Full Length Research Paper

Effect of maturity and harvest season on antioxidant activity, phenolic compounds and ascorbic acid of *Morinda citrifolia* L. (noni) grown in Mexico (with track change)

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Antioxidant activity diphenylpicrylhydrazyl (DPPH), the ferric-reducing antioxidant power assay (FRAP), nitric oxide (NO)], total polyphenols, phenolic compounds and ascorbic acid of *Morinda citrifolia* L. fruits were investigated as a function of maturity and three seasons patterns in Mexico. Maturity was evaluated in early, middle, sub-mature and mature stages (1 to 4) according to color and firmness. Significant differences were observed in the antioxidant activities and chemical composition of the fruits at different maturity and seasons. During February-March and May- June, fruits from middle and mature stages exhibited the highest antioxidant activities and total polyphenol content compared to other stages, while in November, ripe fruits reached the greatest antioxidant efficacy, total phenolic and ascorbic acid contents. Total polyphenols and ascorbic acid reached the highest amounts during May-June, although antioxidant activities were moderate compared to greater values in February-March or November depending upon maturity. The ability of *M. citrifolia* fruits to inhibit NO production by LPS-activated RAW 264.7 cells was quite comparable to or higher than N-nitro-L-arginine methyl ester (L-NAME). This work shows that season and maturity stages have a profound effect on the antioxidant capacity, phenols and ascorbic acid of *M. Citrifolia* fruits.

Key words: *Morinda citrifolia*, diphenylpicrylhydrazyl (DPPH) radical scavenging, maturity, seasons, total polyphenol and phenolics compounds, reducing power, ascorbic acid, scavenging nitric oxide.

INTRODUCTION

Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants. The majority of the active antioxidant compounds are vitamins, carotenoids, flavonoids, isofla-vones, anthocyanins, coumarins, tannins, lignans, and catechins (Ramasamy and Thomas, 2010). Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatopro-tective agents, with antioxidant and/or radical-scavenging mechanisms partially responsible for these activities (Ruiz et al., 2008).

The search for natural source medicinal products, which have antioxidant and radical- scavenging activities

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Table 1. Changes of fruit skin color and firmness in the course of ripening.

Maturity stage	Degree of maturation	Color	Firmness
1	Immature	Dark green	Very hard
2	Middle	Green-yellow	Very hard
3	Sub-mature	Pale-yellow	Fairly hard
4	Mature	Translucent- grayish	Soft

capable of controlling free radical-induced tissue injury, is on the rise. There is extensive evidence to implicate oxidative stress in the pathogenesis of over one hundred human diseases, including inflammation, metabolic disorders, cellular aging, cardiovascular diseases, diabetes mellitus, neurodegenerative diseases, cancer and HIV/AIDS (Astley, 2003; Atoui et al., 2005). Free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH^-) and nitric oxide (NO) are continuously produced by the body's normal mitochondrial respiration and by some cell- mediated immune functions (Zimniak, 2011; Dyson et al., 2011). Naturally, there is a dynamic balance between the quantity of free radicals generated in the body and the activity of antioxidants, which quench and/or scavenge them and protect the body against their deleterious effects.

Antioxidant principles from natural sources provide enormous scope for correcting imbalances between oxidant production and antioxidant defenses. Among these is *Morinda citrifolia* L. (noni), which was discovered 2000 years ago by ancient Polynesians and can confer health benefits. The genus *Morinda* (Rubiaceae), including the species *M. citrifolia* L., is made up of around 80 species (Furusawa et al., 2003; Wang et al., 2002; McClatchey, 2002; Furasawa et al., 2003; Chan-Blanco et al., 2006).

Traditional islander healers use the noni leaves, fruits, roots, barks, stems and flowers. For example, noni fruits are used as blood purifiers, antiemetic agents, digestive disorders, tuberculosis, urinary-tract ailments, hypertension, depression, diabetes, heart disease, AIDS and cancers, whereas the leaves are often used as a poultice for rheumatic or swelling joints, fish stings, broken bones, wound and ulcers (Pawlus and Kinghorn 2007). Pacific traditional healers commonly use noni leaves and green immature fruit; modern Hawaiian home healers commonly use ripe noni fruits (McClatchey, 2002). Modern noni manufacturers produce processed noni juice from ripe noni as a dietary supplement (Yang et al., 2011). Noni fruits are harvested throughout the year, although there are seasonal patterns in flowering and fruit bearing. In Hawaii, noni plots are usually harvested two to three times per month, although fruit production is lower during winter. The fruits may be harvested at different stages of development and continue to mature. Most processors buy noni harvested at the hard white stage for juice production, as the fruits become soft too quickly (Chan-Blanco et al., 2006).

Processing noni fruits at the sub. mature stage, white hard, can improve functional quality of noni products (Yang et al., 2011). Fermented noni juice obtained from ripe fruit contains higher quantities on total phenolic compounds, flavonoids, condensed tannin and scopoletin, thus exhibited better reductive and free radical-scavenging activities than that of unripe noni juice (Yang et. al., 2007).

The variations of antioxidant properties and chemical composition of noni fruits at various stages of maturity in relationship to the harvest season have not yet been characterized. The purpose of this research was to determine antioxidant capacity, content of total and specific phenolic compounds, and ascorbic acid content of noni fruits at various stages of maturity in relation to season. Moreover, scientific information regarding the bioactive compounds and antioxidant potential of *M. citrifolia* grown in Mexico is still limited. This information may be useful for noni manufacturers, to aid selection of the right stage and season for processing products that can maximally benefit consumers.

MATERIALS AND METHODS

Samples

The fruits were harvested from agricultural cooperative societies in Tepic, Nayarit, Mexico (latitude 21.4167, longitude: -105.1833, altitude: 30 m a.s.l.). Samples were collected in winter-spring (February-March); spring-summer (May-June); and autumn (November) 2010. Three independent sampling were carried out at the beginning, mid-point and at the end of each harvest time. Before performing the analyses, the samples were washed, first with running water and then with distilled water, and residual moisture was evaporated at room temperature. The fruits were agrupated in four groups according maturity stage shown in Table 1.

