

## Effect of Different Methods of Deacidification on the Properties of Noni (*Morinda citrifolia*) Extract

(Kesan Kaedah Berbeza Penyahasidan terhadap Sifat Ekstrak Mengkudu (*Morinda citrifolia*))

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### ABSTRACT

Deacidification of fruit extracts is necessary in certain situations which include consumer preference and product application. Although deacidification of noni extract using ion exchange resin and calcium carbonate addition has been reported, comparison between the two methods is lacking. This study was conducted to compare the effects of different deacidification treatments of ion exchange resin and calcium carbonate on the properties of noni extract. Ion exchange resin (Amberlite IRA-67) (R), calcium carbonate addition (CC), ion exchange resin followed by calcium carbonate addition (RC) and calcium carbonate addition followed by ion exchange resin (CR) were used to deacidify noni extract. Subsequently, analysis for pH, total soluble solids (TSS), total phenolic content (TPC), free radical scavenging ability (DPPH) and ferric reducing power (FRAP) were carried out. Compared to control, TPC, DPPH, and FRAP of noni extract decreased significantly ( $p < 0.05$ ) after deacidification using all different treatments. pH increased significantly ( $p < 0.05$ ) for all samples. TSS decreased significantly ( $p < 0.05$ ) after deacidification using all different treatments except for samples using resin (R) and calcium carbonate (CC). Deacidification using resin (R) produced a higher pH but lower TPC, DPPH and FRAP compared to calcium carbonate (CC). Ion exchange resin followed by calcium carbonate (RC) produced a lower ( $p < 0.05$ ) DPPH compared to calcium carbonate followed by ion exchange resin (CR). Deacidification of noni extract using calcium carbonate (CC) was the best method as it produced the smallest ( $p < 0.05$ ) reduction per change of pH for FRAP and no significant difference for DPPH compared to ion exchange resin (R).

Keywords: Antioxidant activity; calcium carbonate; deacidification; ion exchange resin; noni

### ABSTRAK

Penyahasidan ekstrak buah adalah perlu di dalam keadaan tertentu berdasarkan keperluan pengguna dan aplikasi produk. Walaupun penyahasidan ekstrak mengkudu menggunakan resin penukar ion dan penambahan kalsium karbonat telah dilaporkan, tetapi tiada perbandingan antara dua kaedah ini yang telah dilakukan. Kajian ini dijalankan untuk membandingkan kesan perlakuan penyahasidan yang berbeza iaitu resin penukar ion dan kalsium karbonat terhadap ciri ekstrak mengkudu. Resin penukar ion (Amberlite IRA-67)(R), penambahan kalsium karbonat (CC), resin penukar ion diikuti dengan penambahan kalsium karbonat (RC) dan penambahan kalsium karbonat diikuti resin penukar ion (CR) telah digunakan untuk penyahasidan ekstrak mengkudu. Seterusnya, analisis untuk pH, jumlah pepejal larut (TSS), jumlah kandungan fenol (TPC), penangkapan radikal bebas (DPPH) dan kuasa penurunan ferik (FRAP) telah dijalankan. Berbanding dengan kawalan, TPC, DPPH dan FRAP ekstrak mengkudu berkurangan dengan ketara ( $p < 0.05$ ) setelah penyahasidan menggunakan kesemua perlakuan. pH meningkat dengan ketara ( $p < 0.05$ ) untuk semua sampel. TSS berkurangan dengan ketara ( $p < 0.05$ ) selepas penyahasidan oleh semua perlakuan melainkan sampel menggunakan resin (R) dan kalsium karbonat (CC). Penyahasidan menggunakan resin (R) menghasilkan pH yang lebih tinggi tetapi TPC, DPPH dan FRAP yang lebih rendah berbanding kalsium karbonat (CC). Penyahasidan menggunakan resin penukar ion diikuti kalsium karbonat (RC) menghasilkan DPPH yang lebih rendah ( $p < 0.05$ ) berbanding kalsium karbonat diikuti resin penukar ion (CR). Penyahasidan ekstrak mengkudu menggunakan kalsium karbonat (CC) adalah kaedah yang terbaik kerana menghasilkan penurunan terkecil ( $p < 0.05$ ) untuk setiap perubahan pH bagi FRAP dan tiada perbezaan ketara dengan DPPH berbanding resin penukar ion (R).

Kata kunci: Aktiviti antioksidan; kalsium karbonat; mengkudu; penyahasidan; resin penukar ion

## INTRODUCTION

*Morinda citrifolia*, also known as noni is a well-known herbal plant not only in Malaysia, but also in Polynesia, China, India, and Australia (Maskat & Tan 2011). Noni is native to tropical Asia or Polynesia (Abbott & Shimazu 1985). There are about 80 species of *Morinda* and at least 20 species have significant economic and traditional value as a source of medicine, food, dyes or wood which among them are *Morinda citrifolia* and *Morinda trimera* (Nelson & Elevitch 2006). Noni intake is mostly in the form of extracts from the fruit. However, its leaf, flower, bark and root have also been used (Dixon et al. 1999; McClatchey 2002). Among the bioactive compounds that has been reported in noni, scopoletin has been reported as its characteristic phytochemical along with quercetin and rutin as bioactive flavonoids (Deng et al. 2010).

