Antioxidative Capacities of Phytochemicals in Selected Fruit Peels

Romeo M. Del Rosario* and Nenita D. Palmes
College of Arts and Sciences
Mindanao University of Science and Technology
CM Recto Ave., Lapasan, Cagayan de Oro City, 9000 Philippines
**rmdelr@yahoo.com*

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Abstract

Fruit by-products in the form of peels are generally considered as wastes but they may contain significant amounts of antioxidants which can serve as basis for the alternative uses. The peels of a total of 36 kinds of fruits were subjected to rapid screening for their antioxidative capacities using a revised version of the thiocyanate method. From the results of the rapid screening, the 10 best performing kinds of fruits were selected for further assay of their peels using different extraction solvents (methanol or diethyl ether) and different peel conditions (fresh or dry). The antioxidative capacities are expressed in terms of the Oxidation Protection Efficiency (OPE). The extracts with methanol showed superior OPEs, in general, compared to those with ether indicating the dominant chemical nature of the antioxidants. Drying, on the other hand, generally led to decreases in the OPEs. Topping the list with their very high OPEs are: Garcinia mangostana (mangosteen), Nephelium lappaceum (rambutan), Diospyros blancoi (mabolo), Mangifera indica (mango), and Ananas comosus (pineapple). Their OPEs ranged from almost 100% down to 80%.

Keywords: antioxidant, antioxidative capacity, fruit peel, oxidation protection efficiency

1. Introduction

Exposed to an atmosphere very rich in oxygen and in an environment bombarded continuously with ionizing or oxidizing radiation and an assortment of pollutants, extending the storage lives or usability of materials is an enormous problem. This holds true with living organisms. They are not just exposed to the same oxidizing elements but they really take oxygen into their cells for metabolism. In the process, some highly oxidizing intermediates can leak out into the vulnerable parts of the cells promoting unwanted oxidative reactions. Different forms of stress also make their

contributions to the pool of prooxidants, thus, aggravating the situation (Droge, 2002).

In this light, there is a continuing need to study more about potentially useful antioxidants for use in the food industry and in the management of human health. Earlier studies have shown that antioxidative activities have been exhibited by the following naturally occurring substances: amino acids, ascorbic acid, carotenoids, cinnamic acids, reductones, peptides, phosphatides, polyphenols, tannins, and tocopherols. There may still be many others. Various plants, fruits, and food systems have been studied by many researchers yielding very interesting results (Dunlap *et al.*, 1997; Bright *et al*., 1999; Gorinstein *et al.*, 1999; Stewart *et al*., 1999; Guan and Whiteman, 2002).

Fruits are exposed not only to a lot of oxygen in the air but also to a good dose of light energy and to a cocktail of environmental pollutants. As a natural recourse, fruit biosurfaces or peels have developed complex integrated extracellular and intracellular defense systems against stresses brought about by reactive oxygen species and related species (Cross *et al*., 1998). While some studies have been already done on the antioxidative power of fruits (Yoshikawa *et al*., 1994; Widyasari, 2002; Di Mauro, 2003), only very few have been done on fruit by-products especially the fruit peels.

This research surveyed the antioxidative capacities of the peels of a number of different kinds of local fruits in the Philippines in order to establish a scientific basis for the alternative uses for these materials that are generally considered as scrap. The specific objectives were: (1) to carry out a preliminary screening of the antioxidative capacities of the peels of as many kinds of local fruits as are available, (2) to conduct full antioxidant assays of the peels of the 10 kinds of fruits exhibiting the highest antioxidative capacities based on the preliminary screening and compare these capacities according to the fruit peel condition (fresh or dry) and the extraction solvent used (methanol or diethyl ether), and (3) to rank the different combinations of fruit-solvent-condition from highest to lowest antioxidative capacity as over-all reference.