Chemicals

All the chemicals used in this work were analytical reagents and grade HPLC for standards and purchased from Sigma Aldrich Co (USA), J. T. Baker (USA) and Faga-Lab (Mexico).
Milli-Q system (Ultrapure Water) processed water was used for these experiments.

Extracts preparation

Noni fruit (stages 1 to 4) aqueous extracts were obtained by homogenizing 2.5 g of noni tissue (pulp and peel) in 25 ml of deionized water until uniform consistency was achieved, using a homogenizer (IKA, Ultra Turray). The homogenates were filtered

using a Whatman no. 4 filter paper and then stored at -20°C until analysis.

Determination of antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging capacity as previously reported by Brand-William et al. (1995) was modified as follows. At 500 µl of different concentrations (50-1000 µg/ml) of the extracts, 500 µl of (0.29 mM) 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol was added and the mixture was incubated in microtiter plate for 30 min in the dark at room temperature. Absorbance of the mixture was measured using microplate spectrophotometer reader Thermo Scientific at 517 nm. Mixture without extract served as absolute control (A blank). Ascorbic acid (35 µM), BHA (6.95 µM), and Rutin (125 µM) were used as antioxidant standards. The free radical scavenging of DPPH was calculated according to the following formula:

$$\% \text{ Inhibition} = (A_{blank} - A_{sample} / A_{blank}) * 100;$$

Where, A_{blank} is the absorbance of the control reaction (containing DPPH solution adequately diluted with ethanol) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. A lower IC_{50} value indicates higher the antioxidant activity of the examined compound.

Ferric-reducing antioxidant power assay (FRAP)

The ferric-reducing antioxidant power assay was carried out as previously described by Benzie and Strain (1996). The FRAP reagent was prepared in acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in water bath prior to use. 5 µl of samples at 0.5 to 2 mg/ml diluted with 20 µl of distilled water were added to 150 µl of FRAP reagent. The absorbance of the mixture was measured using microplate spectrophotometer reader Thermo Scientific at 595 nm after 4 min. The standard curve was prepared by iron (II) sulfate solution, and the results were expressed as µM Fe (II)/ mg polyphenol-rich extract.

Nitric oxide (NO) inhibition activity

RAW 264.7 cells were activated with LPS according to previously described procedures (Kim et al., 1999). Briefly, cells were plated in 96-well plates (2×10^5 cells/well). After preincubation for 24 h, LPS (1 µg/ml) was added to the cells, and incubated for 4 h. N-nitro-L-arginine methyl ester (L-NAME) at 1 mM was used as positive control to inhibit inducible nitric oxide synthase (iNOS) enzyme activity. Test aqueous extracts of fruits in different maturity stages were dissolved in DMSO and were diluted with DMEM with 5% FBS into appropriate concentrations, and were incubated for another 48 h. To measure NO production, the stable conversion product of NO, nitrite (NO^{2-}), was measured using Griess reagent [1:1 mixture (v/v) of 1% sulfanilamide (in 60% of acetic acid) and 0.1% naphthyl-ethylenediaminedihydrochloridein], and the optical density was measured using microplate spectrophotometer reader Thermo Scientific at 550 nm. A standard curve of sodium nitrite was used to find the concentrations of nitrites produced by the RAW 264.7 cells (Green et al., 1982). The activity was expressed as percentage of

inhibition and IC_{50} (extract concentration to inhibit 50% of nitrites.

Determination of total polyphenols

About 1 g of pulps at various degrees of maturation were homogenized and extracted with 10 ml distilled water at ambient temperature. The complex was centrifuged (5000 rpm, 5 min). The supernatant was collected and it was taken as aqueous extract. 500 µl of extract at different concentrations (0.5 to 2 mg/ml) were determined by the Folin-Ciocalteu method as described by Singleton and Rossi (1965) using gallic acid as a standard and the results were expressed as µg gallic acid equivalent (GAE)/g fresh weight (FW).

Analysis of phenolic compounds

The extraction and HPLC analysis for phenolic compounds was modified from Rodríguez-Delgado et al., (2001). 1 g of fresh pulp was mixed with 10 ml of ethyl acetate; the mixture was shaken slightly for 30 min and then centrifuged at 5000 rpm for 5 min (HERMLE mod 236HK). The supernatant was removed and the extraction process was twice repeated. The combined filtrates were evaporated to dryness under vacuum using a rotatory evaporator (Buchi, Switzerland) and reconstituted in 2 ml of methanol: water (50:50 v/v). Finally, the extracts were filtered through a 0.22 µM nylon filter (Millipore) and stored at -20°C until analysis. HPLC was performed on a Varian 240 at a detection wavelength of 280 nm, at a flow rate of 1 ml/min, at a sample size of 20 µl, and on a column C-18 (Varian Microsorb 100, 5 µM, 4.6 × 150 mm) coupled with a C-18 guard cartridge (Varian, 5 µM, 4.3 × 10 mm), with mobile phase A (Acetic acid: water 2% v/v) and B (methanol) in gradient. Gallic acid, catechin, aesculetin, scopoletin, rutin and quercetin standards were injected for identification of these compounds in the samples by comparison of retention times, and calibration standards curves were used to calculate concentrations present in *Morinda citrifolia* (noni). The contents of phenolic compounds were expressed in µg per g fresh weight respectively.

Ascorbic acid analysis

The ascorbic acid determinations were based on the method described by Doner and Hicks (1981) with some modifications. For extraction, the mixture was prepared with 1 g of fruit pulp and 10 ml of metaphosphoric acid and acetic acid. The solution was shaken slightly for 30 min in absence of light, and then centrifuged at 5000 rpm for 5 min (HERMLE mod 236HK). The supernatant was filtered through filter a 0.22 µM nylon filter (Millipore) and stored at -20°C for later analysis. 20 µl were injected onto HPLC systems that consisted of a Varian ProStar model 240 solvent delivery module, and a Varian ProStar 325 UV-Vis detector with Galaxie software, version 1.9. The separation was performed on a reverse-phase C-18 analytical column (Varian Microsorb 100, 5 µM, 4.6 × 150 mm) coupled with a C-18 guard cartridge (5 µM, 4.3 × 10 mm) at room temperature. An isocratic method was used, with KH_2PO_4 (0.05 N): acetonitrile: (99:1 v/v) as the mobile phase at a flow rate of 1.0 ml/min. Ascorbic acid was detected by UV at 268 nm. The concentrations of AA were calculated using a standard curve and the results were expressed as mg/ 100 g of fresh weight (FW).

