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Interaction of antioxidants and organic acid from noni (*Morinda citrifolia L.*) juice with ion exchange resins during deodorization via deacidification

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ABSTRACT

The unpleasant odour of noni (Morinda citrifolia L.) fruit has been a longstanding problem which is caused by octanoic acid. Deodorization of that compound have been performed using deacidification process. Deacidification of the octanoic acid using ion exchange resin was able to reduce the unpleasant odor but resulted in a reduction of beneficial antioxidant activities. Thus, this study was conducted to determine the effects of different types and weight of resin on the adsorption of octanoic acid and antioxidant compounds in noni juice during deacidification. Three types of weak base anion exchange resins (Amberlite IRA 67, Duolite A7 and Amberlite IRA 96) of different resin weight (0, 5, 10%, w/v) were used. The treated noni juice was analyzed for pH, antioxidant activity, total phenolic content (TPC), individual phenolic compounds and octanoic acid content. Deacidification of noni juice using the resins significantly ($p<0.05$) decreased the octanoic acid content compared to fresh juice where Amberlite IRA 67 gave the maximum reduction of octanoic acid followed by Duolite A7 and Amberlite IRA 96. The results indicated that deacidification of noni juice using 10% of resin weight (w/v) gave higher percentage of antioxidant activity (DPPH and FRAP) and total phenolic content (TPC). Results also showed a similar trend when different weight of resin was used where Amberlite IRA 67 > Duolite A7> Amberlite IRA 96 for DPPH, FRAP, TPC and antioxidant compounds.

Keywords: Noni fruit (*Morinda citrifolia*), ion exchange resins, antioxidants, organic acid, deodorization.

INTRODUCTION

Noni *L.* is a Rubiaceous plant widely distributed in many tropical areas [1]. Commonly called noni, it is used traditionally to treat a broad range of diseases reportedly for over 2000 years [2]. Noni juice is widely consumed globally. It has been accepted in the European Union as a novel food [3]. However, many people avoid to consume the juice because of its unpleasant odor. Deodorization will reduce the undesirable odor of noni juice which have been contributed by medium chain fatty acids such as capric (decanoic acid), caproic (hexanoic acid) and caprylic acids (octanoic acid) [4]. The volatile compounds of noni extract consist of carboxylic acid (83%), alcohol (5%) and esther (3%) [5].

Deodorization of noni extract have been done using activated charcoal [4] and deacidification using calcium carbonate [6,7] or ion exchange resin [8]. Deacidification has been used to reduce the level of acid in food systems. Ion exchange and adsorbent resins have shown promising results in the modification of acids in fruit juices. According to [9], they were able to reduce the acidity in citrus fruits using ion exchange resins. Several studies to

deacidify fruit juices using ion exchange resin have also been performed on passion fruit [10]. However, resin has the tendency to adsorb antioxidant compounds resulting in reduced antioxidant activity [11]. In the deacidification of the citrus juice by [12], weak base anion resins are preferred as they are best able to attract acid ions of the juice. Deodorization via deacidification of noni juice has been attempted previously using Amberlite IRA 67 anion exchange resin [8] which even though resulted in reduced undesirable odor, also resulted in lower antioxidant activity. Thus, it is important to understand the interaction of antioxidants and octanoic acid with ion exchange resins to maximize the adsorption of octanoic acid while minimizing the loss of antioxidants.

Antioxidants are compounds that contribute to good health. Antioxidants can be classified as primary or long-term antioxidants and as secondary or processing antioxidants. The primary antioxidants are active radical scavengers or hydrogen donors or chain reaction breakers while the secondary antioxidants are such as peroxide decomposers [13]. Antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative diseases [14]. Recently, more attention has been focused on the role of natural antioxidants, in particular, phenolic compounds, which may act both by reducing the content of toxic compounds in foods and by supplying the human body with exogenous antioxidants [15]. In this study, we focused on three types of phenolic compounds present in noni fruit as reported by researchers as listed in Table 1.

Table 1 Antioxidant compounds in different part of noni

Antioxidants (Phenolic Compounds)	Plant part	Researchers
Scopoletin, rutin, ursolic acid, ß-sitosterol, asperuloside, damnacanthal	Fruit	[16-18]
Damnacanthal, scopoletin, morindone, alizarin, aucubin, nordamnacanthal, rubiadin, rubiadin-1-methyl ether	Juice	[2,19-21]
Damnacanthal, morindone, morindin, aucubin, asperuloside, scopoletin	Whole plant	[21]
Scopoletin, quercetin, rutin, dimetylmorindol	Fruit, commercial juice	[22]

Due to the beneficial role of antioxidants, it is important that deodorization did not reduce the antioxidant activity. Therefore, the purpose of the present study was to deodorize the noni juice by determining the effect of different types and weight of ion exchange resin on the adsorption of antioxidant compounds and octanoic acid from noni juice.

MATERIALS AND METHODS

2.1 Material

Plant material used in this study was noni fruits that were harvested in Bangi, Selangor, Malaysia at a maturity index of 4 with pale yellow, whitish in color [23]. The fruit was ripened at room temperature for three days.

2*.2 Preparation of Noni Juice*

The soft, ripened noni fruits were sorted and washed with running tap water to remove dirt. The fruits were chopped into pieces, followed by blending (7011S, Waring Blender, Torrington, USA) with the addition of distilled water at a ratio of 1:1 w/v for 1 min. The noni juice was filtered using a muslin cloth, then centrifuged at 8000 rpm (7871 x g) for 30 min. The juice was filtered again using filter paper (Sartorius 1288) [7].

2.3 Resins

Three weak base anion exchange resins were obtained from Sigma-Aldrich (St. Louis, MO,USA). Weak base resins present higher ion exchange capacities and lower consumption of regeneration reagents for deacidification juices [24-26]. Amberlite IRA 67 is a gel type resin while Duolite A7 and Amberlite IRA 96 are macroporous. The resins consisted of different matrix types which were crosslinked acrylic gel (Amberlite IRA 67), phenol-formaldehyde (Duolite A7) andstyrene divinylbenzene (Amberlite IRA 96). Table 2 shows the chemical and physical properties of the resins.

