葉位與採收期對葉菜甘藷葉片之總酚含量及 DPPH 自由基清除能力之影響

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摘要

甘藷葉部富含生物活性化合物，如維生素、蛋白質、礦物質、抗氧化物質、膳食纖維、必需脂肪酸等有益於健康的成分，其中抗氧化物質之總酚日漸受到重視。本研究旨在調查葉菜甘藷台農 71 號不同葉位及採收期對總酚及 DPPH 自由基清除能力之影響。研究結果顯示，春作及秋作皆以頂芽及展開葉第一葉擁有最大含量之總酚物質，其餘葉位則次之。而 DPPH 自由基清除能力亦有相似之變化情形，以頂芽及展開葉第一葉擁有最佳清除率，採收期僅對春作 DPPH 自由基清除能力有顯著影響，對總酚含量則無顯著影響。

關鍵詞：甘藷葉、葉位、採收期、總酚、DPPH 自由基清除能力。

Effects of Leaf Position and Harvest Time on Total Phenolics Content and Free Radical Scavenging Activity of DPPH of a Leafy Sweet Potato

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ABSTRACT

Leaves of sweet potato are rich in bioactive compounds, including vitamins, proteins, minerals, antioxidants, dietary fibers, essential fatty acids and other healthful elements. In particular, the total phenolics content has received much attention worldwide recently. This study aimed to investigate the effects of leaf positions and harvest time on the total phenolics content and the free radical scavenging activity of DPPH. Results indicated that apical bud and the first fully expanded leaf had the maximum total phenolics content in both spring and fall crops. Similar results were found in free radical scavenging activity, both apical bud and the first fully expanded leaf had the best scavenging activity of DPPH. Further, free radical scavenging activity of DPPH in Spring Crop was significantly affected by harvest time, but not in total phenolics content.

Key words: Sweet potato leaf, Leaf position, Harvest time, Total phenolics content, Free radical scavenging activity of DPPH.

前言

甘藷 *Ipomoea batatas* (L.) Lam.適合在臺灣氣候條件下生產，不受季節限制。近年由於消費型態改變及科學研究進步，過去未被重視的甘藷莖葉在許多報告中皆被提及富含生物活性化合物(bioactive compounds)，包括維生素、蛋白質、礦物質、抗氧化物質、

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投稿日期：2012 年 5 月 18 日
接受日期：2012 年 6 月 20 日
作物、環境與生物資訊 9:98-107 (2012)
Crop, Environment & Bioinformatics 9:98-107 (2012)
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膳食纖維、必需脂肪酸等(Huang *et al*. 2006, Liao *et al*. 2011)。臺灣葉菜用甘藷台農 71 號可週年栽培，適合機械採收，且採收期長，約 2 至 4 週即可開始採收，嫩梢葉質地細柔適口性佳(Lee *et al*. 1996, Lai 2007)。

隨著養生風氣的盛行，許多學者乃進行抗氧化物質之研究，而甘藷莖葉即為一富含多種生物活性化合物的蔬菜，此類抗氧化物質在許多研究中更顯示與抗氧化作用呈現正相關。經食用後可在人體內轉化並維持生理機能，所以食物中含有的抗氧化物可以直接被吸收，具有降低人體內自由基氧化傷害的效果(Kano *et al*. 2005, Huang *et al*. 2006, Teow *et al*. 2007, Carvalho *et al*. 2010, Johnson and Pace 2010, Liao *et al*. 2011)。然而作物在生長發育中有不同表現，研究顯示葉位對作物的抗氧化物質含量、自由基清除能力在不同葉位存有差異，如萵苣及茶葉之報告皆顯示其抗氧化物質含量及活性受到葉位及採收期影響，且差異顯著(Chen *et al*. 2003, Cano and Arnao 2005, Chiu and Lin, 2005, Mamati *et al*. 2006)。