According to the ECSCF (2002), the European Union (EU) approved and registered noni juice as a novel food in 2002. The high nutritional value of noni fruit has made it increasingly popular around the world. In recent years, the scientific community has shown interest in *Morinda citrifolia* L. and its products due to its known benefits (Zin et al. 2002). Nowadays, there are companies that produce noni products in the form of juices, powders and capsules (Maskat & Tan 2011). Although the health benefits of noni products have been acknowledged through numerous studies, consumer acceptance is very low due to, among others, the sour taste of noni.

Deacidification has been used in the food industry to reduce acid levels in food products. Although acids in food systems facilitate the preservation of foods by reducing the rate of microbial growth, in certain situations high acid in foods is not preferable and even detrimental to the consumers. It has been reported that acids may affect the strength of plastic food packages. Satish et al. (2012) observed an increased amount of plastic extractives from 0.4 ppm to 4.9 ppm in 1 liter bottled distilled water when pH was reduced to less than pH 5 to simulate a high acid drink. However, the amount was still within the permissible limit. In addition, it was reported that lactic acid in salmon muscle was able to partially degrade polypropylene films even though the films were free from defects thus producing microcrackings (Zumelzu et al. 2017). In certain situations, fruit acids need to be reduced in order to fulfil certain product specifications. A system was patented for deacidification of acidic orange juices to produce reduced acid orange juice for consumers experiencing gastral distress and/or digestive difficulties to high acid juices (Lineback et al. 2006). Vera et al. (2009) deacidified tropical fruit juices of passion fruit, castilla mulberry, najanrilla and araza using electrodialysis to reduce its usage limitations in

other food systems.

There have been several reported methods of deacidification. Among them are by using ion exchange resin, calcium carbonate addition and activated charcoal (Khalafu et al. 2017; Sin et al. 2018). Ion exchange resin has been reported to be used for the reduction of oxalic acid in star fruit juice (Fong et al. 2017). In addition, noni juice has been deacidified using ion exchange resin (Amberlite IRA-67) which was reported to be more practical on an industrial scale as this process can be carried out on a continuous basis (Haslaniza et al. 2015). However, although reduction in the acidity of noni juice was achieved using ion exchange resin, it also reduced the antioxidant capacity. As phenolic compounds contributed to the antioxidant capacity of noni juice, it was suggested that the adsorption of phenolic compounds to ion exchange resin through hydrophobic interactions resulted in the reduced antioxidant capacity (Ain et al. 2021). Reduced level of titratable acidity (Hafiza et al. 2010) and reduced level of total phenolic content in noni juice was observed with the use of calcium carbonate addition for deacidification (Nur Hafiza et al. 2008). The reaction between phenolic acids and calcium carbonate through neutralization reaction may have contributed to the reduction in total phenolic content of noni juice.

Although ion exchange resin and calcium carbonate methods of deacidification resulted in the reduction of antioxidant capacity and total phenolic content, respectively, there is a need to determine which method of deacidification has a lesser reduction effect. Currently there has not been any studies that compared the effects of using ion exchange resin and calcium carbonate on the properties of noni extract, nor has there been any studies that combines both deacidification methods. The selection of an appropriate deacidification method is important because it may affect the properties and subsequently determine the overall acceptance of noni extract products. Thus, this study was carried out to compare the effects of using Amberlite IRA-67 resin and calcium carbonate and also in combination on the properties of noni extract.

## MATERIALS AND METHODS

### MATERIALS

Noni fruits used in this study was obtained from Kajang, Selangor, Malaysia. The fruits used was at maturity stage four, where the fruits were whitish yellow, and the texture of the contents was slightly hard. The identification of noni fruit at maturity stage four was based on the definition of Chan-Blanco et al. (2006). The noni fruits were stored at room temperature for 3 days for ripening

before being used for juice extraction (Haslaniza et al. 2015). The ion exchange resin used was a polymer-based resin, Amberlite IRA-67 obtained from Fluka, Rohm and Haas Company, France. The resin was a weak-based anion converter resin and has acrylic and tertiary amine ( $-NH_2$ ) bonds as functional groups. Amberlite IRA-67 was round bead-shaped and translucent white in colour. Food-grade calcium carbonate was used in this study.

#### NONI EXTRACTION

Noni juice extraction was carried out according to Haslaniza et al. (2015). Ripened noni fruits at maturity stage four were cleaned with distilled water and dried with a cloth. The fruit was cut into small pieces about 2 to 4 cm, added with distilled water at a ratio of 1:1 (weight:volume) and then ground using a blender (Cornell, Malaysia). The resulting juice was filtered using a muslin cloth. The filtered juice was then centrifuged using a centrifuge (5810R - Eppendorf Centrifuge, Germany) at 4000 rpm ( $3220\times g$ ) for 25 min to remove the pulp and foreign matter remaining in the noni juice. The extracted extract was then filtered again using Whatman No. 4 filter paper.