Table 2 Chemical and physical properties of resins used for deacidification of noni juice

	Amberlite IRA 67	Duolite A7	Amberlite IRA 96
Chemical matrix	Crosslinked acrylic gel	Crosslinked phenol-formaldehyde	Styrene divinylbenzene copolymer
Functional group	Tertiary amine	Secondary amine	Tertiary amine
Total exchange capacity (eq/L)	≥ 1.60 (FB form)	2.1meq/ml (FB form)	1.25eq/L (FB form)
Physical form	Translucent white spherical beads	Cream colored granules	White to amber opaque spherical beads
Particle size (mm)	0.50 – 0.75	0.60 – 0.80	0.55 – 0.75

Source: Rohm and Haas

2.4 *Deacidification*

The noni juice was deacidified using three types of weak base anion exchange resins (Amberlite IRA 67, Amberlite IRA 96 and Duolite A7) at different weights (5 and 10%, w/v) in Erlenmeyer flasks. For pH determination, the samples were deacidified at 0, 2,4,6,8 and 10 % resin weights (w/v). The flasks were agitated in an orbital shaker (WiseCube, Daihan Scientific, Korea) at a constant speed of 120 rpm for 15 min. The samples were then filtered using filter paper (Sartorius 1288) and kept at -20°C in amber bottles before analysis.

2.5 pH Determination

pH determination were carried out on all noni juices (fresh and treated) using (Model PB-10, Sartorius Basic Meter, Germany). The pH meter were calibrated using pH 4.0 and 7.0 buffer. The juice was stirred before measuring the pH values. The reading was taken at room temperature.

2.6 Determination of Free Radical Scavenging using DPPH Method

The antioxidant activity of all juices were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [27]. Two ml of 0.1mM DPPH methanolic solution was added into 200 µl of sample juice and 0.8 ml methanol. The mixture was prepared by mixing 2 ml of DPPH and 1 ml methanol. The absorbance was measured at 517 nm using spectrophotometer. Samples were measured in three replications. Percentage of free radical scavenging activity was calculated based on the formula below:

% inhibition of DPPH = [Abs control – Abs sample/ Abs control] x 100.

The results were then normalized based on the free radical scavenging activity of fresh noni juice as shown below:
Normalized % inhibition of DPPH = [% of DPPH deacidified juice / % of DPPH fresh juice] x 100.

2.7 Determination of Ferric Reducing Assay (FRAP)

FRAP assay was conducted according to the method of [28] with some modification. FRAP reagent was prepared from acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ solution in 0.04 M HCl and 0.02 M iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and incubated at 37°C in waterbath prior to use. A total of 50 µl samples juice were added to 1.5ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using spectrophotometer (Spectronic 200, Madison, WI USA) after 4 min time. Samples were measured in three replications. A standard curve was prepared using a series of standard solution of iron (II) sulphate (200 – 1000 µM). The results were expressed as µmol/ g fresh weight (FW) sample. The results were then normalized using the formula: [% of FRAP deacidified juice / % of FRAP fresh juice] x 100.

2.8 Determination of Total Phenolic Content

Total phenolic content of noni juice was determined using Folin-Ciocalteau reagents [29]. Samples were inserted into different test tube and mixed thoroughly with 5ml Folin-Ciocalteau reagent (previously pre-dilute 10 times with distilled water). After 5 min, 4 ml of 7.5% sodium carbonate (Na_2CO_3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measured at 765 nm using spectrophotometer in three replications. Standard curve of gallic acid solution (0, 10, 25, 50 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/g FW. The results were then normalized using the formula: [% of TPC deacidified juice / % of TPC fresh juice] x 100.

2.9 Determination of Phenolic Compounds using HPLC

Chemicals and Standards

Methanol (MeOH) and chemical standards of scopoletin, rutin hydrate and quercetin were obtained from Sigma-Aldrich (St. Louis, MO,USA). The standards were accurately weighed and then dissolved in appropriate volume of MeOH/ deionized water to produce corresponding stock standard solutions. Working standard solutions for calibration curves were prepared by diluting stock solutions with MeOH at different concentrations. All stock and working solutions were maintained at 0°C. Deionized water was used throughout. Samples were kept at -20°C before analysis and filtered through a 0.2 µm membrane filter (Iwaki Glass) and injected directly into the HPLC.

The HPLC chromatogram demonstrating the separation of a standard mixture of the phenolic compounds studied is shown in Figure 1. The order of the retention time was scopoletin, rutin and quercetin with a relative retention time of 16.51, 18.47 and 20.37, respectively. The calibration curves were obtained with concentration in five increments. The curves were plotted after linear regression of the peak areas versus concentrations. The linear regression equation was calculated as: $y = ax + b$, where x is the concentration, and y is the peak area of the standard as showed in Table 3. The results showed acceptable linearity with correlation coefficient higher than 0.99 within the range of concentration for all phenolic compounds investigated.