2. Methodology

There were 36 kinds of fruits included in this research. The fruits were

sourced from the fruit markets or from some accessible fruit trees grown in the Province of Misamis Oriental. No special sampling procedure was followed. It was just seen to it that there would be about 3 fruits or more (in case of small size) for each kind of fruit and that there would be enough peels that could be used for the entire testing. All fruits were at the right stage of ripeness for eating.

A revision of the modified thiocyanate method of Osawa and Namiki (Anggadiredja *et al.*, 1997) was developed and employed. This is a spectrophotometric antioxidative activity determination based on the thiocyanate method. For the preliminary screening, all the fruits were processed according to the procedure established but the extraction was done on the 'as is' or fresh samples only (soon after milling) and using one kind of solvent—methanol. The extraction was carried out by simply soaking the ground peels in the solvent overnight at about 15^{o}C at a fixed ratio of peel (dry basis) to solvent. In the assay portion, absorbance monitoring was done for only one day—at the start and after about 24 hours of incubation. The preliminary screening results were expressed as percent oxidation (% oxidation = sample absorbance/control absorbance x 100). Full evaluation was then conducted on the top 10 fruits. The prepared fruit peels were evaluated as fresh material and as dry material using two solvents, methanol and diethyl ether, and using the same extraction conditions as employed for the preliminary screening. In the assay proper, the absorbance monitoring was done daily for a period of at least 120 hours or 5 days. The results of the full evaluation were expressed as Oxidation Protection Efficiency [OPE = (control curve area – sample curve area)/control curve area x 100].

2.1 Substrate-Sample Extract Preparation

About 1000 µL of the clear supernatant from the extraction was placed in an 80-mL test tube. (When solvent is ether, this is first evaporated completely before adding the substrate below).

The components of the substrate for the stability test were added (with shaking after each component is added):

1) 5.00 mL ethanol
2) 5.00 mL of 0.2M phosphate buffer (pH = 7.0 at 22^{o}C)
3) 2.50 mL of distilled water
4) 0.075 mL linoleic acid

5) 0.100 mL 0.016M hydrogen peroxide
6) 0.100 mL of 0.016M iron (II) sulfate in 0.015M EDTA.

The substrate-sample extract mixture was mixed again for a few seconds using a vortex mixer and incubated at 40^{o}C. The same thing was done with the control which is the substrate plus 1000 µL of the extraction solvent instead of the sample extract.

2.2 Absorbance Measurement

For preliminary screening purposes, absorbance measurement was done on the incubated substrate-sample extract mixture and the control at 0 hour and after only about 24 hours of incubation. For the full evaluation, absorbance measurement was done daily for 5 days or about 120 hours starting at 0 hour. This was carried out as follows:

1) A 50-microliter substrate-sample extract mixture was added to 9.70 mL of 75% by mass ethanol in an 80-mL test tube.
2) The mixture was homogenized by brief shaking.
3) Next, 0.100 mL 30% (w/v) ammonium thiocyanate was added followed by another brief shaking to mix the reagents.
4) Then, 0.100 mL of 0.02M iron (II) sulfate heptahydrate in 3.5% by mass HCl was finally added.

Thorough mixing was done by shaking for a few seconds using a vortex mixer. The absorbance was then measured at 490 nm using a spectrophotometer after 30 min but not beyond 70 min from the addition of the last reagent. The four steps above were also followed for the control except that in step 1 it is 50 µL of the control in lieu of the substrate-sample extract mixture. Matched glass cuvettes of 1-cm path length were used. The instrument was zeroed using distilled water before every sample reading.

2.3 Antioxidative Activity Calculations

2.3.1 Preliminary Screening: Percent Oxidation (Based on Fixed-time Absorbance)

The absorbance readings were adjusted or corrected so that the 0-hour readings are all equal to zero (0.0000). This was done by simply subtracting

the 0-hour readings from themselves and from the subsequent readings after about 24 hours.

From the adjusted or corrected readings, the absorbances after exactly 24 hours were obtained by interpolation or extrapolation depending on whether the subsequent readings were within or beyond 24 hours.

