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Volume 1 Issue 1 *Journal of Nutrition and Diet Supplements*

# Effect of Different Drying Methods on Antioxidant Activity of Star Fruits *(Averrhoa Carambola L.)*

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**Citation:** Ruvini L, Dissanayaka WMMMK, Chathuni J, Rizliya V, Swarna W, et al (2017) Effect of different drying methods on antioxidant activity of star fruits *(Averrhoa carambola L.)*. J Nutr Diet Suppl 1(1): 101

**Received:** June 07, 2017; **Published:** August 24, 2017

## Abstract

Star fruit *(Averrhoa carambola L.)* is a highly perishable seasonal fruit with a high level of antioxidants giving protection from many non-communicable diseases. In this study, the effect of dehydration, oven-drying and sun-drying on antioxidant activity, total phenolic content and ascorbic acid content was evaluated in two star fruit *(Averrhoa carambola)* cultivars; Honey sweet and Arkin grown in Sri Lanka. Antioxidant activity, total phenolic content (TPC) and ascorbic acid content were analysed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method, Folin-Ciocalteu's method and 2, 6-dichloroindophenol (DCP) dye method, respectively. Antioxidant activity in dehydrated Honey sweet and Arkin samples was not significantly different compared to the fresh fruit samples from both cultivars. Dehydrated samples from both cultivars had significantly higher ($P<0.05$) antioxidant activity and ascorbic acid content compared to the sun dried and oven dried samples. Sundried samples from both cultivars had the lowest phenol content compared to other samples. The results indicate dehydration as the best amongst the evaluated drying methods to preserve antioxidant activity and ascorbic acid content in star fruits.

***Keywords:*** *Averrhoa carambola; antioxidants; dehydration; Sri Lanka*

## Introduction

Star fruit *(Averrhoa carambola L.)* is a nutrient-rich tropical fruit native to Sri Lanka, Indonesia and India [1,2]. This fruit has received increased interest worldwide due to its nutritional composition and the presence of biologically active compounds that provide health benefits and reduce the risk of certain diseases [3,4]. It possesses a high amount of natural antioxidants, including polyphenols and ascorbic acid [5,6]. Phenolic compounds were found to be the major antioxidants in star fruit. It is proven that consumption of fruits rich in natural antioxidants reduces the risk of chronic diseases such as cancer, cardiovascular diseases, brain and immune dysfunction [7,8]. The rising market for nutraceuticals and functional foods has significantly increased the focus on natural sources of antioxidants and their potential for nutraceuticals and functional foods [9]. The star fruit is usually consumed fresh or made into fruit juice or juice drinks. However, the star fruit is reported as one of the underutilised tropical fruits [10].

The ripe star fruit has digestive and biliousness properties. Preliminary phytochemical analysis has indicated the presence of saponins, tannins, alkaloids and flavonoids. It is also a good source of vitamin C and is used to treat headache, vomiting, cough, hangovers, and eczemas [11]. Furthermore, it is used as an appetite stimulant, diuretic, anti-diarrheal, and febrifugal agent. Insoluble fiber-rich fractions derived from *A. carambola* fruit have shown in vitro hypoglycemic effects [12]. Fresh fruits of carambola are highly perishable and are bulky as they contain more than 80% of moisture. Therefore drying is used as a widespread and economical fruit preservation method [13]. Also, dried fruits are widely used in confectionery, baking and sweet industries. Drying is an important food-processing technique and is one of the oldest methods of food processing. In developing countries, it is possible to practice methods such as sun drying, oven drying and dehydration as they are cost-effective [14].

It is evident that the composition and activity of some antioxidants of the fruits are affected by drying. The main objective of this study was to evaluate the effect of different drying methods on selected antioxidant related parameters (total phenols, ascorbic acid content) and antioxidant activity of two cultivars of *A. carambola*; Arkin and Honey sweet grown in Sri Lanka.

## Materials

### Chemicals

Folin-Ciocalteu's phenol reagent, 2,2-Diphenyl-1-picrylhydrazyl Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, methanol, 2,6-Dichlorophenolindophenol (DCP) dye solution, metaphosphoric acid and sodium carbonate were purchased from Sigma-Aldrich, USA. All chemicals used were of analytical grade.

### Fruit Samples

Fresh and healthy *A. carambola* fruits were selected from two cultivars, Arkin and Honey sweet, two cultivars recommended by the Department of Agriculture Sri Lanka. Fresh fruits were harvested from orchards of Department of Agriculture, Sri Lanka. In order to ensure the homogeneity, randomly selected fresh and healthy fruits were washed thoroughly under running tap water followed by distilled water to remove surface foreign materials. The cleaned fruits were cut into 0.5 cm thickness along the horizontal axis and homogenised. Three replicates were maintained.