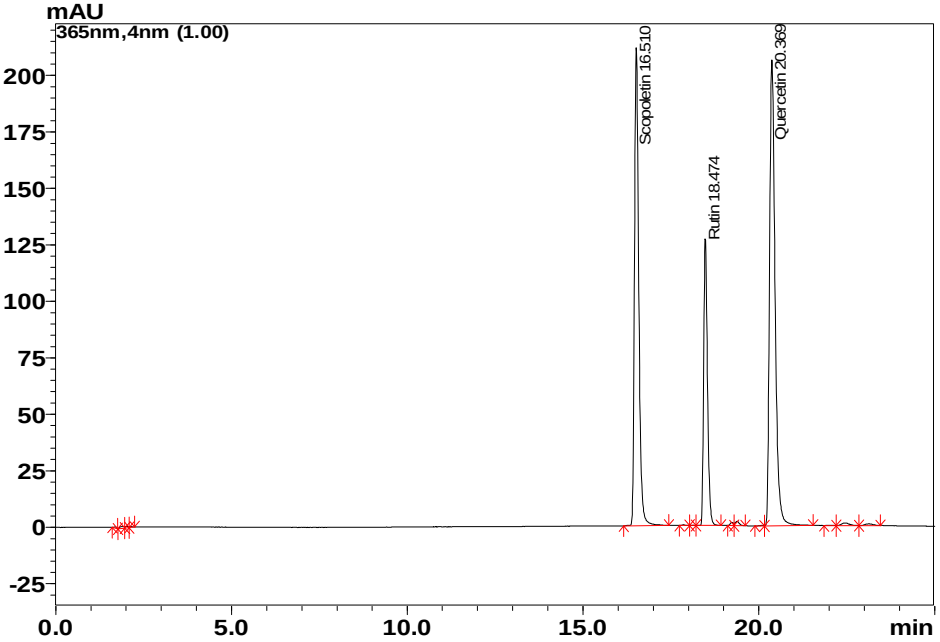

Fig.1 RP-HPLC chromatographic profiles of the phenolic reference compounds at 100 ppm; scopoletin (Rt = 16.510), rutin (Rt = 18.474) and scopoletin (Rt = 20.369). The *x*- and *y*- axis represent the running time (min) and peak absorbance (mAU), respectively

Table 3 The linear regression equation and correlation coefficient, r of phenolic reference compounds

Phenolic compounds (Standard)	Regression Equation ($y = ax + b$)	Correlation Coefficient, r
Scopoletin	$y = 18029.53x$	0.9993
Rutin	$y = 9586.03x$	0.9995
Quercetin	$y = 22701.09x$	0.9957

Analysis of Antioxidant Compounds

Scopoletin, rutin and quercetin were the antioxidant compounds determined in this study. All three phenolic compounds were consistently reported in several studies [16-18,22]. The HPLC analysis on antioxidant compounds were performed according to the modified method of Analytical HPLC Application 031481, Merck, USA (2008). The system consisted of chromatographic separation performed on a Shimadzu Chromatography 20A with photodiode array detector (PDA), and equipped with Chromolith Performance RP-18 endcapped, Merck, UK (Cat. No. 1.02129) for establishing phytochemical fingerprints of different resins used for deacidification. The pump was connected to a mobile phase system composed of two solvents: A; Methanol/ deionized water (2.5: 97.5, v/v) and B;

Methanol/ deionized water (50:50, v/v). The mobile phase was programmed consecutively in linear gradient as follows: 0-10 min, 100% A, 0% B; 10-15 min, 65% A, 35% B; 15-20 min, 0% A, 100% B; 20-22 min, 100% A, 0% B; and 22-25 min, 100% A, 0% B. The elution was ran at a flow rate of 2.1 mL/ min at 25 min. The gradient was selected as it afforded a good separation and symmetrical peak shape of target analytes in the HPLC chromatograms. The UV spectra was monitored in the range of 210 to 450 nm for the quantitative analysis. Sample peaks in the chromatograms derived from the photodiode array were integrated at 365 nm. The injection volume was 20 µL for each of the sample and standard solutions. The column temperature was maintained at 30°C. Quantification was based on the peak area measurement. Characterization of the three phenolic compounds were achieved by comparing the HPLC retention time and absorption of target peaks in the samples with those of the standards. Data collection and integration were performed using Shimadzu Lab Solution software.

2.10 Volatile Compounds of Noni Juice

Volatile compounds were extracted using Gas Chromatography Solid Phase Microextraction (GC-SPME). Temperature and time for the sampling were 53°C for 12 min according to the previous study [30].

About 1 ml of sample was added into a headspace vial and sealed with silicone septum layered with Teflon faced silicone septa (Supelco, USA) and heated in a waterbath (Memmert, Germany). SPME needle which contained a divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fiber (*StableFlex, Supelco*) was injected through the septum into the vial for 10 min. After the extraction, the needle was removed from the vial and inserted immediately into the injection port of a Gas Chromatography Mass Spectrometry (Agilent, Model HP6890, USA) equipped with Flame Ion Detector (FID) and splitless injector using an inlet SPME 0.75 mm (*Supelco*). A capillary column HP-5 (30m x 0.25 i.d., 0.25µm film thickness, J&W Scientific Pte Ltd, USA) was used. Nitrogen (N_2) was used as carrier gas. Oven temperature was programmed according to the method of [31] with some modifications. Initial temperature was 50°C for 2 min before raised to 80°C at 20°C/min for 1 min, then heated to 100°C at 20°C/min for 1 min. When it reached 100°C, the temperature finally raised to 250°C at 30°C/min and held for 2 min. The gas flow rate was 40 cm/s. The total time for separation for each samples were 13.5 min. Percentage of peak area were determined by comparing the peak retention time for the standard of octanoic acid with the peak retention time for deacidified samples. The analysis were expressed as percentage of peak area. The results were then normalized using the formula: [% of octanoic acid in deacidified juice / % of octanoic acid in fresh juice] x 100.

2.11 Statistical Analysis

Three replications were used for all parameters measured. Analysis of the data was analyzed using Excel (Microsoft Inc.) and SAS version 6.12. Statistical tests used were ANOVA and Duncan's Multiple Range test. Data obtained were reported as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

3.1 pH

To obtain the most appropriate resin with the best adsorption capacities, three types of weak base resins with different physical properties were assessed and the results were shown in Figure 2. Noni juice without deacidification (0% of resin) gave a pH value of 4.39. According to [32], in the characterisation of noni fruit, found the pH to be 3.72.

