Evaluation on Oxidative Stress Total Phenolic Compound Content and Antioxidation Inhibition Power in Variety Colored Vegetables

Pornpimol Muangthai [1], Rasita Wanwe, Natta Cholpitakwong, Peeranut Youpaisarn

Department of Chemistry, Faculty of Science, Srinalharinwirot University, Bangkok, THAILAND.

[1] pornpi @g.swu.ac.th

ABSTRACT

The work aims to study the effect of season change on the oxidative stress of vegetables by analysis the quantity of malondialdehyde.The malondialdehyde was monitored as a marker in varieties colored vegetables. The total phenolic compound content and antioxidant inhibition power in all vegetable samples were also analysed. The varieties colored vegetables were sampled from local market in Thailand. The experimental results presented that malondialdehyde could be detected in all vegetables as low level in the range of 5.76 ± 1.20 – 906.56 ±10.23 ng/g in summer season, 5.04 ± 0.98 – 332.93 ±5.66 ng/g in rainy season and 5.77 ± 1.05 – 771.88 ± 21.54 ng/g in winter season. The total phenolic compound were in the range of of *56.33±7.89 – 1357.60± 25.45 µg/g.All vegetables showed the difference in antioxidation inhibition power in the range of 2.76-22.65 %.The result showed total phenolic compound in vegetables suppressed the occurrence of oxidative stress in vegetables,more over the total phenolic compound content also depend on color of vegetables too. However, this work proved that the oxidative stress also occurred in varieties color vegetables but in low level.*

Keywords: Antioxidant inhibition power, Colored vegetables, Malondialdehyde, Oxidative Stress, Total phenolic compound

INTRODUCTION

Oxidative stress was defined as an imbalance in the homeostatic of cell[1].In general the stress effect may breaks cells and nearby tissue which was known as the stress from the oxidation. Thus the oxidative stress effect lead to the degeneration of human organs such as eyes, kidney, brain and arteries [2-3]and may involves in the development of many diseases such as cancer[4], Parkinson's disease[5], Alzheimer'sdisease[6], atherosclerosis, heartfailure[7],myocardial infarction[8-9],fragile syndrome[10], Sickle Cell Disease[11], lichen planus[12],vitiligo[13], autism[14],infection[15], and chronic fatigue syndrome[16].There are many factors to cause an oxidative stress such as the environment, food and water,radiation,drought ., etc. The oxidative stress also increased the production from oxidizing species or a significant decrease in the effectiveness of antioxidant defenses. The malondialdehyde(MDA) is an important marker that used to evaluate the oxidative stress[15,17]. Malondialdehyde(MDA) is also the products from lipid peroxidation in the system containing lipid.Fruit and vegetables are an important source of many interesting compounds especially phenolic compounds which was referred as the reducer of the risk of referred chronic disease[18]. Antioxidants are the compounds that detoxify reactive oxygen species(ROS) and prevent their damage to cellular macromolecules and organelles through complex mechanisms[19]. Many plants which contain the phenolic compound exhibit antioxidant properties for protection the occurrence of oxidation[20]. Frequently plants grow in many stress situation such as salt stress[21], drought, cooling, flooding[22].In the referred situation effect on proteins damage[23] that change from the common status. There were many research works report about the study of oxidative stress in