Statistical analysis

Experimental results are expressed as the mean ± SD from three independent sampling in triplicate. A multifactorial analysis of the variance (ANOVA), statistical significance, lineal regression and
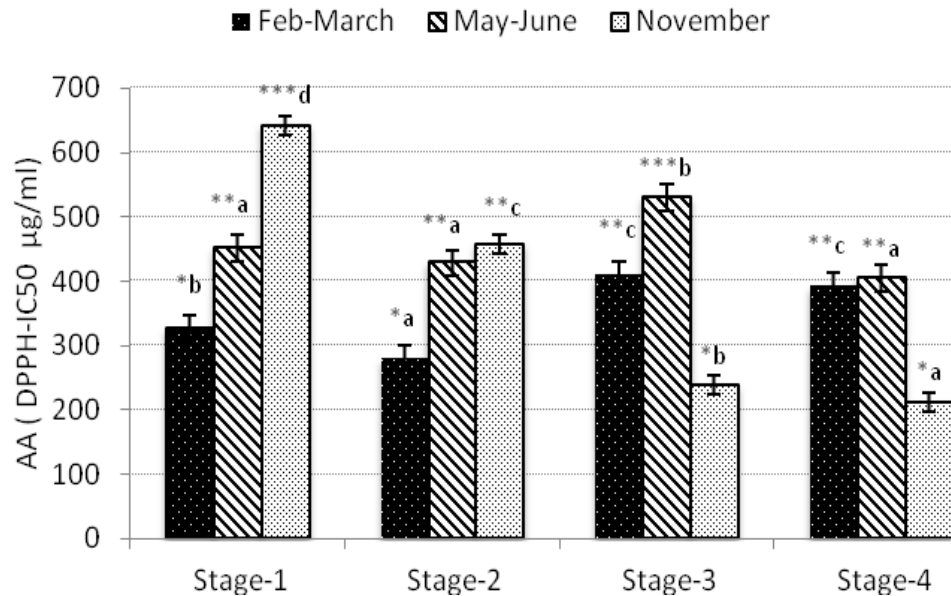
Figure 1. Antioxidant activity by DPPH at various stages and seasons patterns. Data were the means of three independent sampling each one with three replicates in triplicates (n=9). Means with different letters indicate significantly different between stages in the same season (P<0.05, Tukey test). *, **, ***Means are significant at P<0.05. Tukey test in the same maturity stage but different seasons. IC_{50} of ascorbic acid and Rutin were 35 µM and 125 µM respectively.

Pearson correlation coefficient analysis was performed in NCSS, 2007. P values < 0.05 were regarded as significant by Tukey multiple-range test.

RESULTS AND DISCUSSION

DPPH radical scavenging activity is shown in Figure 1. Radical scavenging activity varied irregularly with degree of maturity and seasonal harvest. For the month of February through March, antioxidant activity increased significantly (P < 0.05) at stage 2, and decreased significantly (P < 0.05) at stage 3 and 4. In the month of May through June, antioxidant activity remained fairly constant between ripeness stages; the only significant difference was a significant decrease detected in the stage 3. For this time, antioxidant activity was moderate but steady, when compared with higher activity for immature fruit found in February-March and mature fruits collected in November.

Antioxidant activity can vary considerably with the changes in environmental conditions within the same maturity stage. The biggest significant differences (P < 0.05) in the antioxidant activity were obtained in fruit stages 1 and 3.

Coincidentally, in these stages generally, antioxidant activity and phenolic compounds decreased significantly. In stages 2 and 4, the variations of DPPH radical scavenging activity with respect to growing season were minor.

Yang et al. (2011) and Chan-Blanco et al. (2007) reported values of antioxidant capacity (ORAC, ABTS and DDPH assays) in ripe soft noni significantly greater than those in immature green noni fruits. We obtained similar results with fruits collected in November. In contrast, Yang et al. (2007) did not find significant differences in the DPPH radical scavenging activity of fermented noni juice between ripe and unripe fruits; our results with fruits harvested in May-June are in accord with this report.

Potent antioxidants found in noni fruits include neolignan, americanin A, 3,3'-bisdemethylpinoresinol, morindolin, and isoprincepin, which inhibit copper-induced low-density lipoprotein oxidation (Kamiya et al., 2004).

This study indicates that the antioxidant capacity of noni fruits could be markedly influenced by environmental conditions; this finding could be pertinent, as noni antioxidants may have the potential to prevent a range of chronic degenerative diseases -including cancer, heart disease, and neurological disorders- by mitigating oxidative stress in the human body. In the reducing power assay, the presence of antioxidants in the fruits reduced Fe^{3+}/ TPTZ complex to the ferrous form. The reducing capacity of compounds could serve as indicator of potential antioxidant properties.

