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Change in Phenolic Compound Content, Reductive Capacity and ACE Inhibitory Activity in Noni Juice during Traditional Fermentation

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ABSTRACT

Morinda citrifolia L., also known as noni, has a long history of traditional use in the Hawaiian and Taihitian islands as cure-all medicine. Recently, commercial noni juice products have been imported from different countries and delivered directly to consumers with claims of multiple health benefits, including anti-cancer and immuno-stimulating activities. In this study, phenolic compounds content, free radical and superoxide anion radical scavenging activities were determined in two noni juices with different extent of maturity from traditional fermentation process. Ripe noni fruits gave higher juice yield with a lighter color. In general, juice from ripe fruits contained higher total quantities of phenolic compounds, condensed tannins, flavonoids and scopoletin, and thus exhibiting better scavenging capacity for free radical, superoxide anion radicals and H_2O_2 and higher ACE inhibitory activity. Fermentation processing gave better juice recovery, whereas prolonged fermentation time reduced the reductive phytochemical content and some related bioactivities.

Key words: noni juice, phenolic compound, flavonoid, condensed tannin, scopoletin

INTRODUCTION

Noni is the name for the fruit of the *Morinda citrifolia* tree. This product has been accepted around the globe. Apart from this name, there are many local names also widely used in respective countries. Noni is native to Southeast Asia and Australia, and is cultivated in Polynesia, India, the Caribbean, Central and northern South America[1]. The tree has been introduced and cultivated by farmers in Taiwan.

Noni belongs to the Rubiaceae family of plant. The noni plant is evergreen, and may grow up to 7 meters high. The leaves are dark green, shiny and up to 30 cm long. Small white flowers appear on small young noni fruit that are still hard and green. The mature fruit has a creamy-white color. The fruit has a bitter and characteristic rancid taste. It also has a foul smell[2]. This plant is therefore not eaten as nourishment except during time of famine.

The noni is used in native medicine in the south and central Pacific Islands. The plant parts used include the bark, leaves, flowers, fruit and seeds. The noni juice is the most widespread noni product and is in high demand as an alternative medicine for various illnesses such as arthritis, high blood pressure, muscle pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction[3]. Various brands of noni juice have been imported to Taiwan from different areas, including the Philippines, Okinawa, Tahiti, South Pacific Islands, Australia and Indonesia. Noni juice is delivered directly to consumers as a health food in Taiwan. The internet is full of sites that make false, misleading and exaggerated claims for noni juice. One example is the elusive compound "Xeronine". Xeronine, its existence declared by Dr. Ralph-Heinicke of the University of Hawaii[4], is the miracle ingredient responsible for the purported cure-all properties of noni. The amount of free Xeronine left in the blood is so minute that chemical analytical tools are unable to detect and therefore its chemical formula has not yet been revealed. Up to date, none of independent laboratories has identified or quantified Xeronine in any noni product. However, more and more curing activities of noni products have been scientifically confirmed in the past two decades[5-10]. More recently, commercial noni juice products are gaining popularity as dietary supplements with claims of anti-cancer and immuno-stimulant activities. Currently, a freeze-dried noni fruit extract is in phase I clinical trials at the Cancer Research Center, University of Hawaii[11].

Scopoletin, belonging to a group of compounds called coumarins, has been found widespread in plants. This phytochemical exhibits multiple curing activities. In a variety of scientific studies, scopoletin has been

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reported to exhibit hepato-protective activities[12,13], the inhibitory activity of *Escherichia coli* growth in the gut[14] and antibacterial activities against *Staphylococus aureus*, *Streptococcus pneumoniae, S. sp., Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Haemophilus influenza*[15]. These bacteria are responsible for disorders such as food poisoning, septicemia, pneumonia, nephritis, urogenital infections, endocarditis, respiratory infections gastroenteritis and so on. In addition, scopoletin has exerted a strong anti-inflammatory effect that is particularly useful in the treatment of bronchial illnesses and asthma[16]. Other studies have shown scopoletin useful in topical applications[17], such as antipyretic, analgesic, antiseptic[18] and hypotensive applications[19].

More scientific evidence on the beneficial constituents of noni juice needs to be established to inform consumers. To yield the best biological activity in noni juice, it is necessary to characterize noni juice in the course of its fermentation. This study was aimed to investigate the changes in contents of phenolic compounds and biological activity of noni juice during traditional fermentation processing.