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animal or human[24-25]. There was a few reports about the measurement of oxidative stress as malondialdehyde in plant leaves as the work of YunSheng Wang .et. al(2013) [26].However, the oxidative stress study in vegetables are also rarely found. Thus, the aim of this work was to evaluate the oxidative stress in vegetables that sold in 3 seasons(summer, rainy and winter) by analysis the marker as malondialdehyde content in varieties colored vegetables to monitor the variation of oxidative stress. An important substance in plant such as total phenolic compound and antioxidation inhibition power were also studied to estimate an important nutrient in vegetables.The vegetable samples which containing the varieties colored of leaves or skin such as dark green color, pale green color, violet and red color, orange color and yellow color were brought in each seasoning from local market in Thailand. The quantity of malondialdehyde, total phenolic compound and antioxidantion inhibition power in all colored vegetables were analysed to evaluate the effect of seasoning change on the content of each parameters.This result may also concern the temperature effect of wheather on an important substance such as total phenolic compound in edible vegetables.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu reagent, sodium carbonate(AR grade) , Gallic acid(AR grade) , 2, 4-dinitrophenylhydrazine(ARgrade) were purchased from Sigma Chemical Co.,Ltd (St.Louis, Mo,USA).1,3,3-tetramethoxypropane (AR grade) , Ascorbic acid (AR grade) , 1,1-Diphenyl-2-picrylhydrazyl radical, 2,2-Diphenyl-1- (2,4,6-trinitrophenyl) hydrazyl(DPPH) and 2,6-bis (1,1-dimethylethyl) -4-methylphenol(BHT) were purchased from Fluka. 2-thiobarbituric acid(AR grade) , Trichloroacetic acid(AR grade) ,Thiourea (AR grade) were purchased from Carlo Ebra.

Vegetable Samples and Preparation samples

The vegetable samples were divided depend on different color such as

Darkgreen vegetables: ivygour (*Coccinia grandis)L.) Voigt*)(S1) , Swamp Morning Glory(*Ipomcea aquatica Forsk*)(S2) , spinach (*Amaranthus Lividus Linn*.)(S3), Kale (*Brassica alboglabra*) (S4)

Green vegetables: Green Oak Lecttuce (*Lactuca sativa var.crispa L.*) (S5), Cross lecttuce (S6) , Butter Head (S7) , green ceylon spinach (*Basella alba Linn.*)(S8), green eggplant (*Solanum melongena Linn.*)(S9), green oak (S9), cabbage (*Brassica oleracea* L. var. *capitata* L.) (S10) , Chinese Cabbage*(Brassica pekinensis*)(S11) ,Leaf Lectuce(*Lactuca sativa*)(S12) ,GreenYard long Bean(*Vigna unguiculata Hc*)(S13)

Violet and Red vegetables: red coral (*Lactuca sativa var.crispa L.*) (S14), violet eggplant (*Solanum melongena Linn.*)(S15). red cabbage (*Brassica oleracea* L. var. *capitata* L.)(S16), red oak (*Lactuca sativa var.crispa L.*) (S17), Red Yard long bean (*Vigna unguiculata Hc*) (S18) ,red ceylon spinach (*Basella rubra Linn.*)(S19)

Yellow and Orange vegetables: pumpkin (*Cucurbita moschata Decne.*)(S20), carrot (*Daucus carota L.*)(S21), cherry tomato (*Lycopersicon esculentum*) (S22)

All above vegetables were brought from local market in Thailand and immediately placed into an ice box before arriving the laboratory. They were also stored at 4 ^{o}C in the refrigerator before analysis.Those vegetables were chopped into small pieces and weighed approximately 5.0 g. The trichloroacetic acid (TCA) (25 ml of 1% w/v) was added into those vegetables, sonicated for 10 min. The residue plants were filtered out and the filtrated was collected for analysis.

Analysis of Malondialdehyde Content

The method for analysis of malondialdehyde content followed Dourerdjou and Koner, B. C,2008.[30] The 0.5 ml of filtrate was pipetted and mixed with 1.0 ml of 0.5% w/v 2-thiobarbituric acid in 20% TCA. The mixture was heated with control constant temperature at 95 ^{o}C in water bath for 30 min. and immediately cooled in ice bath to room temperature. The mixing solution was centrifuged at10,000 g x for 5 min, and the absorbance of supernatant was recorded by Ultraviolet Visible Spectrophotometer (UV -VIS Shimadzu Model UV100) at 532 nm. The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances (TBARS) and 1,3,3-tetramethoxypropane was used as standard. The content of TBARS was calculated by comparison with the standard curve, and the level of lipid peroxides was expressed as malondialdehyde content in unit of nanogram per gram of wet vegetables.

