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IN VITRO ANTIOXIDANT ACTIVITY OF BLACK GRAM, COWPEA, DESI CHICKPEA AND YELLOW MUSTARD AS AFFECTED BY SPROUTING

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Abstract

To assist our choice of sprouts as healthy foodstuffs, we examined and compared the antioxidant activities of various sprouts. β-carotene, ascorbic acid, total antioxidant activity, total phenol and flavonoid content of black gram (Vigna mungo), cowpea (Vigna unguiculata), desi chickpea (Cicer arietinum), and yellow mustard (Brassica alba) seeds and their sprouts (48 h) were determined. The parameters chosen reflect the antioxidant capacity with respect to the dietary antioxidants (β-carotene, ascorbic acid) which were noted to be highest in chickpea sprouts and black gram sprouts respectively. The highest antioxidant activity in terms of % DPPH inhibition and flavonoid content was observed in cowpea sprouts 48.42±0.30 % DPPH inhibition and 205.00±5.06 mg/100g respectively. The highest total phenol content was noted in yellow mustard sprouts (57.33±6.67 mg/100g).

Key words: Ascorbic acid, β-carotene, DPPH, flavonoid, sprouts, total antioxidant activity.

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INTRODUCTION

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals which can start chain reactions that cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reaction by being oxidized themselves. Antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Moreover, oxidative stress is both the cause and the consequence of disease.

The importance and health benefits of legume consumption in the prevention of chronic diseases such as cancer and heart disease have been well documented[1,2,5,9,]. This is because they contain phytochemicals that combat oxidative stress in the body by helping to maintain a balance between oxidants and antioxidants. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias[10.]

Nutrition has a significant role in the prevention of many chronic diseases such as cardiovascular diseases (CVD), cancers, and degenerative brain diseases. The consumption of food-based antioxidants like β-Carotene is found to be useful for the prevention of macular degeneration and cataracts [1] .Health benefits of carotenoids that may be related to their anti-oxidative potential include enhancement of immune system function, protection from sunburn[2] and inhibition of the development of certain types of cancers[9]. Vitamin A deficiency is a major public health problem and thus β-carotene supplementation of the diet decreases morbidity and mortality related to several pathological conditions[5]. Ascorbic acid functions as an antioxidant and minimizes free radical damage in cells. In its antioxidant role, ascorbic acid reduces the risk of chronic diseases such as heart diseases, certain forms of cancer and cataracts. In addition to working independently as an antioxidant, it helps recycle oxidized Vitamin E for re-use in the cells. It also stabilizes the reduced form of the folate coenzyme[12].

Flavonoids constitute an unavoidable component of the diet. Of the many actions of flavonoids, antioxidant and anti-proliferative effects stand out[14].Thus, the current study aims to determine the antioxidant activity by way of estimating the biochemical parameters such as total antioxidants, flavonoids and phenols also highlighting the importance of the dietary antioxidants β-carotene, ascorbic acid on sprouting legumes such as black gram *(Vigna mungo)*, desi chickpea *(Cicer arietinum)* and cowpea (*Vigna unguiculata)* and the oilseed yellow mustard (*Brassica alba)*. The objectives of this study were to compare the antioxidant activity (total antioxidants, phenols, flavonoids) of the selected dry seeds and their germinated samples (48 h) and to identify by comparison, the sample having maximum antioxidant activity within the selected sprouted samples.

MATERIALS AND METHODS

Plant material

Dry seeds of legumes black gram- *(Vigna mungo* var Rashmi*)*, Desi Chickpea *(Cicer arietinum* var JG-11*)* and Cowpea (*Vigna unguiculata* var PK-B6*)* were purchased from the University of Agricultural Sciences (UAS), Bangalore, India. The oilseed yellow mustard (*Brassica alba)* was purchased from Namdhari Seeds, Bangalore, India. As a first step, healthy seeds of similar size and shape were chosen. The seeds were not chemically treated in any way as this would slow down the germination rate.

Sprouting process

The seeds were washed thoroughly in tap water and then soaked overnight in fresh water for 8-10 hours. The seeds were then rinsed and the water drained off. They were allowed to germinate for 48 hours and used for further experiments.

β-carotene

The β-carotene content was estimated using the method described by Mangels[7]. β-carotene was extracted from 0.5 g of fresh tissue in 10 ml of 1:1 petroleum ether and acetone. The extract was then filtered through Whatman No. 1 filter paper and left undisturbed overnight. The extract was centrifuged and the centrifugate evaporated till half its original volume. A pinch of sodium sulphate was added to all the solutions and centrifuged. β-carotene thus obtained was read at 470 nm in a calorimeter.