MATERIALS AND METHODS

I. *Reagents*

Folin-Ciocalteus phenol reagent, gallic acid, quercetin, vanillin, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butyl hydroxyl anisole (BHA), phenazine-methosulfate (PMS), nitroblue-tetrazolium(NBT), NADH, ferrozine, hippury-His-Leu (HHL), hippuric acid (HA) and angiotensin converting enzyme (ACE from rabbit lung, EC 3.4.15.1) were purchased from Sigma-Aldrich (USA). Al $(NO_3)_3$ and H_2O_2 were from Merck (Germany).

II. *Fermentation of Noni Juice and Samples Preparation*

Hard yellow-white noni fruits were purchased from Tung-Feng Company, Yun-Lin county, Taiwan. After washing, the noni fruits were air dried overnight. Softened ripe fruits, including partially softened ripe, were separated from hard unripe ones and placed into juice separation and collection plastic vessels for 12 weeks at room temperature. The noni juice collects inside the containers and ferments as it gradually leaks out of the fruit. The juice appearance is initially an amber or golden color liquid that gradually darkens during aging. Every two-weeks, the juice samples were drained from spigots at the bottom of the containers and centrifuged to recover the supernatant for measurements of pH, color, phenolic compounds, antioxidant capacity and ACE inhibitory activity. The pH was recorded using a pH meter. The color was measured using SZ-Σ90 color measuring system (Nippon Denshoku, Japan)

III. *Measurement of Total Phenolic Compounds*[20]

Fifty microliters of noni juice was diluted with 1 mL of water; 0.5 mL of Folin-Ciocalteu phenol reagent was added and the tube was vigorously shaken. Immediately after that, 2.5 mL of 20% sodium carbonate was pipetted and shaken thoroughly. After 20 min, the absorbance was read at 735 nm using a spectrophotometer (UV 2100, Shimadzu). Gallic acid was used as standard and treated in the same manner. The total phenolic compound content was expressed as mg of gallic acid equiv/per mL sample.

IV. *Condensed Tannin Determination with Vanillin-HCl*[20]

Noni juice (0.1 mL) was placed into tubes covered with aluminum foil. One milliliter of 4% vanillin (w/v) in methanol was added and the tubes were shaken vigorously. Immediately after that, 0.5 mL of concentrated HCl was pipetted and the tubes were shaken again. The absorbance was read at 500 nm after the mixture was allowed to stand for 20 min at room temperature. The results were plotted after a catechin standard was made in the same manner. The condensed tannin content was expressed as mg of catechin equi/mL of juice.

V. *Flavonoid Determination*[21]

Deionized water (1.4 mL) was added to a tube containing noni juice (250 μL), 10% Al $(NO_3)_3$ (50 μL) and 1 M CH_3COOK (50 μL). The absorbance was read at 410 nm after the mixture was shaken and allowed to stand for 40 min at room temperature. The results were plotted after a quercetin standard was made in the same manner. The flavonoid content was expressed as μg of quercetin equi/mL of juice.

VI. *Measurement of Scopoletin*[22]

Noni juice was centrifuged (10,000 ×g, 10 min) to remove the precipitate. After passing through a 0.45-μm filter membrane, the filtrate was analyzed by RP-HPLC on a Synergi Fusion-RP80 column (250 × 4.6 mm, 4 U, Phenomenex) in the flow rate of 1 mL/min. The linear gradient elution was effected as follows: 0 min: 100% H_2O; 75 min: 75% H_2O + 25% acetonitrile; 85 min: 100% H_2O. The effluent was monitored using UV-VIS detector S-3702 (Soma, Japan) at 345 nm and a programmable Fluorescence Detector (FD-500, Groton Technology, MA, USA) (λex: 335 nm, λem: 455 nm) in connection.

VII. *Measurement of Hydrogen-Donating Activity*[23]

After 1 mL of juice was added to 1 mL of 0.008% DPPH in 50% ethanol, decoloration of DPPH donated H^+ was followed by measuring the absorbance at 528 nm.