Using the absorbances after exactly 24 hours of incubation for the control and the sample, the Percent Oxidation is calculated as follows:

$$\% \text{ oxidation} = \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100 \qquad (1)$$

2.3.2 Full Evaluation of Antioxidative Capacity

The best-fit equation for the absorbance-incubation hour's relationship was determined using available polynomial regression program.

The area bounded by the absorbance-incubation hours curve and the line y = y-intercept, from time equals 0 hr to time equals 120 hr, was determined by integration. These were carried out for the control and the sample.

From the areas obtained, the Oxidation Protection Index (OPI) is calculated according to equation (2) and interpreted as shown in Table 1.

$$\text{OPI} = \frac{\text{control curve area} - \text{sample curve area}}{\text{control curve area}} \qquad (2)$$

Table 1. Interpretation of OPI values

OPI	Interpretation
0.0	absence of antioxidative activity
1.00	100% antioxidative activity relative to control
between 0.00 and 1.00	antioxidative activity between very weak to very strong
less than 0.00	some amount of prooxidative activity
more than 1.00	an oxidation curve that dips below the y-intercept and means exceedingly high antioxidative activity that includes reducing power so that reducible substance(s) is/are even acted on almost immediately

The OPI can be converted into the Oxidation Protection Efficiency (OPE) expressed in percentage by simply multiplying the OPI by 100. This quantity offers the advantage of being easier to understand.

3. Results and Discussion

3.1 Preliminary Screening: Percent Oxidation

The results of the preliminary screening revealed percent oxidation values that are spread widely from below 0% to above 100%. Three kinds of fruit peels gave below 0% oxidation values indicating very high antioxidative ability and even some reducing power. On the other hand, there were 6 kinds of fruit peels that registered percent oxidation values beyond 100%. The 10 most antioxidative kinds of fruit peels, those 10 with the lowest % oxidation values, are: *Anacardium occidentale*/cashew (-14.3%), *Mangifera indica*/mango (-6.50%), *Garcinia mangostana*/mangosteen (-2.09%), *Ananas comosus*/pineapple (1.85%), *Nephelium lappaceum*/rambutan (8.33%), *Diospyros blancoi*/ velvet apple "mabolo" (12.9%), *Annona squamosa*/sugar apple "atis" (34.0%), *Syzygium cumini*/black plum "lomboy" (51.9%), *Persea americana*/avocado-red brown (62.2%), and *Citrus microcarpa*/ "calamansi" (68.2%). Figure 1 shows some of these fruits and their peels.

3.2 Full Antioxidative Capacity Evaluation: OPEs.

The complete results of the assay of the antioxidative capacities of the selected 10 fruits under different conditions are summarized in Table 2.