The results show that reducing power varied significantly (P < 0.05) between fruits of different maturity, and within the same stage according to the growing season (Figure 2). The same maturity stages presented significant
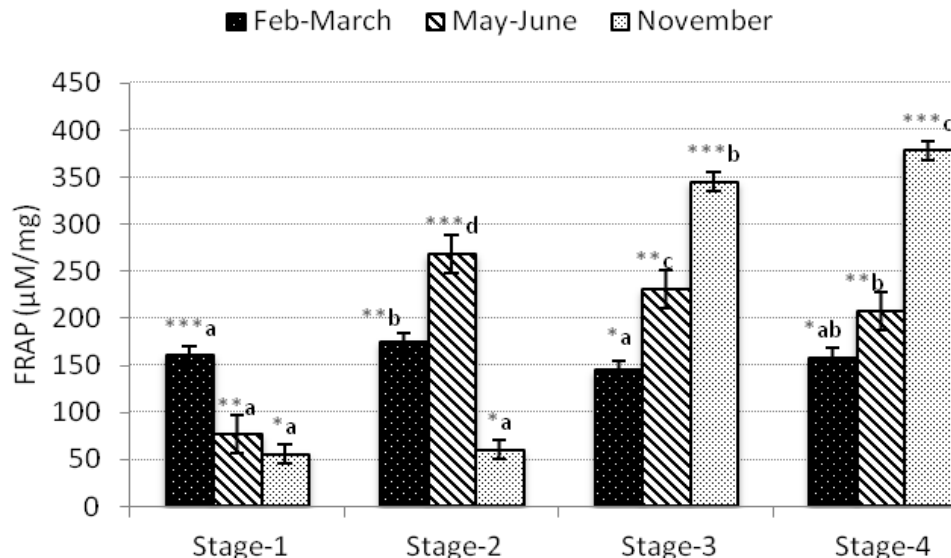
Figure 2. Reducing power (FRAP) in different maturity stages and season. Data were the means of three independent sampling each one with three replicates in triplicates (n=9). Means with different letters indicate significantly different between stages in the same season (P<0.05, Tukey test). *, **, ***Means are significant at P<0.05, Tukey test in the same maturity stage but different seasons.
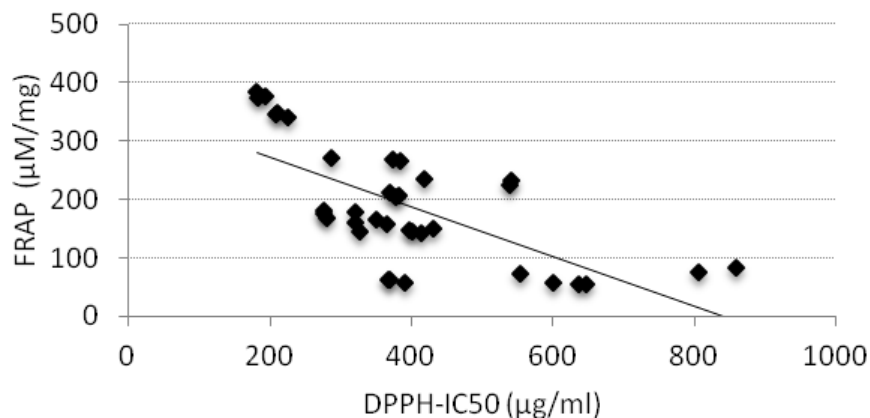
Figure 3. Correlation pearson between power reducing (FRAP μM/mg) with radical scavenging activity (DPPH-IC$_{50}$μg/ml) in different maturity stages and season.

variation in reducing power according to seasons. The most dramatic seasonal variation was presented in ripe fruits where FRAP values in November showed almost 3-fold increase compared to February-March. During February-March and May-June, fruit in stage 3 had lower reducing power than in stage 2; similar results were observed in antioxidant activity measured by DPPH assay. Maximum reducing power was observed in November fruit, in the stages 3 and 4 while the minimum reducing power were found at the first stages of maturity in the same time. Fruits in the stage-1 presented the lowest reducing power for all harvest time.

In our study, DPPH and FRAP assays showed good correlation with slight differences between different fruit development (Figure 3). Correlation between DPPH (IC$_{50}$) and reducing power (FRAP) was moderately negative (r= -0.684, P < 0.01) in noni at various degree of maturity in different seasons. (Note that, the lower the IC$_{50}$, the higher the antioxidant activity). Thaipong et al. (2006) found high correlation between DPPH and FRAP assays in guava fruit.

Aqueous extracts of fruit of different maturity stages were analyzed for their inhibitory effects on NO production from LPS-treated RAW macrophages. The NO

Table 2. The IC $_{50}$ values of fruits at different maturity stages and season and standards on nitric oxide inhibition activity.

Season	Nitric oxide (NO) inhibition activity (IC $_{50}$ µg/ml)			
	Stage 1	Stage 2	Stage 3	Stage 4
Feb-March	>600[a*]	178.21 ± 61.04[b*]	553.26 ± 55.41[c*]	276.12 ± 100.04[b*]
May-June	226.78 ± 2.91[a**]	187.87 ± 45.25[a*]	211.58 ±22.69[a***]	166.58 ±45.05a*
November	94.50 ±12.44[a***]	23.65± 10.75[b**]	47.60 ± 4.16[b***]	94.30± 7.46[a**]
% cell viability	99 ± 1.73	100 ± 0.0	94.3 ± 6.03	98.3 ± 2.89
Catechin	107.75 ± 8.76 µg/ml	(% cell viability = 100 ± 3.65)		
% Inhibition of L-NAME (1 mM/ 269.7 µg/ml) 62.41 ± 4.65 %	(% cell viability = 100)			

Values are given as mean of three independent sampling each one with three replicates in triplicates (n=9). Means with different letters indicate significantly different between stages in the same season (P<0.05, Tukey test). *, **, ***Means are significant at P<0.05, Tukey test in the same maturity stage but different seasons.

inhibition of samples tested exhibited activity in a dose-dependent manner. The corresponding IC$_{50}$ values are presented in Table 2.

It was noted that in February-March, the extracts showed the greatest differences in inhibitory activity between degrees of maturation. For stages 2 and 4, the NO inhibition increased compared to stage-1 and stages-3. Similar findings regarding the antioxidant activity measured by DPPH and FRAP were discussed above.

According to Tsai et al. (2007) IC$_{50}$ values below 200 µg/ml indicate good NO-inhibitory activity, whereas IC$_{50}$ values between 200 to 400 µg/ml indicate moderate activity. May-June fruit showed good inhibitory activity during all stages. This activity could be correlated with the high levels of phenolics present in this season. On the other hand, the fruits harvested in November presented a strong ability to inhibit nitric oxide, with values better than L-NAME. In this season the best antioxidant activity was found, mainly in mature stages.