本試驗主要在探討葉位及採收期對葉菜用甘藷台農71號葉片總酚含量及DPPH自由基清除能力的影響，期供未來決定較佳採收時間及分級分類之參考。

材料與方法

一、試驗材料及取樣方法

本試驗以由行政院農業委員會農業試驗所(以下簡稱'農試所')選育之葉菜用甘藷品種台農 71 號(TNG 71)為試驗材料，春作於 2011 年 4 月種植，秋作則於同年 9 月種植，兩期作皆栽植於農試所嘉義農業試驗分所之試驗田區，試區共三塊。栽培及管理採用一般慣行法，扦插初期灌溉後，視田間土壤濕度進行適度灌溉，以不超過田間容許量為度。生長期間不施用肥料，試區之土壤 pH 值介於 4.79~5.05，有機質含量介於 1.83~1.93 %，有效性磷、鉀、鈣及鎂分別介於 45~55 mg kg^{-1}、20~27 mg kg^{-1}、488~509 mg kg^{-1} 及 59~63 mg kg^{-1}。種植 4 wk 後採摘一次，經隔 4 wk 再採摘一次，共採摘 2 次。採集以逢機方式進行，為完全隨機設計，三重覆。

二、甘藷葉檢品溶液之製備

分別秤取甘藷不同葉位之葉片鮮重 3 g，並加入 15 mL 80% 甲醇 (liquid chromatography, Merck)溶液且置於超音波儀器中震盪 15 min，取上清液離心 30 min，加速度為 400 g，再取一次上清液以 0.22 μm 微孔過濾膜過濾後，將 80%甲醇萃取液定量至 5 mL 存於樣品瓶中並儲藏於-20℃冰箱中。

三、總酚含量分析

參考 Taga *et al.* (1984)方法，檢測甘藷葉片 80%甲醇萃取液之抗氧化物質含量：取 200 μL 甘藷葉片萃取液於抽風櫥內加入 200 μL Folin-Ciocalteus (2N, Merck)，振盪後置於室溫下作用 5 min，再加入 3 次水稀釋後加入 600 μL 的 20%碳酸鈉(GR, Merck)水溶液，室溫下靜置 10 min 後再離心 10 min，加速度為 400 g，同時以 gallic acid (HPLC, Fluka)作標準曲線於波長 730 nm 測吸光值。

四、DPPH 自由基清除能力試驗

參考 Shimada *et al.* (1992)方法，檢測甘藷葉片 80%甲醇萃取液對 DPPH (≧90, Sigma)之清除效率：取 200 μL 甘藷葉片甲醇萃取液加入 600 μL DPPH 甲醇溶液中，均勻混合 30 min 後，同時以 Trolox (≧98%, Aldrich)作標準曲線於波長 517 nm 下測吸光值。

結果與討論

一、葉位對總酚含量及 DPPH 自由基清除能力之影響

2011 年葉菜用甘藷春作之葉位總酚含量變方分析結果如 Table 1，由變方分析結果發現葉菜甘藷台農 71 號葉位對於總酚含量及

DPPH 自由基清除能力均有顯著性影響，而秋作之葉位總酚含量變方分析結果如 Table 2，亦發現葉位對於總酚含量及 DPPH 自由基清除能力均有顯著性影響，顯示葉菜用甘藷無論在春作或秋作，總酚含量及 DPPH 自由基清除能力皆受不同葉位之顯著影響。

春作葉位間之總酚含量及 DPPH 自由基清除能力差異顯著性測驗結果如 Table 3，就春作採收之葉菜用甘藷總酚含量而言，以頂芽之 54.2 mg gallic acid 100 g^{-1} 最高，其次為第一葉之 50.4 mg gallic acid 100 g^{-1}，而以第六葉之 2.40 mg gallic acid 100 g^{-1} 最低，次低者為第三葉之 3.72 mg gallic acid 100 g^{-1}、第五葉之 3.79 mg gallic acid 100 g^{-1}、第四葉之 3.88 mg gallic acid 100 g^{-1} 及第二葉之 3.95 mg gallic acid 100 g^{-1}，而此五者與前二者(頂芽、第一葉)有顯著差異存在。而秋作之總酚含量及 DPPH 自由基清除能力差異顯著性測驗結果如 Table 4，表現相似。

