Original Research Article

Effect of Storage Temperature and Duration on the Antioxidative Property of *Atuna racemosa* Raf. Fruits

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ABSTRACT

Introduction: The fruits of *Atuna racemosa* Raf. has been utilized in the preparation of "kinilaw" which is a Philippine delicacy. However, not the whole fruit is consumed and the unused part is stored and conserved. **Objectives:** In this study, the antioxidative property of aqueous acetic acid extracts of *Atuna racemosa* Raf. halved fruits stored at various conditions was determined. **Materials and Methods:** Extraction of *Atuna racemosa* Raf. halved fruits stored at various conditions: ambient temperature (25°C) and 4°C for 1, 2 and 3 days, was carried out using 5% aqueous acetic acid. The antioxidative properties of the aqueous acetic acid extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging and Reducing Power Assay. **Results and Discussion:** The results of the determination of DPPH inhibition, expressed as microgram L-ascorbic acid equivalent (AAE) per gram sample, show relatively higher DPPH inhibition of the fruits stored at 4°C in the order of 21.16 (day 1) > 17.59 (day 2) > 8.52 (day 3) µg AAE/g compared to those stored at ambient temperature (25°C) in the order of 15.99 (day 1) > 11.8 8 (day 2) > 4.43 (day 3) µg AAE/g, respectively. On the other hand, the results of the determination of reducing power, expressed as milligram L-ascorbic acid equivalent (AAE) per gram sample, reveal higher reducing power in the fruits stored at 4°C in the order of 70.25 (day 1) > 61.25 (day 2) > 54.08 (day 3) mg AAE/g compared to those stored at ambient temperature in the order of 61.42 (day 1) > 41.75 (day 2) > 36.17 (day 3) mg AAE/g, respectively. **Conclusion:** The present findings imply that the antioxidative properties of the fruits of *A. racemosa* are dependent on the storage time and temperature.

Key words: DPPH inhibition, Reducing power, *A. racemosa,* Storage conditions, Temperature.

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INTRODUCTION

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest.[1] Epidemiological studies have suggested positive association between the consumption of antioxidant-rich foods and the prevention of diseases.[2] Antioxidants are compounds that eliminate undesirable effects of Reactive Oxygen Species (ROS) and free radicals which damage the human body.[3] These antioxidants protect the cells from the potentially damaging oxidative stress, which is a result of an imbalance between the formation of the ROS and the body antioxidant defense. Antioxidants may occur naturally or synthetically. Natural antioxidants can be derived from plant materials such as vegetables, fruits,[4] leaf spices and herbs.[5]

Atuna racemosa Raf. locally known as "tabon-tabon" is among the promising plants. Studies have reported that the methanolic extract of *A. racemosa* fruits has high phenolic content and exhibit antioxidant properties.[5,6] This colored brown pulpy fruit is used to add sour-sweet flavor to the Philippine local delicacy "kinilaw"[7] and remove the "fishy" smell of the fish meat. However, during preparation, not the whole fruit of *A. racemosa* is consumed and most likely the unused part of the fruit is conserved and stored. This practice may have

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an effect on the antioxidative properties of the fruit. In the present study, investigation on the effect of storage conditions on the antioxidative properties, specifically DPPH radical scavenging activity and reducing power of *A. racemosa* fruits was conducted.

MATERIALS AND METHODS

Sample Collection

Intact, healthy and mature fruits of *Atuna racemosa* Raf. were randomly collected by hand in Purok 2, Mibantang, Municipality of Quezon, Bukidnon, Philippines. Plant samples including the leaves, flowers and fruits were also submitted to the Central Mindanao University (CMU) Herbarium, Musuan, Bukidnon for identification and authentication. The collected fruits were washed with tap water to remove dirt, sorted randomly for the six treatments (storage at 4°C and at ambient temperature at 25°C for a period of 1, 2 and 3 days) and were cut halved using a stainless knife. The halved fruits were stored at the various conditions with the endosperm exposed to the atmosphere. After the storage, the exposed endosperm was scraped out for extraction.

