Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 140–147
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation

Jin-Yuarn Lin *, Ching-Yin Tang

Nutritional Immunology Laboratory, Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC

Received 24 August 2005; received in revised form 21 December 2005; accepted 17 January 2006

Abstract

This study selected 13 fruits and vegetables to determine their total phenolic and flavonoid contents and their stimulatory effects on splenocyte proliferation from female BALB/c mice. The highest total phenolic content was observed in mulberry (1515.9 ± 5.7 mg gallic acid equivalents (GAE)/100 g fresh matter (FM)) among four selected fruit species. The highest total phenolic content was observed in a variety of red onions (310.8 ± 4.9 mg GAE/100 g FM) among nine selected vegetable species. The highest total flavonoid content was observed in mulberry (250.1 ± 6.3 mg quercetin equivalents (QE)/100 g FM) among the selected fruits. The highest total flavonoid content was observed in ceylon spinach (133.1 ± 26.2 mg QE/100 g FM) among the selected vegetables. The mulberry, strawberry and red onion demonstrated an immuno-modulatory potential via stimulating splenocyte proliferation. Bitter melon showed a significantly ($P < 0.05$) negative correlation with splenocyte proliferation. Their immuno-modulatory components are highly correlated with phenolics, including flavonoids. The total phenolic contents in all selected fruits and vegetables significantly correlated with splenocyte proliferation in vitro.

Keywords: Phenolics; Flavonoids; Splenocyte proliferation; Strawberry; Mulberry; Red onion; Bitter melon

1. Introduction

Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruits, and vegetables and the risks for chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Chen et al., 2005; Middleton, Kandaswami, & Theoharides, 2000; Prior, 2003; Saleem, Husheem, Harkonen, & Pihlaja, 2002; Zhang, Vareed, & Nair, 2005). These physiological functions of fruits and vegetables may be partly attributed by their abundance of phenolics. Deep-colored vegetables and fruits are good sources of phenolics, including flavonoids and anthocyanins, and carotenoids (Cieslik, Greda, & Adamus, 2006; Qian, Liu, & Huang, 2004; Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005; Trappey, Bawadi, Bansode, & Losso, 2005). More than 4000 known flavonoids, which are one class of plant polyphenols, have been found (Merken & Beecher, 2000). Dietary flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones, and flavonols which responsible for the orange, red and blue colors in fruits and vegetables (Merken & Beecher, 2000). Traditionally, deep-colored fruits, vegetables or foods are recognized as more healthy to human body, especially in the oriental countries. There has been a growing interest in pigment components of fruits and vegetables, which may promote human health or lower the risk for disease.

Recent studies have focused on health functions of phenolics, including flavonoids and anthocyanins, and carotenoids from fruits and vegetables. It has been found

* Corresponding author. Tel./fax: +886 4 22851857.
E-mail address: jinlin@nchu.edu.tw (J.-Y. Lin).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.01.014

that the crude extract and the phenolics of *Terminalia chebula* retz. fruit, commonly known as black Myroblans, inhibit the growths of human cancer cell lines (MCF-7, HOS-1, PC-3, and PNT1A) and mouse breast cancer cell line (S115) in vitro (Saleem et al., 2002). Polyphenols, including anthocyanins and hydroxycinnamic acids, isolated from both blueberry and cranberry are found to protect endothelial cells against H_2O_2 and TNF-α induced up-regulation of oxidative and inflammatory insults (Youdim, McDonald, Kalt, & Joseph, 2002). The main phenolic compounds in different polar extracts from boxthorn (*Lycium chinense* Mill) fruits, such as rutin (a flavonol), chlorogenic acid, protocatechuic acid, serve as a free radical scavenger against DPPH (Qian et al., 2004). Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibit an inhibitory effect on the migration and invasion of the human lung cancer cell line (Chen et al., 2006). Anthocyanidins, the aglycones of anthocyanins, which impart brilliant colors to many fruits and vegetables also inhibit the growth of human cell lines, including AGS (stomach), HCT-116 (colon), MCF-7 (breast), NCI H460 (lung), and SF-268 (central nervous system, CNS) (Zhang et al., 2005). A human intervention study concerning the consumption of carotenoid-rich vegetables shows that a low-carotenoid diet reduces T-lymphocyte functions and supplementation of tomato juice restores these functions, such as cell proliferation and the secretion of immuno-reactive cytokines (Watzl, Bub, Brandstetter, & Rechkemmer, 1999). Many researches have unraveled the health functions of pigment components from fruits or vegetables, however, the study on immuno-modulatory potentials of deep-colored fruits and vegetables is still limited.