L-NAME inhibited NO production by 62.4% at the concentration of 269.7 µg/ml (1 mM). Similar NO inhibitory activities were obtained in noni extracts for almost maturity stages during February - March and May-June, except in stage-1 and stage-3 in February-March. The most potent NO inhibition was seen in November fruit. The powerful NO inhibition of noni fruits at different levels of maturation could reflect either radical-scavenging activity and/or inhibition of iNOS gene expression (Oskoueian et al., 2011). This effect has received considerable attention, as noni has dual therapeutic potential with antioxidant and anti-inflammatory properties.

In order to compare the inhibitory effect of noni fruits on inducible NO production catechin as flavonoid standard control was provided. When RAW 264.7 macrophages were treated with different concentration of catechin, significant concentration dependent inhibition of nitrite production was detected. The IC$_{50}$ value was 107.75 µg/ml, better inhibitions were seen in November in stages-2 and 3, nevertheless the fruits harvested in the others seasons displayed less inhibition activity than catechin, mainly for stages 1 and 3 during February to March.

Examination of cytotoxicity of fruits and catechin by MTT assay indicated that all compounds did not decrease cell viability in RAW 264.7 cells. Therefore, inhibition of LPS-induced nitrite production by noni fruits in different maturity stages and catechin was not the result of their cytotoxicity on cells.

It has been reported that total phenols, ascorbic acid and carotenoids can contribute to antioxidant activity (Zhang and Hammauzu, 2003). Many phenolic compounds have high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. The delocalization of electrons over the aromatic phenolic rings allows resonance stabilization of phenolic compounds after they donate an electron, and hence enables them to function efficiently as quenchers of free radical chain reactions (Tsao and Akhtar, 2005). Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation.

Total phenolic content of noni fruit varied with different ripeness stage and seasons, in the range of 1426.5 to 3647.0 µg GAE/g FW (Figure 4). The total content of phenolic changed irregularly. During February-March and May-June, total phenols increased significantly (P< 0.05) at stage 2, decreased significantly (P < 0.05) at stage 3 and then increased again in stage 4. These findings agree in part with the results reported by Yang et al. (2011), which reported that total phenol in sub-mature white hard fruit was 1.3 times greater than those of ripe fruits. Our research showed that in all seasons' patterns ripening fruit rates had higher values than immature ones.

In November, this behavior changed; the phenolic content of unripe fruits was lowest, and increased significantly throughout development (P < 0.05) to twice the initial level. This greater level of phenols was associated with higher scavenging activity and reducing power. These results suggest that antioxidant activity was
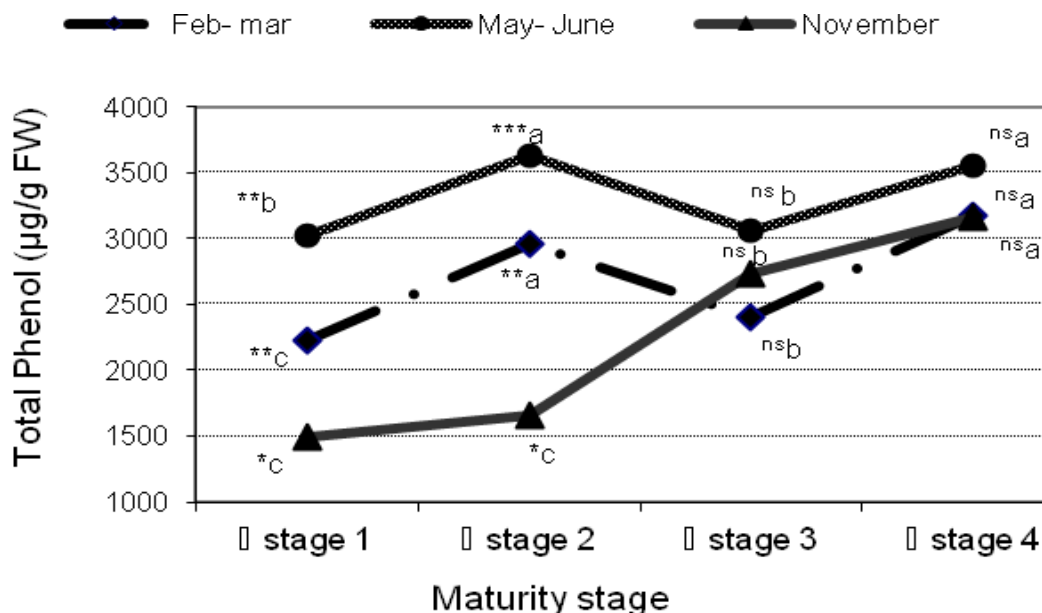
Figure 4. Changes in total phenol during ripening and season. Data were the means of three independent sampling; each one with three replicates in triplicates (n=9). Means with different letters indicate significantly different between stages in the same season ($P<0.05$, Tukey test). [ns] = not significant and *, **, ***Means are significant at $P<0.05$, Tukey test in the same maturity stage but different seasons.

related to the level of phenolic compounds, and that these values varied according to the season.

Chan-Blanco et al. (2007) reported levels of total phenols of 410 and 510 µg GAE/ g for unripe and ripe noni, respectively, cultivated in Costa Rica. In our research, total phenolics values were much greater than those reported by this author. Folin -Ciocalteu assay may over-estimates the content of phenolic compounds due to the interference from other reducing agents present in food (that is, ascorbic acid) in this paper.

The contents of specific polyphenols - catechin, scopoletin and rutin as they varied during fruit develop-ment and season, are listed in Table 3. Scopo-letin is one of the most important compounds in noni juice. Scopoletin showed homogenous content during the ripening process. In noni, ripe fruit tends to be slightly higher in scopoletin than unripe fruit, but this difference was not notable, as reported by Yang et al. (2007) in noni juice during fermentation. In May-June, the content of scopoletin was relatively low in all maturity stage. Cate-chin and rutin values showed changes during ripeness and seasons. Generally, catechin and rutin pre-sented the high amounts in stages 2 and 4; this profile appears to be independent of season, and relates powerfully with total phenolic content. Our study found average values (µg/g) for catechin, scopoletin and rutin were nearly similar between February-March and Novem-ber. Nevertheless this result was not observed in May-June; in this season, catechin and scopoletin were present at low amounts, whereas rutin was particularly high in

unripe fruit. This fact could indicate that phenolic compounds depend on seasonal patterns.

