Effect of soaking on total phenolic content and antioxidant activities assessed by different *in vitro* assays of cashew nut

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Abstract

The cashew tree (*Anacardium occidentale* L.) yields one of the world's most popular edible nuts containing proteins, fats, fiber, vitamins and minerals. This research evaluated soaking at room ambient in water on the antioxidant activity and total phenolic content (TPC) of cashew nuts. The samples were soaked for 2, 12, and 24 h. Results showed that a longer soaking time exhibited greater DPPH, ABTS and FRAP values from 2 to 24 h and with the highest at 24 h. Total phenolic contents of all samples significantly decreased ($p < 0.05$) after soaking. Results supported the concept that antioxidants were acquired while consuming processed cashew nuts without soaking should be recommended.

Keywords: Cashew nut, soaking, TPC, antioxidant activity, processing

Introduction

The cashew tree (*Anacardium occidentale* L.) is native to Brazil and now grown extensively in Africa, India, and Vietnam, producing one of the world's most popular edible nuts containing proteins, fats, fiber, vitamins (vitamins B5 and B6, riboflavin, and thiamin), and minerals (magnesium, potassium, and copper) [1]. Cashew nuts have been consumed for a long time as a very popular snack. They can be eaten roasted, lightly salted or sugared, dipped in chocolate, or as ice cream topping. Cashew nuts are also a rich source of antioxidant vitamins and phenolic compounds. Recently, the antioxidant activities of various bioactive compounds such as phenolics, flavonoids, phospholipids, sterols, and tocopherols were reported in cashew nuts [2, 3]. The pretreatment of cashew nut may be done in different ways. For instance, removal of cashew kernel from the shell is a labour intensive operation involving cleaning/grading, pre-treatment by roasting or steam-boiling, shelling, separation, drying, and peeling [4, 5].

Soaking in water is a traditional preparation method prior to cooking for most nuts. This starts the germination process which activates the readily enzymes, vitamins, minerals, proteins and essential fatty acids and alters the biochemical properties of the nuts.

Cashew nuts are not normally eaten raw and thermal processing methods are applied to achieve the desirable sensory and nutritional properties. Soaking cashew nuts prior to thermal processing is a common practice to shorten the cooking time.

However, little information is available in the literature regarding the change in phenolic contents and antioxidant activity initiated by the soaking process. Therefore, this study aimed to determine the effect of soaking on the phenolic contents and antioxidant activities of the cashew nut.

Materials and methods

Cashew nut samples

Cashew nuts were supplied by Kunya Community Enterprise (Krabi, Thailand). All nuts were shelled using a hand operated knife cutter and separated whole kernels. Moisture content was calculated. Results were reported on a dry weight basis. They were stored in dry condition under refrigeration.

Soaking of cashew nuts

Raw cashew nuts were soaked in water (1:10 w/v) for 2, 12, and 24 h in the dark at room temperature [6]. After soaking, the cashew nuts were blanched in water at 98 °C for 1 min, then cooled in cold water for 1 min, drained and blotted with paper towels to remove excess water. The moisture content of the soaked cashew nuts was calculated and the samples were stored at −20 °C prior to analysis.

Extraction

The soaked cashew nuts were extracted for 3 h with 80% ethanol (1:10, w/v) at room temperature on a shaking incubator at 150 rpm [7]. The solution was filtered through filter paper and the filtrate was used to determine the total phenolic contents and antioxidant activity.

Determination of total phenolic content

Total phenolic content (TPC) was determined according to the method of Kubola and Siriamornpun [7] . Briefly, 0.3 ml of standard gallic acid and the cashew nut extract was reacted with 2.25 ml of 10% (v/v) Folin-Ciocalteu reagent and left for 5 min. Then, 2.25 ml of 6% sodium carbonate solution was added to the mixture and kept for 90 min in the dark at room temperature. Absorbance was measured at 725 nm using a spectrophotometer. The results were reported as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

DPPH free radical scavenging activity

DPPH scavenging activity was measured according to the method of Xu and Chang [8] with slight modifications. Extract solutions (0.1 ml) were mixed with 0.1 mM DPPH solution in ethanol (3 ml). The mixtures were shaken and kept in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. The percent inhibition of DPPH radical was calculated as $[(A_o - A_e)/A_o]100$ (A_o = absorbance of control; A_e = absorbance of sample).

ABTS free radical scavenging activity

The ABTS assay was conducted following the method of Wootton-Beard et al [9] with a slight modification. The ABTS radicle cation ($ABTS^{\bullet+}$) was produced by the oxidation of 7 mM ABTS solution with 2.45 mM potassium persulfate, and the mixture was incubated for 12 – 16 h in the dark at 4 °C. The solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Reaction mixtures containing 20 µl of sample added to 2 ml of $ABTS^{\bullet+}$ solution were shaken and kept at room temperature for 6 min in the dark. The absorbance was detected at 734 nm using a spectrophotometer. The percent inhibition of ABTS radical was calculated as $[(A_o - A_e)/A_o]100$ (A_o = absorbance of control; A_e = absorbance of sample).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted according to the method of Luo et al [10]. FRAP reagent was freshly prepared from 0.3 M acetate buffer pH 3.6 (100 ml), 10 ml of TPTZ solution (10 mM TPTZ dissolved in 40 mM HCl) and 20 mM $FeCl_3 \bullet 6H_2O$ (10 ml). Then, 12 ml of distilled water was added to the reagent and the mixture was incubated at 37 °C. Briefly, 60 µl of the extract was mixed with 180 µl distilled water and 1.8 ml of FRAP solution. The mixtures were shaken and incubated in a water bath at 37 °C for 4 min. The absorbance was read at 593 nm in a spectrophotometer and results were expressed as µmol$FeSO_4$ per g dry weight (µmol$FeSO_4$/g DW).