It has been found that most of the Chinese medicinal ingredients promote cellular or humoral immune responses by promoting lymphocyte proliferation and serum antibody titer (Kong, Hu, Rui, Wang, & Li, 2004). We have found that *Amaranthus spinosus* Linn. (red-colored thorny amaranth), a plant that grows in the wild fields of Taiwan and is extensively served as Chinese traditional medicine or wild vegetables to treat diabetes, leads to B lymphocyte activation and subsequent T cell proliferation in vitro (Lin, Chiang, & Lin, 2005). Searching for immuno-modulatory materials from dietary fruits and vegetables and characterizing the immune enhancement effects may have great potential for future practical applications for human health and immunopharmacology.

We hypothesized that deep-colored food materials are phenolic-rich, especially flavonoid-rich, and have potential in immuno-modulation. Therefore, 13 varieties of deep-colored fruits and vegetables cultivated in Taiwan were selected to determine their total phenolic and flavonoid content and evaluate their stimulatory effects on the proliferation of splenocytes from female BALB/c mice. This work also attempted to find the relationships between the total phenolic and flavonoid contents in fruits and vegetables and their immuno-modulatory effects on splenocyte proliferation.

2. Materials and methods

2.1. Collection and preparation of deep-colored fruit and vegetables samples

Four deep-colored fruit species were collected for this study. These fruits included one variety of *Fragaria ananassa* (strawberry), one variety of *Eriobotrya japonica* (loquat), one variety of *Morus alba* (mulberry), and one variety of *Prunus salicina* (oriental plum). The selected fruits were purchased from a supermarket in Taichung, Taiwan in December 2002.

Nine deep-colored vegetable species were collected for this study. These vegetables included three varieties of *Allium cepa* L. (two varieties of red onion and one variety of white onion (using as a color control)), one variety of *Basella rubra* L. (ceylon spinach), one variety of *Beta vulgaris* L. (beetroot), one variety of *Monordica charantia* L. (bitter melon), and three varieties of *Capsicum annuum* L. (including green, red and yellow colors of sweet pepper). Nine deep-colored vegetable species were cultivated in Taiwan and collected in November 2002. Two varieties of red onions (seed Nos. 215–216) were provided by Yuh-Long seed Co., Ltd., Pingtung, Taiwan, ROC. The remainders of the selected vegetables were purchased from a supermarket in Taichung, Taiwan.

The fresh samples were purchased and immediately, without storage, squeezed to juices. The edible portions of deep-colored fruits and vegetables were weighed, washed and chopped, respectively, to squeeze fruit and vegetable juices by a manual stainless screw squeezer (Vegetable and Fruit Grinder, manual type, Mei-Er-Then Co., Ltd., Taipei, Taiwan, ROC). The juices were centrifuged at 10,000*g* (4 °C) for 30 min, and then the supernatants were collected using suction filtration through filter papers (Toyo No. 5B). The filtrates were measured, lyophilized, and stored at −20 °C for future use.

2.2. Determination of total phenolic and flavonoid contents

Total phenolic contents of fruit and vegetable samples were determined by the Folin–Ciocalteu method (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, aliquots of 0.1 g lyophilized powder of fruit and vegetable samples were, respectively, dissolved in 1 ml deionized water. This solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture absorbance was measured at 750 nm against a deionized water blank on a spectrophotometer (Hitachi, Model 100-20). Gallic acid (GA) was chosen as a standard. Using a seven point standard curve (0–200 mg/l), the levels of total phenolic contents in fruits

and vegetables were determined in triplicate, respectively. The data were expressed as milligram gallic acid equivalents (GAE)/g lyophilized powder. Finally, the data were converted to milligram gallic acid equivalents (GAE)/100 g fresh matter of fruit or vegetables based on the moisture contents of lyophilized powder and fresh fruit and vegetable materials. The moisture content in fresh fruits and vegetables. The moisture content of lyophilized powder from fruits and vegetables were determined according to the AOAC method (AOAC, 1984). The moisture contents in fresh fruits and vegetables were referred from the data bank of nutritional compositions of foods in Taiwan. The data bank of nutritional compositions of foods in Taiwan is an official press published by the government. The data in the press are exact and referred by many institutes in Taiwan. To avoid overspending on time, we referred the moisture content of fruits and vegetables cultivated in Taiwan from the data bank.

The total flavonoid content was determined according as the aluminum chloride colorimetric method described by Chang, Yang, Wen, and Chern (2002). Briefly, aliquots of 0.1 g of vegetable and fruit samples were, respectively, dissolved in 1 ml deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionized water blank on a spectrophotometer (Hitachi, Model 100-20). Quercetin was chosen as a standard. Using a seven point standard curve (0–50 mg/l), the levels of total flavonoid contents in fruits and vegetables were determined in triplicate, respectively. The data were expressed as milligram quercetin equivalents (QE)/g lyophilized powder. The data were then converted into milligram quercetin equivalents (QE)/100 g fresh matter from fruit or vegetables based on the moisture content of lyophilized powder and fresh fruit and vegetable materials.