Chan-Blanco et al. (2007) reported rutin (6.06 µg/g FW) and scopoletin (27.9 µg/g FW); Muñoz-Jáuregui et al. (2007) reported rutin (60.23 µg/g FW). Our results are in accordance with the result reported by Muñoz-Jáuregui et al. (2007), but disagree in part with the result reported by Chan-Blanco et al. (2007). We found 10 times greater rutin content than those reported in noni of Costa Rica; for scopoletin, we obtained average value similar in May-June.

During the ripening, the ascorbic acid increased (Figure 5).The level of ascorbic acid varies from 100.00 mg in unripe and 182.42 mg in ripe for each 100 g FW, respectively. The level differed significantly between green, yellow and grayish fruit; ripe fruit had the highest level of ascorbic acid, while green noni had the lowest. The content in fruits harvested in May-June was found to be very high, while in November, it was just slightly lower than in February-March. Yang et al. (2011) reported low amounts of ascorbic acid in ripe soft fruit compared with the white hard one, however in their study, the amount obtained for ascorbic acid was 173.77 mg/100 g which is comparable to the result of the present study.

The literature reported that fresh noni contained ascorbic acid from 32 to 316 mg/100 g FW. The different values may be due to different cultivars, different extraction methods, and different ways of expressing the results. In our study the ripe soft fruits were found to exhibit much higher values in ascorbic acid than immature

Table 3. Phenolic contents (HPLC) at different stage of development and season's post- harvest in *Morinda citrifolia*.

Maturity	Phenols content	Seasons harvest		
		February-March	May-June	November
		(µg/g FW)		
Stage 1	Catechin	36.88 ± 6.1^b	26.16 ± 2.2^b	38.58 ± 0.7^b
	Scopoletin	$40.59 \pm 7.1^{*/**}$	$26.67 \pm 6.0^{ns**}$	$55.65 \pm 8.8a^*$
	Rutin	$29.61 \pm 4.0^{b**}$	$116.76 \pm 24.6^{a*}$	$29.86 \pm 0.6^{c**}$
	Total phenols	$2596.8 \pm 240.6^{b**}$	$3022.8 \pm 257.3^{b**}$	$1426.5 \pm 159.5^{b*}$
Stage 2	Catechin	$48.34 \pm 3.9^{a*}$	$28.15 \pm 1.3^{ab**}$	$41.05 \pm 7.8^{b*/**}$
	Scopoletin	$58.47 \pm 7.1^*$	$24.26 \pm 2.2^{ns**}$	$43.52 \pm 0.6^{b**}$
	Rutin	$74.13 \pm 14.4^{a*}$	$47.18 \pm 0.4^{bc**}$	$59.28 \pm 1.9^{b**}$
	Total phenols	$2930.6 \pm 177.5^{a**}$	$3557.0 \pm 107.8^{a***}$	1663.2 ± 208.6^b
Stage 3	Catechin	$33.73 \pm 2.3^{b\ ns}$	$31.63 \pm 5.4^{ab\ ns}$	$38.18 \pm 4.1^{b\ ns}$
	Scopoletin	$51.21 \pm 1.2^*$	$25.03 \pm 3.8^{ns**}$	$47.54 \pm 6.6^{b*}$
	Rutin	43.20 ± 5.2^{abns}	$28.70 \pm 6.2^{c\ ns}$	$35.29 \pm 5.3^{c\ ns}$
	Total phenols	2372.2 ± 167.3^b	3074.9 ± 85.0^b	2729.6 ± 248.4^a
Stage 4	Catechin	$42.67 \pm 4.9^{a\ ns}$	$45.47 \pm 2.4^{a\ ns}$	$^{ns}53.75 \pm 3.9^a$
	Scopoletin	$45.76 \pm 8.1^{**}$	$31.67 \pm 0.7^{ns**}$	$^*65.16 \pm 5.4^a$
	Rutin	$72.98 \pm 11.1^{a\ ns}$	$65.22 \pm 13.9^{b\ ns}$	$^{ns}81.34 \pm 7.6^a$
	Total phenols	3099.9 ± 250.2^a	3647.0 ± 281.0^a	3053.2 ± 248.6^a
Average in 4 stage of maturity	Catechin	40.41 ± 6.46	31.15 ± 8.69	40.43 ± 7.29
	Scopoletin	48.88 ± 8.47	26.90 ± 3.33	52.96 ± 9.57
	Rutin	54.98 ± 22.45	64.47 ± 36.38	51.44 ± 23.68
	Total phenols	2749.88	3325.43	2218.13

Data were the means of three independent sampling each one with three replicates in triplicates (n=9). Means with different letters in the same column indicate significantly different between stages in the same season (P<0.05, Tukey test). *, **, ***Means are significant, ^{ns} not significant at P<0.05, Tukey test in the same maturity stage but different season.

green and sub-mature fruit in all season patterns. Noni represents an important source of ascorbic acid for human consumption, presenting values as high as 182 mg100g^{-1}, higher than those of mango (60.5 mg100 g^{-1}) and papaya (92.9 mg100 g^{-1}), and in range of guava (174.2-396.7 mg100 g^{-1}) (Thaipong et al., 2006).