Sample Extraction

A 100-gram portion of sample for each treatment was extracted with 200 mL of 5% aqueous acetic acid. After extraction, the mixture was centrifuged for 15 mins and filtered through a Buchner funnel. The residue was discarded while the filtrate was freeze-dried.

DPPH Radical Scavenging Assay

In the determination of the DPPH radical inhibition, a 1.00 mL aliquot of the freeze-dried extract solution (2 g/mL) was diluted to 15.00 mL with methanol (dilution factor is 15). The diluted test solution was then used for the analysis. In a test tube containing 3.00 mL of test solution, 1.00 mL of 0.1 mM DPPH in methanol was added. The reaction mixture was thoroughly mixed and incubated for 30 mins in the dark at room temperature. The absorbance of the reaction mixture was measured at 517 nm using visible spectrophotometer.[8] The same procedure was done for the 2.00 mg/L L-ascorbic acid standard solution and methanol as a blank. The DPPH inhibition, expressed as mg L-ascorbic acid equivalent (AAE) per liter test solution (DPPH Inhibition 1) and µg L-ascorbic acid equivalent (AAE) per gram sample (DPPH inhibition 2) was calculated using Equations 1 and 2, respectively.

DPPH Inhibition (1), mg AAE/L =
[(Abs M-Abs B)/(Abs M-Abs S)] × 2mg/L Eq. 1

where:

Abs M = absorbance of the blank
Abs B = absorbance of the test solution
Abs S = absorbance of the standard L-ascorbic acid

DPPH Inhibition (2), µg AAE/g =
[DPPH Inhibition (1) × Df]/2 g/mL Eq. 2

where

Df (Dilution factor) = 15.00

Reducing Power Assay

A 200 mg/L stock solution of L-ascorbic acid was prepared by dissolving 0.0500 gram of the standard and was diluted to 250 mL with distilled water. From the stock solution, various concentrations (40, 50, 60, 70, 80, 90 and 100 mg/L) was prepared as working standards for the calibration curve. For the sample test solution, a 50 µL aliquot of 2 g/mL was diluted to 100 mL with methanol. The reducing power of the extracts were determined by employing the spectrophotometric method.[9,10] In a test tube containing 2.50 mL of sample test solution, a 2.50 mL of phosphate buffer solution (0.2 M, pH 6.6) and 2.50 mL of 1% potassium ferricyanide was added. The mixture was incubated for 20 mins at 50°C. After cooling, the mixture was added with 2.50 mL of 10% trichloroacetic acid. The mixture was centrifuged for 10 mins at 4500 rpm. An aliquot of 2.50 mL of the supernatant of the mixture was added with 2.50 mL distilled water and 0.50 mL of 0.1% ferric chloride. After allowing to stand for 20 mins at room temperature, the absorbance of the mixture was measured at 700 nm using visible spectrophotometer. The same procedure was done for the blank and L-ascorbic acid working standard solutions. From the calibration curve data, the mg L-ascorbic acid per liter of sample test solution (mg AAE/L solution) was determined using linear regression equation of the line. The reducing power expressed as mg L-ascorbic acid equivalent (AAE) per gram sample was calculated using Equation 3.

Reducing Power, mg AAE/g = [(mg AAE/L solution) (1L/1000 mL)(Df)]/2 g/mL Eq. 3

where:

Df (Dilution Factor)=2000

Statistical Analysis

Significant differences between the means for storage temperature (ambient and 4°C) was determined by *T*-test. The data on storage days (1, 2 and 3 days) were

subjected to one-way analysis of variance (ANOVA) at 0.05 level of significance. Significant differences among the means were determined using Tukey's test.

RESULTS

Physical Description

The physical appearance of the *Atuna racemosa* halved fruits at various storage conditions is presented in Figure 1.

DPPH Radical Scavenging Activity

The results for the determination and of DPPH radical scavenging activity of the *A. racemosa* halved fruits extracted with 5% acetic acid exposed at various storage conditions are shown in Table 1.