Analysis of Total Phenolic Compound Content

The analysis method modified from Vasco, C .,et al.,2008 [31],the clear filtrate from part 2.2 in 2.2.1 and 2.2.2 were accuraltely pipetted as 0.4 ml and mixed with 2 ml of 10 % Folin Ciocalteau reagent and 1.6 ml of 7.5 % Na_2CO_3 and kept at room temperature for 30 min. The mixing solution was measured an absorbance at 765 nm by Ultraviolet Visible Spectrophotometer and calculated the Total phenolic compound content as gallic acid equivalent.

Analysis of Antioxidant Inhibition Power

The method for analysis of antioxidant inhibition power was modified from Kapur, A.et. al,2012 [32], the clear filtrate were pipetted 600 µL mixed with 600 µL of 0.1 mM DPPH then measured an absorbance of the complex color from the reaction at 517 nm. The inhibition power was calculated by comparing with standard BHT.

Statistical Analyses

All determinations were carried out at least in triplicate and values were averaged. For all statistics, SPSS and Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

Malondialdehyde Analysis

After the colored vegetable samples in each season were brought and taken to the laboratory to analyse the malondialdehyde marker content as showed in the figure 1.

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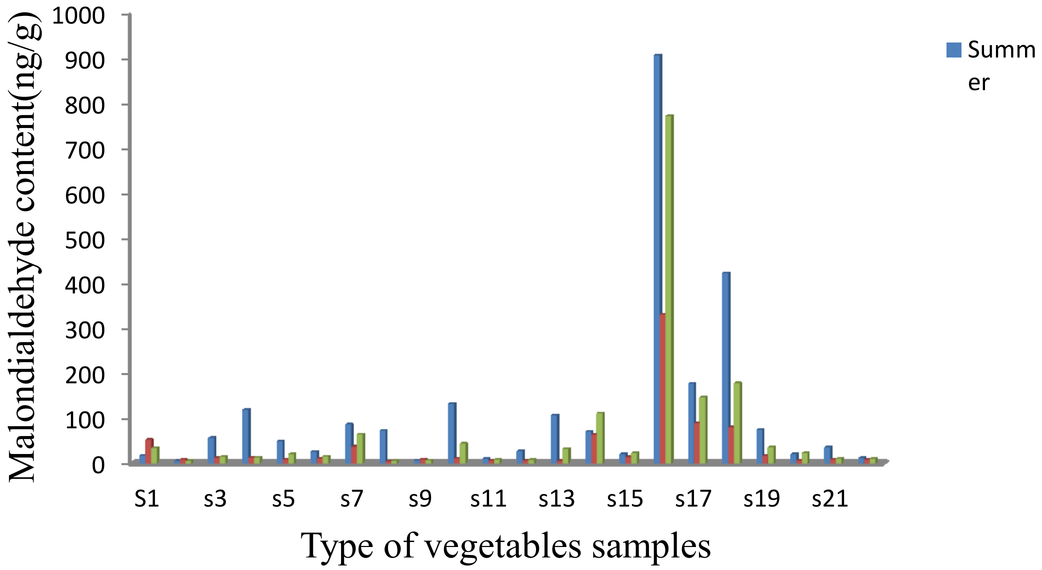

Figure 1. Malondialdehyde content in varietie colored vegetable samples in 3 seasons

Note:

S1= Ivy gour, S2 = Swamp Morning Glory, S3 = Spinach, S4 = Kale,

S5 = green Oak Lecttuce, S6=Cross lecttuce, S7=Butter Head, S8 = green ceylon spinach, S9=green eggplant, S10=Cabbage, S11= Chinese Cabbage, S12=Leaf Lectuce,S13=greenYard long Bean, S14= red coral, S15= violet eggplant,S16 = red cabbage,

S17 = red oak, S18 = red Yard long bean, S19 = red Ceylon spinach, S20 = Pumpkin,