## Methods

### Drying Methods

Dehydration, oven drying and sun drying were applied in this experiment to compare the effects of each drying process on antioxidant activity, phenolic content and ascorbic acid content. Prepared fruit samples were subjected to dehydration, oven drying and sun drying until moisture content reached 10%. In dehydration, samples were placed on the trays of the dehydrator (Laboratory model dehydrator, Japan) in a single layer at 65°C and 60 m3s-1 air flow rate for 4 hours. In oven drying, samples were placed on a mesh in a single layer and dried at 65°C for 4.5 hours (Shibata, SPF-600, Japan) at a constant air velocity(0.6m/s). In sun drying samples were arranged on a mesh in a single layer and kept under direct sunlight for two days. Each drying method was replicated three times for each variety.

### Determination of Ascorbic Acid Content

The analysis of ascorbic acid content of the samples was done according to the method given in the Association of Official Analytical Chemists [15]. Solutions of 2, 6 Dichlorophenolindophenol (DCP) dye, 2.5% metaphosphoric solution and the standard ascorbic acid solution was prepared. The dye solution was standardised. Accurately weighed 5 g of the sample was macerated using mortar and pestle with a little amount of 2.5% metaphosphoric acid solution, filtered into a 50 ml volumetric flask through a muslin cloth and made up to the volume with 2.5% metaphosphoric acid. A volume of 10 ml of the sample was pipetted out and placed in a conical flask. The solution was titrated with the dye solution until a pink colour appears. The ascorbic acid content of the sample was calculated. Six replicates were maintained.

### Determination of Antioxidant Activity

Antioxidant activity was measured by the DPPH radical scavenging activity method with few modifications [16]. The samples were extracted with 70% methanol. Dilutions series were prepared by adding 70% methanol and DPPH solution (0.004%) to the sample. Mixtures were vortexed for 1 minute and were incubated at room temperature for 30 minutes and the absorbance was measured at 512 nm using a UV- visible spectrophotometer (UV-VIS 2460, Shimadzu,Kyoto,Japan). The concentration of sample required to scavenge 50% of the DPPH radical (IC50) was obtained from a graph plot of percentage inhibition and extract concentrations, using ascorbic acid as standard. Six replicates were maintained.

### Determination of Total Phenolic Content

The total phenolic content was determined by the previously reported Folin-Ciocalteu method with slight modifications [17]. The mean (±SD) results of quadruplicate analyses were expressed as mg of gallic acid equivalents per gram of sample (mg GAE/g). The calibration equation for gallic acid was $y=0.040x+0.159$ ($R^2=0.996$), where x is the gallic acid concentration in mg/g, and y is the absorbance reading at 765 nm on UV-visible spectrophotometer (UV-VIS 2460, Shimadzu, Kyoto, Japan). Six replicates were maintained.

## Statistical Analysis

Data were analysed using the SAS statistical software version 9.1.3 (SAS Institute Inc., Cary, NC). Results were calculated and expressed as mean ± standard deviation (SD) of 3 independent analyses. P values of ≤0.05 were considered to be significant.

# Results

## Total Ascorbic Acid Content

Drying methods significantly reduced (P<0.05) the ascorbic acid content of *A. carambola* extracts (Table 1). Dehydration method preserved the highest amount of ascorbic acid while sun-dried samples from both cultivars had the lowest ascorbic acid content and cultivar Arkin retained negligible amount of ascorbic acid.

| Drying method | Honey sweet (mg/g) | Arkin (mg/g) |
|---|---|---|
| Fresh (control) | $5.07\pm0.74^{A,a}$ | $4.14\pm0.17^{A,a}$ |
| Dehydrated | $2.45\pm0.05^{A,b}$ | $2.38\pm0.11^{A,b}$ |
| Oven dried | $0.65\pm0.05^{A,c}$ | $0.60\pm0.08^{B,c}$ |
| Sun dried | $0.29\pm0.01^{A,c}$ | $0.02\pm0.05^{B,d}$ |

Different letters of the upper index within a column and different letters of the lower index within a row indicate significant difference at P<0.05

**Table 1:** Total ascorbic acid content of ethanolic extracts of A.Carambola fruits from different drying treatments (mg/g)