Other fruits and vegetables also show varying levels of total phenol and ascorbic acid content at different stages of maturation and ripening. For example, ripening increases the ascorbic acid content and total phenols of sweet cherry (Serrano et al., 2005), but decreases total phenols of blueberry (Castrejon et al., 2008) and red pepper (Zhang and Hammauzu, 2003), and lowers the ascorbic acid content of *Rubus coreanus* fruit (Park et al., 2008).
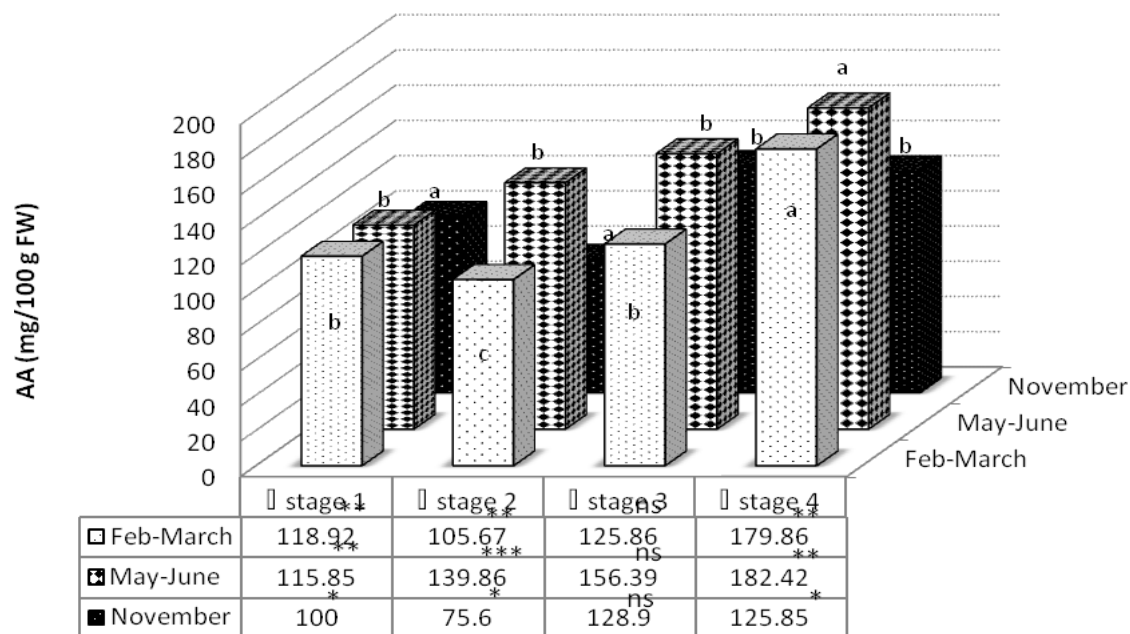
Knowledge of the seasonal and maturity effects on the chemical and antioxidant activities of noni will contribute to the characterisation and standardisation of this natural product; this could be important for practical applications of noni in pharmaceuticals and food supplements manu-facture. Correlations between antioxidant activities obtained

by DPPH, FRAP, NO inhibition, total phenols, ascorbic acid, catechin, scopoletin and rutin were moderate (Table 4).

Most techniques, including these, used for determining antioxidant activity, showed high correlation with total phenols in different crops. In our study, DPPH and FRAP assays had significant correlation with phenols.

The reducing power of noni fruits in various maturity stages and different seasons exhibited a significant linear correlation with total phenols (r= 0.526; P< 0.01). Gil et al. (2002) found a high correlation (r > 0.9, P ≤ 0.05) between antioxidant activities as determined by DPPH or FRAP assays and total phenols in nectarines, peaches and plums. Yang et al. (2007) reported that the antioxidant activity of heat-treated noni juice exhibited a significant linear correlation with total phenols (r=0.64, P < 0.01). Others had reported varying correlation coefficients between DPPH and total phenols (-0.78 to0.30). The differences between these studies and ours may be attributable to the changes of bioactive contents and antioxidant activities of poly-phenols during fruit development, in addition to the seasonal effect.

Afr. J. Biotechnol.

	stage 1	stage 2	stage 3	stage 4
Feb-March	118.92	105.67	125.86	179.86
May-June	115.85	139.86	156.39	182.42
November	100	75.6	128.9	125.85

Figure 5. Ascorbic acid content of noni fruits at various stages of maturity. Data were the means of three independent sampling, each one with three replicates in triplicates (n=9**)**. Means with different letters indicate significantly different between stages in the same season (P<0.05, Tukey test),*, **, ***Means are significant at P<0.05, Tukey test in the same maturity stage but different seasons. ns not significant

Table 4. Pearson´s correlation coefficients of antioxidant activities, ascorbic acid, total and phenolics compounds in noni fruits at different maturity stages in all season.

Trait	FRAP	NO	A.A	TP	Catechin	Scopoletin	Rutin
DPPH (IC$_{50}$)	-0.684 **	0.037 ^{ns}	-0.065 ^{ns}	-0.278*	-0.418*	-0.369*	0.267^{ns}
FRAP		-0.207^{ns}	0.331 ^{ns}	0.526**	0.234^{ns}	0.140^{ns}	-0.151^{ns}
NO			0.121 ^{ns}	-0.064^{ns}	-0.105^{ns}	-0.108^{ns}	-0.235^{ns}

DPPH, free radical antioxidant activity; FRAP, power reducing; NO, nitric oxide inhibition; TP, total phenolic; A.A, ascorbic acid; ^{ns}, not significant and *, ** = significant at P< 0.05 or 0.01, respectively.

Both catechin and scopoletin showed moderate significant negative correlations with DPPH, while there was no correlation with FRAP and NO. Scopoletin and catechin show antioxidant activity more potent than rutin owing to their chemical structures.

There was no correlation between ascorbic acid and antioxidant activities assays. Gil et al. (2002) found similar results in nectarines, peaches and plums, whereas high correlation between antioxidant activity and ascorbic acid was likely to be found only in fruits that contain high ascorbic acid, such as orange and guava (174.2 to 396.7 mg/100 g) (Gardner et al., 2000; Thaipong et al., 2006). The ascorbic acid in noni fruits changes with maturation (75.60 to 182.42 mg/100 g, Figure 5), and is relatively low compared to guavas.

It is important to bear in mind that these correlations do not depend solely on concentration and antioxidant quality but also on its interaction with other compounds and the method used to determine the antioxidant activity.

Conclusion

The seasonal effect modulates the biological activity and the chemical composition in different way in noni fruits at various degree of maturity. Climatic change like high temperatures found in May-June promote production of total polyphenol and ascorbic acid, which is related to the moderate but stable antioxidant activities throughout the development cycle. The maturation increases the ascorbic acid content, independent of the season patterns. Nevertheless the content of phenols and the activity antioxidant change irregularly according to the time of harvest.