2.3. Experimental animals for primary splenocyte cultures

The BALB/c mice (female, 6-week-old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained in the Department of Food Science and Biotechnology at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The mice were housed and kept on a standard chow diet (Experimental mouse feed CI021010010, Fwusow Industry Co., Ltd., Taiwan). The animal room was kept on a 12-h-light and 12-h-dark cycle with constant temperature (25 ± 2 °C) and humidity. The animals (8–10-week-old) were sacrificed using CO_2 inhalation to obtain spleens. The abdominal cavities were opened aseptically and the spleens were removed.

2.4. In vitro proliferation assay of fruit and vegetable samples in mouse splenocytes

The primary splenocyte culture preparation and assay of in vitro proliferation using vegetable and fruit samples were manipulated as described by Lin et al. (2005). Briefly, the mice were anesthetized with diethyl ether, exsanguinated by retro-orbital venous plexus puncture and immediately euthanized by CO_2 inhalation. The splenocytes were prepared by aseptically removing spleens from BALB/c mice. Spleens were homogenized with help of a syringe piston. Single spleen cells were collected and treated by lysing the red blood cells. Splenocytes were isolated from each animal and adjusted to 1×10^7 cells/ml in TCM medium. Splenocytes (50 μl/well) without or with mitogens (50 μl/well), such as phytohemagglutinin (PHA, 20 μg/ml in TCM medium, Sigma), lipopolysaccharide (LPS, 10 μg/ml in TCM medium, Sigma), fruit or vegetable samples (10, 25, 50, 250, 500 μg lyophilized powder of raw squeezed juices/ml in TCM medium, respectively) were plated in 96 well microtiter plates. The plates were incubated at 37 °C in a humidified incubator with 5% CO_2 and 95% air for 72 h. Aliquots of 10 μl (5 mg/ml) 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS were then added to each well. The plates were incubated at 37 °C in a humidified incubator with 5% CO_2 and 95% air for another 4 h. After incubation, the plates were centrifuged at 200*g* for 10 min. The culture medium was then discarded. The plates were carefully washed with PBS buffer three times. Aliquots of 100 μl dimethyl sulfoxide (DMSO) were added to each well and oscillated for 30 min. The absorbance was measured at 550 nm on a plate reader (ELISA reader, ASYS Hitech GmbH, Austria). The cell proliferation results were described as mean absorbencies.

2.5. Statistical analysis

Data were analyzed by the Windows SAS program (Version 8.0). Data were expressed as mean ± SE using ANOVA, if justified by the statistical probability ($P < 0.05$), by Duncan's new multiple range test. The relationship between total phenolic or flavonoid contents in fruit and vegetable samples and cell proliferation was described as Pearson product–moment correlation coefficient (r). Differences were considered statistically significant if $P < 0.05$.

3. Results

3.1. Total phenolic and flavonoid contents in the selected fruits and vegetables

The results showed that the total phenolic and flavonoid contents in the selected fruits and vegetables varied considerably. Total phenolic contents from different fruit species ranged from 1515.9 ± 5.7 to 199.4 ± 13.1 mg GAE/100 g FM (Table 1). This study showed that total phenolic

Table 1
Total phenolic and flavonoid content in the selected fruits and vegetables

Materials	Total phenolic content (mg GAE/100 g FM)	Total flavonoid content (mg QE/100 g FM)
Fruits		
Strawberry	363.7 ± 6.7^{c}	14.6 ± 3.0^{c}
Oriental plum	668.0 ± 8.0^{b}	37.6 ± 7.0^{b}
Mulberry	1515.9 ± 5.7^{a}	250.1 ± 6.3^{a}
Loquat	199.4 ± 13.1^{d}	14.2 ± 0.9^{c}
Vegetables		
Green pepper	206.0 ± 5.4^{e}	$7.5 \pm 1.6^{e,f}$
Yellow pepper	$191.2 \pm 18.2^{e,f}$	4.1 ± 0.9^{g}
Red pepper	180.3 ± 1.6^{f}	10.4 ± 2.9^{e}
Ceylon spinach	269.0 ± 3.1^{b}	133.1 ± 26.2^{a}
White onion	216.7 ± 1.4^{d}	30.6 ± 6.8^{c}
Red onion I	310.8 ± 4.9^{a}	56.4 ± 10.3^{b}
Red onion II	253.6 ± 4.4^{c}	36.5 ± 7.6^{c}
Bitter melon	143.6 ± 8.4^{g}	15.0 ± 2.4^{d}
Beetroot	257.2 ± 0.7^{c}	62.8 ± 0.7^{b}

Each value in the table is represented as mean ± SE ($n = 3$).
Superscript letters with different letters in the same column of fruits and vegetables, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

contents in the selected fruits as: mulberry (1515.9 ± 5.7 mg GAE/100 g FM) > oriental plum (668.0 ± 8.0 mg GAE/100 g FM) > strawberry (363.7 ± 6.7 mg GAE/100 g FM) > loquat (199.4 ± 13.1 mg GAE/100 g FM). Total phenolic contents in the selected vegetables ranged from 310.8 ± 4.9 to 143.6 ± 8.4 mg GAE/100 g FM. The highest total phenolic content was observed in a variety of red onions and the lowest was observed in bitter melon among the selected vegetables.

