



Scientia Research Library

ISSN 2348-0408
USA CODEN: JACOGN

Journal of Applied Chemistry, 2014, 2 (1):144-152

<http://www.scientiaresearchlibrary.com/archive.php>

The antioxidant content in different dietary item used in Nepal

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ABSTARCT

In Nepal, different plant and animal based diets are used for edible purpose . Plant based diets protect against chronic oxidative stress related diseases. Dietary plants contain variable chemical families and amount of antioxidant. Our objective was to develop a comprehensive food database consisting of total antioxidant content of typical foods as well as other dietary items such as traditional medicine plants, herbs and spices and dietary supplements. Thus, database is intended for use in a wide range of nutritional research, from in vitro and cell and animal studies to clinical trials and nutrition epidemiological studies. We procured samples from the local market and then assayed the samples for their total antioxidant content using the FARP assay. Our result shows large variation in antioxidant activity between various categories of food. Antioxidant content of spices, herbs and supplements were found maximum and introduce significantly more antioxidants into human diet than animal based or mixed foods

Keywords: Antioxidants , FARP assay , Medicinal herbs , food data base , Nepal

INTRODUCTION

Free radicals and antioxidants are considered so vital to our understanding of the origin of cancer, aging, illness, and diseases that they have become a profound are of research. An important concern to human today is environmental hazards and its adverse effects to the health. Environmental pollutants, pesticides, smoke, UV-radiation create free radicals. Free radicals can attach various substrates in the body and contribute to the disease development. Oxidative modified DNA play important role in carcinogenesis, cataract results from the free radicals generated from photosensitization of the protein. Such distortion of biomolecules and cells that results from the reaction of free radical is one of the most important factors related to chronic diseases. Prevalence of such chronic degenerative diseases is growing in this modern era. Thus, people have started paying much attention in their food consumption which will prevent them from the diseases and improve their health as well as economic status. We are well known about the various nutritional factors in the food that protect us from diseases and promote our healthy life. Our best way for the protection form free radicals is to keep our antioxidant levels high, Antioxidants are the nutrients that come from out diet. The major sources of antioxidants are the dietary sources and supplements. Dietary items contain variable chemical families and amount of antioxidants. It is

hypothesized that antioxidants originating from food may work as antioxidant in their own right in vivo as well as bring beneficial health effect through antioxidants defense, longevity, cell maintenance and DNA repair.

In context of Nepal, people have different food habits. Our approach was to assay antioxidant property and to develop a comprehensive food database consisting of total antioxidant content of food, beverages, spices, herbs and supplements that are used in our country. Antioxidant property in many varieties of western fruits and vegetables has been studied. Results demonstrated large variation in antioxidant property even within the same category of food. Our aim was to develop a database revealing antioxidant content of commonly used food in the country with the intention that it will prove as an essential research tool for further studies and research.

MATERIALS AND METHODS

Method of FRAP

Ferric reducing- antioxidant power assay method is based on the reduction of the Fe³⁺-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 620nm. A modified FRAP was used as it is inexpensive, reagents are simple to prepare, results are highly reproducible and the procedure is straight forward and speedy. The FRAP assay offers index of antioxidants or reducing potential of biological fluids within the technological reach of every lab and research interested in oxidative stress and its effects.

Samples were commonly used food in daily life of the Nepalese people. Most of the food samples were procured from the local markets and stores. Commercially procured samples were stored at 4°C and analyzed within 3 days. Fruits (n=20) vegetables (n=34), dry fruits (n=7), spices (n=18) were collected from the local markets. They were at first chopped into small pieces and then extracted with MiliQ water.

For beverages (n=14), cold drinks and alcoholic drinks and locally brewed alcoholic drink chyang were purchased from the local markets. Tea and coffee were prepared by brewing an infusion (1gm in 50ml boiling water for 5 minutes). Similarly, supplements for infants (n=4) were prepared by dissolving 1g in 50ml boiling water. Before analysis beverages and supplements were diluted in MiliQ water.

Oils (n=9), sweet and chocolates (n=13) and processed foods (n=6) were purchased from local stores. Fats and oils were directly dissolved in isopropanol whereas sweets, chocolates and junk foods were extracted in isopropanol. Further, all samples were homogenized in a homogenizer for 10-12 minutes. Three replicates were taken for each sample in microfuge tubes of 1.5ml each and all samples were kept in ice bath for 30 minutes for sonication. After sonication, samples were centrifuged at 10,000rpm for 10 minutes. After this pellet were discarded and triplicate of supernatant were taken for the measurement of antioxidants.

The FRAP assay was used with minor modification. Colorimeter was used for the measurement of absorbance that appears when TPTZ-Fe³⁺ complex reduce to TPTZ Fe²⁺ form in presence of antioxidants. An intense blue colour develops and absorbance was taken at 620nm.