Ascorbic acid

The Ascorbic acid content was estimated using the method described by Osborne and Boggt[11]. 0.5 g of the sample was homogenized with 10 ml of oxalic acid-acetic acid mixture and filtered through a muslin cloth. The filtrate was then centrifuged at 4000 rpm for 5 minutes and the resultant supernatant collected, and used for the estimation of ascorbic acid. To 2.5 ml of the supernatant taken in a conical flask, 5 ml of the oxalic acid- acetic acid mixture was added and titrated against indophenol dye. The volume of indophenol dye used to neutralize the acid was noted and was used for the calculation of the amount of ascorbic acid in the sample.

Total antioxidant

The total antioxidant activity of the samples was estimated by the method described by Braca[4]. Stock solution of ascorbic acid in distilled water (1000 µg/ml) was diluted suitably in order to obtain different concentrations ranging from 10µg-100µg. 0.1 ml of each of the above prepared concentrations was taken in clean dry test-tubes. The volume in each tube was made up to 3 ml with DPPH. The test tubes were incubated for 10 min at room temperature. The contents of each tube were

mixed well and the absorbance read at 517 nm against a suitable blank. 3.1 ml DPPH was used as the control. A standard curve was plotted to determine the total antioxidant content in the samples. The % DPPH inhibition by the samples was calculated as follows:

$$\% \text{ Inhibition of DPPH by the sample} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is the Optical Density (O.D.) of the control,
A_s is the Optical Density (O.D.) of the sample.

Total Phenol

The total phenol content of the samples was estimated by the method described by Singleton and Rossi [13.] 0.1 to 0.5 ml aliquots of standard gallic acid (1 mg/1 ml methanol) were used for the estimation. The volume in each of the tubes was made up to 2 ml with distilled water. 0.2 ml of 1:4 diluted Folic-Ciocalteu (FC) reagent was added to each of the test tubes followed by 0.5 ml 7.5 % sodium carbonate solution. The tubes were then incubated for 15 min at room temperature. The contents of each tube were mixed well and the absorbance was read at 760 nm against a suitable blank. A standard curve was plotted to determine the phenol content in the samples.

Flavonoid

The flavonoid content of the samples were estimated using the method described by Yang[15]. 0.5 to 2.0 ml aliquots of standard quercetin solution (1 mg/1 ml) was pipetted out into different test tubes. The volume in each of the tubes was made up to 2 ml with methanol. 2 ml of methanol served as the blank. 0.1 ml 10 % aluminium chloride reagent was added to each of the tubes followed by 0.1 ml of 1M potassium acetate solution and 2.8 ml of distilled water. The test tubes were incubated for 30 min at room temperature. The contents of each tube were mixed well and the absorbance was read at 670 nm against the blank. A standard curve was plotted to determine the flavonoid content in the sample.

RESULTS AND DISCUSSION

Carotenoids are a widespread group of naturally occurring fat-soluble colorants. β-carotene, as an antioxidant, has been shown to act as an immune modulator, quench singlet oxygen, and reduce peroxyl radicals at a low partial oxygen pressure. There is an increase in the β –carotene content of all the selected seeds on sprouting (Table 1). The highest increase in β –carotene content was noted in chickpea (57.05 µg/100g) on sprouting, indicating the significance of sprouting on the nutritive content of this specific legume. This was followed by yellow mustard sprouts in which an increase of 26.88 µg/100g was noted. A moderate increase of 13.66 µg/100g and 6.41 µg/100g in the other two selected legumes sprouts; black gram and cowpea was noted respectively. The richest source of β –carotene are generally considered to be yellow orange fruits and green leafy vegetables, but as observed, on germination the β –carotene levels also increase on sprouting, thus making sprouts a moderately good source of β –carotene. It has been observed that upon germination the concentration of β-carotene steadily increases with increasing germination time[15].

The vitamin ascorbic acid, an important dietary antioxidant was found to increase in all selected seeds on sprouting (Table 1). The ascorbic acid content of the selected seeds was minimal in dry seeds and on sprouting it increases manifold especially in black gram sprouts which showed the maximum increase of 22.9 mg of ascorbic acid/100g when compared to its dry seed. The ascorbic acid content of cowpea sprouts also showed a notable increase of 11.25 mg/100g upon sprouting. A moderate increase in ascorbic acid was noted in yellow mustard and chickpea sprouts which exhibited an increase of 7.5 mg/100 g and 5 mg/100 g respectively. Thus, sprouted seeds hold excellent potential as sources of dietary antioxidants in the form of ascorbic acid and pose an important role in disease prevention. It is a known fact that ascorbic acid, which is either completely absent or present in negligible amounts in dry legumes is synthesized during the germination process [3] and hence the ascorbic acid content of legumes increases manifold on sprouting. Mao[8] observed that increase in the ascorbic acid level is a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination.