Reducing Power

The results for the determination of reducing power in the aqueous acetic acid extracts of *A. racemosa* halved fruits stored at various conditions are summarized in Table 2. Figure 2 shows the effect of storage conditions on the DPPH inhibition and reducing power.

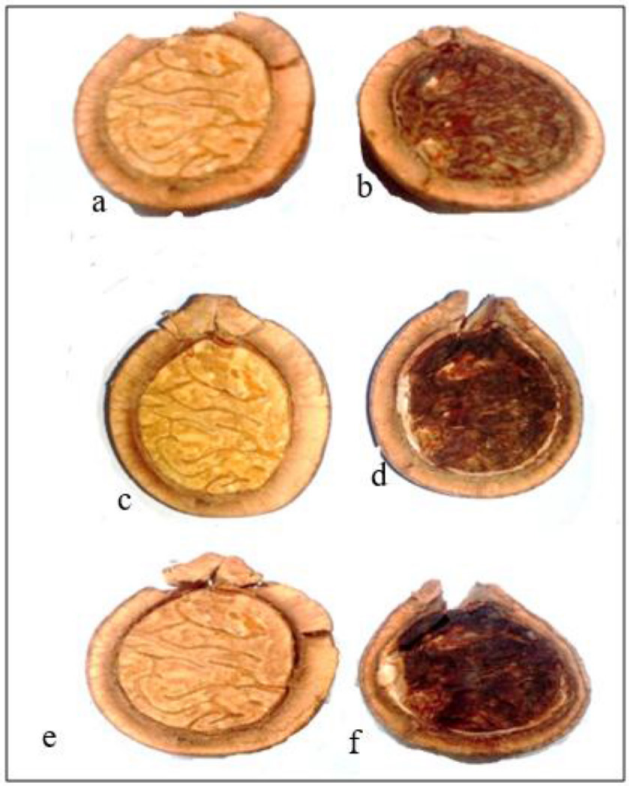

Figure 1: Physical appearance of *A. racemosa* halved fruits stored at various conditions: (a) Day 1 at 4°C, (b) Day 1 at ambient temperature, (c) Day 2 at 4°C, (d) Day 2 at ambient temperature, (e) Day 3 at 4°C and (f) Day 3 at ambient temperature.

Table 1: Mean DPPH Inhibition, µg L-ascorbic acid equivalent (AAE) per gram sample, of the endosperm of *Atuna racemosa* Raf. halved fruits exposed at various storage conditions.

TIME (day)	DPPH Inhibition, µg AAE/g (%RSD)	
	Ambient Temperature (25°C)	4°C
0	22.66 (11.63)^{a}	22.66 (11.63)^{a}
1	15.99 (3.7)$^{b, x}$	21.16 (7.4)$^{a, y}$
2	11.88 (4.97)$^{c, x}$	17.59 (3.28)$^{b, y}$
3	4.43 (12.43)$^{d, x}$	8.52 (12.5)$^{c, y}$

$^{a, b, c, d}$ Means of the same letter superscript within a column are not significantly different at 0.05 level of significance by Tukey's Test.
$^{x, y}$ Means of the same letter superscript within a row are not significantly different at 0.05 level of significance by *T*-test.

Table 2: Mean reducing power, mg L-ascorbic acid (AAE) per gram sample, of the endosperm of *Atuna racemosa* Raf. halved fruits exposed at various storage conditions.

TIME (day)	Reducing Power, mg/g (%RSD)	
	Ambient Temperature (25°C)	4°C
0	90.92 (4.91)^{a}	90.92 (4.91)^{a}
1	61.42 (2.55)$^{b, x}$	70.25 (2.19)$^{a, y}$
2	41.75 (3.25)$^{c, x}$	61.25 (1.41)$^{b, y}$
3	36.17 (1.60)$^{d, x}$	54.08 (2.42)$^{c, y}$

$^{a, b, c, d}$ Means of the same letter superscript within a column are not significantly different at 0.05 level of significance by Tukey's Test.
$^{x, y}$ Means of the same letter superscript within a row are not significantly different at 0.05 level of significance by *T*-test.

