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Sprouting Exert Significant Influence on the Antioxidant Activity in Selected Pulses (Black Gram, Cowpea, Desi Chickpea and Yellow Mustard)

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ABSTRACT

Pulses are a vital source of plant-based proteins and amino acids for people around the globe and should be eaten as part of a healthy diet to address obesity, as well as to prevent and help manage chronic diseases such as diabetes, coronary conditions and cancer; they are also an important source of plant-based protein for animals. Pulses provide protein and fibre, as well as a significant source of vitamins and minerals, such as iron, zinc, folate, and magnesium, and consuming half a cup of beans or peas per day can enhance diet quality by increasing intakes of these nutrients. The present attempt deals with comparison antioxidant activities of various sprouts of selected pulses. The 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 selected 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 (49.837 ± 0.61 %) and flavonoid content (211.06 ± 8.17 mg/100g) was observed in cowpea sprouts. The highest total phenol content was noted in yellow mustard sprouts (58.45 ±6.67 mg/100g). Inclusion of pulses in the diet is a healthy way to meet dietary recommendations and is associated with reduced risk of several chronic diseases including cancer.

Long-term randomized controlled trials are needed to demonstrate the direct effects of pulses on the cancer like diseases.

***Keywords*:** Ascorbic acid; β-carotene; DPPH; flavonoid; sprouts; total antioxidant activity

1. INTRODUCTION

Pulses are annual leguminous crops yielding between one and 12 grains or seeds of variable size, shape and colour within a pod, used for both food and feed. The term "pulses" is limited to crops harvested solely for dry grain, thereby excluding crops harvested green for food, which are classified as vegetable crops, as well as those crops used mainly for oil extraction and leguminous crops that are used exclusively for sowing purposes (based on the definition of "pulses and derived products" of the Food and Agriculture Organization of the United Nations). Pulse crops such as lentils, beans, peas and chickpeas are a critical part of the general food basket. Pulses are a vital source of plant-based proteins and amino acids for people around the globe and should be eaten as part of a healthy diet to address obesity, as well as to prevent and help manage chronic diseases such as diabetes, coronary conditions and cancer; they are also an important source of plant-based protein for animals. In addition, pulses are leguminous plants that have nitrogen-fixing properties which can contribute to increasing soil fertility and have a positive impact on the environment.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or vitamin C, vitamin A and vitamin E obtained by ingestion. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer or coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large trials including beta-carotene, vitamin A, and vitamin E singly or in different combinations indicated that supplementation has no effect on mortality or might increase it. Randomized clinical trials of taking antioxidants including beta carotene, vitamin E, vitamin C and selenium have shown no effect on cancer risk or have increased cancer risk. Supplementation with selenium or vitamin E does not reduce the risk of cardiovascular disease. By these examples, oxidative stress can be considered as either a cause or consequence of some diseases, stimulating drug development for potential antioxidant compounds to treat diseases. Antioxidants have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

Pulses are a great tasting addition to any diet. They are rich in fibre and protein, and have high levels of minerals such as iron, zinc, and phosphorous as well as folate and other B-vitamins. In addition to their nutritional profile and links to improved health, pulses are unique foods in their ability to reduce the environmental footprint of our grocery carts. Put it all together and these sensational seeds are a powerful food ingredient that can be used to deliver the results of healthy people and a healthy planet. Pulses come in a variety of shapes, sizes and colours and can be consumed in many forms including whole or split, ground in to flours or separated into fractions such as protein, fibre and starch. Pulses do not include fresh beans or peas. Although they are related to pulses because they are also edible seeds of podded plants, soybeans and peanuts differ because they have a much higher fat content, whereas pulses contain virtually no fat (Amal, *et al*, 2008; Agate and Tarwadi, 2010).

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 (Carlo, *et al*, 2000; Bibi, *et al*, 2008 and Sakdeo and Khyade, 2013). 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. 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 (Mangels, *et al*, 1993; Nishino, 1998; Mao, *et al*, 2005 and Jian, 2007).