ACKNOWLEDGEMENTS

REFERENCES

Astley SB (2003). Dietary antioxidants-past, present and future?. Trends in Food Sci. Technol. 14:93-98.

Atoui AK, Mansouri A, Boskou G, Kefalas P (2005). Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chem. 89:27-36.

Benzie IFF, Strain JJ (1996). The ferric reducing abillity of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239:70-76.

Brand-William W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenchaft and Technology 28:25-30.

Castrejon ADR, Eichholz I, Rohn S, Kroh LW, Huyskens-Keil S (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vacciniumcorymbosum* L.) during fruit maturation and ripening. Food Chem. 109:564-572.

Chan-Blanco Y, Vaillart F, Pérez AM, Belleville MP, Zuniga C, Brat P (2007). The ripening and aging of noni fruits (*Morinda citrifolia* L.): micro-biological floral and antioxidant compounds. J. Sci. Food Agric. 87:1710-1716.

Doner LW, Hicks KB (1981). High-performance liquid chromatographic separation of ascorbic acid, erythorbic acid, dehydroascorbic acid, dehydroerythorbic acid, diketogulonic acid and diketogluconic acid. Anal. Biochem. 115:225-230.

Dyson A, Bryan SB, Fernandez O, Garcia MS, Saijo F, Mongardon N, Rodriguez J, Singer M, Feelisch M (2011). An integrated approach to assessing nitroso-redox balance in systemic inflammation. Free Radic- Biol. Med. 51:1137-1145.

Furusawa E, Hirazumi A, Story S, Jenson J (2003). Antitumor potential of polysaccharide-rich substance from the fruit juice of Morinda citrifolia (noni) on sarcoma 180 ascites tumor in mice. Phytother. Res. 17:1158-1164.

Gardner PT, White TAC, McPhail DB, Duthie GG (2000). The relative contributions of ascorbic acid, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem. 68:471-474.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, Kader AA (2002). Antioxidant capacities, phenolic compounds, carotenoids, and ascorbic acid contents of nectarine, peach, and plum cultivars from California. J. Agric. Food Chem. 50:4976-4982.

Green LC, Wanger DA, Glowgowski J, Skepper PL, Wishnok JS, Tamebaum SR (1982). "Analysis of nitrate, nitrite and [15N] nitrate in biological fluids". Annals Biochem. 1236:131.

Kamiya K, Tanaka Y, Endang H, Umar M, Satake T (2004). Chemical constituents of Morinda citrifolia fruits inhibit copper-induced low-density lipoprotein oxidation. J. Agric. Food Chem. 52:5843-5848.

Kim HK, Cheon BS,, Kim YH,, Kim SY, Kim HP (1999). Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. Biochem. Pharmacol. 58:759-765.

McClatchey W (2002). From Polynesian healers to health food stores: changing perpectives of *Morinda citrifolia* (Rubiaceae). Integr. Cancer Ther. 1:110-120.

Muñoz-Jáuregui AM, Ramos-Escudero F, Alvarado-Ortiz CY, Castañeda-Castañeda B. (2007). Evaluación de la capacidad antioxidante y contenido de compuestos fenólicos en recursos vegetales promisorios. Rev. Soc. Quím. Perú. 73(3):142-149.

Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Hendra R, Ho YW (2011). Antioxidant, anti-inflamatory and anticancer activities of methanolic extracts from *Jatrofacurcas* Linn. J. Med. Plants Res. 5(1):49-57.

Park Y, Kim SH, Choi SH, Han J. Chung HG (2008). Changes of antioxidant capacity, total phenolics, and ascorbic acid content during Rubus coreanus fruit ripening. Food Sci. Biotechnol. 17:251-6.

Pawlus AD, Kinghorn AD (2007). Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). J. Pharm. Pharmacol. 59:1587-1609.

Ramasamy TN, Thomas S (2010). In vitro anti-oxidant studies on ethanolic extracts of leaves and stems of *Nyctanthes arbor-tristis* L. (night-flowering jasmine). Int. J. Biol. Med. Res. 1(4):188-192.

Rodríguez-Delgado MA, Malovana S, Perez JP, Borges T, García MFJ (2001). Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. J. Chromatogr. A. 912:249-257.

Ruiz TF, Mendrano MA, Navarro OA (2008). Antioxidant and free radical scavenging activities of plant extracts used in traditional medicine in Mexico. Afric. J. Biotech. 7(12):1886-1893.

Serrano MF. Guillen D, Martinez-Romero, Castillo S, Valero D (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stage. J. Agric. Food Chem. *53*:2741-2745.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Viticulture 16:144-158.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins BD (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruits extracts. J. Food Compos. Anal. 19:669-675.

Tsai PJ, Tsai TH, Yu CH, Ho SC (2007). Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea. Food Chem. 103(1):181-187.

Tsao R, Akhtar MH (2005). Nutraceuticals and functional foods I: Current trend in phytochemical antioxidant research. Agric. Food Chem. Environ. 3:10-17.

Wang MY, West BJ, Jensen CJ, Nowicki D, Su, Ch, Palu A, Anderson G (2002). *Morinda citrifolia* (noni): A literature review and recent advances in noni research. Acta Pharmacol. Clinica. 23:1127-1141.

Yang J, Gadi R, Thomson T (2011). Antioxidant capacity, total phenols, and ascorbic acid content of noni (*Morinda citrifolia*) fruits and leaves at various stages of maturity. Micronesica 41(2):167-176.

Yang J, Paulino R, Janke-Stedronsky S, Abawi F (2007). Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage. Food Chem. 102:302-308.

Yang S, Chen T, Li K, Tsai T (2007). Change in phenolic compound content, reductive capacity and ACE inhibitory activity in noni juice during traditional fermentation. J. Food Drug Anal. *15*(3):290-298.

Zhang D, Hammauzu Y (2003) Phenolic compounds, ascorbic acid, carotenoids and a antioxidant properties of green, red and yellow bell peppers. Food Agric. Environ. 1(2):22-27.

Zimniak P (2011). Relationship of electrophilic stress to aging. Free Radic. Biol. Med. 51:1187-1105.