This study showed that total flavonoid contents in the selected fruits as: mulberry (250.1 ± 6.3 mg QE/100 g FM) > oriental plum (37.6 ± 7.0 mg QE/100 g FM) > strawberry (14.6 ± 3.0 mg QE/100 g FM) > loquat (14.2 ± 0.9 mg QE/100 g FM). However, total flavonoid contents in the selected vegetables ranged from 133.1 ± 26.2 (ceylon spinach) to 4.1 ± 0.9 mg QE/100 g FM (yellow pepper) (Table 1).

3.2. Cell stimulatory effects on splenocyte proliferation by the selected fruits and vegetables

Splenocyte proliferation from inbred BALB/c mice species cultured in the presence of different species of fruits and vegetables was determined to evaluate cell stimulatory effects on splenocyte cultures by fruit and vegetable samples. The results showed that the fruit samples from mulberry, loquat, and strawberry at concentration of 500 μg/mL significantly increased splenocyte proliferation, however, the oriental plum did not (Fig. 1A). The vegetable samples from white onion, red onion I, and red onion II at concentration of 500 μg/mL also significantly increased splenocyte proliferation, but bitter melon significantly inhibited splenocyte proliferation in a dose response manner (Fig. 1B). The vegetable samples from green pepper, red pepper, yellow pepper, ceylon spinach, and beetroot at the indicated concentration of 500 μg/mL did not significantly affected splenocyte proliferation (Fig. 1C).

3.3. Correlation between total phenolic or flavonoid contents in the selected fruits and vegetables and their stimulatory effects on splenocyte proliferation

The results showed that total phenolic contents in the selected fruits and vegetables significantly ($P = 0.0078$) correlated ($r = 0.3272$) with splenocyte proliferation (Fig. 2A). However, total flavonoid contents in the selected fruits and vegetables did not significantly ($P = 0.0992$) correlate ($r = 0.2063$) with splenocyte proliferation (Fig. 2B). The correlation of total phenolic or flavonoid content in individual fruit or vegetables was correlated with splenocyte proliferation. The results showed that the correlation coefficient for individual fruits and vegetables varied considerably and demonstrated differential effects on splenocyte proliferation. Both total phenolic and flavonoid contents of strawberry, mulberry, and red onion I showed a significantly ($P < 0.05$) positive correlation with splenocyte proliferation, but bitter melon showed a significantly ($P < 0.05$) negative correlation with splenocyte proliferation (Table 2).

4. Discussion

Fruits and vegetables have health benefits and are good sources of phenolics, flavonoids, anthocyanins, and carotenoids (Cieslik et al., 2006; Qian et al., 2004; Sass-Kiss et al., 2005; Trappey et al., 2005). We presumed that deep-colored fruits and vegetables are phenolic-rich, especially flavonoid-rich. However, we found total phenolic and flavonoid contents in the selected fruits and vegetables varied considerably among 13 species of deep-colored fruits and vegetables in this study. The ranges of total phenolic contents from different fruit and vegetable species varied up to 7.6 times (from 1515.9 ± 5.7 to 199.4 ± 13.1 mg GAE/100 g FM) and 2.16 times (from 310.8 ± 4.9 to 143.6 ± 8.4 mg GAE/100 g FM), respectively (Table 1). The ranges of total flavonoid contents from different fruit and vegetable species varied up to 17.6 times (from 250.1 ± 6.3 to 14.2 ± 0.9 mg QE/100 g FM) and 32.4 times (from 133.1 ± 26.2 to 4.1 ± 0.9 mg QE/100 g FM), respectively (Table 1). The black-colored fruit (mulberry fruit, oriental plum) and red- or green-colored vegetables (red onion, beetroot, ceylon spinach) seemed rich in total phenolics and flavonoids. Green vegetables such as pepper, broccoli, and spinach extracted with 80% aqueous methanol have been found rich in total phenolics (Turkmen, Sari, & Velioglu, 2005). In this study, we directly determined the total phenolic and flavonoid contents from fruit and vegetable juices. Although the absolute phenolic content might be slightly lower than that of 80% aqueous methanol extract, we found green pepper, ceylon spinach, onion (either white or red colors), and beetroot were rich in total phenolic content. However, yellow pepper, red pepper, and