Black gram, *Vigna mungo*, is a bean grown in the Indian subcontinent. At one time it was considered to belong to the same species as the mung bean. The product sold as black lentils is usually the whole urad bean, whereas the split bean (the interior being white) is called Minumulu in Telugu, Urad Dalin Hindi, or white lentils. Black gram originated in India, where it has been in cultivation from ancient times and is one of the most highly prized pulses of India and Pakistan. The coastal Andhra region in Andhra Pradesh is famous for black gram after paddy. The Guntur District ranks first in Andhra Pradesh for the production of black gram. Black gram has also been introduced to other tropical areas mainly by Indian immigrants. It is an erect, sub-erect or trailing, densely hairy, annual herb. The tap root produces a branched root system with smooth, rounded nodules. The pods are narrow, cylindrical and up to six cm long. The plant grows 30-100 cm with large hairy leaves and 4-6 cm seed pods. While the urad bean was, along with the mung bean, originally placed in *Phaseolus*, it has since been transferred to *Vigna*. Black gram is very nutritious as it contains high levels of protein (25 g/100 g), potassium (983 mg/100 g), calcium (138 mg/100 g), iron (7.57 mg/100 g), niacin (1.447 mg/100 g), Thiamine (0.273 mg/100 g), and riboflavin (0.254 mg/100 g). Black gram complements the essential amino acids provided in most cereals and plays an important role in the diets of the people of Nepal and India. Black gram has been shown to be useful in mitigating elevated cholesterol levels.

Chickpea, *Cicer arietinum* is a nutrient-dense food, providing rich content (> 20% of the Daily Value, DV) of protein, dietary fibre, folate, and certain dietary minerals such as iron and phosphorus. Thiamin, vitamin B_6, magnesium, and zinc contents are moderate, providing

10-16% of the DV. Chickpeas have a protein digestibility corrected amino acid score of about 0.76, which is higher than many other legumes and cereals. Compared to reference levels established by the United Nations Food and Agricultural Organization and World Health Organization, proteins in cooked and germinated chickpeas are rich in essential amino acids such as lysine, isoleucine, tryptophan, and total aromatic amino acids. A 100 g serving of cooked chickpeas provides 164 kilocalories (690 kJ). Carbohydrates make up 68% of calories, most of which (84%) is starch, followed by total sugars and dietary fibre. Lipid content is 3%, 75% of which is unsaturated fatty acids for which linoleic acid comprises 43% of total fat. Malnutrition and insufficient micronutrient supply have been reported in many regions where chickpeas are a major part of the diet. However, this nutritional lack is not due to the consumption of chickpeas but due to the overall inadequate food supply for people. In some parts of the world, young chickpea leaves are consumed as cooked green vegetables. Especially in malnourished populations, it can supplement important dietary nutrients. Chickpea leaves have a significantly higher mineral content than cabbage and spinach. In natural settings, environmental factors and nutrient availability could influence mineral concentrations. Nevertheless, consumption of chickpea leaves is recommended for areas where chickpeas are produced as food for humans. Preliminary research shows that chickpea consumption may lower blood cholesterol.

The cowpea, *Vigna unguiculata* is one of several species of the widely cultivated genus *Vigna*. Four subspecies are recognised, of which three are cultivated (more exist, including *V. textilis*, *V. pubescens*, and *V. sinensis*). Cowpeas are one of the most important food legume crops in the semiarid tropics covering Asia, Africa, southern Europe, and Central and South America. A drought-tolerant and warm-weather crop, cowpeas are well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the useful ability to fix atmospheric nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus. In addition, it is shade tolerant, so is compatible as an intercropwith maize, millet, sorghum, sugarcane, and cotton. This makes cowpeas an important component of traditional intercropping systems, especially in the complex and elegant subsistence farming systems of the dry savannas in sub-Saharan Africa. In these systems the haulm (dried stalks) of cowpea is a valuable by-product, used as animal feed. Research in Ghana found that selecting early generations of cowpea crops to increase yield is not an effective strategy. Francis Padi from the Savannah Agricultural Research Institute in Tamale, Ghana, writing in *Crop Science*, suggests other methods such as bulk breeding are more efficient in developing high-yield varieties. According to the USDA food database, the leaves of the cowpea plant have the highest percentage of calories from protein among vegetarian foods. The Cowpeas provide a rich source of proteins and calories, as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors. This diet complements the mainly cereal diet in countries that grow cowpeas as a major food crop. The amount of protein content of cowpea's leafy parts consumed annually in Africa and Asia is equivalent to 5 million tonnes of dry cowpea seeds, representing as much as 30% of the total food legume production in the lowland tropics.