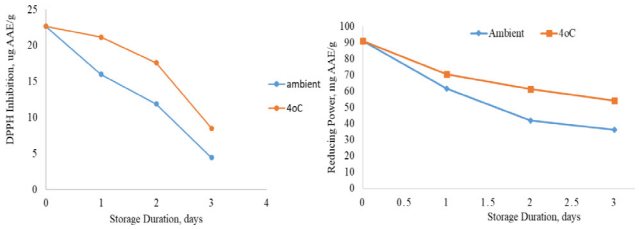

Figure 2: Effect of storage condition on the DPPH inhibition and reducing power of the endosperm of the halved fruit of *Atuna racemosa* Raf.

DISCUSSION

Physical Description

The color of the endosperm of *Atuna racemosa* Raf. fruits were milky white to yellowish in color. When the halved fruits were exposed to various conditions, their physical appearance changed. Generally, the fruits stored at ambient temperature (Figures 1b, 1d and 1f) were darker in color than those stored at 4°C (Figures 1a, 1c and 1e). Moreover, it was observed that as the storage time was lengthened, the color of the endosperm of the

halved fruits stored at ambient temperature darkened intensely from brown to dark brown while those stored at 4°C underwent slight color change. The darkening in color of the endosperm can be attributed to enzymatic action of polyphenol oxidase (PPO) upon exposure to atmospheric oxygen.[11] When oxygen is present in cells, PPO enzymes in the chloroplasts rapidly oxidize phenolic compounds into highly reactive quinones. Polymerization of PPO-derived quinones then causes the postharvest browning of cut or bruised fruit.[11,12] Lastly, shriveling of the endosperm stored at ambient temperature was more pronounced than those stored at 4°C. Post -harvest water loss has a great impact on fruit and vegetable quality and is one of the main causes of deterioration.[13] Water loss causes fruit's shriveling after losing a percentage of the fruit's original weight.[14]

DPPH Radical Scavenging Activity

As shown in Table 1, *A. racemosa* halved fruits exhibited DPPH radical scavenging activity. This present finding may as well imply presence of antioxidant compounds responsible for the fruits' bioactivity. The present findings provide scientific basis to support the medicinal value of the *A. racemosa* fruits as a potential source of anti-oxidative compounds. Results of the analysis of variance indicate that there are significant differences in the DPPH inhibition of the *A. racemosa* halved fruits stored under various conditions. Post Hoc Tukey's Test shows that the DPPH inhibition of *A. racemosa* halved fruits stored at ambient temperature for 1, 2 and 3 days are statistically different in the order of Day 1> Day 2> Day 3. Those stored under 4°C varied significantly in their DPPH radical scavenging activity in the same order as those at ambient temperature. These findings suggest that the DPPH radical scavenging activity of *A. racemosa* fruits is affected by storage duration.

Comparison of the means of the DPPH inhibition activity of the *A. racemosa* halved fruits by *T*-test revealed significant differences between the samples stored at ambient temperature and 4°C for all the storage days. The DPPH Inhibition of *A. racemosa* fruit stored at ambient temperature are lower than those at 4°C. These findings imply that the DPPH radical scavenging activity of the halved fruits of *A. racemosa* is affected by storage temperature.

The difference on the extent of drop of DPPH inhibition between the samples stored at ambient temperature and at 4°C is observable (Figure 2). The decrease in DPPH inhibition with respect to storage time is more pronounced at ambient temperature than at 4°C storage temperature. These findings may be attributed to the influence of temperature with respect to the stability of the antioxidant compounds in the *A. racemosa* fruits. A significant decrease in the phenolic content was observed in the yacon chips after storage. Accordingly, an increase in temperature may also cause a loss of thermo-sensitive antioxidant compounds.[15] Moreover, known antioxidant such as phenolics, when exposed to oxygen are highly unstable and they rapidly oxidize to ortho-quinone which in turn polymerize quickly to brown or black pigments.[12] Samples stored at ambient temperature are more exposed to oxygen compared to those stored under 4°C. This may explain the faster rate of browning of the endosperm of the fruits of *A. racemosa* stored at ambient temperatures than at 4°C.