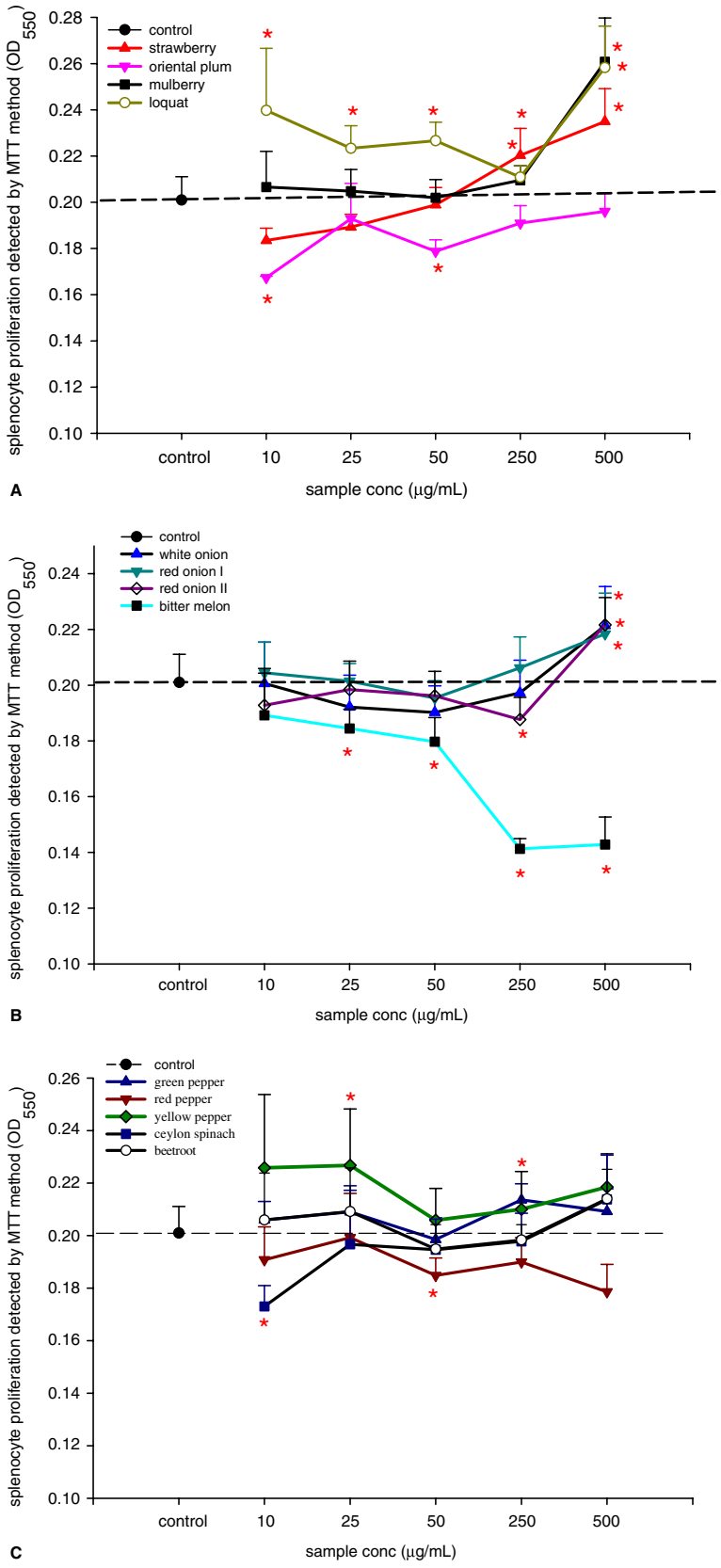

Fig. 1. Effects of different varieties of (A) fruits and (B and C) vegetables on splenocyte cell proliferation from female BALB/c mice. Each values are represented as mean ± SE ($n = 3$ individual mice). *, means significantly different ($P < 0.05$) from the control analyzed by Duncan's multiple range test.