Yellow mustard, *Brassica alba* is cultivated for its seeds, mustard, as fodder crop or as a green manure, it is now widespread worldwide, although it probably originated in the Mediterranean region. The whole, ground, cracked, or bruised mustard seeds are mixed with water, salt, lemon juice, or other liquids, and sometimes other flavorings and spices, to create a paste or sauce ranging in color from bright yellow to dark brown. The tastes range from sweet to spicy. Commonly paired with meats and cheeses, mustard is a popular addition to sandwiches, salads, steaks, tofu, hamburgers, corn dogs, and hot dogs. It is also used as an ingredient in many dressings, glazes, sauces, soups, and marinades; as a cream or a seed, mustard is used as a condiment and in the cuisine of India and Bangladesh, the Mediterranean, northern and southeastern Europe, Asia, the Americas, and Africa, making it one of the most popular and widely used spices and condiments in the world. Mustard is most often used at the table as a condiment on cold meats. It is also used as an ingredient in mayonnaise, vinaigrette, marinades, and barbecue sauce. Mustard is also a popular accompaniment to hot dogs, pretzels, and bratwurst. In the Netherlands and northern Belgium it is commonly used to make mustard soup; which includes mustard, cream, parsley, garlic and pieces of salted bacon. Mustard as an emulsifier can stabilize a mixture of two or more immiscible liquids, such as oil and water. Added to Hollandaise sauce, mustard can inhibit curdling. In the eastern state of India, namely West Bengal, mustard oil is extensively used in cooking, and also as household remedies. Dry mustard is used in food preparation, and can be mixed with water to use as a condiment. In its dry form, powdered mustard lacks potency; the addition of water releases the pungent compounds. The pungency of mustard is always reduced by heating; if added to a dish during cooking, it gives less pungency than if added afterwards. The amounts of various nutrients in mustard seed are to be found in the USDA National Nutrient Database. As a condiment, mustard averages approximately 5 calories per teaspoon. Some of the many vitamins and nutrients found in mustard seeds are selenium and omega 3 fatty acid. For a food that is high in carbohydrates, pulses have a low glycemic index which means they do not cause a fast rise in blood sugar after eating. Studies have shown that eating pulses is a good way to manage blood sugar levels which is particularly important for people with diabetes.

Pulses are available as whole seeds, and can also be turned into ingredients like flours, fibre, proteins and starches. Their versatility gives endless options to add more pulses to the diet and to meet the recommended weekly amounts of several important nutrients. Many health organizations recommend eating pulses to maintain good health and prevent chronic diseases like diabetes, heart disease and cancer. Eating Well with Canada's Food Guide states "Have meat alternatives such as beans, lentils and tofu often" and suggests that regularly choosing beans and other meat alternatives such as lentils can help minimize the amount of saturated fat in the diet. The 2010 Dietary Guidelines for Americans says that "beans and peas are unique foods. Bean and peas are the mature forms of legumes. They include kidney beans, pinto beans, black beans, garbanzo beans, lima beans, black-eyes peas, split peas, and lentils. Beans and peas are excellent sources of protein. They also provide other nutrients, such as iron and zinc, similar to seafood, meat, and poultry. They are excellent sources of dietary fiber and nutrients such as potassium and folate, which also are found in other vegetables. Because of their high nutrient content, beans and peas may be considered both as a vegetable and as a protein food." Pulses are also listed in "foods and nutrients to increase "Choose a variety of protein foods, which include seafood, lean meat and poultry, eggs, beans and peas, soy products, and unsalted nuts and seeds.

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 (Agate and Tarwadi, 2010).Health benefits of carotenoids that may be related to their anti-oxidative potential include enhancement of immune system function, protection from sunburn (Amal, *et al*, 2008) and inhibition of the development of certain types of cancers Mao, *et al* , 2005). 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 (Braca, *et al*, 2002). 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 (Osborne and Boogt, 1978; Oke and Hamburger, 2002). Flavonoids constitute an unavoidable component of the diet. Of the many actions of flavonoids, antioxidant and anti-proliferative effects stand out (Singleton and Rossi, 1965). 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 the present attempt 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.