#### RESIN CONDITIONING

The resin used, Amberlite IRA-67, was conditioned according to Haslaniza et al. (2015) before being used in the deacidification process of noni juice. A total of 0.5 g of resin was soaked in 5.0 mL of HCl (5%) for 45 min. Then, the mixture was filtered with Whatman No. 4 filter paper and subsequently soaked in 15 mL of deionized water for 2 h. The resin was rinsed again with deionized water to remove excess residue. Finally, the mixture was filtered, and the resin dried in a desiccator for 24 h.

#### NONI EXTRACT DEACIDIFICATION

Four deacidification methods consisting of ion exchange resin (R), calcium carbonate addition (CC) and two combinations of deacidification treatments which were ion exchange resin treatment followed by calcium carbonate (RC) and calcium carbonate addition followed by ion exchange resin (CR) was carried out. For deacidification using ion exchange resin (R), noni extract was mixed with 8% (w/v) conditioned Amberlite IRA-67 resin in a 250 mL Erlenmeyer flask and shaken using an incubator shaker (Model WIS 20 - WiseCube, Korea) for 2 h and 120 rpm at room temperature. After that, the mixture was filtered using Whatman No. 4 filter paper.

Deacidification using calcium carbonate (CC) was carried out by adding  $CaCO_3$  (Sigma, UK) directly to the noni extract until it reached maximum solubility, stirred

and left for 3 min. Subsequently, the treated extracts were filtered using Whatman No. 4 filter paper prior to analysis. For the combined deacidification methods of ion exchange resin treatment followed by calcium carbonate (RC), the noni extract was subjected to ion exchange resin treatment as described for R and followed by calcium carbonate treatment as described for CC. Similarly, for the combined deacidification methods of calcium carbonate followed by ion exchange resin treatment (CR), the noni extract was subjected to calcium carbonate treatment as described for CC and followed by ion exchange resin treatment as described for R. Non-deacidified noni extract was taken as control.

#### DETERMINATION OF PH VALUE

A pH meter (Model PHM 210 - MeterLab) was used to measure the pH value. Before sample measurements were performed, the pH meter was calibrated with pH 4.0 and pH 7.0 buffer solutions. Measurements of pH values were performed using 10 mL of sample at room temperature. The sample was stirred before the pH reading was taken.

#### DETERMINATION OF TOTAL SOLUBLE SOLIDS (TSS)

Total soluble solids (TSS) were measured using a hand-held refractometer (ATAGO, Japan). As many as 2 to 3 drops of sample were dropped on the surface of the hand refractometer. Readings were taken and the values were expressed in  $^{\circ}$ Brix.

#### TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content was determined using Folin-Ciocalteu method (Slinkard & Singleton 1977; Yang et al. 2011). Dilution of noni extract was carried out by mixing 0.1 mL of noni extract with 0.9 mL of distilled water. Solution absorption readings were measured using UV-Vis microplate reader (Biotech model 259037) at a wavelength of 765 nm. Standard curve of gallic acid was done and phenolic activity was determined and expressed as mg gallic acid equivalent (GAE/L). The calculation of the total phenolic content was as follows (1):

$$\text{Total phenolic content in noni extract (mg GAE/L)} = R \times D \quad (1)$$

where R is the reading on standard curve (gallic acid); and D is the dilution factor = 10.

#### FREE RADICAL SCAVENGING ABILITY (DPPH)

The free radical scavenging ability of noni extract was measured based on the method of Akowuah et al. (2005). The 0.1 mM solution of DPPH (2,2-diphenyl-

1-picrylhydrazil) was used as a standard stock solution and prepared fresh each time it was used. Absorption was read at a wavelength of 517 nm using UV-Vis microplate reader (Biotech model 259037). The free radical scavenging ability of noni extract was calculated using the following formula (2):

$$\text{Percentage of DPPH} = \frac{[AC - AS] / AC \times 100\%}{(2)}$$

where AC is the absorption readings of control samples (DPPH solution); and AS is the absorption readings of extracted noni samples after 1 hour.

#### FERRIC REDUCING POWER (FRAP)

The method of ferric reducing power determination was carried out based on the method of Delgado-Andrade et al. (2010). Before the analysis was carried out, the noni extract was diluted to 100 times using distilled water. Next, a total of 900  $\mu\text{L}$  of freshly prepared FRAP reagent was mixed with 100  $\mu\text{L}$  of noni extract and the mixture was incubated in the dark at 37 °C for 30 min. Thereafter, 200  $\mu\text{L}$  of the mixture was pipetted into the microplate groove and absorption readings were taken at a wavelength of 595 nm using an Epoch-branded UV-Vis microplate reader (Biotech model 259037). Standard curve was made with  $\text{FeSO}_4$  and the results were expressed as  $\mu\text{mol Fe (II)}$  equivalent ( $\mu\text{mol Fe (II)}/\text{mL}$ ). The calculation was as follows (3):

$$\text{Ferric reducing power } (\mu\text{mol Fe (II)}/\text{mL}) = R \times D \quad (3)$$

where R is the readings from standard curve; and D is the dilution factor = 100.