就春作採收之葉菜用甘藷 DPPH 自由基清除能力而言，以頂芽及第一葉之 118.8 mg Trolox 100 g^{-1} 及 113.7 mg Trolox 100 g^{-1} 最佳，其次為第四葉之 61.7 mg Trolox 100 g^{-1}，而以第六葉之 27.8 mg Trolox 100 g^{-1} 最差，次差者為第五葉之 43.1 mg Trolox 100 g^{-1}、第三葉之 43.6 mg Trolox 100 g^{-1} 及第二葉之 51.0 mg Trolox 100 g^{-1}，而此五者與前二者(頂芽、第一葉)有顯著差異存在。而秋作表現相似。無論在春作或秋作，其最大總酚含量及最佳 DPPH 清除率皆存於頂芽及第一葉，與其餘葉位均有顯著差異存在。

葉位之相關研究中，Cano and Arnao (2005)調查三種不同萵苣品種之抗氧化活性的差異，以及不同葉位(莖部、內葉、中葉、

Table 1. ANOVA of total phenolics content and free radical scavenging activity of DPPH in different leaf positions and harvest times in Spring Crop.

SOV	DF	MS	
		Total phenolics content	Free radical scavenging activity of DPPH
Leaf position (L)	6	3416.6***	7795.1***
Harvest time (H)	1	0.709	488.4**
L x H	6	3.272	472.9***
Error	28		
Total	41		

*, ** and *** denote significant at 5%, 1% and 0.1%, respectively.

Table 2. ANOVA of total phenolics content and free radical scavenging activity of DPPH in different leaf positions and harvest times in Fall Crop.

SOV	DF	MS	
		Total phenolics content	Free radical scavenging activity of DPPH
Leaf position (L)	6	3892.7***	6069.5***
Harvest time (H)	1	4.76	5.23
L x H	6	13.07***	482.9
Error	28		
Total	41		

*, ** and *** denote significant at 5%, 1% and 0.1%, respectively.

Table 3. Significant tests of differences among leaf positions in total phenolics content and free radical scavenging activity of DPPH in Spring Crop.

Leaf position	Total phenolics content (mg Gallic acid 100 g^{-1} fw)	Free radical scavenging activity of DPPH (mg Trolox 100 g^{-1} fw)
Apical bud	54.2[a*]	118.8[a]
1st leaf	50.4[b]	113.7[a]
2nd leaf	3.95[c]	51.0[c]
3rd leaf	3.72[cd]	43.6[c]
4th leaf	3.88[c]	61.7[b]
5th leaf	3.79[cd]	43.1[c]
6th leaf	2.40[d]	27.8[d]
$LSD_{0.05}$	1.47	9.13

* Values with the same letters in each column are not significant difference at P ≦0.05 by LSD test.

Table 4. Significant tests of differences among leaf positions in total phenolics content and free radical scavenging activity of DPPH in Fall Crop.

Leaf position	Total phenolics content (mg Gallic acid 100 g^{-1} fw)	Free radical scavenging activity of DPPH (mg Trolox 100 g^{-1} fw)
Apical bud	60.4[a*]	117.8[a]
1st leaf	50.3[b]	108.1[a]
2nd leaf	5.49[c]	61.2[b]
3rd leaf	3.20[d]	52.1[bc]
4th leaf	3.40[d]	41.6[c]
5th leaf	3.40[d]	50.5[bc]
6th leaf	2.22[d]	41.9[c]
$LSD_{0.05}$	1.78	12.7

* Values with the same letters in each column are not significant difference at P ≦0.05 by LSD test.