VIII. *Measurement of Superoxide Scavenging Activity*[24]

Point five milliliter of phenazine methosuphate (40 μM), NADH (312 μM) and NBT (100 μM) in 0.1 M phosphate buffer pH 7.4 were added into tubes containing equal volumes of noni juice. After 4 min of incubation at room temperature the color was read at 560 nm against blank samples which contained no phenazine methosulphate.

IX. *Measurement of H_2O_2-Scavenge*[25]

Three milliliters of 4 mM H_2O_2 in phosphate buffer saline (pH 7.4) was added into tubes containing 2 mL of noni juice. The mixture was incubated for 10 min at room temperature after mixing. The color was read at 230 nm against BHA.

X. *Assay for ACE Inhibitory Activity*

ACE inhibitory activity was analyzed spectrophotometrically using HHL as the substract according to the method of Cushman and Cheung[26]. HHL was prepared in 0.1 M sodium borate buffer (pH 8.3) containing 0.4 M NaCl. ACE from rabbit lung was dissolved in the same buffer at a concentration of 60 mU/mL. A mixture containing 225 μL of HHL solution and 25 μL of noni juice was incubated at 37°C for 5 min. Seventy five microliter of ACE solution was then added in and the incubation was extended for 30 min. The reaction was stopped with 20 μL of 0.1% trifluoroacetic acid (TFA). Hippuric acid (HA) liberated by ACE was determined by RP-HPLC on a LiChrospher C18 column (4 × 250 mm, Merck, Germany). The mobile phase was 0.1% TFA in 50% methanol with a flow-rate of 0.8 mL/min. The effluent was monitored with an ultraviolet detector at 228 nm. ACE inhibitory activity (%) was expressed as

ACE Inhibitory Activity (%) = *(Ho-Hp) x 100/ Ho*

Where *Ho* is the height of HA peak without noni juice; *Hp* is the height of HA peak with noni juice.

XI. *Statistical Analysis*

Experimental results were expressed as means ± SD (standard deviation) of three parallel replicates. ANOVA and correlation analysis were performed. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSIONS

At the "hard white" stage, noni fruits are able to withstand transportation in baskets or containers. Exposure of the fruit to light or high temperatures immediately after harvest does not affect their overall quality[27].

About 160 phytochemical compounds have been identified in the noni plant. The major micro-nutrients are phenolic compounds, organic acids and alkaloids. Among the phenolic compounds, the most important ones reported are anthraquinones, aucubin, asperuloside and scopoletin[28]. The main organic acids are caproic and caprylic acids[2], while the principal reported alkaloid is Xeronine[4].

However, the chemical composition of noni differs largely with the plant parts[26]. Although the crude composition of the fruit has been characterized, the complete chemical composition of the fruit has not yet been reported and only limited information is available on noni fruit[28]. This is also true for the juice because its constituents depend on a number of factors including species, soil, climate, fruit maturity, production and storage. Addition of other fruit juices to mask the original foul flavor of noni juice would lead to a more complicated juice composition. Although many beneficial health properties have been claimed for noni juice in commercial bulletins, only few of these claims have been scientifically proved.

Dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, present in vegetables contribute to both the first and second defense lines against oxidative stress. As a result, they protect cells against oxidative damage, and may therefore prevent chronic diseases, such as cancer, cardiovascular disease, and diabetes. Polyphenolic compounds are believed to be responsible for the healthy effects of moderate wine consumption due to their antioxidant properties to minimize oxidative stress damage[29]. It has been found that a linear relationship exists between the phenolic content and its antioxidant activity of *Maydis stigma* extracts[30]. Tea drinking is associated with an improved antioxidant status in vivo which may contribute to lower the risk for certain types of cancer, coronary heart disease and stroke. Catechins are the chief polyphenols of green tea[31]. To yield best biological activity in noni juice, it is necessary to characterize noni juice in the course of its fermentation.