#### NORMALIZED CHANGE OF TPC, DPPH AND FRAP

In order to determine which method has the highest ability to deacidify with minimal reduction of TPC, DPPH, and FRAP of the noni extract, the degree of reduction needed to be normalized for change of pH as shown in the formula below (4):

$$\text{Normalized change of TPC/DPPH/FRAP} = \quad (4)$$

$$\frac{(\text{TPC/DPPH/FRAP of treatment} - \text{TPC/DPPH/FRAP of control})}{(\text{pH of treatment} - \text{pH of control})}$$

#### STATISTICAL ANALYSIS

All experiments were conducted in three replications. Data obtained was analysed using ANOVA and Duncan test using Statistical Analysis System (SAS) software version 9.3, 2011. The confidence level used was 95% ( $p < 0.05$ ).

#### RESULTS AND DISCUSSION

##### pH VALUE

Figure 1 shows the pH value of control noni extract and deacidified noni extract using ion exchange resin (R), calcium carbonate (CC) and two other combinations

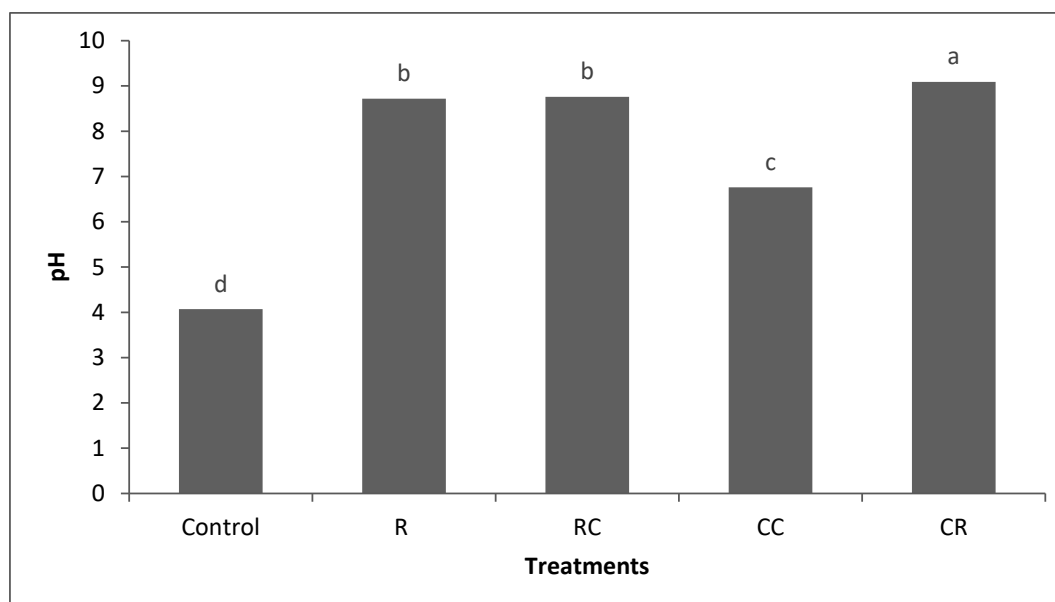


FIGURE 1. The pH value of noni extract deacidified using ion exchange resin (R), calcium carbonate (CC), ion exchange resin followed by calcium carbonate (RC) and calcium carbonate followed by ion exchange resin (CR)

<sup>a-d</sup> Means with different alphabets showed significant difference ( $p < 0.05$ )

of treatments with different sequences which were ion exchange resin treatment followed by calcium carbonate (RC) and calcium carbonate addition followed with ion exchange resin (CR). The control noni extract had a pH value of 4.07 (Figure 1). These results were similar to the study of Satwadhari et al. (2011) who found that noni extract was acidic with a pH of 4.16.

The results showed that there were significant differences ( $p < 0.05$ ) between the pH value of the control sample with the pH value of all deacidified samples. The pH value of noni extract increased significantly ( $p < 0.05$ ) after deacidification in all different treatments compared to the control sample. These results were similar to the study by Haslaniza et al. (2015) and Hafiza et al. (2010), where deacidification using Amberlite IRA-67 resin and calcium carbonate resulted in an increase in the pH of noni juice. Deacidification using Amberlite IRA-67 resin reduced the acidity of noni juice and caused the pH to increase. OH<sup>-</sup> from the ion exchange resin was exchanged with dissociated anions from the acids which were then adsorbed to the resin. Subsequently, the dissociated H<sup>+</sup> from the acids reacted with OH<sup>-</sup> to form water thus reducing the pH (Haslaniza et al. 2015). For deacidification using calcium carbonate, the increase in pH was due to acid neutralization, as reported by

Herjavec et al. (2003) and Vera et al. (2003).

Comparison between calcium carbonate addition (CC) and ion exchange resin (R) showed that R produced higher deacidification effect ( $p < 0.05$ ) compared to CC. Haslaniza et al. (2019) reported that at higher pH, interaction between organic acids and Amberlite IRA-67 ion exchange resin increased. This showed that CC had a lesser effect in changing the pH compared to R. For deacidified samples using different combined treatment sequences of ion exchange resins and calcium carbonate, CR gave a slightly higher although significant ( $p < 0.05$ ) pH value compared to RC.