300mM acetate buffer(pH=3.6) was prepared by dissolving 0.82g sodium acetate in 8.1ml glacial acetic acid and the volume as made 500ml by adding distilled water. 10mM TPTZ solution was prepared by mixing 0.031g TPTZ solution in 10ml 40mM HCl and dissolved at 50°C in water bath. 20mM FeCl₃ solution was prepared by dissolving 0.032g of FeCl₃ in 10ml distilled water. Finally, all three solutions were mixed in the ration of 10:1:1 to make the working FRAP reagent at the time of assay.

Standard

1000uM/1.7H₂O was prepared by dissolving 0.027g FeSO₄ in 100ml of distilled water. The colorimeter was set at 0 by the blank. i.e., 3ml of FRAP reagent. 10ml sample was mixed with 3ml of working FRAP reagent and absorbance was taken at 620nm. Thereafter, samples were allowed for 4 minutes and absorbance was again taken in 595nm.

FRAP value of sample (uM)

$$= \frac{\text{change in absorbance of sample form 0 to 4 minutes}}{\text{Change in absorbance of standard form 0 to 4 minutes}} \times \text{concentration of standard}$$

RESULTS AND DISCUSSIONS

Our result shows large variation in antioxidant activity between various categories of food. Samples within the same category also have shown differences in their antioxidant content. There is large variation in the chemical families and amount of antioxidants in various categories of diet. The assay demonstrates the differences in antioxidant content of foods. Fruits, spices and herbs are found to contain relatively high amount of antioxidants. Other categories such as beverages, chocolates, sweets contain medium to high antioxidant value. Similarly, plant based foods and generally rich sources of antioxidant content than animal based foods or mixed foods. This systematic measurement enable us to calculate total antioxidant content of complex diet identify and rank potentially good sources of anti oxidant and provide comparable data on the relative antioxidant capacity of wide range of foods.

Categories	No of samples (N)	Mean value	Minimum value	Maximum value
Fruits	20	2.33	0.36	9.09
Medicinal Plants	23	1.34	0.18	6.63
Spices	20	1.44	0.02	4.36
Cereals and pulses	21	0.83	0.09	3.1
Beverages	11	1.24	0.09	3.09
Vegetables	31	0.56	0.01	3
Infant supplements	5	2.1	1	3
Fats and Oils	9	1.23	0.36	2.7
Dry fruits	9	0.50	0.09	1.72
Dairy product	6	0.56	0.09	1.18
Eggs and meats	5	0.11	0.09	0.18

Antioxidant concentration of Fruits

Fruit Samples	Botanical Names	Antioxidant content (mM/100g)
Apple	Malus pumila	1
Lemon	Citrus lemon	1.8
Papaya	Carica papaya	3.7
Malta orange	Citrus sinensis	3.5
Banana	Musa sapientum	1.5
Pear	Pyrus communis	1.8
Mango	Mangifera indica	3.09
Pomegranate	Punica granatum	1.6
Pineapple	Ananas comosus	0.8
Watermelon	Citrullus lanatus	0.36
Carrot	Ducus carota	2

Nepalese hog pulm	Choerospondias axillaries	1.5
Sugarcane	Saccharum officinarum	1
Guava	Psidium guajava	2.1
Amala (Juice preserved)	Emblica officinalis	5.9
Amala (Juice fresh)	Emblica officinalis	9.09
Custard apple	Annona reticulate	1.18
Persimon	Diospyros kaki	3
Pummelo	Citrus maxima	0.81
Cucumber	Cucumin sativus	0.7

Antioxidant content of Vegetables

Vegetable samples	Botanical Names	Antioxidant content (mM/100g)
Tomato	Lycopersicum esculentum	0.9
Cabbage	Brassica oleraceae var capitata	0.8
Chilly	Capsicum annum	1.1
Okra	Hibiscus esculentus	1.1
Potato	Solanum tuberosum	0.6
Brinjal	Solanum melongena	0.45
Onion	Allium cepa	0.01
Pointed gourd	Trichosanthes dioica	0.01
Pumpkin	Cucurbita maxima	0.01
Colocasia	Colocasia esculenta	0.15
Mushroom	Agaricus sps.	0.27
Raddish	Raphanus sativus	0.7
Bottle gourd	Lagenaria siceraria	0.6
Cocoyam	Colocasia antiquorum	1.08
Coriander	Coriandrum sativum	1
Chayote	Sechium edule	0.09
Asparagus	Asparagus racemosus	0.8
Chatel	Momordica cochinchinensis	3
Sponge gourd	Luffa cylindrical	0.27
Bitter gourd	Momordica charantia	0.18
Indian rape	Brassica compestris var toria	0.09
Spinach	Spinacea oleracea	0.18
Cow pea	Vigna sinesis	1.1
Leaf mustard	Brassica juncea	1.1
Snake gourd	Trichosanthes anguina	0.09s
Cauliflower	Brassica oleraceae var. botrytis	0.18
Bell pepper	Capsicum frutescense var. grossum	0.27
Pumpkin green	Benincasa hispida	0.09
Soybean	Glycine max	0.36
Bamboo shoots	Dandrocalamus sps.	0.7
French bean		0.27