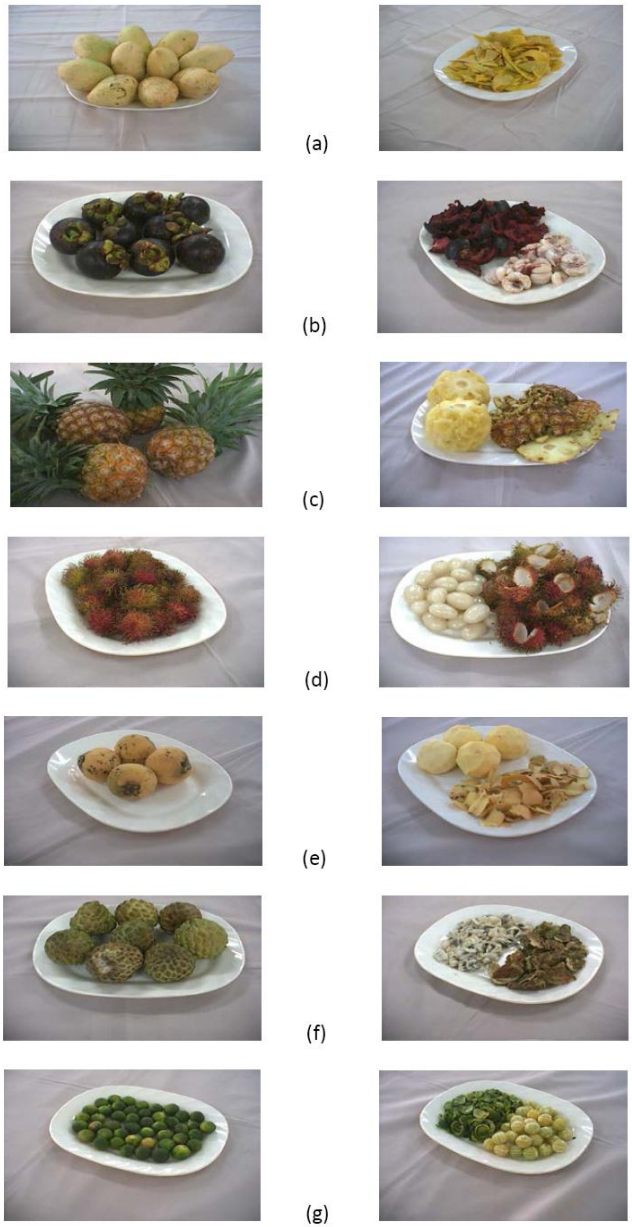


Figure 1. Some of the fruits and their peels which exhibited substantial antioxidative capacities as indicated by the low % oxidation values that were obtained with their extracts: (a) *M. indica* (mango), (b) *G. mangostana* (mangosteen), (c) *A. comosus* (pineapple), (d) *N. lappaceum* (rambutan), (e) *D. blanco* (velvet apple), (f) *A. squamosa* (sugar apple), and (g) *C. microcarpa* ("calamansi").

Table 2. Oxidation Protection Efficiencies (OPEs) based on 5 replicates of the 10 kinds of fruit peels selected out of the 36 screened

Fruit Peel		Extraction Solvent		
		Methanol (Mean ± SD)	Diethyl ether (Mean ± SD)	Statistical Notes
N. lappaceum (rambutan)	Fresh	104.1 ± 0.354	55.1 ± 2.049	1
	Dry	104.0 ± 0.329	9.26 ± 4.050	1,2(Ether)
G.mangostana (mangosteen)	Fresh	100.5 ± 0.045	100.5 ± 0.101	
	Dry	100.5 ± 0.110	100.4 ± 0.158	
D. blancoi (velvet apple)	Fresh	94.4 ± 0.797	23.1 ± 3.202	1,2(MeOH)
	Dry	67.6 ± 1.121	22.1 ± 3.712	1
M. indica (mango)	Fresh	92.9 ± 0.436	65.7 ± 1.742	1,2(MeOH)
	Dry	87.2 ± 0.394	-5.07 ± 2.905	1,2(Ether)
A. comosus (pineapple)	Fresh	79.8 ± 0.880	10.1 ± 2.936	1,2(MeOH)
	Dry	11.2 ± 4.531	-7.73 ± 4.938	1,2(Ether)
A. squamosa (sugar apple)	Fresh	67.9 ± 2.077	28.2 ± 6.742	1,2(MeOH)
	Dry	15.2 ± 1.753	16.8 ± 2.719	2(Ether)
A. occidentale (cashew)	Fresh	40.1 ± 1.420	4.27 ± 3.536	1,2(MeOH)
	Dry	5.31 ± 0.890	1.50 ± 4.325	
P. americana (avocado)	Fresh	29.2 ± 3.782	64.1 ± 1.949	1,2(MeOH)
	Dry	40.6 ± 2.947	35.2 ± 4.219	1,2(Ether)
C. microcarpa ("calamansi")	Fresh	24.1 ± 3.054	17.8 ± 2.037	1
	Dry	21.1 ± 1.540	9.23 ± 2.464	1,2(Ether)
*S. cumini (*black plum)	Fresh	9.54 ± 5.358	-2.27 ± 5.899	1
	Dry	9.68 ± 2.037	2.76 ± 3.729	1