外葉)間之總酚含量，其結果顯示葉位之脂溶性抗氧化物質有差異存在，推估其對成熟之葉有保護作用；而茶葉研究中亦發現抗氧化成份受不同葉位之影響，Chiu and Lin (2005)採摘茶樹青心烏龍之一心二葉(幼葉)及第 10 至 15 片完全展開葉(老葉)，以 HPLC 分析茶多酚(polyphenolic)之含量，指出在青心烏龍幼葉中含有較高之表沒食子兒茶素及沒食子酸酯 (epigallocatechin gallate; EGCG)；Mamati *et al.* (2006)指出在小葉種茶樹中之表沒食子兒茶素(epigallocaechin; EGC)及表兒茶素沒食子酸酯(epicatechin gallate; ECG)含量隨葉齡增加而下降，而 EGCG 在第一葉及第二葉含量最高；本試驗結果顯示，無論春作或秋作，頂芽總酚含量皆為最高，其次為第一葉，相較之下，下位葉之總酚含量較低。推估可能之因素有二：(1)此顯著差異存在之原因乃是初生之嫩芽及第一葉對環境耐受性較差，易受栽培環境影響，故植體將酚類物質累積於此以增加其對環境之耐受性；(2)頂芽及第一葉易受環境刺激，進而誘導酚類物質合成，隨成熟葉之表皮發育逐漸完全而使總酚物質逐漸被分解而減少。惟目前尚無相關之研究，仍有待未來進行試驗以進一步探討。

二、採收期及葉位與採收期交感對總酚含量及 DPPH 自由基清除能力之影響

春作葉菜用甘藷不同採收期之總酚含量變方分析結果如 Table 1，結果顯示採收期對總酚含量未有顯著影響，然採收期對 DPPH

自由基清除能力則有顯著影響。而葉位與採收期交感對春作之 DPPH 自由基清除能力(Table 1)，以及秋作之總酚含量，則有顯著影響(Table 2)。

春作不同採收期之總酚含量及 DPPH 自由基清除能力差異顯著性測驗結果如 Table 5，以春作第二次採收之 69.1 mg Trolox 100 g^{-1} 較佳，其次為第一次採收之 62.3 mg Trolox 100 g^{-1}，兩者間有顯著差異存在。春作葉位及採收期之交感對總酚含量及 DPPH 自由基清除能力之差異比較試驗結果如 Table 6，DPPH 自由基清除能力之最佳交感條件為頂芽之第一次和第二次採收及第一葉之第一次和第二次採收，再者為第四葉之第一次和第二次採收、第二葉之第一次和第二次採收及第三葉之第二次採收，而以第三葉及第五葉之第一次採收、第六葉之第一次及第二次採收之 DPPH 自由基清除能力最差。

秋作葉位及採收期之交感對總酚含量及 DPPH 自由基清除能力之差異比較試驗結果如 Table 7，秋作總酚含量之最佳交感條件為頂芽之第一次和第二次採收，其次為第一葉

Table 5. Significant tests of differences between harvest time in total phenolics content and free radical scavenging activity of DPPH in Spring Crop.

Harvest time	Total phenolics content (mg Gallic acid 100 g^{-1} fw)	Free radical scavenging activity of DPPH (mg Trolox 100 g^{-1} fw)
1st harvest time	17.3	62.3^{b*}
2nd harvest time	17.6	69.1^{a}
$LSD_{0.05}$	ns	4.88

* Values with the same letters in each column are not significant difference at P ≦ 0.05 by LSD test.

Table 6. Significant tests of differences among leaf position and harvest time combinations in phenolics content and free radical scavenging activity of DPPH in Spring Crop.