#### TOTAL SOLUBLE SOLIDS (TSS)

Based on Figure 2, there was no significant difference for TSS resulting from deacidification of noni extract either using resin (R) or calcium carbonate (CC) when compared to control. However, the TSS of noni extract decreased significantly ( $p < 0.05$ ) after being deacidified using both combination of ion exchange resin and calcium carbonate (RC and CR) compared to the control sample.

The significant decrease ( $p < 0.05$ ) of TSS in noni extract when treated with the combination of ion exchange resin and calcium carbonate (RC and CR) may be caused by the additional filtration step due to the combined treatments. This argument is further supported

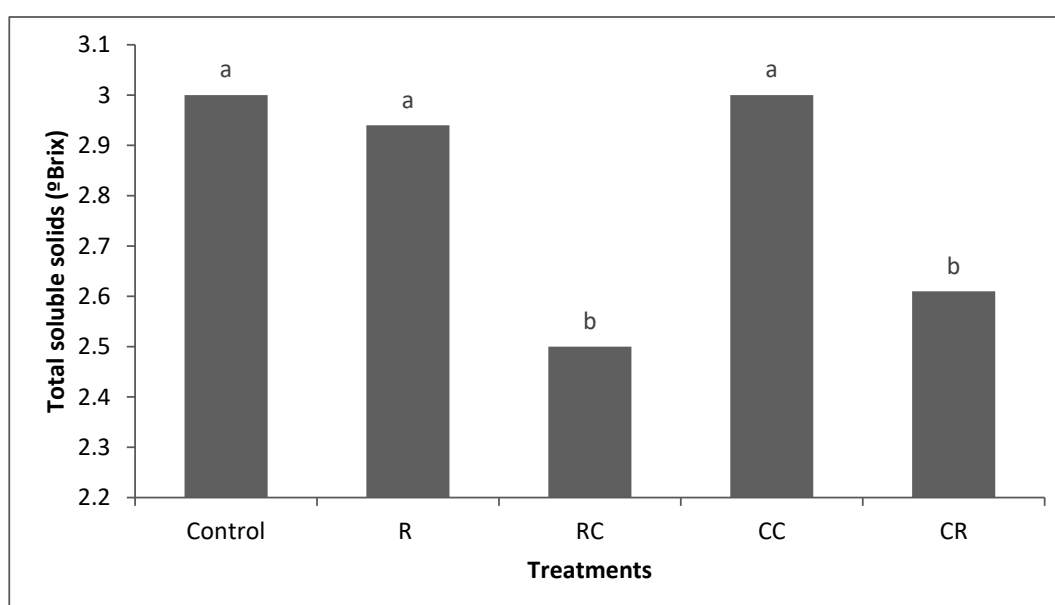


FIGURE 2. Total soluble solids (TSS) of noni extract deacidified using ion exchange resin (R), calcium carbonate (CC), ion exchange resin followed by calcium carbonate (RC) and calcium carbonate followed by ion exchange resin (CR)

<sup>a-b</sup> Means with different alphabets showed significant difference ( $p < 0.05$ )

by the results of the combined methods where there was no significant differences for TSS values between the deacidified samples using different ion exchange resin and calcium carbonate treatment sequences (RC and CR).

#### TOTAL PHENOLIC CONTENT (TPC)

Figure 3 shows the total phenolic content of control noni extract and deacidified noni extract using different treatments. Based on Figure 3, the total phenolic content of control noni extract was the highest ( $p < 0.05$ ) which was  $1106.09 \pm 85.14$  mg GAE/L. All deacidified samples were significantly lower ( $p < 0.05$ ) in TPC compared to control. Reduction of TPC in noni extract after deacidification using ion exchange resin has been suggested to be caused by the ion exchange of phenolic acids and adsorption of non-polar phenolic compounds through hydrophobic interactions and van der Waals force to the hydrophobic matrix structure of the resin

(Haslaniza et al. 2015). The decrease in TPC resulting from calcium carbonate may be due to the neutralizing of phenolic acid in noni extract as stated previously. The decrease in total phenolic content resulting from resin treatment (R) was more significant ( $p < 0.05$ ) compared to calcium carbonate (CC). This pattern was in parallel to the pH result where the resin treatment (R) gave a greater deacidification effect compared to calcium carbonate (CC). Comparing Figure 3 to Figure 1 shows the loss of TPC was closely related to pH. Among the deacidified samples, there were no significant differences for total phenolic content between the deacidified samples between both combinations of resin and calcium carbonate treatment (RC and CR). These results indicate that the sequence of deacidification using ion exchange resin and calcium carbonate did not have a significant effect on the TPC of noni extract when used in combination.

