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TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOL CONTENT OF PULSES AND ROOT VEGETABLES COMMONLY USED IN INDIA

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ABSTRACT

With increasing importance of the role antioxidants, as quenchers of free radicals, in the prevention and treatment of diseases, knowledge on the antioxidant capacity of foods becomes important. Very scarce data is available on the antioxidant capacity of Indian foods especially pulses and root vegetables which form an integral part of Indian diet. Commonly used pulses namely, Bengal gram (*Cicer arietinum*), Black gram (*Phaseolus mungo*), Green gram (*Phaseolus aureus*), Red gram (*Cajanus cajan*), Rajmah(*Phseolus vulgaris*) and root vegetables namely Carrot, Beetroot, Potato, Onion, Yam, Radish, Turnip, Taro(*Colocasia esculenta*), Koorkankilangu (*Plectanthrus rotundifolius*) were selected for the study. Estimation for Total Antioxidant Capacity (TAC) was done by Oxygen Radical Absorbance Capacity (ORAC) assay and Ferric Reducing Antioxidant Power (FRAP) assay and Total Phenol (TP) content was estimated by Folin Ciocalteau method. The selected food samples were subjected to one method of cooking, pulses were pressure cooked and root vegetables were steamed, to know the effect of heat on the TAC and TP of these foods. Soybean had the highest antioxidant capacity among the selected pulses which was 5246.81µmols TE/100g(by ORAC) and green gram whole had the highest phenolic content of 193.59 mgGAE/100g. Among root vegetables Koorkankilangu (*Plectanthrus rotundifolius*) had the highest antioxidant capacity and phenolic content. Cooking had a profound influence on both groups of foods. The TAC of pulses decreased by 30-65%, whereas, the TAC of root vegetables significantly increased.

Key words: Total Antioxidant Capacity, Total Phenols, Pulses, Root Vegetables.

INTRODUCTION

Antioxidants play a very important role in the body's defence system against Reactive Oxygen Species (ROS), which are the harmful by products generated during normal cell aerobic respiration (Gutteridge et.acl., 2000). Antioxidants are also quenchers of free radicals, one of the causative factors for the development and progression of degenerative diseases. Increasing intake of dietary antioxidants may help to maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system and play an important role in preventing diseases.

Many compounds have been isolated from plant foods, mainly fruits, green leaves and vegetables, which have been identified to play a protective role in the human system. Phenols, polyphenols, flavonoids, terpenes in fruits and vegetables (Scalbert et.al., 2002) and specific components like lycopene in tomatoes and its products(Basu and Imrhan, 2002) and green tea polyphenols (Ahmad and Mukhtar, 1999) prevent degenerative diseases, offer chemoprotection and reduce oxidative stress. Despite the association of fruits and vegetables in preventing diseases, the intake of fruits and vegetables varies globally (Hall and Moore) and is generally less than recommended levels in developing countries including India (Yadav and Krishnan, 2008). Of the cereals, which constitute the staple food in India, whole grains and millets also have unique phytochemicals different from fruits and vegetables and the bran and germ of whole grains are found to be rich in antioxidants and vitamins (Marquart et.al., 2002).

Pulses and root vegetables also form an integral part of Indian diet and some of these foods have been identified to be rich in antioxidants. Soybean, rich in isoflavones is also rich in phenolic acids and has a high antioxidant capacity (Malencic et.al., 2007). Roots and tubers like carrot, beetroot and turnip have been studied for the carotenoids, pigments and vitamins, especially vitamin C, which contribute to the antioxidant capacity of these vegetables and red beetroot extracts have shown high antioxidant capacities in different test systems (Kugler et.al., 2007). There are other pulses and root vegetables conventionally used in India and the data on the antioxidant content of these foods is scarce. The data on the antioxidant capacity of these foods would help in including them in daily diet and may contribute significantly to the dietary antioxidant capacity as a whole. Moreover, estimations on individual antioxidants in foods are complicated due to chemical diversity of these compounds along with their synergistic reactions with other compounds. Hence data on total antioxidants and phenols in foods will help in evaluating the antioxidant capacity of foods.