Statistical analyses

All soaking processes were performed in triplicate and the data were expressed as mean ± standard deviation. Statistical analyses were performed using SPSS version 11.5 for Windows. Analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine significant differences at $p < 0.05$.

Results and discussion

Total phenolic contents (TPCs) of raw and soaked cashew nuts are shown in Figure 1. TPC of raw cashew nuts was 11.22 mg GAE/g DW, significantly higher ($p < 0.05$) than that of soaked cashew nuts. Significant decrease in the total phenolic content of cashew nuts was observed as a result of the soaking process. TPC decreased from 11.22 mg GAE/g DW in raw cashew nuts to 1.18, 0.97 and 0.95 mg GAE/g DW in samples soaked for 2, 12, and 24 h, respectively. Similar decreases were observed for TPCs of soaked beans [8, 11]. Kataria et al [12] found that the total phenolic contents of mung bean decreased after soaking, and Siah et al [13] and Khandelwal et al. [11] reported that soaking of faba beans and Indian pulse reduced the phenolic content. The reduction in TPCs during soaking may be due to the leaching of some components into the water and the activation of the enzyme polyphenol oxidase affecting degradation and loss of polyphenols [14].

DPPH radical scavenging of raw and soaked cashew nuts is shown in Figure 2. Raw cashew nuts showed significantly higher DPPH values than the soaked samples. Unlike TPC, a longer soaking time increased the DPPH value from 2 – 24 h with the highest at 24 h (45% inhibition). This may be caused by after soaking some of inactive phenolic contents were lose therefore the total content was decreased. On the other, the remaining as well as resultant enhanced phenolic from treatments could be more active hence increasing DPPH activity. ABTS values significantly decreased ($p < 0.05$) during soaking for 2 and 12 h and then increased after 24 h (Figure 3). The antioxidant activity measured by the FRAP method ranged from 64.09 – 153.1 mmol $FeSO_4$/g (Figure 4). FRAP values were highest in raw cashew nuts. During the soaking process FRAP values significantly increased, but soaked samples at 12 and 24 h were not significantly different ($p < 0.05$). Xu and Chang [8] reported that after soaking the DPPH free radical scavenging capacities of soaked cool season food legumes (CSFL's) significantly

reduced compared to raw CSFL's. Siah et al. [12] studied antioxidant activities of five faba bean genotypes with different seed coat colors. Their result showed that FRAP values of all soaked faba beans reduced after soaking.

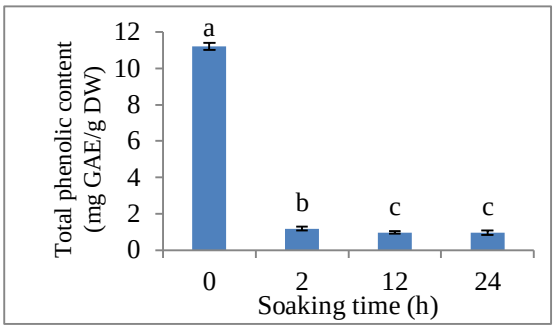

Figure 1. Total phenolic content of raw and soaked cashew nuts

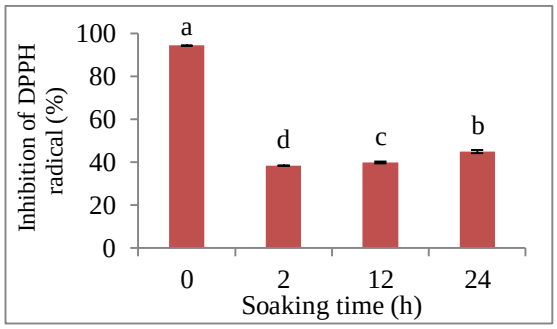

Figure 2. Inhibition of DPPH radical (%) of raw and soaked cashew nuts

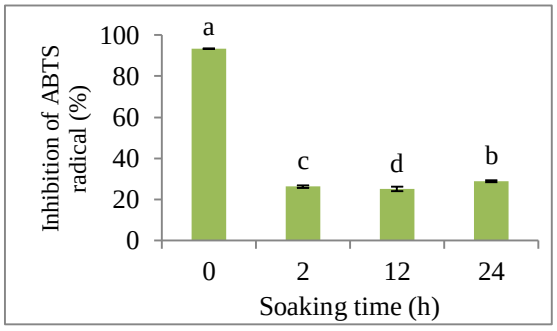

Figure 3. Inhibition of ABTS radical (%) of raw and soaked cashew nuts

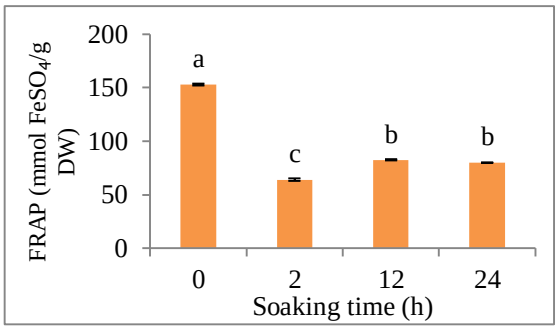

Figure 4. FRAP values of raw and soaked cashew nuts

Conclusions

The soaking process significantly affected the total phenolic contents and antioxidant activities of cashew nuts with changes dependent on soaking time. TPC decreased while DPPH, ABTS, and FRAP increased with increased soaking time. This study generated useful information for consumers to utilize cashew nuts as a source of natural antioxidants.

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