It was observed that the selected seeds on sprouting showed an increase in % DPPH inhibition (Table 1) which implies that the ability of the components in sprouts (antioxidants) to scavenge free radicals increases after the germination process, thus giving sprouts a significant physiological role to help quench free radicals and ward off degenerative diseases. The % DPPH inhibition of yellow mustard showed maximum increase of 14.81 % DPPH inhibition on sprouting. The increase in % DPPH inhibition by chickpea sprouts is 11.23 %. The % DPPH inhibition of cowpea seeds although being the highest amongst all samples at 48.42 %, showed moderate increase upon sprouting (8.33 %). The increase in % DPPH inhibition by black gram on sprouting for 48 hours proved to improve antioxidant activity by 9.5 % This signifies the importance of sprouting as a convenient method to improve the antioxidant activity of seeds.

There are problems to drawing general conclusions regarding a health effect common to all phenolic compounds as their structures and hence biological effects are extremely diverse, some being beneficial while others being harmful to the health of human beings. At the same time, the possible health benefits of a specific phenolic compound remain most of the time unproved. A distinct increase in the amount of phenols in all selected samples was observed on sprouting for 48 hours (Table 1). The highest phenol content amongst all the selected samples was noted in yellow mustard sprouts with 57.33 mg/100g as compared to the seeds that contained 42.60 mg phenol/100g. The highest increase was exhibited by black gram seeds on sprouting with a percentage increase of 15.93 % of phenol content from 25.03 mg/100g (seeds) to 40.96 mg/100g (sprouts). Cowpea seeds on sprouting showed a moderate increase of 8.37 % from 44.53 mg/100g to 52.90 mg/100g of the sample. A minimal increase was noted in desi chickpea seeds on sprouting with only a 3.23 % increase of phenols.

Flavanoids, a class of secondary plant metabolites with significant antioxidant and chelating properties were found to increase significantly on sprouting (Table 1). The highest flavonoid content was noted in cowpea sprouts (205 mg/100g) followed by black gram sprouts (76.6 mg/100g). A moderate increase of 34.7 mg/100g of the sample was noted in yellow mustard sprouts on comparison with yellow mustard seeds. A minimal increase of flavonoid content was observed on sprouting desi chickpea seeds from 96.66 mg/100g to 126.66 mg/100g. The secondary metabolites of plants provide humans with numerous biologically active products. These plant secondary metabolites which include several classes such as terpenoids, flavonoids, and alkaloids, have diverse chemical structures and biological activities. Therefore, these natural compounds as dietary components have considerable impact on human health[6.]

CONCLUSION

When comparing the nutritive value of all sprouted seeds, cowpea sprouts (very closely followed by mustard sprouts) was found to have an increase amongst all sprouted seeds of selected samples owing to its high nutritive content with the highest antioxidant and flavonoid content. Thus cowpea sprouts can be considered as effective nutraceutical foods amongst the selected samples. Germination brought about significant increases in the micronutrient, phytonutrient content of all selected seeds, thus proving that there is marked increase in the nutritive value of the seeds on sprouting. This ultimately signifies that sprouts should be considered a vital component of the diet and can be incorporated into the menu plan with a wide variety of variations.

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TABLE 1: A summary of the changes in nutrient content of selected seeds on sprouting (Expressed as Mean ± standard deviation)

Parameter	Time of germination (48 h)	Black Gram	Chickpea	Cowpea	Yellow mustard
β- carotene (µg/100g)	Dry seed	38.68±0.57	146.67±4.80	12.91±5.46	162.21±5.20
	Sprouts	52.34±3.46	203.72±5.60	19.32±3.29	189.09±7.50
Ascorbic acid (mg/100g)	Dry seed	5.00±0.09	6.25±0.03	7.50±0.23	7.50±0.10
	Sprouts	27.9±0.72	11.25±0.98	18.75±0.04	15.00±0.68
Total antioxidants (% DPPH inhibition/100g)	Dry seed	23.13±0.82	23.55±0.44	40.09±2.65	32.68±0.46
	Sprouts	32.63±0.10	34.788±0.4	48.42±0.30	47.49±0.36
Total phenols (mg/100g)	Dry seed	25.03±3.00	19.23±0.35	44.53±0.70	42.6±3.29
	Sprouts	40.96±1.23	22.46±0.35	52.90±0.80	57.33±6.67
Flavonoids (mg/100g)	Dry seed	100.00±8.60	96.66±2.75	101.66±2.89	78.33±2.83
	Sprouts	176.6±2.80	126.66±2.65	205.00±5.06	113.33±2.80

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