MATERIALS AND METHODS

SELECTION OF SAMPLES

Commonly used pulses namely, Bengal gram (*Cicer arietinum*), Black gram (*Phaseolus mungo*), Green gram (*Phaseolus aureus*), Red gram(*Cajanus cajan*), Rajmah(*Phseolus vulgaris*) and root vegetables namely Carrot(*Daucus carota*), Beetroot(*Beta vulgaris*), Potato(*Solanum tuberosum*), Onion(*Allium cepa*), Yam(*Typhonium trilobatum*), Radish(*Raphanus sativus*), Turnip(*Brassica rapa*), Taro(*Colocasia esculenta*), Koorka kilangu (*Plectanthrus rotundifolius*) were selected for the study. Each food sample was purchased from five different outlets in the local market of Coimbatore city, Tamilnadu, India, thoroughly cleaned, inedible parts removed and mixed and the mixture was considered to represent the food sample.

COOKING OF SAMPLES

The pulses were washed well, but not soaked, before cooking and pressured cooked for 10 minutes with the right amount of water. The root vegetables were steamed for seven minutes. Cooking methods that involve soaking and draining water result in decreasing the antioxidant capacity of foods (Kugler et.al., 2007) and hence the above methods were chosen

PREPARATION OF EXTRACTS

100 g of the sample was powdered (for dry samples) or homogenised (for wet samples) using a blender. 2g of the powdered or homogenised sample was used for extraction (Vicas et.al., 2009) as represented in figure 1. 80% methanol was used for extraction as it facilitated maximum extraction of compounds (Shabir et.al., 2011).

TOTAL ANTIOXIDANT CAPACITY (TAC)

OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY

TAC of foods was measured as Oxygen Radical Absorbance Capacity (ORAC) using method by Ou et al (2001). Though the TAC of foods are measured using various methods, ORAC assay is gaining importance as it measures the antioxidant capacity of the food using biologically relevant peroxide radical and the experiment is conducted at 7.4 PH. The ORAC assay is more sensitive and reflects antioxidant properties even of such a low quantity of polyphenols (Prior et.al., 2002). The advantage of the ORAC assay is that it combines both the inhibition

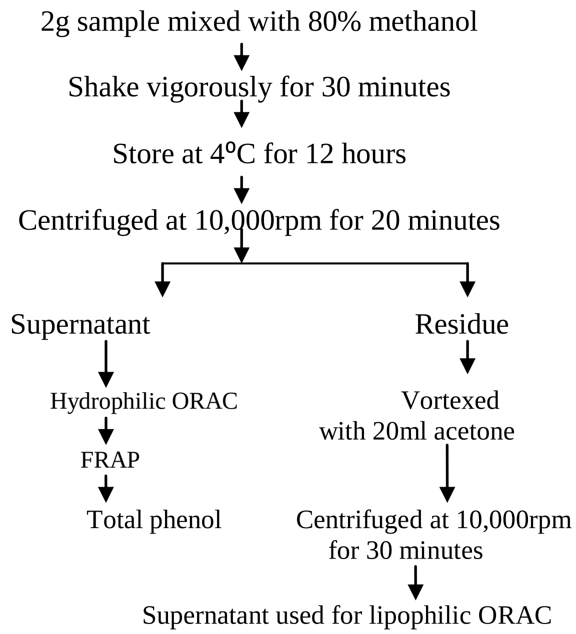


Figure 1 – Method of Extraction

time and inhibition degree of the radical generation, as it takes the oxidation reaction to completion and uses the area under the curve to quantify the antioxidant capacity. Fluoroscein sodium salt was used for imparting fluorescence. The working solution and the solution for dilution was sodium phosphate buffer with a Ph of 7.4. Trolox, a water soluble analogue of vitamin E was used as the standard and measurements were taken using a spectrofluorometer (Jasco FP 777, Tokyo, Japan). The fluorescence was measured every minute after the addition of 2,2-azobis(2-methyl propionamidine) dihydrochloride (AAPH). All fluorescent measurements are expressed relative to the initial reading. The final results were calculated using the difference of areas under the fluoroscent decay curves between the blank and sample and were expressed as micromole Trolox equivalents per 100 g of food (µmol TE/100g). Total ORAC value for the samples was calculated by adding the values of the hydrophilic and lipophilic extracts.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Ferric Reducing Antioxidant Power (FRAP) of the sample extracts was estimated using the method by Benzie and Stain (15). It estimates the ability of the sample to reduce ferrous ion to ferric ion. In presence of TPTZ, a blue colour was formed, the intensity of which was measured at 593 nm using a UV spectrophotometer (optima). Trolox was used as the standard and results expressed as micromole Trolox equivalents per 100 g of food (µmol TE/100g).