Antioxidant content of Medicinal Plants

Medicinal Plant Samples	Botanical Name	Antioxidant content
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		(mM/100g)
Ghiukumari	Aloe vera	2.3
Neem	Azadirachta indica	2.36
Aswagandha	Withania cogulans	0.18
Tulsi	Ocimum sanctum	3.18
Mint	Mentha piperita	0.18
Kuchila	Strychnos nuxvomica	0.27
Sarpagandha	Rawalfia serpentine	1.27
Liqorice (Jethimadhu)	Glycyrrhiza glabra	0.13
Majitho	Rubia manjith	1.27
Padamchal	Rheum austral	2.27
Nisoth	Operculina turpethium	0.72
Sugandhawaal	Valeriana jatamansi	0.27
Grujo	Tinospora sinensis	0.18
Nirmansi	Delphinium denadatum	1.27
Chiraito	Swretia chirayita	0.54
Pakhanved	Bergenia ciliate	1.09
Chitu	Plumbago zeylanica	0.18
Asuro	Justicia adhatoda	0.18
Barro	Terminalia bellirica	6.63
Chutro	Berberis asitica	0.63
Khair	Acacia catechu	4.45
Jatamansi	Nardostachys grandiflora	0.69
Pipla	Piper longum	0.18

Antioxidant content of Spices

Spice samples	Botanical Name	Antioxidant content 9mM/100g)
Clove (Lwang)	Syzygium aromaticum	3
Indian colza (Sarseu)	Brassica compestris var. glauca	3.6
Indian rape (Tori)	Brassica compestris var. toria	0.09
Cumin seed (Jeera)	Cumimum cynium	1.63
Lovage (Jwano)	Trachyspermum ammi	1.81
Fenugreek (Methi)	Trigonella foenum graecum	2.45
Nepal pepper (Timur)	Zanthoxylum armatum	3
Black pepper (marich)	Piper nigrum	2.18
Pepper (Red chilly)	Solanum frutescens	1.27
Small cardamom (Sukmel)	Elettari cardamomum	2.02
Cardamom	Ammomum subuletum	0.09
Coriander (Dhaniya)	Croiandrum sativum	0.03
Cinnamon Bark (Dalchini)	Cinnamomum zeylanicum	0.03
White Sesame (Steo til)	Seasum indicum	0.45
Black sesame		0.36
Wild garlic (Jimmu)	Allium wallichii	3.27
Turmeric (Besar)	Curcuma domestica	0.39
Cinnamon leaf (Tejpat)	Cinnamomum tamala	0.45
Asafetida (Hing)	Ferula asafetida	0.27

Maize	Zea mays	0.45
Rice/Rice flour/beaten rice	Oriza sativa	0.18
Gram	Cicer arietinum	2
Lentil (Musuro)	Lens culinaris	3.1
Black gram	Phaseolus mungo	0.63
Res gram	Cajanus canjan	0.09
Wheat flour	Triticum aestivum	0.45
Dried pea	Pisium sativum	0.72
Fresh pea	Pisium sativum	0.63
Kidney bean	Phaseolus vulgaris	1.63
Black bean		1
Horse gram	Dolichos biflorus	0.09
Broad bean	Vicia faba	1.63
Soyabean	Glycine max	1.45
Cow pea	Vigna sinensis	1.54
Gram flour	Cicer arietinum	0.53
Puffed rice (Murai)		0.09
Suji		0.27

Dry fruits

Cashew nut (Kaju)	Anacardium occidentale	0.09
Almond	Terminalis catappa	0.36
Raisins	Vittis vinifera	1.72
Dry coconut	Cocus nucifera	0.36
Walnut	Juglans regia	0.09
Dried fig	Ficus carica	0.54
Dried apricot	Prunus ar.....	0.36
Date	Phonix dactylifera	0.36
Pistachio	Pistacia vera	0.63

Vinegar	1.8
Whisky	0.19
Brandy	0.36
Black tea	2.27
Herbal tea	2.54
Coffee	2.9
Milk tea	2.2
Green tea	3.09
Coke	0.27
Vodka	0.09
Homemade alcohol	0.00
Almond oil	0.72
Cocounut oil	0.36
Sunflower oil	0.54
Soyabean oil	1.36
Amala hair oil	2.45