Figure 2 shows that all treated samples gave higher pH value compared to control. During deacidification ion exchange resins exchanges OH^- with anion from dissociated acids leaving the dissociated H^+ in the juice. Subsequently, the H^+ will react with OH^- from the resin to form water. Reduction of the acid will cause pH to increase [33]. A similar phenomenon was observed during deacidification of passion fruit juice where the citric ions were exchanged with the OH^- ions of the resin resulting in pH increase [10]. Previous studies also reported that in ion exchange deacidification, when the citrus juice interacts with the resins, ions from the juice were exchanged with those of the resins [12]. Accumulation of a relatively high concentration of the ions (adsorption) on resin pore surface resulted in the juice to become less acidic. The pH value for noni juice treated with the three types of resins significantly ($p<0.05$) increased when the weight of resins increased. Increasing the weight of resins allowed more vacant sites for ion exchange. As discussed by other researchers on different ion exchange resins for the deacidification of passion fruit juice, the increase in pH was obtained by reduction of the citric ions that were exchanged by OH^- ions of the resin [33]. Noni juice treated with Amberlite IRA 67 gave the highest pH value

compared to other resins followed by Amberlite IRA 96 and Duolite A7. When the percentage of resins were increased (6 to 10%), Amberlite IRA 67 resin significantly ($p<0.05$) produced the highest pH value compared to Amberlite IRA 96 and Duolite A7. Significant differences ($p<0.05$) existed between different types of resin. For all types of resin, pH value increased with increasing amount of resin but different resin showed different rate of increase. At 2 and 4% of resin, the difference in pH value were lower than at 6 to 10% of resin. At 6 to 10% of resin, Amberlite IRA 67 produced the highest pH among all samples.

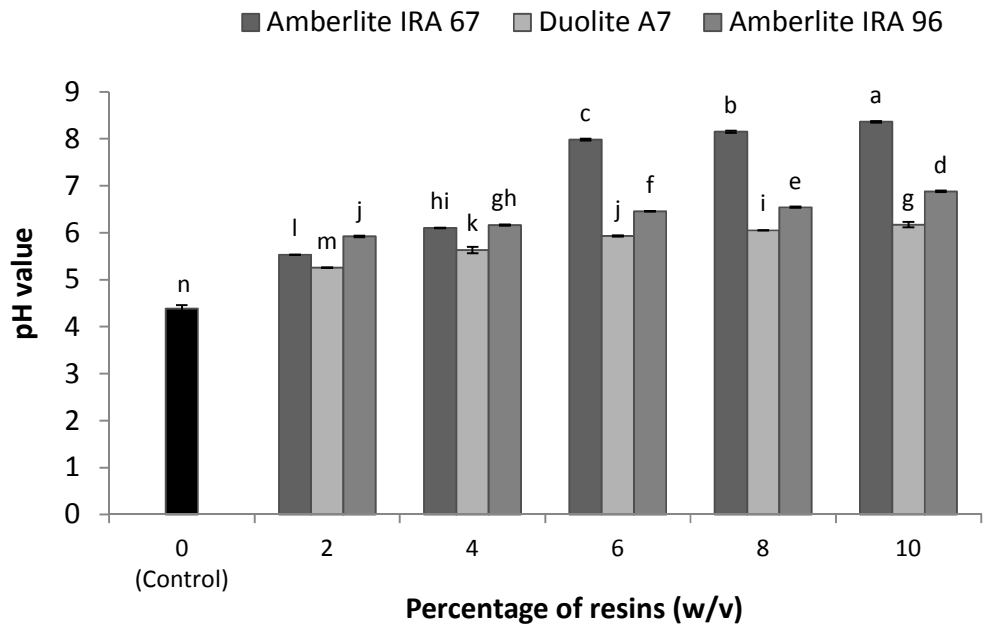

Fig. 2 pH value of noni juice treated with different types of ion exchange resins at different weight of resins (w/v)
[a-f]Means with different letters are significantly different ($p<0.05$)
Note: Error bars represent standard deviation

The different results of the three types of resins may be due to the different functional groups, matrix structures, ion exchange capacities, hydrogen bonding and hydrophobic interaction. The higher pH after treatment using Amberlite IRA 67 may be related to its stronger affinity compared to Amberlite IRA 95 and Duolite A7. Amberlite IRA 67 is an effective adsorbent for the adsorption of some organic compounds for example lactic acid, citric acid and tartaric acid [34]. This might be due to the matrix compounds and functional groups of the resins. In the studies done by [33], they also reported that Amberlite IRA 67 showed the highest ion exchange capacity, followed by Amberlite IRA 95 (similar functional group with Amberlite IRA 96) and Duolite A7. Their experimental work regarding ion exchange capacities of some resins can be observed in Table 4. Interaction involving hydrogen bonding between nitrogen of the tertiary amine (Amberlite IRA 67 and Amberlite IRA 96) and secondary amine (Duolite A7) and also the oxygen of hydroxyl group of the noni juice will also contribute to effectiveness of adsorption process. The superior performance of Amberlite IRA 67 among these resins also can be attributed to the van der Waals forces due to hydrophobic interaction. The same phenomenon occured in the study conducted by [12] where they observed that the capacity of Amberlite® resin (Amberlite IRA 68; 65% capacity) was better than Duolite® resin (Duolite A7; 44% capacity) during the deacidification of orange juice).

Table 4 Ion exchange capacities of three different resins tested

Resin	Ion exchange capacity (eq/L)	
	Reported by manufacturer	Experimental (for juice at pH 4)[a]
Amberlite IRA 67	1.60	1.15
Amberlite IRA 95 (similar as Amberlite IRA 96)	1.25	1.07
Duolite A7	2.1	0.93

[a] *Constant flow rate and velocity*
Source: [33]

3.2 Octanoic Acid

Figure 3 shows the concentration of octanoic acid content of fresh and treated noni juice using different types of ion exchange resin. The three types of resin significantly ($p<0.05$) decreased the amount of octanoic acid in treated noni juice compared to fresh juice. In comparison, the juice treated with 5% of resin (w/v) showed higher octanoic acid content than 10% (w/v) of resin. Noni juice treated with Amberlite IRA 67 gave the lowest octanoic acid content for both amount of resin. The results were similar to [30] where, the percentage of octanoic acid significantly ($p<0.05$) decreased with the addition of resin. In the present study, weak base anion exchange resin of Amberlite IRA 67 showed the most effective resin that reduces acids from noni juice. As expected, there were no significant ($p<0.05$) difference among these three types of resin at 5% of resin (w/v). The lower concentration of octanoic acid in the deacidified noni juice can be explained by losses due to deacidification. Deacidification of noni juice with Amberlite IRA 96 significantly ($p<0.05$) gave higher octanoic acid concentration compared to Amberlite IRA 67 at 10% of resin (w/v). This might be due to the different physical properties as explained in the previous section. From the results, a significant ($p<0.05$) decrease in octanoic acid concentration in noni juice treated with Duolite A7 and Amberlite IRA 96 was observed as the resin amount increased. When a higher quantity of resin was used, more ions were trapped onto the resins within a certain period of time resulting in the juices being less acidic [12].