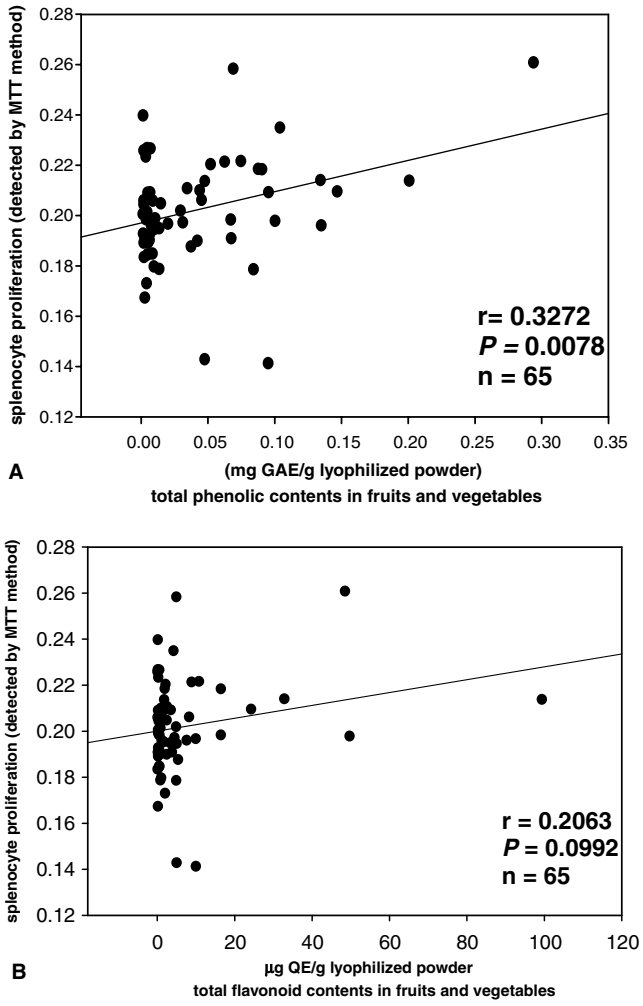

Fig. 2. The relationship between (A) total phenolic contents or (B) total flavonoid contents in the selected fruits and vegetables and stimulatory effects on splenocyte proliferation.

Table 2
The correlation analyses between total phenolic and flavonoid contents in the selected individual fruit or vegetables and their stimulatory effects on splenocyte proliferation

Samples	Splenocyte proliferation vs.			
	Total phenolic contents		Total flavonoid contents	
	r	*P* value	*r*	*P* value
Fruits				
Strawberry	0.9656*	0.0076	0.9658*	0.0076
Oriental plum	0.6553	0.2299	0.6548	0.2304
Mulberry	0.9171*	0.0283	0.9171*	0.0283
Loquat	0.5047	0.3858	0.5029	0.3878
Vegetables				
Green pepper	0.4539	0.4426	0.4535	0.4431
Yellow pepper	−0.7224	0.1681	−0.7189	0.1711
Red pepper	−0.1991	0.7481	−0.1989	0.7484
Ceylon spinach	0.8031	0.1017	0.8031	0.1017
White onion	0.8646	0.0586	0.8643	0.0588
Red onion I	0.8841*	0.0466	0.8825*	0.0475
Red onion II	0.7244	0.1663	0.7247	0.1660
Bitter melon	−0.9141*	0.0298	−0.9141*	0.0298
Beetroot	0.4399	0.4585	0.4388	0.4598
All selected samples	0.3272*	0.0078	0.2063	0.0992

The correlation analyses between total phenolic or flavonoid content in individual fruit ($n = 1$ species × 5 dosages = 5), vegetables ($n = 1$ species × 5 dosages = 5) or all selected samples ($n = 13$ species × 5 dosages = 65) were described as Pearson product–moment correlation coefficient (r). *, Differences were considered statistically significant if $P < 0.05$.

bitter melon were low in total phenolic content. Each pigment color is an overall expression of phenolics, including flavonoids and anthocyanins, or/and carotenoids (Cieslik et al., 2006; Qian et al., 2004; Sass-Kiss et al., 2005; Trappey et al., 2005). We could not only attribute a single chemical component (such as phenolics) to fruit and vegetable colors. It is reported that plant genotype affects total contents in fruits (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005). The flavonol and selected phenolic acid contents in strawberries and *Vaccinium* species (blueberry, wild bilberry, wild bog whortleberry) are influenced by cultivar, cultivation site and technique (Hakkinen and Torronen, 2000). Our results suggest that the total phenolic and flavonoid content in fruits and vegetables varied considerably. To screen phenolic-rich, including flavonoid-rich fruits and vegetables, direct determination of each fruit and vegetables, but not only colors, is a practical method.

