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Studies on Antioxidant Activities of Papaya (Carica papaya) and Pineapple (Ananas cosmosus) Fruits Using Ferric Reducing Antioxidant Power and Rancimat Methods

Teshome Degife[1*] Solomon Libsu[2]
1.Department of Chemistry, Dire Dawa University, Po box 1362, Dire Dawa, Ethiopia
2.Department of Chemistry, Bahir Dar University, Po box 79, Bahir Dar, Ethiopia

Abstract
Most health-benefits of fruits and vegetables have often been attributed to their antioxidant contents. This paper reports attempts made to look into the antioxidant activities of papaya (Carica papaya) and pineapple (Ananas cosmosus) fruits purchased from fruit shops in Bahir Dar City, North-West Ethiopia, using ferric reducing antioxidant potential (FRAP) and Rancimat methods. The antioxidant activity of the fruits found in the FRAP assay is expressed in terms of µg of ascorbic acid (AA) equivalent per gram of edible flesh part of ripen fresh fruit while that found in the Rancimat method is expressed in terms of protection factor. The FRAP value of papaya fruit (13.6 µg AA/g sample) was found to be higher than that of pineapple fruit (8.6 µg/g AA /g sample). Similarly the protection factor of papaya (1.103) was found to be greater than that of pineapple fruit (1.036). In both the assays employed, it is seen that both papaya and pineapple fruits do exhibit antioxidant properties with the former showing greater reducing power.
Keywords: Antioxidants, Antioxidant capacity, Oxidative stress, Ferric-ion reducing antioxidant power, Rancimat method

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are oxidants that originate in the human body from endogenous sources such as essential metabolic processes. These chemical species also enter the human body from exogenous sources including, amongst others, environmental pollutants, medication, radiation and cigarette smoke. ROS and RNS contain unpaired electrons and can be molecules such as hydrogen peroxide, singlet oxygen and nitric oxide or molecular fragments like hydroxyl and alkyl peroxyl free radicals. At low or moderate concentrations, free radicals perform useful functions such as controlling the flow of blood through arteries, fighting infections and keeping the brain alert and focused. Excessive amounts of free radicals in the body, however, lead to a phenomenon called oxidative stress wherein chain reactions triggered by these reactive species seriously alter the cell membranes and other structural proteins, lipids and DNA, leading to tissue damage which ultimately results in a host of health problems in the human body (Cui *et al*., 2004; Boots *et al*., 2008; Pham-Huy *et al*., 2008; Yang and Omaye, 2009).

Fortunately, the human body is endowed with endogenous enzymatic as well as non-enzymatic antioxidants that scavenge excessive free radicals through mechanisms that include hydrogen atom donation, single electron transfer, adduct formation, etc. and thus protect the body from the oxidative damage induced by them. An antioxidant is defined as "any substance that when present at low concentrations compared with that of an oxidizable substrate significantly delays or inhibits oxidation of that substrate". Under conditions of oxidative stress where antioxidants are overwhelmed by free radicals, the body's built-in antioxidant defense system may just not be good enough to shield the body from free radical-induced oxidative damage. It thus becomes necessary to supplement the body's defense system with dietary antioxidants which are now widely believed to counteract the deleterious effects induced by oxidants in the body (Halliwell, 1994; Sies, 1997**;** Benzie, 2003; Carocho and Ferreira, 2013).

The last few decades have witnessed a surge in investigations of antioxidant properties of vegetables and fruits using a variety of analytical methods **(**Prior and Cao, 1999; Tai *et al*., 2011). Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants increases the antioxidant capacity of the plasma and reduces the risk of chronic and degenerative diseases induced by oxidative stress. Plant-derived antioxidants in particular have been shown to function as singlet oxygen quenchers, free radical scavengers and peroxide decomposers. The commonly observed inverse relationship between the consumption of fruits and vegetables on the one hand and the incidence of a host of human health problems including cancers, heart diseases, inflammation, cataracts, brain dysfunction, etc on the other, is attributed to the antioxidant constituents such as vitamins, phenolics and carotenoids found in the plants (Ramarathnam *et al*., 1995; Toit *et al*., 2001; Krishnaiah *et al*., 2011).