2. MATERIALS AND METHODS

The 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 procured from the University of Agricultural Sciences (UAS), Bangalore, India through the local dealer. The oilseed yellow mustard (*Brassica alba*) was procured from Namdhari Seeds, Bangalore, India through the local dealer. As a first step, healthy seeds of similar size and shape were selected. The seeds were not chemically treated in any way as this would slow down the germination rate. For the Sprouting process, 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.

The β-carotene content was estimated using the method described by Mangels, et al (1993). β-carotene was Soxhlet extracted through the use of 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.

The Ascorbic acid content was estimated using the method described by Osborne and Boggt (1978). 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, *et al* (2008). Stock solution of ascorbic acid in distilled water (1000 μg/ml) was diluted suitably in order to obtain different concentrations ranging from 10-100 μg. 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: % Inhibition of DPPH by the sample = Ac-As x 100 Ac where, Ac is the Optical Density (O.D.) of the control, As is the Optical Density (O.D.) of the sample.

The total phenol content of the samples was estimated by the method described by Singleton and Rossi (1965). 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.

The flavonoid content of the samples were estimated using the method described by Yang, *et al* (2001). 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. The experimentations were repeated for three times for consistency in the results. The data obtained was subjected for statistical analysis (Norman and Baily, 1995).

3. RESULTS AND DISCUSSION

Carotenoids are a widespread group of naturally occurring fat-soluble colorants. B-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/100 g) 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/100 g was noted. A moderate increase of 13.66 μg/100 g and 6.41 μg/100 g 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 (Tapas, *et al*, 2008). 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/100 g when compared to its dry seed. The ascorbic acid content of cowpea sprouts also showed a notable increase of 11.25 mg/100 g 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 Khyade, *et al*, 2013). And hence the ascorbic acid content of legumes increases manifold on sprouting. Mao, *et al* (2005) observed that increase in the ascorbic acid level is a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination.

Table 1. Effect of sprouting on the contents antioxidant activity in selected seeds of pulses (*Vigna mungo*, *Vigna unguiculata*, *Cicer arietinum* and *Brassica alba*).

Parameter	Time of germination (48 h)	Black Gram	Chickpea	Cowpea	Yellow mustard
β- carotene (µg/100 g)	Dry seed	42.616±0.78	159.88±5.82	14.071±6.58	176.82±5.20
	Sprouts	57.051±3.46	221.05±5.60	21.05±1.83	206.11±7.51
Ascorbic acid (mg/100g)	Dry seed	5.45±0.21	6.812±0.88	8.175±0.72	8.912±0.93
	Sprouts	31.413±0.72	12.263±0.89	20.437±0.13	16.359±0.87
Total antioxidants (% DPPH inhibition/ 100 g)	Dry seed	26.214±0.88	25.669±0.48	40.09±3.63	35.683±0.69
	Sprouts	36.638±0.10	37.918±0.4	49.837±0.34	51.764±0.53
Total phenols (mg/100 g)	Dry seed	27.283±3.48	21.961±0.38	48.537±0.76	51.634±4.33
	Sprouts	40.96±1.23	22.46±0.35	52.90±0.80	58.450±6.67
Flavonoids (mg/100 g)	Dry seed	109.89±8.60	97.76±3.13	102.63±2.89	79.36±2.88
	Sprouts	178.6±2.83	129.67±2.63	211.06±8.17	113.33±2.80

- Each value is the mean of three replications.
- Figures in parenthesis with ± signs are the standard deviations.

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 (Khyade and Lonkar, 2013). 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 (Khyade, et al,2014; Khyade and Mote, 2014). 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/100 g as compared to the seeds that contained 42.60 mg phenol/100 g. 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/100 g (seeds) to 40.96 mg/100 g (sprouts). Cowpea seeds on sprouting showed a moderate increase of 8.37 % from 44.53 mg/100 g to 52.90 mg/100 g 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/100 g) followed by black gram sprouts (76.6 mg/100 g). A moderate increase of 34.7 mg/100 g 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/100 g to 126.66 mg/100 g. The secondary metabolites of plants provide humans with numerous biologically active products (Khyade, et al, 2015; Khyade and Deshpande, 2015). These plant secondary metabolites which include several classes such as terpenoids, flavonoids, and alkaloids, have diverse chemical structures and biological activities Khyade and Sancer, 2016). Therefore, these natural compounds as dietary components have considerable impact on human health 6.

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.