In this study, the fruit species of mulberry, and strawberry and the vegetable species from red onion demonstrated stimulatory effects on splenocyte proliferation and significantly correlated with their total phenolic and flavonoid contents (Fig. 1 and Table 2). Mulberry fruit, strawberry, and red onion have been found to have many physiological functions. Mulberry fruit extract inhibits the development of atherosclerosis in cholesterol-fed rabbits (Chen et al., 2005). It is found that wild strawberries and cultivated strawberries are high phenolic contents and demonstrate strong antioxidant activities (Scalzo et al., 2005). In strawberries, the most abundant bioactive compounds are ellgic acid, and certain flavonoids, such as anthocyanin, catechin, quercetin and kaempferol, and they may lower risk of cardiovascular diseases (Hannum, 2005). An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents (Yamada, Naemura, Sawashita, Noguchi, & Yamamoto, 2004). Onion juices exert antioxidant and antihyperglycemic effects and alleviate liver and renal damage caused by alloxan-induced diabetes of rats (El-Demerdash, Yousef, & El-Naga, 2005). Ethyl acetate extracts from white, yellow and red onions demonstrate the antimutagenicities and antioxidant properties and are related to their phenols, including flavonoids (Shon, Choi, Kahng, Nam, & Sung, 2004). In this study, our results suggest that mulberry, strawberry and red onion demonstrate an immunomodulatory potential via stimulating splenocyte proliferation and their immuno-modulatory components are highly correlated with phenolics and flavonoids. However, the

immuno-modulatory compounds in mulberry, strawberry and red onion remain to be further clarified.

In this study, we found that bitter melon (*Momordica charantia*) inhibited splenocyte proliferation in vitro and significantly correlated with its total phenolic and flavonoid contents ($r = -0.9141$, $P = 0.0298$) (Table 2). Bitter melon (*M. charantia*) supplementation exhibits a potent serum and liver triglyceride-lowering activity in rats (Senanayake et al., 2004). It also has been found that an antiviral protein, MAP30, from bitter melon improve the efficacy of anti-HIV therapy (Bourinbaiar & Lee-Huang, 1995). Our study suggests that bitter melon demonstrates an immuno-modulatory potential via inhibiting splenocyte proliferation and its immuno-modulatory components are also highly correlated with phenolics, including flavonoids. Immuno-modulatory compounds in mulberry, strawberry, red onion and bitter melon were highly correlated with phenolics and flavonoids, but bitter melon showed a significantly ($P < 0.05$) negative correlation with splenocyte proliferation (Table 2). We presumed that different fruits and vegetables may contain different types of phenolics, such as flavonoids. However, different types of phenolics, including flavonoids, may demonstrate differentially immuno-modulatory effects, such as increase or decrease of splenocyte proliferation. However, the immuno-modulatory compounds in bitter melon remain to be elucidated.

A correlation was observed between total phenolic or flavonoid contents in the selected fruits and vegetables and their stimulatory effects on splenocyte proliferation. The results revealed that total phenolic contents in the selected fruits and vegetables significantly ($P = 0.0078$) correlated ($r = 0.3272$) with splenocyte proliferation (Fig. 2A). However, total flavonoid contents in the selected fruits and vegetables did not significantly ($P = 0.0992$) correlate ($r = 0.2063$) with splenocyte proliferation (Fig. 2B). The correlation line might be not a best fit for the data, although the correlation is statistically significant ($P < 0.05$) between total phenolic contents in the selected fruits and vegetables and splenocyte proliferation. The magnitude of the immune cell response (such as splenocyte proliferation) generally depends on the dose of stimulant (such as phenolics) administered, however, very low or very high doses of stimulant may induce specific unresponsive states. Thus, acquired low-zone or high-zone tolerance of immune response, respectively, are known under some conditions. We suppose that low-zone or high-zone tolerance might result in the lack of linear correlation between total phenolic contents and splenocyte proliferation. However, the best fit curve between total phenolic contents and splenocyte proliferation remains to be further elucidated.

Phenolics might have polyphyletic effects on immuno-modulation, especially flavonoids. It is found that dietary isoflavones suppress endotoxin-induced inflammatory reaction in liver and intestine (Paradkar, Blum, Berhow, Baumann, & Kuo, 2004). Baicalin, a flavonoid compound from the medicinal plant *Scutellaria baicalensis* Georgi, exhibits anti-inflammatory activity by binding to chemokines (Li et al., 2000). We suggest that some flavonoids in the selected fruits or vegetables in this study might inhibit splenocyte proliferation via their anti-inflammatory activities. Therefore, total flavonoid contents in the selected fruits and vegetables could not be significantly correlated with splenocyte proliferation.

5. Conclusion

In this study, 13 selected fruits and vegetables were examined concerning their total phenolic and flavonoid contents and their stimulatory activities on proliferation of splenocytes from female BALB/c mice. The results suggest that mulberry, strawberry and red onion demonstrate an immuno-modulatory potential via stimulating splenocyte proliferation. However, bitter melon showed a significantly negative correlation with splenocyte proliferation, and their immuno-modulatory components are highly correlated with phenolics, including flavonoids. Total phenolic contents in all selected fruits and vegetables significantly ($P < 0.05$) correlated with splenocyte proliferation in vitro.