I. *Physical Characteristics*

Noni juice collects inside containers and ferments as it gradually leaks out from the fruits. The juice appearance from ripe fruit is initially an amber or golden colored liquid that gradually darkens during age. The color of unripe noni is quite dark (L = 21.95%, a = 22.20 and b = 13.83) due to the presence of trace chlorophyll, whereas the color of noni juice from ripe fruit is dark brown (L = 26.22%, a = 21.33 and b = 16.25) which is similar in appearance and texture to soy sauce after 12-week fermentation[32]. The pH is around 4.0 (table 1), lending a characteristically sour taste to aged noni juice. In general, the juices from ripe fruits showed lower acidity than that of

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Table 1. The change of color and pH values in noni juices during 12-week fermentation

Fermentation time (weeks)	L		a		b		pH	
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
2	34.84 ± 4.17	35.14 ± 7.93	14.30 ± 1.00^{c}	12.54 ± 2.30^{c}	20.28 ± 2.67	18.78 ± 3.06	4.21 ± 0.13	4.18 ± 0.13
4	27.45 ± 6.94	31.02 ± 8.61	18.98 ± 2.60^{b}	17.16 ± 0.42^{b}	16.63 ± 4.66	17.46 ± 4.39	4.07 ± 0.16	4.03 ± 0.12
6	30.70 ± 8.44	34.35 ± 11.19	22.17 ± 1.08^{a}	19.62 ± 0.76^{a}	19.15 ± 5.57	19.86 ± 5.99	4.12 ± 0.14	4.07 ± 0.11
8	28.27 ± 9.31	32.52 ± 11.87	23.20 ± 1.73^{a}	20.78 ± 0.33^{a}	17.89 ± 6.32	19.38 ± 7.01	4.13 ± 0.13	4.09 ± 0.09
10	26.93 ± 7.18	31.10 ± 11.31	24.42 ± 2.19^{a}	22.16 ± 0.59^{a}	17.19 ± 5.03	19.05 ± 7.08	4.11 ± 0.12	4.08 ± 0.08
12	21.95 ± 5.92	26.22 ± 9.63	22.20 ± 3.10^{a}	21.33 ± 2.10^{a}	13.83 ± 4.31	16.25 ± 6.46	4.05 ± 0.08	4.03 ± 0.06

Each value is the mean ± standard deviation (n = 3).
$^{a\text{-}c}$Mean in the same column with different superscripts are significandy different ($p < 0.05$).

unripe fruits and remained relative constant after 2 weeks-fermentation. The yield of juice leaks out from noni fruits were 13.3 ± 1.0% and 9.5 ± 0.1% for ripe and unripe fruits, respectively. After 12 weeks fermentation, all ripe noni fruit would be soft, while some unripe fruit would still be semi-hard. Softened fermented noni fruit is ideal for juice extraction or compression in subsequent juice processing to obtain more juice.

II. *Change of Reductive Phytochemicals*

(I) *Total Phenolic Compound*

Phenolic compounds are a large group of secondary metabolites widespread in the plant kingdom. Phenolic compounds possess various important biological activities, including antioxidant activity, capillary protective effect and inhibitory effect elicited in various staged tumors. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on their stability in different systems, as well as the number and location of hydroxyl groups. In many *in vitro* studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids[33]. The change of total phenolic compounds content during fermentation was shown in Figure 1. The content of total phenolic compounds remained fairly constant in first 10 weeks and then dropped quickly from 1.95 and 2.41 to 1.36 and 1.47 gallic acid equivalent (mg/mL) for noni juice of unripe and ripe noni fruit in the last two weeks, respectively. Noni juice from ripe fruits contained higher amounts of total phenolic compounds than that from unripe fruits.

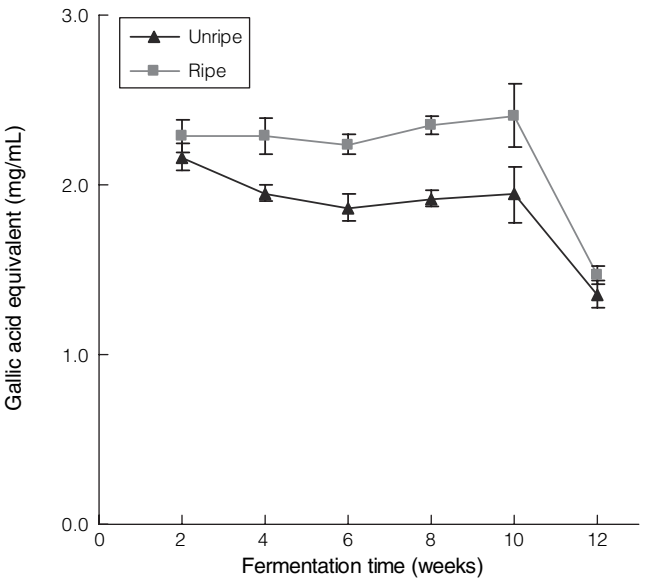

Figure 1. Change of total phenol content in noni juice during fermentation.