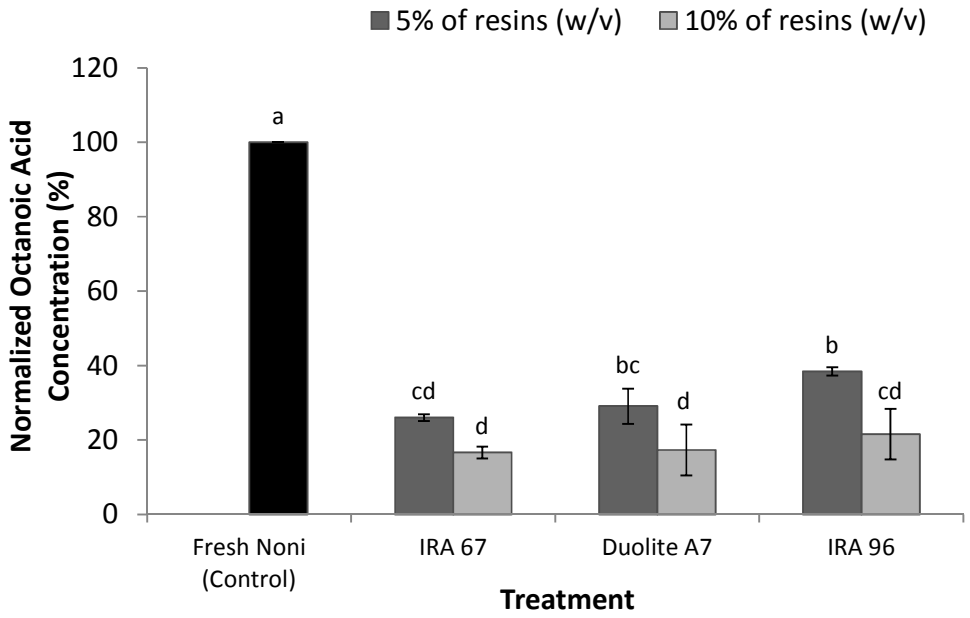

Fig. 3 Normalized octanoic acid content of noni juice treated with different types of ion exchange resins at 5% and 10% of resins (w/v)
[a-c] Means in the same percentage of resin with different letters are significantly different ($p<0.05$)
Note: Error bars represent standard deviation

According to [9], Amberlite IRA 68 which had a polystyrene matrix similar to Amberlite IRA 67, had a negligible affinity for the bitter compounds in orange juice. It might be that the resin had inadequate amount of surface area accessible to the bitter compound molecules compared to the other resins. The same phenomenon might had happened in the deacification of noni juice. Weak base anion exchange resin which was polystyrene copolymer containing a tertiary amine group as functional group have been used in the deacidification of citrus juice and high acid maintenance because of its ability to pull organic acid from the juices [12]. Previous research also reported that Amberlite IRA 67 resin is a promising adsorbent for the adsorption of some organic acids [34].

3.3 Total Phenolic Content (TPC)

The assay of total phenols with Folin-Ciocalteau reagent determined both free phenolics and bound phenolics in the products [35]. As expected, results in Figure 4 indicate that total phenolic content of noni juice treated with three types of resins decreased significantly ($p<0.05$) compared to fresh juice. This might happened due to the hydrophobic interactions between resin and phenolic compounds during deacidification of the juice. It has been reported that most of the phenolic compounds found in noni fruit are non-polar in nature [36]. Thus, it is most possible that the interaction of the phenolic compounds with the resin is not through ion exchange but rather hydrophobic interaction. The polyphenol binding of ion exchange resins is influenced by the functional groups of

the resin and hydrophobic matrix of the resin [37]. Amberlite IRA 96 which is a polystyrene-divinylbenzene matrix gave the lowest total phenolic content which suggested a higher binding capacity compared to the other resins. The polystyrene-divinylbenzene has the highest hydrophobicity among all resin studied which consisted of different groups based on their corresponding monomers: poly(styrene-co-DVB), poly(ester), poly(acrylates), poly(styrene-*co*-butadiene), poly(urethane) and poly(ethylene-*co*-vinyl acetate) [38]. This suggests that hydrophobic interaction maybe the cause of the significant ($p<0.05$) reduction of phenolic compounds in noni juice with Amberlite IRA 96. Total phenolic content significantly ($p<0.05$) increased when the amount of resin was increased from 5 to 10% for Duolite A7 and Amberlite IRA 96. However, increase in the amount of resin from 5 to 10% did not significantly ($p<0.05$) affects the total phenolic content of noni juice treated with Amberlite IRA 67. The results were similar to [8] during optimization of a deacidification process of the same juice using Amberlite IRA 67 resin. An opposite trend is observed when resin weight was increased from 5 to 10% between acid and phenolics. This may be due to the difference in mechanism of interaction between the resin and acid which was via ion exchange and between phenolics and the resin via hydrophobic interaction. Increasing the adsorbent dose of Amberlite IRA 67 increased the adsorption efficiency [34]. As has been stated previously, it is possible that the increased adsorption efficiency resulted in increased adsorption of octanoic acid thus leaving more unadsorbed antioxidant compounds in the juice.

