Oboh, 2005), mustard leaves and cabbage (Padayatty *et al*., 2003), and many fruit such as citrus (Gardner *et al*., 2000; Del Caro et al., 2004), pineapple, rambutan and papaya (Leong and Shui, 2002; Mahattanatawee *et al*., 2006). In Table 1, changes of antioxidant activity of leaves kept in LDPE-20°C, considered on Day 3, appear to be in contrast to the changes in ascorbic acid content. One possible explanation for differences noticed might be explained through variations of leaf samples utilised. However levels of both ascorbic acid content and antioxidant activity measured in all treatments significantly decreased from the levels measured on Day 0, suggesting antioxidant activity of Teaw leaves essentially relates to ascorbic acid content.

There are contrast trends in changes of antioxidant activity and total phenolic content (TPC) (Table 1). Whilst the former continuously decreased, the latter apparently was stable during storage period. The research findings indicate that DPPH inhibition of the radical scavenging may not always be proportional to the concentration of total phenolics. Similar results were reported in for examples berries (Heinonen *et al*., 1998), plant extracts (Kähkönen *et al*., 1999) and fresh-cut apple (Aguayo *et al*., 2010). Such relationship may be attributed to the presence of other components in the extracts for examples vitamin C, aromatic amines, and sulfur containing compounds that possibly react with radicals in addition to phenolic compounds by donating protons (free radical quenching), radical addition, redox reaction (electron transfer) and radical recombination (Yu *et al*., 2002). Lack of changes in the phenolic content relative to the antioxidant activity as reported in fresh-cut apples (Aguayo *et al*., 2010) and Teaw leaves (Table 1) suggests that the level of phenolics is controlled by mechanisms other than ascorbic acid. The results in no significant correlations between TPC and DPPH support the findings aforementioned that ascorbic acid could be the key contributor to antioxidant activity of Teaw leaves.

High rates of losses in the antioxidant activities were noticeable among Teaw leaves packaged in both films stored at 20ºC (Figure 4). These could be attributed to the loss in ascorbic acid content which dramatically decreased under high temperature (Figure 3), due to the fact that ascorbic acid is not stable at high temperature (Nagy and Smooth, 1977). Considered on Day 3 of the storage period, antioxidant activities of Teaw leaves kept at 10ºC were about 1.5-2 fold higher than those of leaves kept at 20ºC, emphasising importance of incorporating the cool chain in Teaw leave storage to delay changes in antioxidant activities.

At 10ºC storage temperature, changes in antioxidant activities of Teaw leaves packaged in FF3 films appeared to be slower than those packaged in

LDPE (apparently observed during 3 days), although there were no significantly different. The pattern of effects of FF3 films on antioxidant activities apparently is similar to that on ascorbic acid. The slow decrease in antioxidant activities in FF3 film accordingly could be attributed to low CO_2 concentration accumulated in the FF3 package headspace whilst the higher CO_2 concentration accumulated in LDPE bags would have influences on antioxidant activity in a manner similar to those discussed on ascorbic acid content (Table 1). Considered on Day 5, it can be observed that antioxidant activities of leaves packaged in both FF3 and LDPE kept at 10ºC slightly increased compared to those measured on Day 3. However there is no significant difference between values of antioxidant activity measured on either Day 3 or Day 5. Similar trend of changes in ascorbic acid contents of leaves in these treatments was observed (Table 1). The research findings support that ascorbic acid is a main cause of antioxidant power of Teaw leaves.

Microbial evaluation

Total viable count and yeast and mould of Teaw leaves in all treatments continuously increased during storage (Figure 3). After 24 h, microbial loads of leaves kept at 20ºC apparently were higher than those kept at 10ºC, regardless of packaging films. The finding supports the existing knowledge on that increasing storage temperature can stimulate microbial proliferations in minimally processed fresh produce (Zagory, 1999; Jacxsens *et al*., 2002). At Day 5 (10ºC), total viable counts of Teaw leaves kept in LDPE bags tended to be higher than those kept in FF3 bags. Boonsiri (2010) reported similar findings on effects of FF3 bags on microbial qualities of rambutan and mangosteen where lower percentages of visual rots were observed among products packaged in FF3 bags compared to those packaged in LDPE bags. The incidence reportedly was attributed to lower humidity accumulated in headspace of FF3 bag than in LDPE bags due to higher WVTR of FF3 film (Boonsiri, 2010).

In Figure 3, there however were unclear difference in yeast and mould counts of Teaw leaves packaged in both FF3 and LDPE bags (considered at Day 5). This may be attributed to the minimal processing technique utilised in the present work that may cause microbial contaminations to the product. Handling during cutting and packing can be important factors among others that can promote yeast and mould proliferation of minimally processed vegetable (Tournas, 2005). Ready-to-eat packaged vegetables such as lettuce, sliced pepper and celery chunk, tend to have high number of yeast and mould population, ranging from 1.6×10^3 cfu/g on iceberg lettuce to 9.2×10^6 cfu/g on sliced tomatoes (Tournas, 2005).

Sensory assessment

Results of sensory assessment for key attributes and overall acceptance of minimally processed Teaw leaves are shown in Table 2. Fresh Teaw leaves packaged in both films kept in 10ºC apparently obtained higher scores particularly in appearances, taste and overall acceptance in comparison to those kept at 20ºC, although there were no significant differences in certain treatments. Teaw leaves kept at 20ºC in LDPE bags had relatively low scores in both appearance and overall acceptance and these were attributed to wilted appearance and condensations on both film surfaces and leaves (data not shown). Leafy vegetables kept under high temperature can have high water loss subsequently causing them wilted (Ezell and Wilcox, 1962; Paull, 1999). High water vapour concentration accumulated in the package headspace caused high equilibrium relative humidity leading to condensation under temperature fluctuations. Both excessive relative humidity inside packages and free liquid water decay since such conditions favour the growth of spoilage organisms (Wills *et al*., 1989).

Visual microbial appearances on Teaw leaves were observed after Day 3 kept at 20ºC making them unmarketable. Considered at 10ºC, fresh Teaw leaves kept in FF3 apparently obtained high scores in all attributes compared to leaves kept in LDPE. Sensory panel studied significantly rated overall acceptances toward Teaw leaves kept in FF3 with higher scores. The findings importantly reflect benefits on FF3 film bags in maintaining quality of minimally processed Teaw leaves.

Conclusion

Active breathable film (FF3 film) packages had significant influences on modified atmosphere conditions at both storage temperatures, i.e. giving high O_2 and low CO_2 accumulations. Such MA condition substantiates the technical knowledge on high permeabilities to O_2 and CO_2 of FF3. The research finding suggests that low CO_2 concentration accumulated in package atmosphere apparently can delay losses of ascorbic acid and antioxidant activities during storage period, especially considered at 10ºC. Utilisations of FF3 bags could lower humidity levels accumulated in package headspace which would be benefits in delaying increases of total viable counts. Such humidity condition however would stimulate mass loss of leaves. Fresh Teaw leaves packaged in FF3 bags obtained higher scores in overall acceptances

from the sensory assessment in comparison to the leaves kept in LDPE bags.

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