(II) *Flavonoids*

Flavonoids occur widely in the plant kingdom and are a major source of the colors of flowers, leaves, stalks, etc. From ancient times, flavonoids have been utilized as natural colors and as active constituents of galenicals/crude drugs and herbal medicines. Many flavonoids have a hydroxyl group in their structure and are called "polyphenols". Recently, polyphenols are in the spotlight for their various anti-oxidative properties and their ability to reduce blood cholesterol levels. The change in total flavonoids in noni juice during fermentation is shown in Figure 2. The total flavonoid content dropped quickly in the first two weeks and then slowly in the remaining fermentation period. In the 12-week fermentation process, the flavonoid contents dropped from 23.39 and 24.82 quercetin equivalent (μg/mL) to 6.60 and 7.69 quercetin equivalent (μg/mL) in noni juice from unripe and ripe noni fruits, respectively. During fermentation some flavonoids might be condensed into polymers with dark color.

(III) *Condensed Tannins*

Tannins are secondary plant metabolites subdivided into condensed and hydrolysable compounds. Condensed tannins are of great interest in nutrition and medicine because of their potent anti-oxidant capac-

ity and possible protective effects on human health[34]. It has recently been hypothesized that the free radical scavenging properties of condensed tannin may reduce risks of cardiovascular diseases, cancer[35] and blood clotting and certain types of condensed tannin may protect against urinary tract infections[34]. The changes in condensed tannin content in noni juice are shown in Figure 3. During fermentation, the condensed tannin content in noni juice gradually increased up to 10 weeks and than dropped. The condensed tannin content in noni juice from unripe fruit and ripe fruit were 1.24 and 1.34 mg/mL, respectively. The increase in condensed tannin content might come from the polymerization of flavonoid and phenolic compounds.

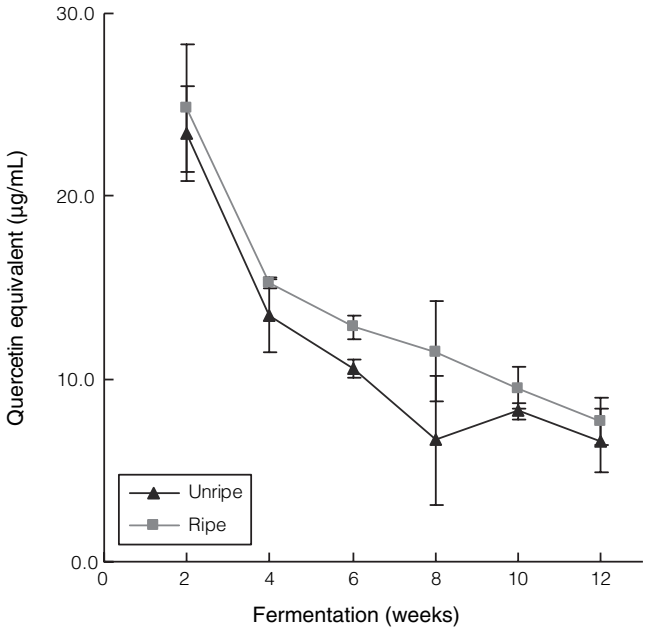

Figure 2. Change of total flavonoid content in noni juice during fermentation.

(IV) *Scopoletin*

Scopoletin is one of the most important phenolic compounds in noni juice. The phenolic exhibited multi-functional activities such as antioxidant capacity, hepatoprotective activity, antimicrobial growth, anti-flammatory effect and hypotensive activity[12-19]. Hypertension is a common chronic disease in many countries. Many synthetic inhibitors have been designed and used as anti-hypertensive medicine, such as Aprovel, Diovan 80, etc. Most of them are derivatives of peptide with ACE inhibitory activity. Undesirable side effects accompany with oral administration of these synthetic peptide derivatives. Few protein hydrolysates were found to contain peptide active enough to inhibit ACE[36]. Isolation of natural peptides with potent ACE inhibitor from protein hydrolysates is a labor-intensive and expensive work.