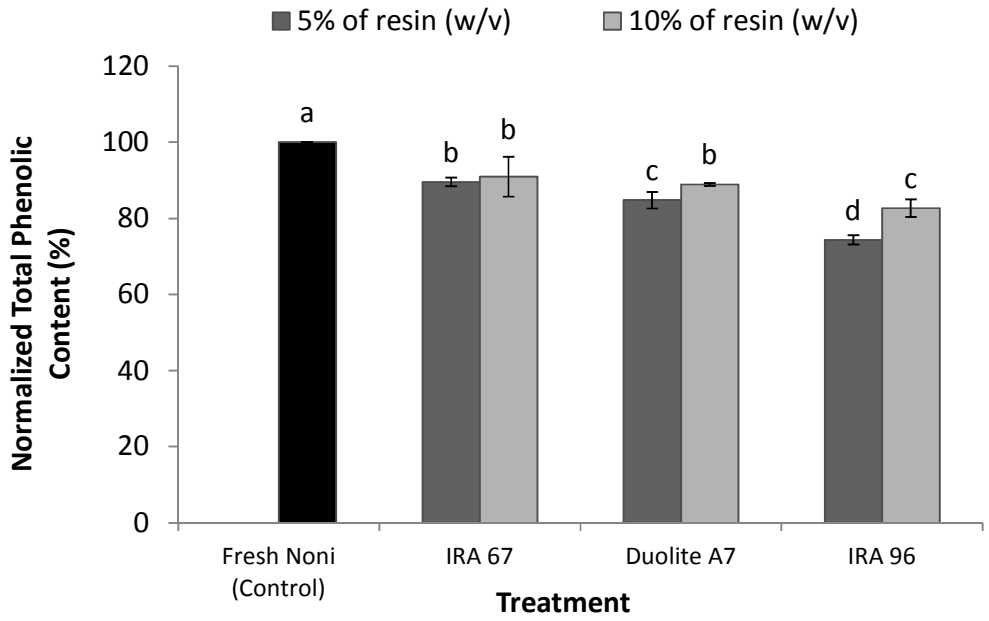

Fig. 4 Normalized TPC of noni juice treated with different types of ion exchange resins at 5% and 10% of resins (w/v)
[a-d] Means with different letters are significantly different ($p<0.05$)
Note: Error bars represent standard deviation

3.4 Ferric Reducing Assay (FRAP)

Figure 5 shows ferric reducing activity of noni juice. From Figure 5, all deacidified noni juice treated with the three different resins were significantly ($p<0.05$) lower compared to control for FRAP. There was no significant difference for FRAP between noni juice treated with 5 and 10 % (w/v) of Amberlite IRA 67 and Duolite A7 resins. However, for Amberlite IRA 96, a significant ($p<0.05$) increase in ferric reducing activity was observed when resin weight was increased from 5 to 10% (w/v). Comparing between different types of resin showed that at both 5 and 10% amount of resin, Amberlite IRA 96 significantly ($p<0.05$) gave the lowest ferric reducing activity of noni juice compared to the juice treated with Amberlite IRA 67 and Duolite A7. There were no significant difference ($P<0.05$) in ferric reducing activity between Amberlite IRA 67 and Duolite A7 for both 5 and 10% (w/v) resin weight.

The results obtained gave significantly different ($p<0.05$) ferric reducing abilities for different types of resin. The observed results may be due to differences in interaction of polyphenols in noni juice with different types of ion exchange resins. The polymeric matrix of weak base anion exchangers significantly contributes to polyphenol binding via hydrophobic interaction, hydrogen bonding and ionic interactions due to the existing functional groups of the resins [37]. Different polymeric matrix (Table 2) of the resins have different interactions to the antioxidant

compounds. As shown in Figure 5, deacidified noni juice treated with Amberlite IRA 96 shows the highest adsorption (lowest ferric reducing activity) among the resins used. As the active compounds that contribute to antioxidative activity of noni fruits are probably non-polar in nature [36], the hydrophobicity of styrene divinylbenzene (Amberlite IRA 96) allows better adsorption of non-polar phenolic compounds through hydrophobic interaction [39] and van der Waals interaction [40] as discussed earlier.

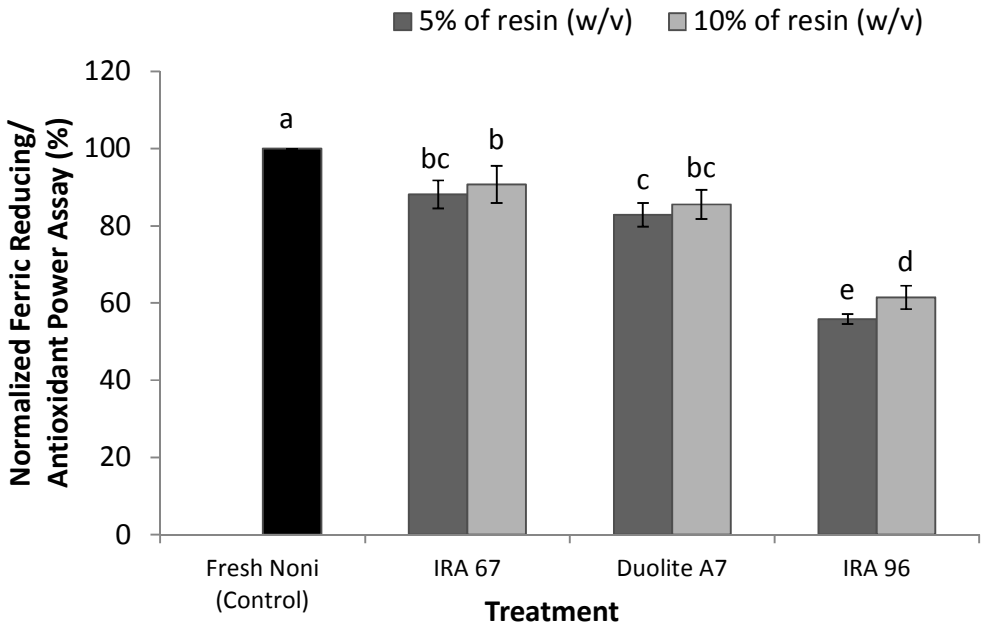

Fig. 5 Normalized antioxidant activity (FRAP) of noni juice with different types of ion exchange resins at 5% and 10% of resins (w/v)
[a-d] Means with different letters are significantly different (p<0.05)
Note: Error bars represent standard deviation