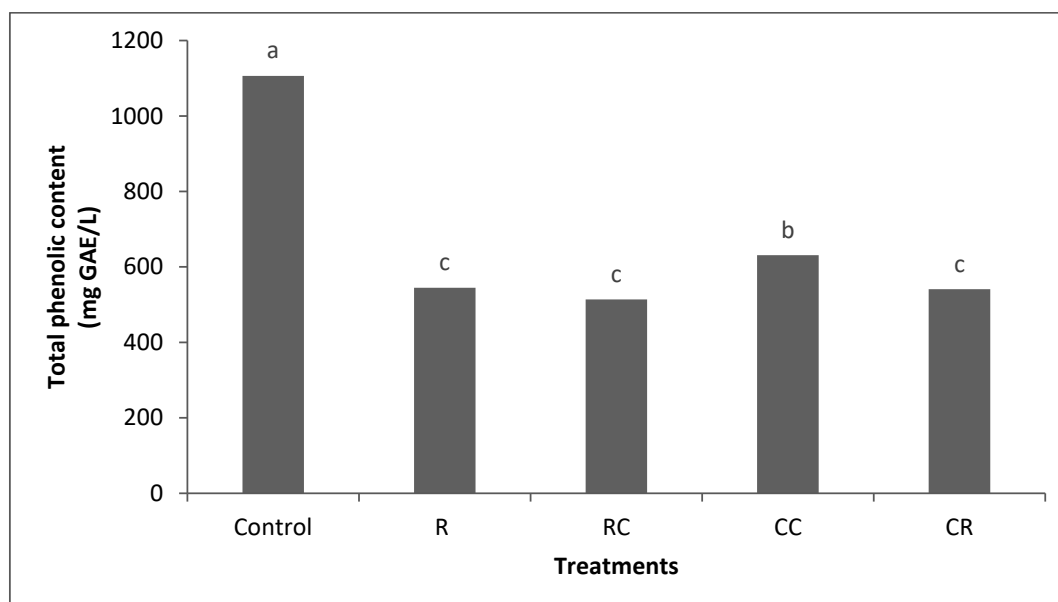


FIGURE 3. Total phenolic content (TPC) of noni extract deacidified using ion exchange resin (R), calcium carbonate (CC), ion exchange resin followed by calcium carbonate (RC) and calcium carbonate followed by ion exchange resin (CR)

<sup>a-c</sup> Means with different alphabets showed significant difference ( $p < 0.05$ )

#### FREE RADICAL SCAVENGING ABILITY (DPPH)

Figure 4 shows the percentage of free radical scavenging ability of noni extract with different treatments. Based on Figure 4, the free radical scavenging percentage of control noni extract was the highest at  $72.11 \pm 2.93\%$ . There was a significant difference ( $p < 0.05$ ) between the percentage of free radical scavenging ability of control

noni extract with deacidified noni extract using all different treatments. The percentage of free radical scavenging of noni extract decreased significantly ( $p < 0.05$ ) after being deacidified using all treatments. Similar studies had also reported that deacidification using ion exchange resin led to a reduction in the percentage of free radical scavenging ability of DPPH in noni extract (Ain et al. 2021).

DPPH values for calcium carbonate treatment was the highest ( $p < 0.05$ ) among deacidified samples. The lower DPPH result for deacidified samples of R, RC and CR compared to CC was in agreement with their lower TPC results (Figure 3). Ain et al. (2021) reported significant ( $p < 0.05$ ) and strong correlation ( $r = 0.943$ ) between TPC and DPPH in noni extract. In addition, several studies on the chemical composition of noni showed that phenolic compounds found in noni juice functioned as free radical scavenger (Chan-Blanco et al. 2006; Dixon et al. 1999). However, the same argument cannot be made when comparing between R with RC or CR, or between RC and CR. Thus, loss of TPC can only serve as a partial explanation to the results of DPPH. This may be due to

TPC measuring a broader range of compounds which include free and bound antioxidant compounds while DPPH predominantly determines only free antioxidant compounds (Slinkard & Singleton 1977).

Comparing between RC and CR, it was observed that using calcium carbonate prior to ion exchange resin produced a significantly higher ( $p < 0.05$ ) DPPH compared to ion exchange resin followed by calcium carbonate. This trend was apparently consistent with the effect of CC and R on DPPH where CC produced a significantly higher ( $p < 0.05$ ) DPPH compared to R. Thus, the DPPH results of the combined treatment was significantly ( $p < 0.05$ ) dependent on the initial method of deacidification.