TOTAL PHENOLS

The total phenolic content of the samples was estimated by Folin Ciocalteau method(16). Gallic acid was used as the standard and results expressed as mg of gallic acid equivalents per 100 g of food (mg GAE/100g).

RESULTS AND DISCUSSION

TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLS IN RAW AND COOKED PULSES

The TAC of pulses ranged from 5246.81 µmol TE/100g in soyabean to 1879.16 µmol TE/100g in red gram dhal by ORAC assay and 563.61 µmol TE/100g in rajmah to 26.97 µmol TE/100g in Bengal gram whole(white) by FRAP assay (Table I). There was no correlation observed between the TAC values by the two methods in both raw (r=0.2174) and cooked (r=-0.0825) pulses. The total phenols ranged from 345.2 mg GAE/100g to 54.43mg GAE/100g (Table I). Rajmah had the highest total phenolic content followed by Green gram whole and Bengal gram (whole, black) while Green gram dhal had the least total phenolic content. Also the total phenolic content of Bengal gram (whole) varieties, Black gram and Bengal gram dhal was higher than the TAC values by FRAP method.

Table I: Total Antioxidant Capacity and Total Phenols in raw pulses

Name of food	Total Antioxidant Capacity (µmol TE/ 100g)		Total Phenols (mg GAE/100g)
	ORAC	FRAP	
Red gram dhal	1879.16 ± 10.0	226.6 ± 1.2	73.49 ± 2.2
Green gram (whole)	2985.48 ± 12.0	325.95 ± .09	193.59 ± 6.3
Green gram dhal	2273.95 ± 21.4	124.23 ± 1.3	54.43 ± 4.2
Bengal gram (whole, white)	2166.16 ± 13.9	26.97 ± 0.5	93.52 ± 3.3
Bengal gram (whole, black)	2298.34 ± 15.2	99.52 ± 1.3	114.33 ± 5.9
Black gram (deskinned)	2573.79 ± 11.9	42.2 ± 1.5	61.64 ± 3.1
Rajmah	3084.65 ± 8.5	563.61 ± 2.2	345.2 ± 4.8
Bengal gram (roasted)	2360.81 ± 4.6	78.05 ± 1.7	102.62 ± 1.7
Bengal gram dhal	2525.73 ± 13.2	63.11 ± 1.9	96.25 ± 2.8
Soya bean	5246.81 ± 14.8	156.38 ± 1.2	97.6 ± 1.4

The TAC values of cooked pulses decreased to a greater extent after cooking (Table II). The decrease by ORAC assay ranged from 9.1 percent in Green gram (whole) to 65 percent in Red gram dhal and by FRAP assay the decrease ranged from 33 percent to 64.3 percent. Among cooked pulses Soybean had the highest TAC value by ORAC and Rajmah had the highest TAC value by FRAP.

Table II: Total Antioxidant Capacity of cooked pulses

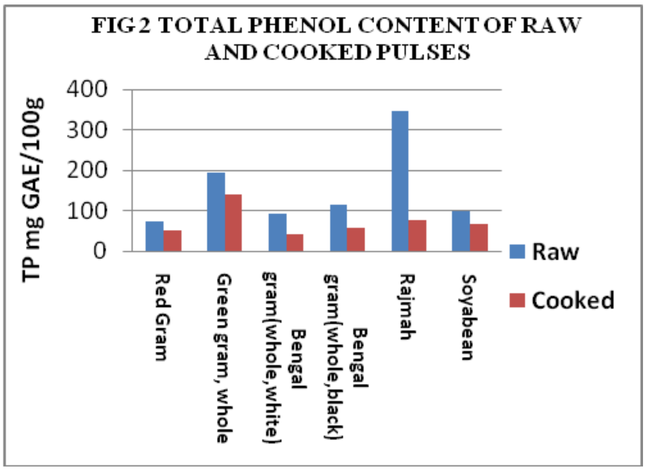
Name of food	ORAC	% Decrease*	FRAP	% Decrease*
Red gram dhal	654.62 ± 14.5	65	136.77 ± 1.5	38.5
Greem gram (whole)	2714.24 ± 17.1	9.1	174.1 ± 2.0	46.6
Bengal gram(whole , white)	1654.64 ± 16.1	23.6	13 ± 0.5	52
Bengal gram(whole , black)	1552.96 ± 9.8	32.5	35.53 ± 1.8	64.3
Rajmah	1548.66 ± 13.4	50	377.59 ± 1.1	33
Soybean	3418.02 ± 4.2	34.8	95.24 ± 1.6	39.1