In the present study, it is very clear that DPPH inhibition is affected by both storage temperature and storage duration. Temperature is an important factor affecting antioxidant activity.[16] Generally, high temperatures cause an acceleration of the initiation reactions and hence a decrease in the activity of the present anti-oxidants in the fruits.[17] Variations in temperature may change the mechanism of action of some antioxidants or affect them in another way. The fruits stored at lower temperature exhibit more stability than those stored at ambient temperature. Moreover, the overall antioxidant status of the plant decreases considerably during storage. These antioxidants are believed to react with free radical, produced by aerial oxygen which consequently led to a decrease in their concentration.[18]

Reducing Power

Significant differences in the reducing power of *A. racemosa* halved fruit stored at ambient temperature and at 4°C was observed at 0.05 level of significance. The results of subsequent Post Hoc Tukey's Test reveal that the reducing power in *A. racemosa* halved fruits exposed at ambient temperature and 4°C for 1, 2 and 3 days are statistically different in the order of Day 1 > Day 2 > Day 3. These findings suggest that the reducing power of the endosperm of the fruit of *A. racemosa* is dependent on the storage duration.

On the other hand, comparison by *T*-test of the means of the reducing power of halved fruit showed significant differences between the samples stored at ambient temperature and 4°C for all the storage days. The reducing power of *A. racemosa* fruit stored at 4°C are higher than those at ambient. These findings imply that the reducing power of the halved fruits of *A. racemosa* is dependent on storage temperature. The decreasing trend in the reducing power of the fruits of *A. racemosa* stored at ambient temperature and 4°C for 1, 2 and 3 days is presented in Figure 2. The similarities in the trend of the DPPH inhibition and reducing power in *A. racemosa*

halved fruits exposed to the various storage conditions may suggest direct relationship between DPPH inhibition and reducing power. This finding is consistent with the results of a study on the antioxidant activity of the buckwheat hulls, bran and flour. It has been reported that the rank correlation coefficient (r_s) between reducing power and DPPH free radical scavenging activity of buckwheat hulls, was 0.76 ($P<0.05$).[19] With this, the reducing power of an extract may serve as a significant indicator of its potential antioxidant activity.[20]

CONCLUSION

The fruit of *A. racemosa* exhibited antioxidant properties in terms of DPPH radical scavenging and reducing power. Further, results of the study showed that the DPPH inhibition and reducing power of the *A. racemosa* fruits are affected by storage conditions, i.e. temperature and storage duration. Higher temperature and longer storage periods caused a decrease in the antioxidative property of the fruits. Thus, these findings may serve as a guide on the proper handling and storage of the fruits of *A. racemosa*.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the subject matter or materials discussed in this manuscript.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **AAE:** Ascorbic Acid Equivalent; **ROS:** Reactive Oxygen Species; **PPO:** Polyphenol oxidase

SUMMARY

The effects of storage conditions, i.e. ambient temperature (25°C) and 4°C for 1, 2 and 3 days, in the antioxidative property of aqueous acetic acid extracts of *Atuna racemosa* Raf. fruits were investigated. The antioxidative properties of the aqueous acetic acid extracts were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging and Reducing Power Assay. Results of the study revealed that decrease in the DPPH inhibition and reducing power is more pronounced at ambient temperature (25°C) than at 4°C. Moreover, fruits stored under 4°C and ambient temperature (25°C) gave values for DPPH inhibition and reducing power activity in the order Day1>Day2>Day3. Thus, antioxidative property of *A. racemosa* fruits is affected by storage temperature and duration.

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