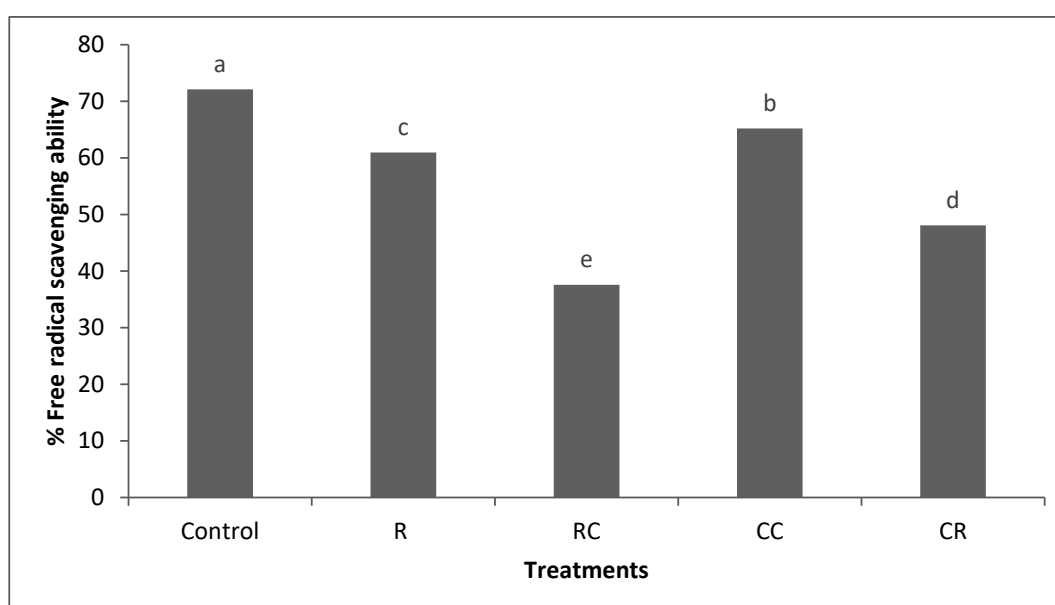


FIGURE 4. Free radical scavenging ability (DPPH) of noni extract deacidified using ion exchange resin (R), calcium carbonate (CC), ion exchange resin followed by calcium carbonate (RC) and calcium carbonate followed by ion exchange resin (CR)

<sup>a-e</sup> Means with different alphabets showed significant difference ( $p < 0.05$ )

#### FERRIC REDUCING POWER (FRAP)

Figure 5 shows the ferric reducing power of noni extract with different treatments of deacidification. Based on Figure 5, the ferric reducing power of control noni extract was significantly the highest ( $p < 0.05$ ). The ferric reducing power of noni extract decreased significantly ( $p < 0.05$ ) after being deacidified using all different treatments including both combinations of resin and calcium carbonate treatments. Based on the results obtained, the FRAP results (Figure 5) has a similar pattern to TPC results (Figure 3) but different from the DPPH results (Figure 4). This maybe because FRAP readings do not include

the antioxidant capacity of antioxidant compounds that are incapable of lowering Fe (III) such as antioxidant compounds that have a sulfhydryl group (Prior & Cao 1999). In addition, the FRAP method can only measure antioxidant compounds that are hydrophilic in nature while the DPPH method measures antioxidant compounds that are hydrophobic in nature (Arnao 2000; Pai et al. 2010; Pulido et al. 2000). The similarity of FRAP results with TPC results indicated the possibility that phenolic compounds contributed to the significantly higher ( $p < 0.05$ ) antioxidative capacity of CC compared to R.

FRAP values for calcium carbonate deacidification (CC) was the highest ( $p < 0.05$ ) among the deacidified samples. There were no significant differences for ferric reducing power between the deacidified samples using

resin (R) and both combinations of resin and calcium carbonate treatments (RC and CR). As for the noni extract samples, deacidified using RC or CR, results showed that for FRAP, the sequence of the different deacidification method did not have any significant effect.

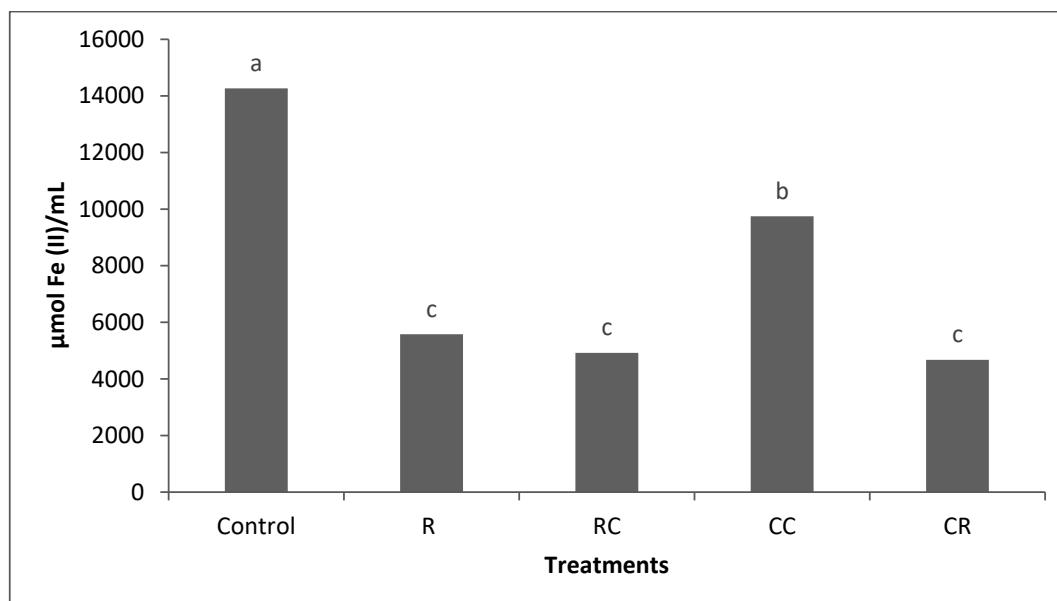


FIGURE 5. Ferric reducing power of noni extract deacidified using ion exchange resin (R), calcium carbonate (CC), ion exchange resin followed by calcium carbonate (RC) and calcium carbonate followed by ion exchange resin (CR)