3.5 Free Radical Scavenging Activity using DPPH

Free radical scavenging activity of DPPH is another important method to evaluate the antioxidant acitivity by which antioxidants inhibit lipid peroxidation [41]. All deacidification process involving the three types of weak base anion exchange resins, free radical scavenging activity decreased significantly ($p<0.05$) compared to untreated juice as shown in Figure 6. Between these three types of resins, Amberlite IRA 67 still exhibited the best free radical scavenging activity. Amberlite IRA 67 showed a significantly ($p<0.05$) higher free radical scavenging activity when the amount of resin was increased from 5 to 10% (w/v). The trend was also similar for Amberlite IRA 96. In free radical scavenging activity, the juice treated with Duolite A7 significantly ($p<0.05$) decreased when the amount of resin was increased from 5 to 10% (w/v). This might happened due to the higher number of functional groups of the resins when the amount of resin increased. Juices treated with Amberlite IRA 67 exhibited a significantly ($p<0.05$) higher scavenging activity (5% of resins, w/v) compared to Amberlite IRA 96 but no significant difference with Duolite A7. Otherwise, treatment with 10% resins (w/v) showed juice treated with Amberlite IRA 67 having significantly ($p<0.05$) higher scavenging activity compared to the other resins. As previously suggested, the higher scavenging activity maybe due to the higher phenolic acids in the compounds and higher affinity of organic acids towards the resin. In general, Duolite A7 and Amberlite IRA 96 exhibits lower free radical scavenging activity than Amberlite IRA 67. The trend was slightly different with total phenolic content due to the different mechanism of reaction. A high phenolic content does not necessarily accompanies high antioxidant activity [42]. The antioxidant activity also depends on the structure and interaction between extracted phenolic compounds [43].

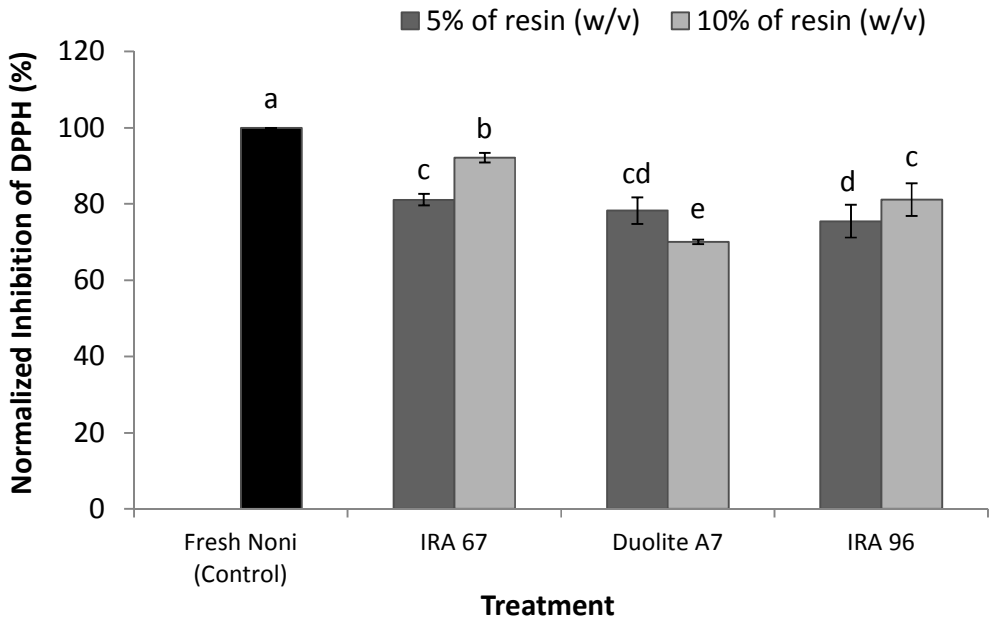

Fig. 6 Normalized inhibition of DPPH of noni juice treated with different types of ion exchange resins at 5% and 10% of resins (w/v)
[a-d] Means with different letters are significantly different (p<0.05)
Note: Error bars represent standard deviation

Similar to FRAP, the crosslinked phenol-formaldehyde matrix for Duolite A7 and styrene-divinylbenzene for Amberlite IRA 96 may be a factor contributing to this observation. Amberlite IRA 67 might have higher selectivity to most of organic acids [34] compared to antioxidant compounds due to the matrix of the resin. On the other hand, even though Amberlite IRA 67 and Amberlite IRA 96 have the same functional group, this phenomenon can be attributed to the decrease in competition between the charged ions. Protonation of these polyphenolic compounds significantly changes the charges and affinity of the resins [44]. The highest antioxidant activity in noni juice treated with Amberlite IRA 67 was expected because it contained the highest total phenolic content compared to the juice treated with Duolite A7 and Amberlite IRA 96. This was probably due to the higher total phenolic contents resulting in stronger antioxidant activities [45].

3.6 Selected Phenolic Compounds

Noni fruits contain complicated mixture of various bioactive compounds including phytochemicals and antioxidants. Even though total phenolics might be a useful marker of nutritional advantage, the actual profile of phenolics within the juices should also studied. Three most common antioxidant compounds which were identified and quantified in noni juice were scopoletin, rutin and quercetin. Scopoletin is a characteristic phytochemical in noni fruit, while rutin and quercetin are bioactive flavonoids [22]. According to the study done by them, scopoletin, rutin and quercetin were detected in all noni fruits and commercial noni juices from different countries all over the world although at different range of concentration.

Figure 7 and 8 shows the normalized HPLC determination of phenolic compounds in noni juice treated with 5 and 10% (w/v) of resin weight. The trend for both 5 and 10% (w/v) resin weight was quite similar for all three different resins used. The concentration of the three phenolic compounds in control sample was significantly ($p<0.05$) higher compared to the treated samples except for quercetin in samples treated with 5% Amberlite IRA 67. Based on Figure 8, phenolic compounds in the sample treated with Amberlite IRA 67 were significantly ($p<0.05$) higher than samples treated with Duolite A7 and Amberlite IRA 96. This suggested that Amberlite IRA 67 which is a crosslinked acrylic gel matrix has lower adsorption ability on phenolic compounds compared to Amberlite IRA 96 (styrene divinylbenzene) and Duolite A7 (phenol formaldehyde). For both 5 and 10% (w/v) of resin weight, similar trends was observed where scopoletin exhibited the highest concentration in the juice, followed by rutin and quercetin. The trend was also similar to [46] during characterization of Costa Rican noni juice. As mentioned in antioxidant activities and total phenolic content analysis (DPPH, FRAP and TPC), noni juice treated with Amberlite IRA 67 showed the highest antioxidant activity due to lower adsorption of antioxidants during deacidification.