*Percentage decrease in TAC values after cooking calculated from mean TAC values of raw and cooked food

Table III: Total Antioxidant Capacity and Total Phenols of root vegetables (raw)

Name of food	Total Antioxidant Capacity (µmol TE/ 100g)		Total Phenols (mg GAE/100g)
	ORAC	FRAP	
Carrot	1337.13 ± 16.7	95.16 ± 1.0	118.96 ± 4.1
Beetroot	3989.52 ± 10.3	472.45 ± 1.6	431.29 ± 4.8
Potato	986.11 ± 6.8	5.34 ± 0.5	151.17 ± 5.8
Yam	1869.86 ± 15.7	602.04 ± 2.6	200.11 ± 3.9
Turnip	124.15 ± 11.7	241.69 ± 1.8	167.97 ± 2.6
Radish	1686.0 ± 7.7	78.54 ± 1.9	127.97 ± 2.8
Onion, small	970.67 ± 5.5	192.5 ± 2.6	86.78 ± 4.1
Onion, big	942.65 ± 15.2	131.46 ± 2.1	49.8 ± 3.8
Seppan kilangu	645.22 ± 10.9	125.96 ± 1.9	120.82 ± 5.6
Koorkankilangu	3635.24 ± 6.4	946.58 ± 2.1	559.39 ± 3.0

The TP values also decreased in all the pulses after cooking (Fig 2). The highest decrease was observed in Rajmah (77percent) and the decrease averaging to 52% in the Bengal gram(whole) varieties. The least decrease was observed in Green gram(whole). Cooking lowers the total phenolic content by 10 to 45 percent and antioxidant capacity by 27 to 68 percent in pulses (Gujral *et.al.*, 2011).



TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLS IN ROOT VEGETABLES

A significant correlation was observed between the TAC values by FRAP and ORAC assay of raw vegetables (r=0.7318, P<0.05), but the vegetables had a different ranking based on their TAC values (Table III). Beetroot had the highest TAC value (3989.52 µmol TE/100g) by ORAC assay and ranked the second highest in TAC by FRAP assay followed by Koorkangkilangu. Raw potato had the lowest antioxidant capacity of 5.34 µmol TE/100g by FRAP assay whereas turnip had the lowest TAC value of 124.15 µmol TE/100g by ORAC assay. Carrots had a TAC of 1337.13 µmol TE/100g by ORAC and 95.16 µmol TE/100g by FRAP which was less than Yam and Beetroot in both assays.

Koorkankilangu had the highest phenolic content of 559.39 mg GAE/100g followed by Yam. The Onion varieties had the lowest phenolic content. Potatoes which had a low TAC value by FRAP assay (5.34 µmol TE/100g) had a phenolic content of 151.17 mg GAE/100g. Compared to some fruits and green leafy vegetables, root vegetables have high phenolic content. Vegetables like Yam, Beetroot and Ginger exhibit high *invitro* antioxidant activity (Tarwadi and agate, 2005).

TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLS IN ROOT VEGETABLES (COOKED)

There was no correlation among the two assays in the TAC values of cooked vegetables (r=0.6301). Contrary to the TAC values of pulses, the TAC values of root vegetables increased considerably after cooking (Table IV). The increase observed was between 12.8 percent and 49.9 percent by ORAC assay, and by FRAP assay the highest increase was observed in potatoes which increased from 5.34 µmol TE/100g to 217.15 µmol TE/100g (3963 percent). Also the TAC value of cooked carrots was 118.84 more than raw carrots by FRAP, an increase of 124 percent.

The increase in TP values was between 19 percent in beetroot and 71.2 percent in carrots. Among cooked vegetables Beetroot and Koorkankilangu had the highest TAC and TP values.