In continuation of our investigation of antioxidant properties of fruits (Osman Ahmed *et al*., 2013) and medicinal plants (Tesfaye Tebeka and Solomon Libsu, 2014), we report herein the reducing properties of papaya (*Carica papaya*) and pineapple (*Ananas cosmosus*) fruits as measured by ferric reducing antioxidant power

(FRAP) and Rancimat methods. Such studies are relevant as they shed light on nutritional information vis-à-vis antioxidant properties which are believed to be of pivotal importance for prevention of diseases caused by oxidative stress. Such information will enable consumers to choose fruits that meet their nutrient and health needs.

2. Materials and Methods

2.1. Chemicals and Reagents

Ascorbic acid, potassium hexacyanoferrate ($K_3[Fe(CN)_6]$), trichloroacetic acid (TCA, CCl_3CO_2H), ferric chloride ($FeCl_3$), sodium phosphate monobasic ($NaH_2PO_4.2H_2O$), sodium phosphate dibasic (Na_2HPO_4) were obtained from Blulux, India. Distilled water was used throughout the FRAP work. Sunflower oil used as a substrate in Rancimat method was obtained from a local small scale edible oil processing unit in Bahir Dar City. A blender (Briliant BX-1002 India) was used for making juice of the fruits. Centrifuge (Centurion, West Sussex. UK), incubator (Tuttling, Germany), UV-Visible spectrophotometer (SANYO, SP75 UV/Vis.), pH meter model 3310 JENWAY (Geneway, UK) and oven drier (Model VO150C, England), Rancimat (Model 743, Metrohm) were used in this work.

2.2 Samples

Papaya and pineapple fruits were purchased from fruit shops in Bahir Dar City in February 2012. The fruit samples were thoroughly washed with tap water to remove dusts and other dirt particles.

2.3 Preparation of Reagent Solutions

From a standard solution of ascorbic acid (0.1 g/100 mL), concentrations of 0.01, 0.02, 0.04, 0.08 and 0.1 mg/mL of ascorbic acid were prepared following standard procedures and used for construction of calibration curve. Phosphate buffer (0.2M, pH 6.6) was prepared by mixing 31.25 mL of 0.1 M monobasic sodium phosphate solution and 18.75 mL of 0.1 M dibasic sodium phosphate solution in 50 mL volumetric flask [Aysun *et al*. (2011)]. The pH of the buffer was checked by pH meter. Freshly prepared solutions of potassium hexacyanoferrate ($K_3[Fe(CN)_6]$, 1%), trichloroacetic (TCA, 10%) and ferric chloride ($FeCl_3$, 0.1%) were used for the analysis.

2.4 Antioxidant Assay

For the FRAP assay, 40 g of the edible part of each fruit was taken and mixed with 200 mL of distilled water. The mixture was homogenized by a blender for one min and filtered by sieve to separate the particulate. The non-particulate juice was filtered and then centrifuged at 3000 rpm for 10 min. The clear supernatant solution was used for evaluation of antioxidant capacity.

The reducing power of the fruit extracts was evaluated according to a literature procedure. **(**Kongsuwan *et al*., 2009). Different concentrations of fruit extracts (1.25, 2.5, 5, and 10 % v/v) were prepared from the centrifuged clear solution. To one milliliter of each sample extract in a test tube were added, sequentially, 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate ($K_3[Fe(CN)_6]$. Following incubation of the solutions at 50^0C for 20 min, 2.5 mL of 10% trichloroacetic acid was added and the solutions centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) of the solution was decanted and mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared aqueous $FeCl_3$ (0.1%) solution. The absorbance of the resulting solution was measured at 700 nm against a blank that lacked the fruits extract. The experiment was conducted in triplicates and the reducing power of the fruits extract was expressed as equivalents of ascorbic acid (μg) per g of extract (Toit *et al*., 2001; Leong and Shui, 2002). A higher absorbance indicates a higher reducing activity.