S21= Carrot, S22 = Cherry tomato

Number of each type of colored vegetable sample in each season = 5

From the figure1, the malondialdehyde content in all colored vegetable could be detected in the range of 5.76 ± 1.20 – 906.56 ±10.23 ng/g in summer season, 5.04 ± 0.98 – 332.93 ±5.66 ng/g in rainy season and 5.77 ± 1.05 – 771.88 ± 21.54 ng/g in winter season.The S16 sample which was Red cabbage showed the highest malondialdehyde content in the range of 332.93 ±5.66 - 906.56 ±10.23 ng/g in all seasons, this may related to the nature of red cabage that has strong violet colored and the important substances in it.The S18 which was a Red Yard long bean also showed the high malondialdehyde content in the level of 80.71± 2.58 – 424.45± 8.77 ng/g. Both of reffered vegetables showed the violet –red shade color so the anthocyananine is an important characteristic which also concerned the method of analysis. However, there was not the literature recorded about the malondialdehyde content in both vegetables.The reason about the malondialdehyde value still showed the significant on the occurrence of oxidative stress in each vegetable that grow in all reasons.The result refered as the value of malondialdehyde may depend on nature of the vegetables and status for growth as refered by Huang.G.T.,et al.,2012 [22].

Total Phenolic Compound Analysis

The Total phenolic compound in colored vegetable samples were analysed by Folin Ciocalteau method and calculated as Gallic acid equivalence (GAE) as in figure2.

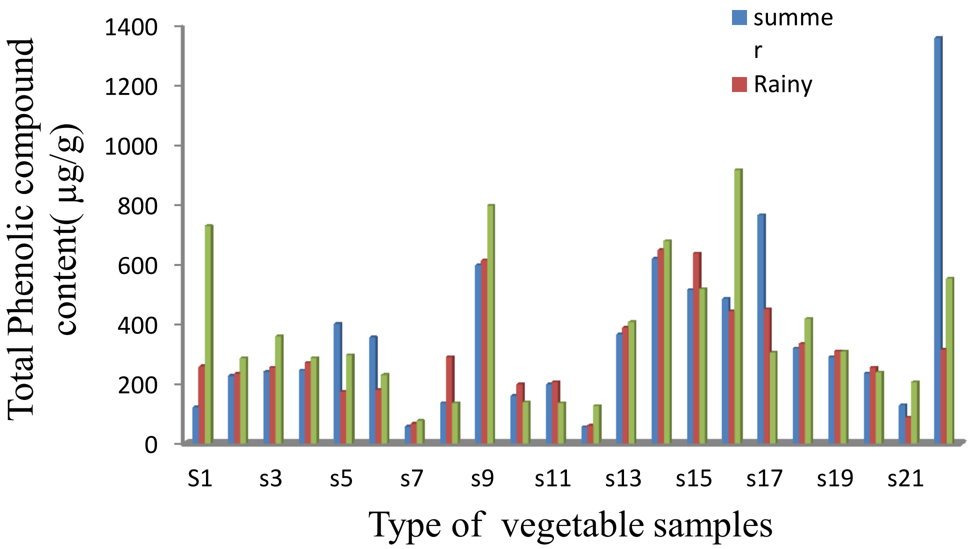

Figure 2. Total phenolic compound content in varietie colored vegetable samples in 3seasons

Note: All abbreviation symbol represented same as in figure1.

Total phenolic compound content in all colored vegetables were found in the range of 56.33±7.89 – 1357.60± 25.45 µg/g.The sample S22 which was Cherry tomato contained the maximum content of Total phenolic compound as 1357.60± 25.45 µg/g, this result supported the nutrient substance in tomato as, phenolic compound mainly chlorogenic acid was the most abundant, others compound such as carotenoids known as lycopene and flavonol as quercetin, rutin[33-35].The factors that effect on the quantity of total phenolic compound in general plants depend on many factors such as type of plant, cultiva, growthing condition, color and time for post harvest[36]. In results from this work also gave the detail about the weather effect of each season since in the figure2,there have the variation of Total phenolic compound content in each colored vegetable. Some vegetable contained the highest Total phenolic compound content in summer season such as Cherry tomato,red Oak, green Oak and Cross, but some sample showed the highest content in winter season such as Ivy gour,Swamp Morning Glory, spinach, Kale, red cabbage, red Yard long bean.