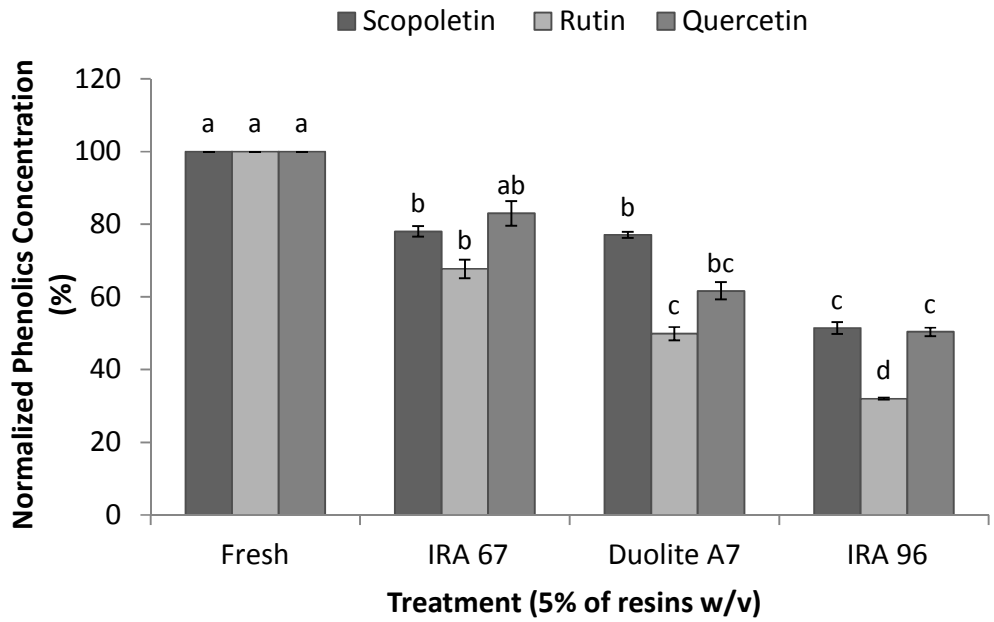

Fig. 7 Normalized RP-HPLC determination of phenolic compounds in noni juice treated with different ion exchange resins at 5% of resins (w/v)

[a-d] Means in the same phenolic compounds (scopoletin, rutin and quercetin) with different letters are significantly different ($p<0.05$)
Note: Error bars represent standard deviation

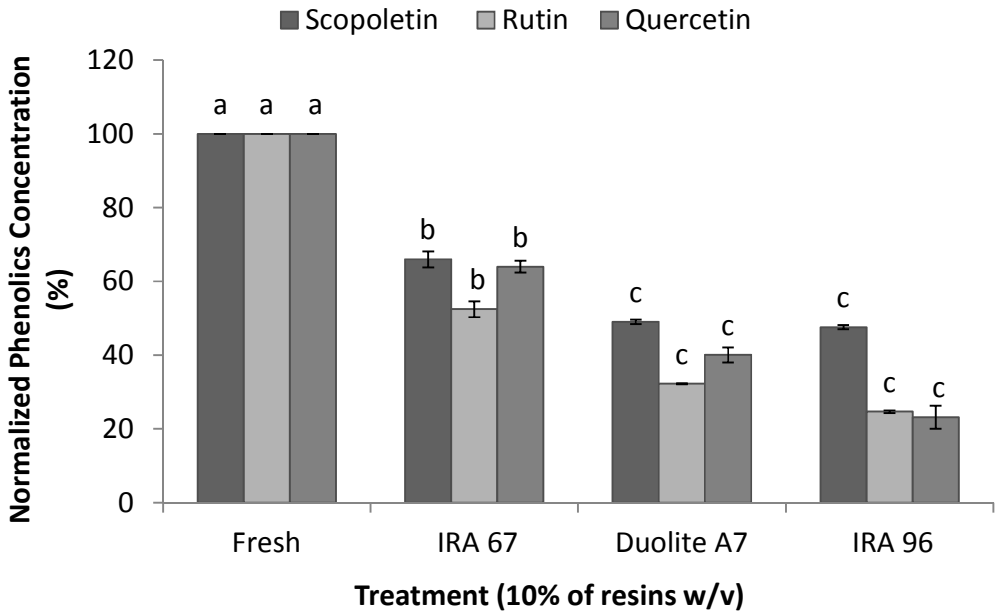

Fig. 8 Normalized RP-HPLC determination of phenolic compounds in in noni juice treated with different ion exchange resins at 10% of resins (w/v)

[a-d] Means in the same phenolic compounds (scopoletin, rutin and quercetin) with different letters are significantly different ($p<0.05$).
Note: Error bars represent standard deviation

CONCLUSION

In this study, deacidified noni juice using Amberlite IRA 67 resin showed the highest antioxidant and the lowest octanoic acid content for all analyses followed by Duolite A7 and Amberlite IRA 96. The results indicated that

deacidification of noni juice using 10% of resin weight (w/v) gave higher percentage of antioxidant activity (DPPH and FRAP) and total phenolic contents (TPC). Results showed similar trends where Amberlite IRA 67 > Duolite A7 > Amberlite IRA 96 for DPPH, FRAP, TPC and antioxidant compounds although at different weight of resins. Noni juice treated with Amberlite IRA 67 showed promising potential to be used for deodorization while it also gave minimal reduction on antioxidant content. The findings of the present study are of utmost importance for further investigation in this field. As a suggestion, more detailed knowledge of resin's behaviour could explain the principles of resin adsorption towards adsorbate.

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