For the Rancimat method, the edible part of the fruits was taken and cut into small pieces and subjected to oven-drying at 80°C for two days until constant weight was achieved. The dried sample was powdered and dispersed in sunflower oil at a concentration of 5% w/w. The mixture was centrifuged to separate the particulates. To 3 g of the centrifugate (the non particulate) of each sample at 120^oC, air was bubbled at a rate of 20 L/h. The volatile oxidation products resulting from the oxidation process were transferred into a conductivity-measuring vessel containing distilled water whereby an oxidation curve depicting conductivity as a function of time is obtained automatically. The time taken for a sharp increase of conductivity to occur is termed the induction time (IT), expressed in hours. The protection factor (PF), calculated using the equation $PF = IT_{inh}/IT_0$, where IT_{inh} is the induction time of the sunflower oil containing the added fruit extract and IT_0 is the induction time of the blank (sunflower oil alone). The induction time is a measure of the extent of protection of the oil from aerial oxidation by each fruit, with a longer IT, i.e. a higher PF, indicating stronger activity of the added extract. Sunflower oil containing no added fruit extract was used as a control and run similarly (Pinedo *et al*., 2007; Arranz *et al*., 2008; Arain *et al*., 2009).

3. Statistical Data Analysis

Data obtained from experiments performed in triplicate were recorded as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA). Significant differences ($\rho < 0.05$) between means of the samples were determined by Tukey's Multiple Range tests by SPSS (version 15.0 for window, SPSS Inc.). A linear absorbance versus concentration calibration curve, Figure 1, was obtained for the standard (ascorbic acid).

4. Results

Numerous analytical methods have been devised for investigation of antioxidant properties of synthetic and natural substances (Duduku *et al*., 2011). The FRAP assay, renowned for being inexpensive, speedy and simple-to-use, measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) caused by reductants (antioxidant) in the test sample. On application of this assay in the present study, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each sample (fruit). Addition of $FeCl_3$ to the resulting ferrous solution produces a Prussian blue colored complex ($Fe_4[Fe(CN)_6]_3$), confirming the reduction of ferric (Fe^{+3}) into ferrous (Fe^{+2}) ions by antioxidant constituents of the fruit samples. As per literature reports **(**Berker *et al*., (2007); Lim *et al*., 2007), measurement of absorbance at 700 nm of this complex is taken as indicative of the reducing power of the test solution, higher absorbance at this wavelength indicating a higher reducing power.

A plot of absorbance at 700 nm versus concentration of the Prussian blue complex of the average of data recorded for experiments conducted in triplicate at all concentrations is depicted in Figure 2. It is seen in the figure that the reducing capacity of the papaya and pineapple fruits differs indicating a difference in the nature or concentration of their antioxidant constituents.

The antioxidant activities of the fruits obtained by the FRAP assay, given as mean ± standard deviation, have also been calculated in terms of ascorbic acid equivalent antioxidant capacity (Coban, 2008). The FRAP value of papaya fruits thus obtained (13.5 ± 0.00017) is higher than that of pineapple fruit (8.6 ± 0.00012). It is thus inferred from the FRAP assay that the antioxidant activity of papaya fruits is significantly greater than that of pineapple fruits ($\rho < 0.05$) suggesting better electron donating capability of the former fruit relative to the latter.