Legend: 1-solvent effect statistically significant at alpha = 0.05, 2-drying effect statistically significant at alpha = 0.05

There was a general decline in the OPEs when the extraction was carried out using ether compared to using methanol. The methanol extracts from the fresh peels exhibited higher antioxidative capacities than the ether extracts, except for *G. mangostana* and *P. Americana*. This can be because in the peels of these fruits there are more of the relatively polar antioxidants than the non-polar ones. Another possibility is that the most potent antioxidants in these fruits are polar. In *G. mangostana*, the capacities of the methanol and ether extracts were equivalent indicative of the equivalent aggregate capacity of the polar antioxidants as compared to that of the non-polar ones. *P. americana* behaved differently in that the non-polar extract showed higher capacity suggesting that relatively non-polar compounds are responsible for the antioxidative activity of this particular fruit peel. In the dry materials, in most cases, the extracts using methanol proved to be still better than those using ether. The exceptions were *G. mangostana*, *A. squamosa*, and *A.*

occidentale. The two kinds of extracts were equivalent as far as these three kinds of fruit peels are concerned.

As far as effect of drying, the following have been observed. Using methanol as the extracting solvent, in 50% of the cases (*D. blancoi, M. indica. A. comosus, A. squamosa,* and *A. occidentale*), drying the fruit peels brought down the antioxidative capacities. The heat exposure under atmospheric conditions could have appreciably destroyed the antioxidizing abilities of the active substances. It was very severe for *A. occidentale* (87% drop), *A. comosus* (86% drop), and *A. squamosa* (78% drop). It was not as bad for *D. blancoi* with only 28% drop. *M. indica* suffered the smallest decline at 6% drop only. The methanol-soluble antioxidants from *G. mangostana* and *N. lappaceum* were most resistant to deterioration brought about by drying. The same thing can be said of *C. microcarpa* and *S. cumini* although the antioxidative powers of these two were not very high in the first place. *P. americana* was most unusual because instead of a drop in antioxidative capacity, or at least a steady one after drying, the OPE picked up by 39%. One possibility is the formation during the drying process of new substances that are themselves antioxidants also. This phenomenon is like the one observed with Maillard reaction products (produced by heating) that exhibit antioxidative activity (Wijewickreme, 1999).

On the other hand, ether extraction from fresh and dry fruit peels revealed declines in OPEs as results of drying for 6 out of 10 kinds of fruits, namely, *N. lappaceum, M. indica, A. comosus, A. squamosa, P. americana,* and *C. microcarpa.* Drying practically eliminated the antioxidative activities of *M. indica* and *A. comosus* while severely reducing that of *N. lappaceum* (83% drop). The reduction in the OPEs of the three others (*A. squamosa, P. americana,* and *C. microcarpa*) ranged from 40% to almost 50%. The antioxidative capacities of the ether extracts from *G. mangostana* and *D. blancoi* had remained unaffected by drying. The same thing is true for those from *A. occidentale* and *S. cumini* but these antioxidative capacities were quite low in the first place (less than 5).

4. Conclusion and Recommendation

Most of the different kinds of fruits included in this research have antioxidative capacities although these range from something low to

something impressively high. Fruits vary in the extent of their need for antioxidants in their peels. This can be due to other defense mechanisms that are available to them. Overall, it can be said that the Philippine fruits are quite promising as sources of antioxidants.

Drying can destroy some of the antioxidants thereby lowering the antioxidative capacities of the resulting extracts. There are exceptions, though, such that when the chemical matrix will allow, new substances may be formed that have antioxidative powers. It is also possible that destruction and formation of antioxidants occur at the same time. Whether the net effect is increase or decrease of antioxidant activity would depend on which process predominates and on the nature of the antioxidants destroyed and generated.