Change in scopoletin content during noni juice fermentation is shown in Figure 4. Scopoletin content in noni juice from ripe fruit was much higher than that from unripe fruit. The ratio of scopoletin content between ripe and unripe fruit ranged from 1.53 to 1.82. The scopoletin in juice from ripe noni fruit increased gradually up to 8 weeks and then decreased, whereas the scopoletin content in juice from unripe fruit increased in first 2 weeks and then decreased gradually over fermentation process. Other than the scopoletin in the fresh juice, some compounds with fluorescence emission as similar as scopoletin were also found in the HPLC profile in noni juice (data not shown). It is speculated that these

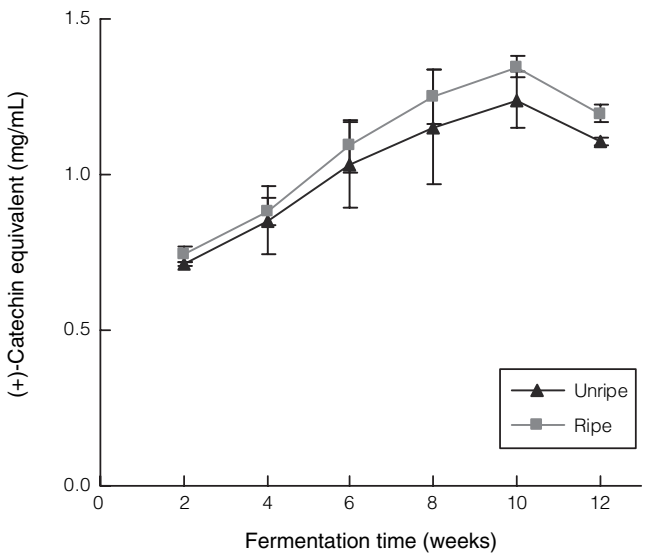

Figure 3. Change of condensed tannin content in noni juice during fermentation.

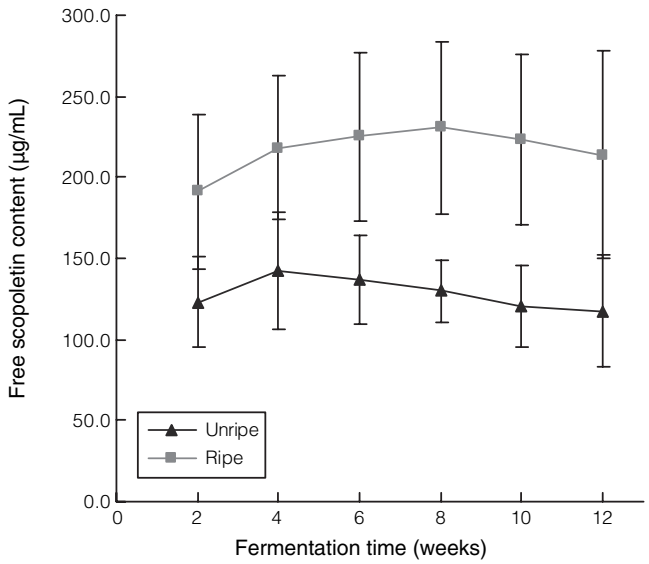

Figure 4. Change of scopoletin content in noni juice during fermentation.

fluorescent compounds could be scopoletin glycoside and might be hydrolyzed to scopoletin gradually, by enzyme or acid during fermentation. Scopoletin has been identified as an active principle in the traditional herbal infusion of the fruit of *Tetrapleura tetraptera* and in gari, a dish made from fresh cassava. It is found to be stable to post processing treatments such as sun-drying, refrigeration and storage[19]. It might decompose gradually at acidic condition such as in the noni juice system.

III. *Reductive Activity*

Small amounts of toxic oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl free radical are formed as by-products of oxidative metabolite in different types of cells[37]. Oxygen radicals and peroxides are capable of damaging lipids, proteins and nucleic acids. The principal targets of the free radicals are the unsaturated lipids in the membranes. Peroxidation of these fatty acid residues lower the membrane fluidity and can lead to cell lysis. The thiol groups of cysteine of proteins can also be oxidized, thereby cross-linking and inactivating proteins. Oxidative damage to DNA may induce mutations. It has been suggested that molecular damage occurred by reactive oxygen compounds may be a factor in a wide variety of diseases, including arthritis, emphysema, and some cancers and even the aging process[37].