There were 3 types of colored vegetable such as green ceylon spinach, violet eggplant and pumpkin showed the highest Total Phenolic compound content in rainy season.This may related the nature of each plant that cultivated and growth in a range of optimum season, since some plant more better growth in rainy season, some growth best in winter season and some plant may drought resistant and not growth in too much water. Total phenolic compound content may relate to the occurrence of oxidative stress, since some colored vegetable that contained the high content of Total phenolic compound such as Cherry tomato (S22) that have Total phenolic compound in the range of 316.30 ±18.23 - 1357.60± 25.45 µg/g showed thelow level of malondialdehyde content in 8.65 ±1.06 – 12.25±2.45 ng/g .This result also supported the report of Mallo,et al.,2013[37]. Another important nutrient as vitamin C in tomato which was reffered by Raffo A.,et al.,2006 in high level as tomato was 0.14±0.001mg/g[38] could reduced the occurrence of oxidative stress so low content of malondialdehyde was detected.

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Antioxidation Inhibition Power

The last parameter as Antioxidant inhibition power in all colored vegetables was analysed as the percentage unit as shown in figure 3.

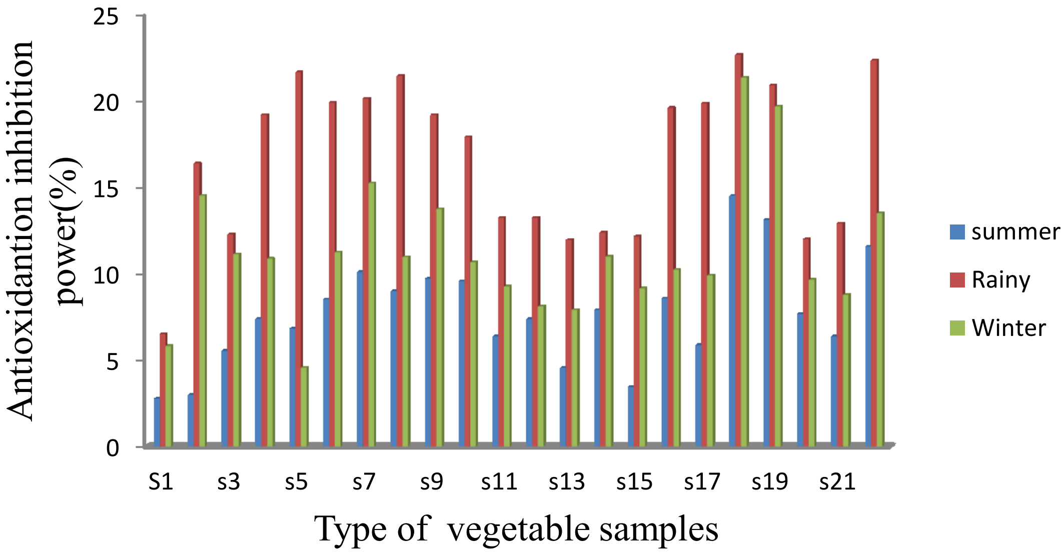

Figure 3. Antioxidation inhibition power in varietie colored vegetable samples in 3seasons

Note: All abbreviation symbol represented same as in figure1.

From the above figure3, almost all colored vegetables showed the highest antioxidant inhibition power in rainy season in the range of 6.5±0.29 – 22.65±0.91%.The 11 types of colored vegetables such as S4-S9,S16-S19 and S22 showed more than 20% of antioxidant inhibition power in rainy season, this also related to the condition for cultivated plant in moisture condition.