Ghee	2.72
Vegetable ghee	1
Rahat Rooh hair oil	0.45
Seasme oil	1.54
Peda	0.27
Soan papadi	0.45
Kalakand	0.36
Rasbari	0.09
Lalmohan	0.09
Laddu	0.00
Jerry	0.1
Gudpak	0.18
Dairy milk	0.92
Jelly	0.52
Chocolate	1.63
Pustakari	0.81
Lays potato chips	0.07
Mayos instant noodles	0.2
Chees balls	0.3
Kurkure	0.23
Popcorn	0.09
Bhujija	0.08
Molasses(Gud)	4.9
Infant supplements	
Cerelac	2.9
Sarvottam lito	1
Lactogen	2.3
Horlicks	1.72
Mother's milk	3
Dairy products	
Yogurt	0.36
Cheese	1.09
Paneer	0.2
Butter	1.18
Milk pasturised	0.09
Milk fresh	0.45
Egg	0.09
Chicken	0.09
Mutton	0.09
Fish	0.18
Buff	0.09

Overall 179 samples were analyzed which were classified into three categories- plant based food, animal based food and mixed food products. These classes were further categorized into 12 different groups of fruits, vegetables, spices, medicinal herbs, beverages, infant supplements, fats

and oils and sweet, chocolates and snacks. With this analysis, there is a comprehensive food database consisting of antioxidant content of commonly used foods in the country.

The result demonstrated that plant based food contain comparatively higher amount of antioxidant content than animal based and mixed food. Thus, diets comprised of animal based food are low in antioxidant content while diet based mainly on variety of plant based food are rich in antioxidant due to presence of thousands of bioactive antioxidant phytochemicals in plants. This study suggested that fruits are rich source of antioxidants. Fruits with highest antioxidant include *Phyllanthus emblica*, persimmon, papaya and mango. Vegetables are found to have medium to high range of antioxidant value.

The spices and herbs analyzed particularly have shown relatively high antioxidant content than other categories. Although spices and herbs are consumed less in our daily life, they are still important contributors to our antioxidant intake.

Beverages especially tea and coffee are almost widely consumed in our day to day life. The analysis in beverages demonstrated that tea and coffee can be good source of antioxidants. This is due to the presence of different compounds such as polyphenols, caffeine and heterocyclic compounds. Particularly, green tea is rich in flavonoids and thus is beneficial in alleviating the oxidative stress. Hard drinks contain least amount of antioxidants among the beverages.

Infant supplements contained higher antioxidant value. Among these mother's milk and lactogen have relatively higher concentration of antioxidants and thus are good sources of antioxidant for infants.

The study in chocolates has shown that antioxidant value is high in the chocolate with high coca content. Since, jelly and sweets do not contain coca they have less antioxidant value.

Though cereals and pulses are major food constituents in Nepal, our result demonstrated that cereals and pulses contain low antioxidant value. Animal based food products have very least antioxidant level.

As concluded from above study, apart from cereals and pulses in our daily life, it is necessary to consume fruits in adequate amount to maintain healthy life. It is also beneficial to add spices in own diet.

CONCLUSION

Free radical is defined as a molecule or molecular species that contain one or more unpaired electron and is capable of independent existence. They attack the nearest stable molecule capturing its electron. When the attacked molecule loses its electron, it becomes a free radical itself beginning a chain reaction. Some free radicals are normal during metabolism and used for intracellular killing of viruses and bacteria by phagocytic cells and granulocytes. Superoxide anion radical O_2^- , hydrogen peroxide radical H_2O_2 , hydroxyl radical (OH^-) are radicals formed during the reduction of water, the other free radicals are hydroperoxy radical, lipid peroxide radical (ROO^-) and peroxy nitrite ($ONOO^-$). Free radicals and other reactive oxygen species that accumulate in the body cause damage on cells, DNA, lipid, sugar and proteins which give rise to the oxidative stress in the body.

To defense against the free radical damage, our body has a defense system called antioxidants. Antioxidants neutralize free radicals by donating one of their own electrons ending the oxidation reaction. The antioxidant themselves do not become free radical by donating an electron because they are stable in their either form. This compound are synthesized in the body or obtained from the diet. They are often reducing agents such as thiols, ascorbic acid, polyphenols or enzyme system as catalase, superoxide dismutase and peroxidase.

Antioxidant assay of food helps us to identify the potentially good sources of antioxidant in our diet. This food database is useful in the nutritional reaseach and provides information of diets rich in antioxidants. FRAP assay was used to determine the antioxidant value. It is easier, faster and

reliable method of assaying. It can be conducted in any laboratory as well as results are more accurate and highly reproducible.

Our results demonstrated that fruits, vegetables, spices, herbs and supplements have relatively high amount of antioxidants. Other categories such as beverages, sweets contain medium to high antioxidant value. Similarly, plant based foods have higher antioxidant content than animal based foods of mixed foods.

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