In the Rancimat method of antioxidant analysis, sunflower oil was used as an oxidizable lipid substrate. A stream of air bubbled at a rate of 20 L/h through a heated sample (120^0C) of sunflower oil in the presence and absence of added fruit extract oxidizes the oil producing volatile compounds whose conductivity is automatically plotted as a function of time giving an oxidation curve. The point of inflection of the oxidation curve so obtained is known as the induction time. The induction time so obtained is a measure of the ability of the added fruit to protect the oil from aerial oxidation. During the oxidation process, a sudden rise in conductivity of the water into which the oxidation products are bubbled is observed and is ascribed to volatile carboxylic acids resulting from the oxidation process (Pinedo *et al*., 2007; Arain *et al*., 2009; Velasco *et al*., 2009). In this assay, a greater induction time was found for papaya fruit (2.15 h) relative to that of pineapple fruit (2.02 h). The induction time measured for sunflower oil (blank) was found to be 1.95 h. As shown in Table 1, calculation of the protection factor (PF) which is defined as the ratio of the induction time of a sample (sunflower oil) containing antioxidant (fruit) to the induction time of the control (sunflower oil alone) using literature reports (Arranz *et al*., 2008), gave values of 1.036 and 1.103 for sunflower oil containing pineapple and papaya fruit extract, respectively. The results indicate that oxidation of the lipid is deferred in the presence of extracts of both pineapple and papaya fruits, with the latter fruit showing greater reducing power.

5. Discussion

It is now common knowledge that high intake of fruits and vegetables is associated with reduced incidence of degenerative diseases, a fact generally attributed to their chemical constituents having antioxidant properties. Indeed, the health-benefiting effects of fruits and vegetables have often been traced to their ability to counteract oxidative stress, a physiological condition wherein oxidants outweigh antioxidants (Wang *et al*., 2011; Wootton-Beard and Ryan, 2011).

Among the fruits widely consumed worldwide for their nutritional and medicinal properties are papaya (*Carica papaya*) and pineapple (*Ananas cosmosus*), both of which are widely cultivated in the tropics and subtropics. In this study, papaya (*Carica papaya*) and pineapple (*Ananas cosmosus*) fruits procured from fruit shops in Bahir Dar City, North-West Ethiopia, have been shown to possess antioxidant properties as measured by ferric reducing antioxidant potential (FRAP) assay and Rancimat methods. As mentioned above, the antioxidant activities of these fruits have been inferred from their ability to reduce ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) in the FRAP assay as well as by their ability to defer aerial oxidation of sunflower oil in the Rancimat assay. The observations made on the antioxidant properties of these fruits are in line with similar studies reported in the literature.

Papaya fruits have been shown to be rich sources of the familiar antioxidants lycopene, β-carotene, β-

cryptoxanthins, as well as vitamins A and C (Oliveria and Vitoria, 2011; Schweiggert *et al*., 2011). It has also been recorded that the antioxidant capacity of papaya fruits measured using different analytical methods correlated with the concentrations of their phenolic compounds, carotenoids and vitamin C (Sancho *et al*., 2011). The antioxidant activity of papaya is not limited to its edible portion. Free radical scavenging properties of papaya seed extracts have also been reported and are attributed to their phenolic compounds, notably *p*-hydroxybenzoic acid and vanilic acid (Zhou *et al*., 2011). It has also been noted, however, that in addition to its health-promoting phytochemicals, the dried leaves of papaya plant also contain cyanogenic glycosides, a potential source of the highly toxic cyanide (Williams *et al*., 2013). Analogous studies conducted elsewhere have also shown that these fruits do exhibit antioxidant activities. **(**Garcia-Alonso *et al*., 2004; Isabelle *et al*., 2010; Fu *et al*., 2011; Martinez *et al*., 2012). In line with literature reports, the findings of the present study also showed that extracts of papaya fruit exhibited antioxidant behavior both by the FRAP as well as Rancimat, a behavior that can be ascribed to the antioxidant constitution of the fruit.