## Total Phenolic Content

Table 2 shows the TPC of the 70% EtOH extracts from the different drying methods expressed as mg GAE/g of extract. According to the results, drying treatments had significant effects (P<0.05) on the phenolic content of *A. carambola* extracts. In both the cultivars, the highest TPC was observed in the fresh sample followed by the oven-dried and dehydrated sample. However, dehydrated samples preserved significantly high (P<0.05) phenolic content compared to sun-dried samples.

| Drying method | Honey sweet (mg/g) | Arkin (mg/g) |
|---|---|---|
| Fresh (control) | $24.92\pm0.98^{A,a}$ | $21.97\pm0.98^{B,a}$ |
| Dehydrated | $4.13\pm0.36^{A,b}$ | $5.40\pm0.36^{B,c}$ |
| Oven dried | $5.57\pm0.36^{A,b}$ | $6.93\pm0.09^{B,b}$ |
| Sun dried | $3.83\pm0.05^{A,c}$ | $2.89\pm0.03^{B,d}$ |

Different letters of the upper index within a column and different letters of lower index within a row indicate significant difference at P<0.05

**Table 2:** Total phenolic conetent of ethanolic extracts of A.Carambola fruits from different drying treatments(mg/g)

## Antioxidant Activity

The antioxidant activity in terms of free radical scavenging activity of the 70% EtOH extracts of the dehydrated, oven-dried and sun-dried *A. carambola* fruit samples were evaluated using ascorbic acid as standard. According to the results, antioxidant activity in dehydrated Arkin and Honey sweet samples was not significantly different (P<0.05) compared with fresh samples and possessed high antioxidant activity compared to other treatments (Table 3). Both oven-dried and sun-dried samples from both cultivars showed significantly low (P<0.05) antioxidant activity compared to their respective fresh and dehydrated samples (Table 1). A poor correlation was observed between TPC and DPPH activity ($R^2$=0.3) while a moderately fair correlation was observed between TAC and DPPH activity ($R^2$=0.5436).

| Drying method | Honey sweet [$IC_{50}$ (ppm)] | Arkin [$IC_{50}$ (ppm)] |
|---|---|---|
| Fresh (control) | $178.89\pm5.43^{A,a}$ | $164.87\pm8.37^{A,a}$ |
| Dehydrated | $196.62\pm4.80^{A,a}$ | $179.27\pm4.58^{B,a}$ |
| Oven dried | $312.27\pm3.88^{A,b}$ | $210.77\pm5.87^{B,b}$ |
| Sun dried | $483.93\pm9.43^{A,c}$ | $395.26\pm17.25^{B,c}$ |

Different letters of the upper index within a column and different letters of the lower index within a row indicate the significant difference at P<0.05

**Table 3:** Antioxidant activity of ethanolic extracts of A.Carambola fruits from different drying treatments(ppm)

## Discussion

In the present study, the effect of different drying methods on the ascorbic acid content, antioxidant activity and TPC of *A. carambola* fruit was studied. According to the results, drying significantly decreased the ascorbic acid content of the fruit samples. This observation is in agreement with previous findings showing that increasing drying air temperature causes more loss in vitamin C in the dried fruits [18,20]. Vitamin C losses can be due to enzymatic and chemical degradation, heating, or leaching [21]. Drying processes have been reported to have a very unfavourable effect on the retention of ascorbic acid [22]. Recently, Demiray., *et al*. studied the kinetics of degradation of lycopene, β-carotene and ascorbic acid in tomato during hot air drying and suggested the drying temperature should be less than 70°C to preserve the ascorbic acid at maximum extent [23]. In vegetables, when cell disruption occurs, L-ascorbic acid is oxidised to form dehydroascorbic acid through enzymatic reactions. Thus, the significantly low ascorbic acid content in sun-dried samples may be due to prolonged exposure to these enzymes.

The antioxidant activity of the fruit extracts was evaluated using the DPPH assay. The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidants [24]. The $IC_{50}$ value is the amount of antioxidant material required to scavenge 50% of free radical in the assay system. Therefore, lower the $IC_{50}$ value, higher is the antioxidant activity. The $IC_{50}$ values obtained through different drying methods were in the order of sun drying > oven drying > dehydration > fresh sample, suggesting that the method of drying could significantly enhance the antioxidant activity of *A. carambola* fruit samples ($P<0.05$). Sun-dried samples from both cultivars showed lowest antioxidant activity compared to other treatments ($P<0.05$) and are in agreement with previous findings for button mushroom fruit bodies [25]. During hot-air drying, thermal degradation of polyphenols is expected. But the decomposition of polyphenols is proven to be dependent on the type of food matrix and processing conditions[26]. Drying process enhances or depletes the antioxidant activity of fruits and vegetables depending on the nature of the substrate [26]. The negative effect of temperature on antioxidant activity could be ascribed to its depleting effect on ascorbic acid and polyphenol contents. Polyphenols undergo thermal degradation upon hot-air drying and thermal degradation of polyphenols may partially result from oxidation due to activation of enzymes such as polyphenol oxidase and peroxidase [26,27]. Therefore, the low antioxidant activity in sun-dried samples might have been caused by exposure to these enzymatic processes for a long time [28]. The effect of antioxidants on DPPH radical scavenging is thought to result from their hydrogen donating ability [29]. Thus the high DPPH scavenging activity exerted by dehydrated samples can be due to the formation of compounds having powerful hydrogen donating ability [28].