L x H^{x}	Total phenolics content (mg Gallic acid 100 g^{-1} fw)	Free radical scavenging activity of DPPH (mg Trolox 100 g^{-1} fw)
Apical bud, lst harvest time	53.2	119.0^{a*}
Apical bud, 2nd harvest time	55.2	118.6^{a}
1st leaf, lst harvest time	51.7	114.5^{a}
1st leaf, 2nd harvest time	49.2	112.9^{a}
2nd leaf, lst harvest time	3.9	49.0^{c}
2nd leaf, 2nd harvest time	4.00	53.1^{c}
3rd leaf, lst harvest time	2.6	25.8^{d}
3rd leaf, 2nd harvest time	4.1	61.4^{bc}
4th leaf, lst harvest time	4.1	68.9^{b}
4th leaf, 2nd harvest time	3.7	54.5^{c}
5th leaf, lst harvest time	3.2	29.7^{d}
5th leaf, 2nd harvest time	4.4	56.4^{bc}
6th leaf, lst harvest time	2.4	29.0^{d}
6th leaf, 2nd harvest time	2.4	26.6^{d}
$LSD_{0.05}$	ns	14.2

* Values with the same letters in each column are not significant difference at P ≦0.05 by LSD test.
x L x H: Interaction of leaf position and harvest time.

Table 7. Significant tests of differences among leaf position and harvest time combinations in phenolics content and free radical scavenging activity of DPPH in Fall Crop.

L x H[x]	Total phenolics content (mg Gallic acid 100 g^{-1} fw)	Free radical scavenging activity of DPPH(mg Trolox 100 g^{-1} fw)
Apical bud, lst harvest time	60.6[a*]	119.3
Apical bud, 2nd harvest time	60.3[a]	116.2
1st leaf, lst harvest time	53.7[b]	108.0
1st leaf, 2nd harvest time	46.8[c]	108.1
2nd leaf, lst harvest time	4.1[e]	48.6
2nd leaf, 2nd harvest time	6.9[d]	73.8
3rd leaf, lst harvest time	3.4[e]	65.9
3rd leaf, 2nd harvest time	3.0[e]	38.3
4th leaf, lst harvest time	3.3[e]	33.5
4th leaf, 2nd harvest time	3.5[e]	36.3
5th leaf, lst harvest time	3.5[e]	58.6
5th leaf, 2nd harvest time	3.3[e]	42.4
6th leaf, lst harvest time	2.1[e]	41.9
6th leaf, 2nd harvest time	2.3[e]	42.0
$LSD_{0.05}$	2.5	ns

* Values with the same letters in each column are not significant difference at P ≦0.05 by LSD test.
[x] L x H: Interaction of leaf position and harvest time.

之第一次採收，再者為第一葉之第二次採收、第二葉之第二次採收，其餘葉位皆次之且無顯著差異。

採收期之相關研究中，Liu *et al.* (2007)調查萵苣在不同採收期之總酚含量及其抗氧化活性，結果顯示其總酚含量與抗氧化活性皆存在顯著差異，以七月採摘萵苣之總酚含量及抗氧化活性較九月採摘為佳，學者推估萵苣之總酚含量及抗氧化活性受到環境之影響。在茶樹採摘時期及抗氧化成分研究中，顯示抗氧化成份受不同採摘時期之影響，Chiu and Lin (2005)發現在四季中兒茶素 EGCG 變化以秋冬季高於春夏季之含量。Huang *et al.* (2003)以青心大冇 四季精製茶為試驗材料，比較不同採收季節兒茶素含量之變化，以 HPLC 調查茶多元酚之含量，發現冬茶(110.6 mg g^{-1})與夏茶(110.4 mg g^{-1})的兒茶素含量較高，而秋茶(105.5 mg g^{-1})及春茶(99.0 mg g^{-1})較低。Wu *et al.* (2007)採摘一心二葉茶菁觀察不同採摘季節間兒茶素含量之變化，發現總兒茶素、EGCG 及 EGC 之含量變化均以夏季含量顯著低於其他季節。本試驗之結果僅發現採收期對春作葉菜用甘藷台農 71 號有顯著影響，且未發現採收期對總酚含量變化之規律。推估葉菜用甘藷台農 71 號之葉片可能對環境變異較不敏感，然目前尚無相關之研究可供比較，未來仍須繼續進一步探討其他葉菜甘藷品種之採收期對總酚含量之影響。