(I) *DPPH Radical Scavenging Activity*

Both noni juices demonstrated satisfied DPPH radical scavenging effects (Figure 5A). The DPPH radical scavenging effects was exhibited relative constant. The scavenging activity ranged from 72% to 83% during the entire fermentation process. No significant difference in DPPH radical scavenging activity between ripe and unripe fruit juices has been found. It might be concluded that fermentation processing could not increase the antioxidative capacities of noni juices.

(II) *Superoxide Anion Scavenging Activity*

Small amounts of toxic oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl free radical are generated as by-products of oxidative metabolism in tissues. The superoxide concentration in the liver is about 10^{-11} M. Both juices exhibited effective superoxide anion scavenging activity, and the results showed that activities decrease as the increase of fermentation time (Figure 5B). It roughly dropped 10% of scavenging activity in 12 weeks fermentation for both noni juices. No significant difference between ripe and unripe juices ($p < 0.05$) has been found.

(III) *Reduction of H_2O_2*

Formation of hydrogen peroxide occurred in catab-

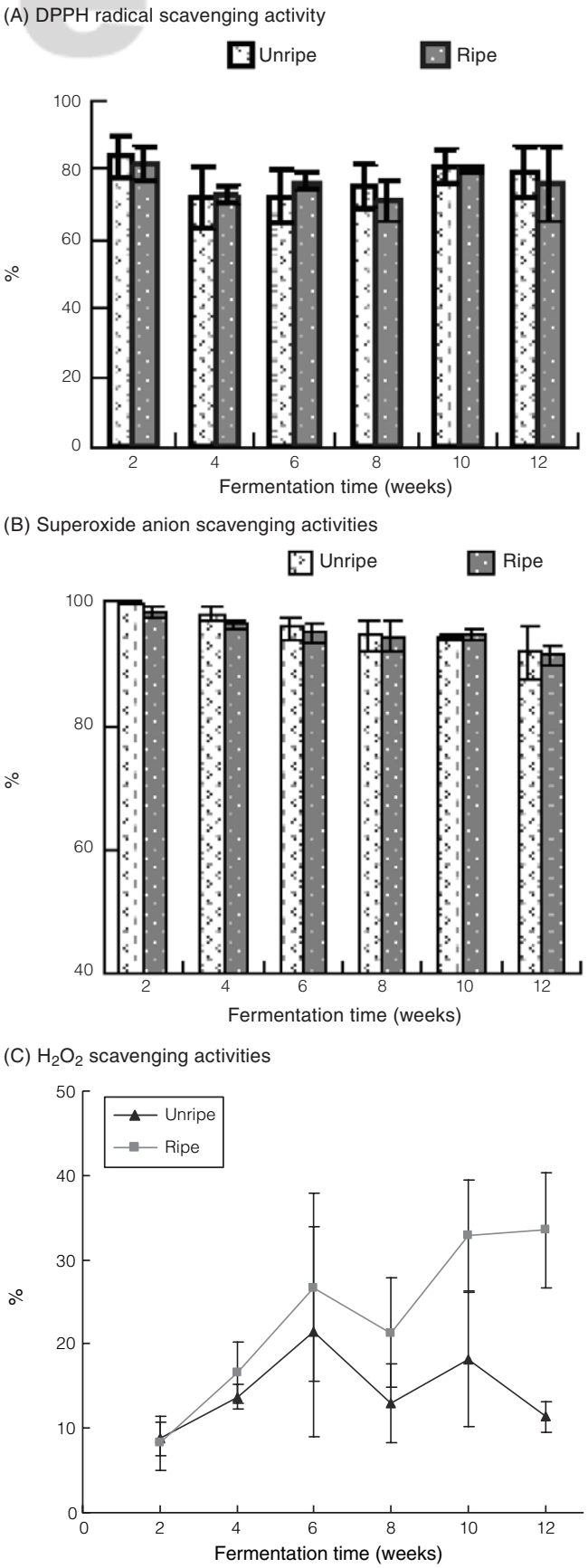

Figure 5. Change of scavenging activities in noni juice during fermentation. (A) DPPH radical scavenging activity; (B) Superoxide anion scavenging activities; (C) H_2O_2 scavenging activities.