As observed for ascorbic acid content and antioxidant activity, dehydrated samples retained highest phenolic content compared to oven-dried and sun-dried samples. Sun drying was the least preferred drying method when the phenolic content was considered and agreed with previous findings [30,32]. Okuda., *et al*. have mentioned that rosmarinic acid was degraded when it is dried under direct sunlight, and in the oven at 60 and 80 $^{o}$C and Mueller-Harvey has reported that some phenolic compounds decompose rapidly in direct sunlight [33,34]. Recent works also demonstrated that the temperature affects the stability of phenolic compounds in herbal infusions [35].

In a study done by Zhang., *et al*. freeze-drying was reported to show the highest TPC in Lentinus edodes, followed by fresh, oven-drying, microwave-drying and sun-drying [28]. The decrease in both TPC and antioxidant activity of the extracts during the process of drying could be attributed to the degradation of heat-sensitive phenolic compounds. Besides, activation of oxidative enzymes (polyphenol oxidase and peroxidase) during drying process may lead to the loss of phenolic complexes [24]. According to Toor and Savage, changes in chemical structure of phenols, such as binding of phenols to proteins could also result in a loss of phenolic content [22]. For the sun-drying method, loss of TPC may be caused due to delayed deactivation of degradative enzymes such as phenolic oxidases, which are able to degrade phenolic compounds before the fruit is completely dry [28]. There was a significant difference in the TPC content of dehydrated and oven-dried samples even though the heating temperature was same. The same observation was seen in a study done by Saini., *et al*. on Moringa oleifera leaves, where the oven-dried sample significantly retained more TPC than the cabinet tray dried sample [36]. Anyhow, as in our study, the DPPH radical scavenging activity was higher for cabinet tray drying than oven drying.

*A. carambola* fruits are a rich source of natural antioxidants and polyphenolics are its main antioxidants [3]. In a study done by Khanam., *et al*. various phenolic acids and flavonoids were found in both aqueous and ethanol extracts of *A. carambola* [37]. Among all the tested flavonoids, quercetin was observed in the highest amount followed by kaempferol, luteolin, naringenin and apigenin in aqueous extract and luteolin was detected in greater percentage as compared to kaempferol, naringenin, myricetin and quercetin in the ethanolic extract. In addition, gallic acid and

vanillic acid were the abundant phenolic acids in *A. carambola*. Larrauri., et al. reported that phenolic antioxidants are not significantly affected when dried at 60 °C although, loss of phenols was higher when drying at 100 and 140 °C [38]. In a study done by Elhamirad and Zamanipoor, quercetin and ellagic acid had the highest thermal stability followed by catechin, tannic acid, caffeic acid and gallic acid [39]. Anyhow, it should be noted that the temperature considered in this study was above 120 °C and the drying temperature in our study was only 65 °C. Some antioxidant compounds like ascorbic acid and carotenoids are very sensitive to heat and storage and are lost during different vegetable processing steps. Ascorbic acid contributes to the total phenols as it is capable of reducing the active reagent used in the analysis of phenols. Hence, the reduction in TPC in dried samples may be mainly due to loss of ascorbic acid [40]. As a whole, among the two cultivars, Arkin retained more TPC and ascorbic acid and exhibited more antioxidant activity, indicating that Arkin can be used commercially for dehydration purposes.

## Conclusion

Drying processes resulted in reduced ascorbic acid, TPC and antioxidant activity in *A. carambola* fruits. Sundried samples from both cultivars had lowest phenol content compared to other samples. The results indicate that dehydration is the best drying method and the cultivar Arkin is best for dehydration purposes in terms of TPC, antioxidant activity, and ascorbic acid content.

## Acknowledgement

This research was funded by the National Institute of Fundamental Studies, Kandy, Sri Lanka. The authors wish to thank National Institute of Fundamental Studies, Hantana, Kandy and Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya for the technical assistance provided.

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