Germination of seeds lead to breakdown of seed reserves (Vanderstoep, 1981) and increased enzyme activity that leads to a loss of totaldry matter and an increase in total protein

(Lorenz, 1980). Mubarak (2005) has reported that during germination the carbohydrate content of mung bean seeds showed a significant decrease and also observed that the decrease could be due to their utilisation as an energy source to start germination. Inyang and Zakari (2008) and Lasekan (1996) had observed that the decrease in the carbohydrate content might be due to the increase in α-amylase activity. The α-amylase breaks down complex carbohydrates to simpler sugars which were utilized by the growing seedlings in the initial stages of germination (Onwuka *et al*., 2009).

Nidaye *et al*. (2008) observed a decrease in the starch content of germinated millet flour. The present study showed a decrease in the carbohydrate content during germination and also well in agreement with Coulibaly and Chen (2011) for germination of foxtail millet. Protein content and ascorbic acid level in germinated seeds were found to be increased and the observation was well in agreement with the studies conducted with germinated seeds. Gernah *et al*. (2011) observed that increase in the protein content of maize during germination could be as a result of mobilization of storage nitrogen to produce the nutritionally high quality proteins needed by the young plant for its development. Taraseviciene *et al*. (2009) has reported the increase in amino acid content in germinated broccoli seeds corresponding with crude protein content increase. Rodriguez *et al*. (2008) reported that seed germination involves mobilization of the protein reserves in cotyledons, coupled with the synthesis of new proteins necessary for sprouts growth. It was observed that water soluble vitamins such as B Complex and vitamin C are synthesized during germination (Bibi *et al*., 2008).

Mao *et al*. (2005) observed that increases in the ascorbic acid level were considered to be a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination. This may be the reason for the increase in the level of ascorbic acid in all three germinated seed varieties. Sangronis and Machado (2007) reported that germination modify the presence of nutrients and antinutrients in legume seeds and increased protein digestibility and ascorbic acid content. Nutritional quality of protein and ascorbic acid level was increased in germinated chick peas (Fernandez and Berry, 1988; Elemo *et al*., 2011). Elevated level of vitamins was observed in germinated soya bean seeds compared to dry seeds (Bau *et al*., 1997).

The caloric content of germinated seeds was decreasing due to the utilization of carbohydrates and the energy might have been utilized for enhancing vitamins and other nutrients. Level of fat and ash content of germinated seeds were not altered. This might be due to the utilization of carbohydrate for initial energy expenditure.

Efficient use of pulses through diet may open a new pharmaceutical avenue for human being. The 68th UN General Assembly declared 2016 the International Year of Pulses (IYP) (A/RES/68/231). The Food and Agriculture Organization of the United Nations (FAO) has been nominated to facilitate the implementation of the Year in collaboration with Governments, relevant organizations, non-governmental organizations and all other relevant stakeholders.

The IYP 2016 aims to heighten public awareness of the nutritional benefits of pulses as part of sustainable food production aimed towards food security and nutrition. The Year will create a unique opportunity to encourage connections throughout the food chain that would better utilize pulse-based proteins, further global production of pulses, better utilize crop rotations and address the challenges in the trade of pulses.

4. CONCLUSIONS

The sprouted legumes, such as black gram- (*Vigna mungo* var Rashmi), Desi Chickpea (*Cicer arietinum* var JG-11) and Cowpea (*Vigna unguiculata* var PK-B6), apart from being a good source of protein also contain useful amounts of beta carotene, ascorbic acid, total phenols and flavonoids. They have had significant antioxidant activity. Pulse sprouts contain an abundance of highly active anti-oxidants that prevent cell destruction and protect us from the ongoing effects of aging. The parameters selected 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 (49.837 ±0.61 %) and flavonoid content (211.06 ±8.17 mg/100 g) was observed in cowpea sprouts. The highest total phenol content was noted in yellow mustard sprouts (58.45 ±6.67 mg/100 g). Inclusion of pulses in the diet is a healthy way to meet dietary recommendations and is associated with reduced risk of several chronic diseases including cancer. Long-term randomized controlled trials are needed to demonstrate the direct effects of pulses on the cancer like diseases. Sprouted pulses may be mixed with other foods and dressings such as lemon juice and rock salt. This live food rejuvenates body cells and tissues and provides energy. It also retards the aging process. They are, on the other hand, excellent, soft food. They contain every known vitamin necessary for the human body in perfect balance.

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