Acknowledgement

This research was kindly supported by grants from the Council of Agriculture, Executive Yuan, ROC.

References

AOAC (1984). *Official methods of analysis* (14th ed.). Washington, DC: Association of Official Analytical Chemists.

Bourinbaiar, A. S., & Lee-Huang, S. (1995). Potentiation of anti-HIV activity of anti-inflammatory drugs, dexamethasone and indomethacin, by MAP30, the antiviral agent from bitter melon. *Biochemical and Biophysical Research Communications, 208*, 779–785.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*, 178–182.

Chen, C.-C., Liu, L.-K., Hsu, J.-D., Huang, H.-P., Yang, M.-Y., & Wang, C.-J. (2005). Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food Chemistry, 91*, 601–607.

Chen, P. -N., Chu, S. -C., Chiou, H. -L., Kuo, W. -H., Chiang, C. -L., & Hsieh, Y. -S. (2006). Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. *Cancer Letters* (in press).

Cieslik, E., Greda, A., & Adamus, W. (2006). Contents of polyphenols in fruit and vegetables. *Food Chemistry, 94*, 135–142.

El-Demerdash, F. M., Yousef, M. I., El-Naga, N. I., & Abou (2005). Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food and Chemical Toxicology, 43*, 57–63.

Hakkinen, S. H., & Torronen, A. R. (2000). Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Research International, 33*, 517–524.

Hannum, S. M. (2005). Potential impact of strawberries on human health: a review of the science. *Critical Reviews in Food Science and Nutrition, 44*, 1–17.

Kong, X., Hu, Y., Rui, R., Wang, D., & Li, X. (2004). Effects of Chinese herbal medicinal ingredients on peripheral lymphocyte proliferation and serum antibody titer after vaccination in chicken. *International Immuno-pharmacology, 4*, 975–982.

Li, B. Q., Fu, T., Gong, W.-H., Dunlop, N., Kung, H., Yan, Y., et al. (2000). The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. *Immuno-pharmacology, 49*, 295–306.

Lin, B.-F., Chiang, B.-L., & Lin, J.-Y. (2005). *Amaranthus spinosus* water extract directly stimulates proliferation of B lymphocytes in vitro. *International Immuno-pharmacology, 5*, 711–722.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*, 571–577.

Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high-performance liquid chromatography: a review. *Journal of Agricultural and Food Chemistry, 48*, 577–599.

Middleton, E., Jr., Kandaswami, C., & Theoharides, T. H. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews, 52*, 673–751.

Paradkar, P. N., Blum, P. S., Berhow, M. A., Baumann, H., & Kuo, S.-M. (2004). Dietary isoflavones suppress endotoxin-induced inflammatory reaction in liver and intestine. *Cancer Letters, 215*, 21–28.

Prior, R. L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *American Journal of Clinical Nutrition, 78*, 570S–578S.

Qian, J.-Y., Liu, D., & Huang, A.-G. (2004). The efficiency of flavonoids in polar extracts of *Lycium chinense* Mill fruits as free radical scavenger. *Food Chemistry, 87*, 283–288.

Saleem, A., Husheem, M., Harkonen, P., & Pihlaja, K. (2002). Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* retz. fruit. *Journal of Ethno-pharmacology, 81*, 327–336.

Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M. M., & Toth-Markus, M. (2005). Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International, 38*, 1023–1029.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition, 21*, 207–213.

Senanayake, G. V. K., Maruyama, M., Shibuya, K., Sakono, M., Fukuda, N., Morishita, T., et al. (2004). The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. *Journal of Ethno-pharmacology, 91*, 257–262.

Shon, M.-Y., Choi, S.-D., Kahng, G.-G., Nam, S.-H., & Sung, N.-J. (2004). Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food and Chemical Toxicology, 42*, 659–666.

Trappey, A., II, Bawadi, H. A., Bansode, R. R., & Losso, J. N. (2005). Anthocyanin profile of mayhaw (*Cretaegus opaca*). *Food Chemistry, 91*, 665–671.

Turkmen, N., Sari, F., & Velioglu, Y. S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry, 93*, 713–718.

Watzl, B., Bub, A., Brandstetter, B. R., & Rechkemmer, G. (1999). Modulation of human T-lymphocyte functions by the consumption of carotenoid-rich vegetables. *British Journal of Nutrition, 82*, 383–389.

Yamada, K., Naemura, A., Sawashita, N., Noguchi, Y., & Yamamoto, J. (2004). An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents. *Thrombosis Research, 114*, 213–220.

Youdim, K. A., McDonald, J., Kalt, W., & Joseph, J. A. (2002). Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *Journal of Nutritional Biochemistry, 13*, 282–288.

Zhang, Y., Vareed, S. K., & Nair, M. G. (2005). Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life Sciences, 76*, 1465–1472.