\*c Means with different alphabets showed significant difference ( $p < 0.05$ )

#### NORMALIZED CHANGE OF TPC, DPPH, AND FRAP

Table 1 shows the normalized change of TPC, DPPH, and FRAP (change of TPC, DPPH, and FRAP relative to pH change). From the results, it can be observed that for TPC, deacidification using calcium carbonate (CC) produced the largest ( $p < 0.05$ ) reduction for each increase of pH. No significant differences in the normalized change for TPC between R, RC, and CR was observed. The reduction of TPC by R was due to the ion exchange and also hydrophobic adsorption of phenolic acids to the resin (Haslaniza et al. 2015) as stated earlier. However, the significantly higher loss ( $p < 0.05$ ) of TPC by CC compared to R may be due to not only the deacidification of phenolic acids but also the destruction of non-phenolic acid compounds such as sugar by CC during deacidification. It has been reported that the Folin-Ciocalteu reagent used in determining TPC is known to react with other non-phenolic compounds such as sugar (Deepa et al. 2006; Matthaas 2002). Cox

et al. (1990) in an investigation regarding carbonation process during sugar refining observed that liming resulted in the destruction of sugars. It is possible that during deacidification using calcium carbonate (CC), apart from neutralization of phenolic acids, destruction of sugar occurred and for this reason CC showed a higher loss of TPC compared to R, RC and CR.

For DPPH, deacidification using ion exchange resin (R) and calcium carbonate (CC) showed the smallest reduction ( $p < 0.05$ ) for each increase of pH. Both combined deacidification methods (RC and CR) resulted in a significantly larger reduction ( $p < 0.05$ ) of DPPH per increase of pH. Thus, it can be suggested that compounds with radical scavenging ability were both similarly decreased by ion exchange (R) and calcium carbonate (CC). It is also observed that the significantly larger ( $p < 0.05$ ) reduction in TPC by CC did not translate into lower DPPH for CC compared to R. The results further support the previous suggestion that the reduction in TPC by CC was due to reactions with non-phenolic acids such as sugar that did not highly contribute to DPPH.



Deacidification using calcium carbonate (CC) produced the smallest reduction ( $p < 0.05$ ) of FRAP per increase of pH compared to the other methods. This result showed that based on pH increase, deacidification of noni extract using calcium carbonate was able to minimise the

loss of FRAP antioxidant activity compared to using ion exchange resin and its combinations. The difference in result for FRAP compared to DPPH was probably due to the different antioxidant activity being measured (Clarke et al. 2013).

TABLE 1. pH normalized change of total polyphenol content (TPC), free radical scavenging ability (DPPH) and ferric reducing power (FRAP) of noni extract deacidified using different treatments

Deacidification treatment	$\Delta$ TPC/pH (mg GAE/L)	$\Delta$ DPPH/pH (%)	$\Delta$ FRAP/pH ( $\mu$ mol Fe (II)/mL)
R	-120.72 <sup>a</sup>	-2.40 <sup>a</sup>	-1867.78 <sup>b</sup>
RC	-126.32 <sup>a</sup>	-7.37 <sup>c</sup>	-1992.38 <sup>c</sup>
CC	-176.55 <sup>b</sup>	-2.57 <sup>a</sup>	-1680.37 <sup>a</sup>
CR	-112.50 <sup>a</sup>	-4.78 <sup>b</sup>	-1909.27 <sup>bc</sup>

<sup>a-c</sup> Means within the same column with different letters are significantly different ( $p < 0.05$ )

R - ion exchange resin; RC - ion exchange resin followed by calcium carbonate; CC - calcium carbonate; CR - calcium carbonate followed by ion exchange resin

### CONCLUSIONS

Results of the study showed an increased pH value and reduced antioxidant activities when noni extract underwent deacidification. Using ion exchange resin (R) showed a significantly higher ( $p < 0.05$ ) deacidification compared to calcium carbonate (CC) but resulted in significantly lower ( $p < 0.05$ ) TPC, DPPH and FRAP values. Combining ion exchange resin followed by calcium carbonate (RC) produced a significantly higher ( $p < 0.05$ ) deacidification compared to calcium carbonate followed by ion exchange resin (CR) but also resulted in a significantly lower ( $p < 0.05$ ) DPPH. Based on the normalized change of DPPH and FRAP with reference to pH increase, deacidification of noni extract using calcium carbonate (CC) was a significantly ( $p < 0.05$ ) better method due to the smaller reduction of FRAP antioxidant activity and no significant difference for DPPH antioxidant activity compared to ion exchange resin (R) relative to increase of pH. A less acidic noni extract with minimal reduction in antioxidant capacity would facilitate its incorporation into products resulting in increased utilization.

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