Generally, methanol produces extracts with higher antioxidative capacities. This implies that the antioxidants in fruit peels are largely polar compounds. Many antioxidants are known to be phenolic in nature and are not very big molecules. But there are appreciable levels of relatively non-polar antioxidants in many fruits that can complement the polar ones in preventing oxidation in the mixed hydrophilic and lipophilic components of the cells of living organisms.

Beyond pointing to the health advantages of fruits, this research opens many interesting areas for further study. Among these are: (1) confirmatory study to assess the averages and ranges of the antioxidative capacities of the very promising fruits through a more thorough and extensive sampling of each kind of fruit, (2) survey of fruits not included in this study, including wild fruits, and other varieties of the fruits that have been included in the study, (3) evaluation of the toxicities of the fruit peels with relatively high level of antioxidative capacities, (4) development of value-added food products from the highly antioxidative fruit peels using technologies that will minimize loss of antioxidative potency, (5) detailed profiling (qualitative and quantitative) of the antioxidants in the very promising fruit peels, and (6) research on other plant materials and parts, e.g., seeds.

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6. References

Anggadiredja, Andyani, Hayati, Muawanah, (1997). Antioxidant activity of Sargassum polycystum (Phaeophyta) and Laurencia obtuse (Rhodophyta) from Seribu Islands. Journal of Applied Phycology, 9.

Bright, Stewart, Patino, (1999). A novel assay for antioxidant potential of specialty malts. Journal of American Society of Brewing Chemists, 57(4).

Cross E.C., van der Vliet, A., Louie, S., Thiele, J.J., Halliwell, B., (1998). Oxidative Stress and Antioxidants at Biosurfaces: Plants, Skin, and Respiratory Tract Surfaces. [On-line].Available:http://ehpnet1.niehs.nih.gov/docs/1998/Suppl5/12411251cross.html

Di Mauro, A., Durst, R.W., Wrolstad, R.E., (2003). Polyphenolic Composition and Antioxidant Properties of Pears. [On-line]. Available: http://ift.conflex.com/Ift/2003/ technoprogram/ paper_20055.htm

Droge, W., (2002). Free radicals in the physiological control of cell function. Physiological Reviews, 82(1).[On-line]. Available: http://physrev.physiology.org /content/82/1/47.long

Dunlap, W.C., Masaki, K., Yamamoto, Y., Larsen, R.M., Karube, I., (1997). A novel antioxidant derived from seaweed. Environmental Biochemistry. [On-line]. Available: http:// www. aims.gov.au/pages/research/ projects/sunscreens/pages/mb-sp07-01.html

Gorinstein, S., Zemser, M., Haruenkit, R., Chuthakorn, R., Grauer, F., Belloso, O.M., Trakhtenberg, S., (1999). Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. Journal of Nutritional Biochemistry, 10. [On-line]. Available: http:// 147.46.94.112/ journals/sej/full/j090608.pdf

Guan, T.Z., Whiteman, M., (2002). Antioxidant Activities of Some Tropical Fruits. [On-line]. Available:http://staff.science.nus.edu.sg/~scilooe/srp2002/sci_paper/ Biochem/research_paper/Tan%20Tze%20Guan1.pdf

Stewart, D., Deighton, N., Davies, H.V., (1999). Antioxidants in soft fruits. Plant Biochemistry and Cell Biology. [On-line]. Available: http://www.scri.sari.ac.uk/ Document/AnnReps/01Indiv/ 15Antiox.pdf

Widyasari, M.A., (2002). Antioxidant Activities of Apples. [On-line]. Available: http://ift. Conflex. com/ift/2002/technoprogram/paper_11588.htm

Wijewickreme, A.N., Krejpcio, Z., Kitts, D.D., (1999). Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model Maillard products. Journal of Food Science, 64(3).

Yoshikawa, M., Harada, E., Miki, A., Tsukamoto, K., Liang, S.Q., Yamahara, J., Murakami, N., (1994). Antioxidant constituents from the fruit hulls of mangosteen (Garcinia mangostana L.) originating in Vietnam. Yakugaku Zasshi, 114(2).