Table IV: Total Antioxidant Capacity and Total Phenols of root vegetables (cooked)

Name of food	ORAC	% increase*	FRAP	% increase*	TP	% increase*
Carrot	1887.07 ± 8.1	41.1	214 ± 2.8	124	203.69 ± 3.3	71.2
Beetroot	5430.2 ± 9.1	36.1	600.68 ± 2.3	27	513.16 ± 3.3	19
Potato	1359.14 ± 4.7	37.8	217.15 ± 1.5	3963	199.73 ± 2.0	32.1
Yam	2109.58 ± 15.2	12.8	748.18 ± 2.4	24.2	310.64 ± 5.2	55.2
Turnip	159.29 ± 6.6	28.3	386.29 ± 2.6	59.8	213.12 ± 1.8	26.9
Radish	1979.84 ± 5.9	17.4	155.87 ± 1.5	98.4	159.45 ± 2.1	24.6
Seppankilangu	967.4 ± 13.7	49.9	256.89 ± 2.3	103.9	199.2 ± 1.6	64.9
Koorkakilangu	4527.68 ± 21.3	24.5	1525.17 ± 3.3	61.1	767.75 ± 4.2	38.2

* Percentage increase in values after cooking calculated from mean values of raw and cooked food

Table V: Percentage contribution of Total Phenols to TAC (ORAC) in pulses before and after cooking

Name of food	Percentage contribution of Total Phenols	
	Before cooking	After cooking
Red gram dhal	3.9	7.9
Greem gram whole	6.5	5.2
Channa white	4.3	2.5
Channa Black	5.0	3.6
Rajmah	11.2	5.1
Soyabean	1.9	1.9

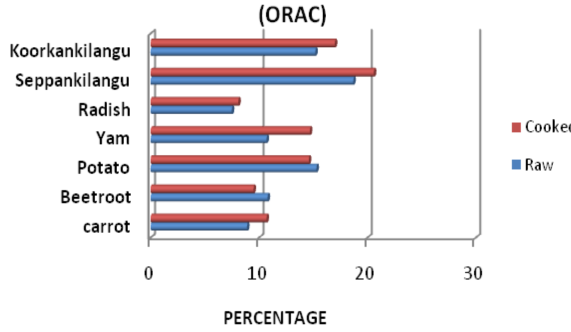
CONTRIBUTION OF PHENOLS TO TOTAL ANTIOXIDANT CAPACITY

Phenolic acids are the major contributors to the TAC of fruits and vegetables. A wide variation was observed with the contribution of phenols to TAC by the two antioxidant assays. ORAC values were considerably higher than FRAP values for all foods but for turnip. The contribution of phenols to TAC, by ORAC assay, ranged between 1.8 percent and 11.2 percent in pulses and 5.3 percent to 18.7 percent in root vegetables except Turnip. But the FRAP values were less than the TP values in

Bengal gram varieties and Black gram(whole) among pulses and in Carrot, Potato and Radish among root vegetables questioning the contribution of other compounds to the TAC of the foods. These results support the recommendation that due to the differences in the test systems investigated; at least two methods are to be used to estimate the antioxidant capacity of foods (Schlesier et.al., 2002).

Cooking had a great influence on the contribution of phenols. As in Table V, the contribution of phenols decreased after cooking in pulses except in red gram dhal. Fig 3 shows the difference in the contribution of phenols to TAC (by ORAC) before and after cooking in root vegetables. The cooked vegetables had a higher percentage of phenols except Yam and Beetroot.

FIG 3 PERCENTAGE CONTRIBUTION OF PHENOLS TO TAC IN RAW AND COOKED ROOT VEGETABLES (ORAC)



CONCLUSION

Among the selected foods Soybean, Rajmah and Green gram whole among pulses and Beetroot, Yam and Koorkakilangu among root vegetables exhibited high antioxidant capacities. Cooking decreased the TAC and TP of pulses whereas it increased that of root vegetables. The TAC of potatoes increases many folds after cooking. There was no correlation observed between the two assays used for TAC analysis except among raw root vegetables. While further studies are needed to evaluate the compounds contributing to the TAC, this data will be useful in choosing foods with high TAC among the selected food groups.

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