Pineapple (*Ananas cosmosus*), a popular non-citrus fruit growing in the tropics and subtropics, is also widely consumed for its nutritional and medicinal properties. As mentioned above, the edible portion of this fruit has also demonstrated antioxidant properties by both FRAP and Rancimat assays, albeit to a lesser extent relative to papaya fruits. These properties can be attributed to the antioxidant substances such as tyrosine, vitamin C and E that constitute the fruit. Analogous studies on the methanolic and ethyl acetate extracts of pineapple fruits have been shown to exert antioxidant activity which has been attributed to the fruit's phenolic substances (Hossain and Rahman, 2011).

In summary, the present work has shown that while both papaya (*Carica papaya*) and pineapple (*Ananas cosmosus*) fruits do exhibit antioxidant properties in both FRAP and Rancimat assays, papaya fruits are apparently more potent in their ability to reduce ferric (Fe^{+3}) ion as well as deter the aerial oxidation of sunflower oil. This indicates the ability of antioxidants contained in these fruits to protect sunflower oil from oxidative deterioration thereby prolonging its shelf life. The observed difference in the antioxidant activities of these two fruits may be linked to the different types of their bioactive compounds.

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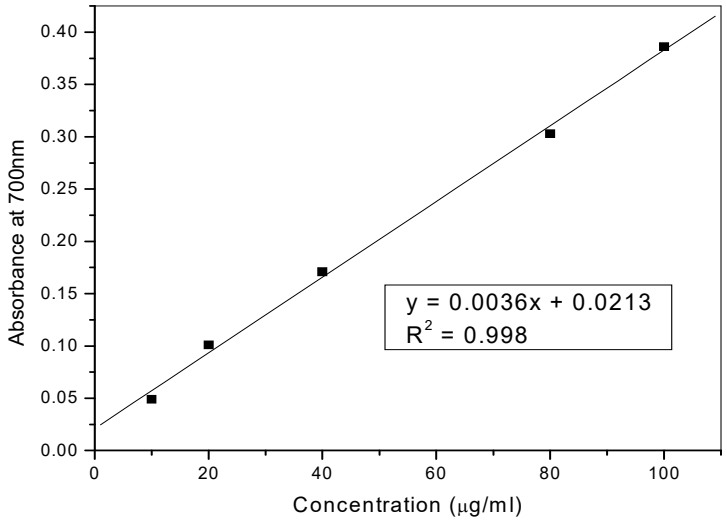

Figure 1: Calibration curve using ascorbic acid.

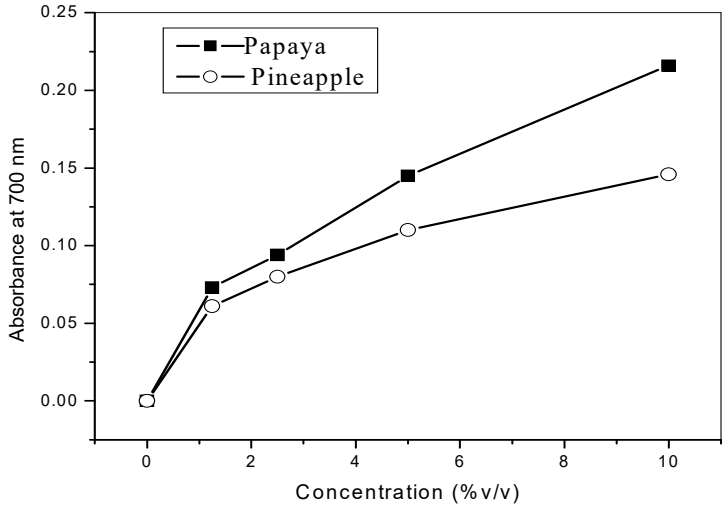

Figure 2. Reducing power of papaya and pineapple fruits at different concentrations.

Table 1: The induction time and protection factor values of papaya and pineapple fruits.

Fruit type	Induction time (IT), h	Protection factor (PF), (ITinh/IT0)
Sunflower oil (blank)	1.95	
Pineapple fruit plus sunflower oil	2.02	1.036
Papaya fruit plus sunflower oil	2.15	1.103