In summer season almost vegetables also contained the low level of antioxidant inhibition power in the range of 2-13%, so in this season some plant was not preferred to growth in that status and produce fewer content of important compound. This may explained that almost plants contained an important not only as Total phenolic compound but also have some colored pigment, some vitamin and some antioxidant metal which were known as non phenolic compound in them that showed the antioxidant activity power of plant too[39]. In general,the phenolic compounds are special characteristics that they have at least one aromatic ring hydroxyl-substituted in theirs chemical structure[40]. However, non- phenolic compounds such as ascorbic acid or vitamin C[41] have antiradical behavior as the phenolic compound that enhance the antioxidant inhibition power of plant.This research also supported the report work from Aoun and Makris(2012) [41]

All results especially the Total phenolic compound in all colored vegetables showed significantly related to the occurrence on oxidative stress in vegetable.The vegetables that contained the high Total phenolic content suppressed the occurrence on oxidative stress.However,not only the Total phenolic compound but also non phenolic compound as vitamin in vegetables enhanced the antiradical activity of vegetable to gain higher potential for resistance to oxidative stress thus the marker as malondialdehyde could be detected in the low level of nanogram per gram of vegetable as compared in table 1.

Table 1. The malondialdehyde and Total phenolic compound content in some colored vegetables that cultivated in summer season

Type of Vegetables	*Malondialdehyde (ng/g)*	*Total phenolic compound (µg/g)*
s1	18.02±0.25	122.6±11.03
s2	5.76±1.02	227.55±12.06
s3	56.93±3.4	241.35± 9.05
s4	120.03±10.2	246.4±7.33
s5	49.72±5.22	402.8± 9.87
s6	26.66± 2.66	357.35±2.22
s7	87.19± 5.88	56.33±2.13
s8	73.5±2.55	136.55±5.2
s9	7.21±0.5	600±12.33
s10	133.31±12.46	160.5±7.61
s11	10.81± 3.5	199.05±2.33
s12	27.38±2.11	55.68±5.69
s13	107.37±8.74	368±10.02
s14	71.34±5.7	620.35±9.54
s15	20.89±2.33	516.6±11.30
s16	906.56±15.32	488.1±9.66
s17	178.72±9.44	766.85±8.33
s18	424.45±12.03	321±7.22
s19	75.67±6.39	290.65±6.3
s20	20.89±5.22	235.95±8.41
s21	36.03±8.21	130.2±3.02
s22	12.25±3.02	1357.6±20.33

Note: All abbreviation symbol represented same as in figure1.

The results from table 1 was presented the comparative data between the content of malondialdehyde and Total phenolic compound in summer, that also support confirm the

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result from Shahidi and Wanasundara (1992) [32] which claim that phenolic compound could protect the oxidative stress in plant.

The antioxidation inhibition power of each colored vegetables also support the result from Aoun and Makris (2012) [41]but the antioxidation inhibition power not showed the relationship to the color of vegetable.

CONCLUSIONS

This work showed that the oxidative stress could be detected in all varieties colored vegatables by measurement the malodialdehyde content as a marker.The oxidative stress in all varieties colored vegetables trend to change with the seasonal change and color of vegetable.Almost vegetables got the oxidative stress situation when they cultivated in the summer season but in rainy season they got the least stress.However, the oxidative stress of vegetables were also depend on theirs nature too. Total phenolic compound content in vegetables also related to the seasonal change.In the winter season, there were 13 types of vegetables that showed the highest Total phenolic compound content.The phenolic compound content depend on nature of each plant too, since the phenolic compound in each plant may be the characteristic of some plant example in tomato, the lycopene is an important phenolic compound in it.Thus, the content of phenolic compound depend on the species of plant example as in Lecttuce which varieties types as Green Oak Lecttuce(*Lactuca sativa var.crispa L.*)(S5) and red coral (*Lactuca sativa var.crispa L.*)(S14) , both vegetables contained Total phenolic compound as 402.8± 9.87 and 620.35±9.54 µg/g, respectively.It was noticed that red coral have higher phenolic compound content than green oak. All of the colored vegetables showed the antioxidantion inhibition power in the range of 2.76 – 22.65 % in all season.The antioxidant inhibition power of almost vegetables depend on season and they showed the highest activity in rainy season.More over, same as the both parameters (malondialdehyde and Total phenolic compound) ,the value of antioxidant inhibition power also depend on nature of plant too.

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