olism of superoxide anions by superoxide dismutase, NADPH by plasma membrane-embedded NADPH oxidase in pentose pathway in stimulated phagocytes and in some reactions related toward hydrogen removal from organic compounds by oxygen[37]. Hydrogen peroxide is a strong oxidant to degrade the nutrients, to denature the enzymes and to damage membranes by oxidizing the glycerophospholipid of membrane components. Hydrogen peroxide is a free radical initiator to autooxidative chain reaction. Hydrogen peroxide could be removed by catalase or glutathione peroxidase in biological tissues.

Hydrogen-donating activity was estimated with scavenging free radicals produce with DPPH reagent. The hydrogen peroxide scavenging capacities in ripe and unripe noni juice during 12-week fermentation are shown in Figure 5C. The capability of reducing hydrogen peroxide increased gradually with the fermentation process in noni juice from ripe fruits, whereas juice from unripe fruits remained relatively constant. After two months fermentation, there is significant difference in scavenging activities between the two juices.

In general, ripe noni juice contained more amount of total phenol, flavonoid and condensed tannin, thus exhibited better reductive and free radical-scavenging activities than that of unripe noni juice.

IV. *ACE Inhibitory Activity*

Noni juice exhibited strong ACE inhibitory activity. The inhibitory effect of juice from ripe fruit is stronger than that from green fruit. Furthermore, single oral administration of the juice reduces the systolic blood pressure spontaneously in hypertensive male rats. It is obvious that the juice contains ACE inhibitory compounds and daily intake of the juice is effective for prevention against hypertension.

ACE inhibitory activities of noni juice during fermentation were shown in Figure 6. The inhibitory activity of noni juice from ripe fruit is significantly stronger than that of unripe fruit. Inhibitory effect remained relatively constant for juice from ripe fruit through the whole fermentation period. Juice of unripe fruit exhibited greater variation in inhibitory effect among collection vessels due to greater variation in ripeness of fruits. Both juices exhibited inhibitory effect in 10-week fermentation. The comparison between Figures 4 and 6 shows a positive corelation between scopoletin content and inhibitory effect and indicates that scopoletin, which has been claimed to reduce blood pressure through a vasodilating effect, might have an ACE inhibitory effect. Other than the higher scopoletin content, ripe noni juice also contained higher amount of total flavonoid and condensed tannin than that of unripe. Some of flavonoids and condensed tannins, such as vitexin, isovitexin, (+)-catechin, isoqucercitrin and (-)-epicatechin, have been found to exhibit ACE inhibitory activity[38].

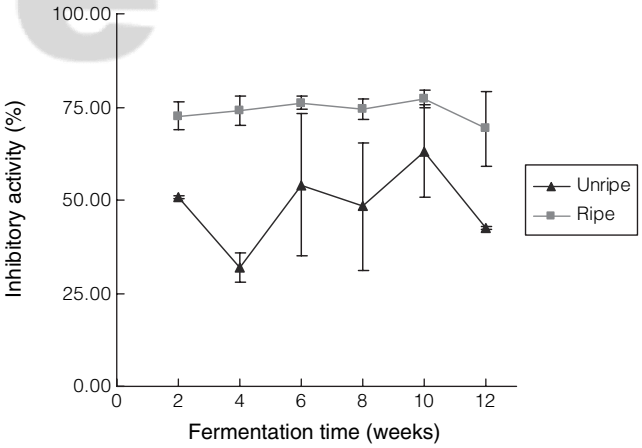

Figure 6. Change of ACE inhibitory activity in noni juice during fermentation.

CONCLUSIONS

Fermented noni juice obtained from ripe fruit is in better yield with lighter color than that from unripe. Fermented noni juice also contains higher quantities on total phenolic compounds, flavonoids, condensed tannin and scopoletin, thus exhibiting higher reductive activity, better superoxide anion and H_2O_2 scavenging activities, and better ACE inhibitory activities. In general, fermentation processing leads to an increase in condensed tannin content, an improvement in H_2O_2 scavenging activities and a decrease in total flavonoid content. The total amounts of phenolic compounds and scopoletin remained relatively constant through the 10-week fermentation experiment.

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