三、總酚含量與自由基清除能力之關係

春作葉菜用甘藷之總酚含量與自由基清除能力之相關分析結果如 Fig. 1，結果顯示總酚含量與抗氧化能力呈正相關，從圖中可得知總酚含量愈高者，自由基清除能力愈佳。相較之下，總酚含量愈低者，自由基清除能力較弱且不穩定，秋作亦有相似之情形(Fig. 2)。Huang *et al.* (2006)指出 DPPH 自由基清

除能力與總酚含量呈現極顯著正相關(r = 0.71**)，而 Padda and Picha (2008)亦指出抗氧化活性與總酚含量呈現極顯著正相關(r = 0.98**)，兩項研究中總酚含量的多寡與抗氧化活性能力之間具有高度相關性，與本試驗之結果相似(Fig. 1 及 Fig. 2)。

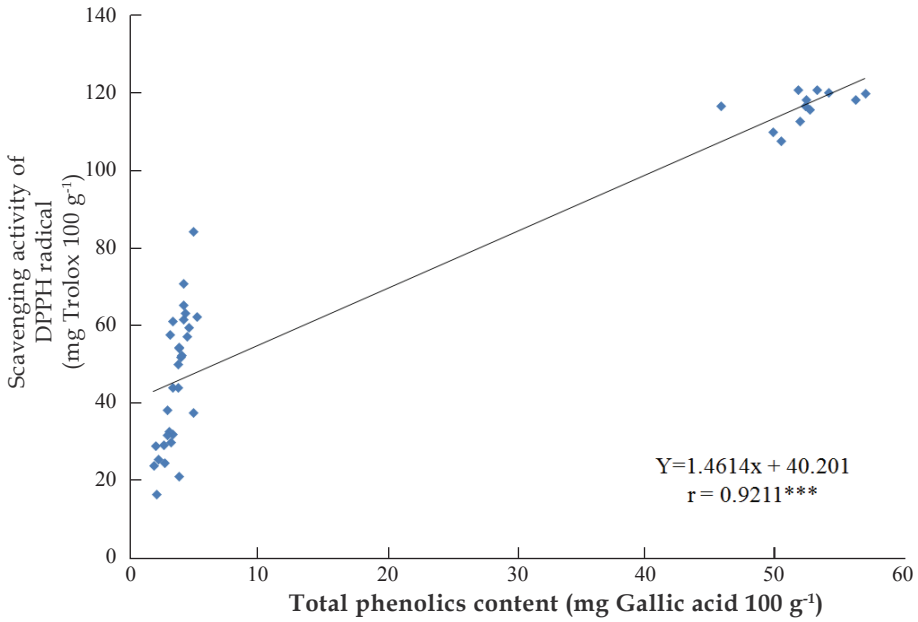


Fig. 1. Correlation between total phenolics content and free radical scavenging activity of DPPH in Spring Crop.

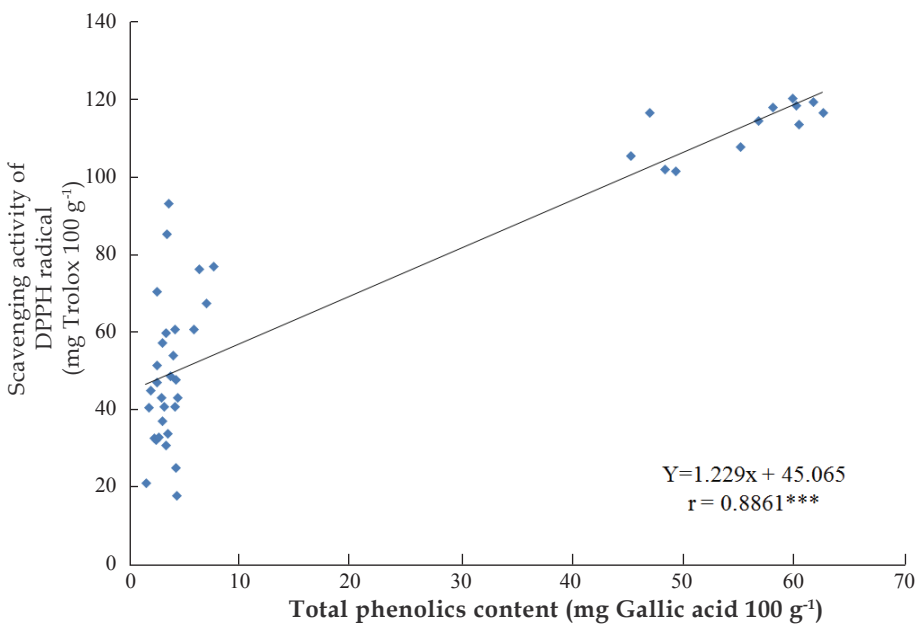


Fig. 2. Correlation between total phenolics content and free radical scavenging activity of DPPH in Fall Crop.

在甘藷葉部抗氧化活性研究中，Huang *et al.* (2006)和 Liao *et al.* (2011)提及除了總酚含量對自由基清除能力有差異之外，水溶性抗氧化物質、脂溶性抗氧化物質及其他酚酸衍生物在自由基清除率及機能性皆有差異存在(Islam *et al*. 2003, Teow *et al*. 2007)，且品種(系)間之抗氧化能力受其他酚類化合物及胡蘿蔔素含量影響，因此具有不同程度的抗氧化能力(Teow *et al*. 2007)。此外，Laura *et al.* (2004)和 Carvalho *et al.* (2010)報告中提及葉片受到強光照射或逆境影響時會啟動合成花青素等，學者推測光照能激活多酚類化合物合成基因之表現，使甘藷莖葉在長時間及長日照處理下，葉片外觀生成紫色斑點，其作用被視為光保護效應，可協助植物產生抗性，進而適應生長環境(Merzlyak and Chivkunova 2000, Ramirez-Tortosa *et al*. 2001, Metwally *et al*. 2003, Albert *et al*. 2009)。本試驗之結果顯示總酚含量愈高者，其 DPPH 自由基清除能力愈佳，而下位葉之 DPPH 自由基清除能力變異大之原因推估有二：(1)下位葉之總酚物質隨表皮發育完善而逐漸轉化成其他酚酸物質，而不同酚酸間之抗氧化能力有差異存在；(2)抗氧化物質之極性可能隨發育時間增加而轉化，進而適應環境之變異，然本試驗萃取時僅使用 80%甲醇作為溶劑，未來可針對抗氧化物質之極性變化加以探討。

許多報告中皆指出蔬菜具有維持人體生理機能之功效，其中酚類物質是研究最廣泛的次級代謝物之一。由於其具有天然抗氧化活性，在許多研究中證實此類物質對人體有益，具有清除自由基、增強免疫系統、降低脂質過氧化、抑制低密度膽固醇、消炎、降低血醣等化學預防作用(chemoprevention)，更可幫助人體代謝致癌物質或其產物(Chen *et al*. 2005, Jung *et al*. 2006, Huang *et al*. 2007, Chen *et al*. 2008)。本試驗結果顯示，葉位對總酚含量及 DPPH 自由基清除能力有顯著影響，採收期則未對總酚含量有顯著影響，而葉位及採收期交感對春作之 DPPH 自由基清除能力及秋作之總酚含量有顯著影響。未來隨著科學及生物技術的進步，逐漸釐清酚類物質的種類、含量、結構、代謝途徑及基因調控模式，將有助於酚類物質的合成及利用。又隨著萃取技術之進步，未來可望增加提取酚類物質之效率與純度並廣泛應用於生活保健用途。

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