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Biochemical Studies on the Germinated Seeds of *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper and *Pennisetum typhoides* (Burm f.) Stapf and C.E. Hubb

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ABSTRACT

The aim of the experiment was to study the effect of germination on the seeds of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides*. Seeds were germinated for 24, 48 and 72 h. After germination the seeds were dried at 40°C and used for the estimation of carbohydrate, protein, fat, ash and ascorbic acid content. Energy value was calculated during the period of germination. Carbohydrate content of the germinated seeds was significantly ($p<0.001$) decreased with the increase in the days of germination. Protein ($p<0.01$, $p<0.001$) and ascorbic acid ($p<0.001$) content of seeds were significantly increased during the period of germination compared with the dry seeds. Fat and ash content of germinated seeds were not altered. Germinating seeds showed a decrease in energy value of all three seeds compared with the dry seeds. This study confirmed the corresponding increase in the nutritive quality of the germinated seeds compared to the dry seeds.

Key words: Carbohydrate, protein, ascorbic acid, fat, germination

INTRODUCTION

Germination improves the nutritive value of cereals and legumes and has been found to decrease the levels of anti nutrients present in cereal and maximizes the levels of utilizable nutrients (Mohamed *et al*., 2007; Inyang and Zakari, 2008). It is suggested as a technological procedure for improving the nutritional quality of legumes and other seeds (Gulewicz *et al*., 2008) and variably affects the proximate composition of seeds (Onwuka *et al*., 2009). During germination metabolic enzymes are activated and utilization or synthesis of wide range of chemical compounds occurs in seeds and results in the enhancement of nutritional quality (Taraseviciene *et al*., 2009). Germinated seeds are rich in vitamins, minerals and are reported to contain important phytochemicals for disease prevention (Fernandez-Orozco *et al*., 2006). An increase in the bioavailability of minerals and vitamins has been observed due to germination (Sulieman *et al*., 2007). Germination is a simple biochemical enrichment tool to enhance the palatability result in increasing the digestibility and nutritive value (Ramakrishna *et al*., 2006). Germination of seeds is a effective practice used in culinary preparations for centuries and *Vigna radiata* L. (green gram), *Vigna mungo* L. (black gram) and *Pennisetum typhoides* (spiked millet or pearl millet) are the common food materials in Southern parts of India (Nene, 2006). *Vigna radiata* and

Vigna mungo belongs to the family Fabaceae (Suriachandraselvan *et al.*, 2005) are called as 'pacchai payaru'/Pasi payir' and 'ulundhu' in Tamil language and are the important pulse crops. They are the rich sources of vegetable protein. Green gram is free of flatulence causing agents. It is consumed in the form of split pulse as well as whole pulse which is an essential supplement of cereal based diet (Puranik *et al.*, 2011). Germination results an appreciable reduction in the factors responsible for flatulence, thus increasing the intake and improving the utilisation of available proteins and carbohydrates (Urbano *et al.*, 2005). It is an acceptable and recommended food for convalescents and feeding babies (Adsule *et al.*, 1986). *Pennisetum typhoides* belongs to Poaceae family called as 'Kambu' in Tamil language. It is used as a cereal food like rice or pounded into a flour or paste to make bread. It has become a staple food for people living in dry and rural regions of India (Nithya *et al.*, 2006).It is one of the most nutritious, high in starch and easily digestible (www.innvista.com). Even though, these seeds were used for centuries, less information is available regarding the biochemical changes occurring during germination. Hence, this experiment was conducted to observe the nutritive quality of seeds during germination.

MATERIALS AND METHODS

Dry seeds of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides* were purchased in Chennai, Tamilnadu, India and the study was conducted during the year 2008. Seeds were handpicked to remove imperfect seeds like broken and empty seeds and washed with running tap water and also for disinfecting with 70% ethanol solution. Then they were washed thoroughly and soaked in distilled water for 4 h and transferred into Petri plate containing moist filter paper and about 5 mL of distilled water was added in it. Then the seeds were kept for germination under dark condition for 24, 48 and 72 h and used for the experiment.

After germination the germinated seeds were dried in air oven at 40°C and milled and analysed. Carbohydrates were estimated by hydrolysing 100 mg of the sample with 5.0 mL of 2.5 N hydrochloric acid and neutralized with solid sodium carbonate until effervescence ceased. The volume was made up to 100 mL and centrifuged supernatant was used for the estimation by anthrone method (Yemm and Willis, 1954). Protein content (Lowry *et al.*, 1951), Fat (Folch *et al.*, 1957), Ash content (AOAC, 1995) and Ascorbic acid (Sadasivam and Manickam, 2009) were estimated. The energy values were calculated using the Atwater factors (Onwuka *et al.*, 2009) of 4, 9 and 4 for protein, fat and carbohydrates respectively.

Statistical analysis: The data were presented as Mean±SEM for three samples in each group and tested statistically using the student's t-test to compare the means of two groups. Differences at $p<0.05$, 0.01,0.001 were considered to be significant.

RESULTS

Carbohydrate, protein, fat, ash, ascorbic acid and energy value of dry and 24, 48 and 72 h germinated seeds of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides* were presented in Table 1. The carbohydrate content was found to be significantly decreased correspondingly with the increase in the germination time in comparison with the dry seed. This was observed in all the three tested seeds. The level of significance was $p<0.001$ at 72 h when compared with the dry seeds. However the level of carbohydrate was found to be higher in *Pennisetum typhoides* (17.4±0.9 g/100 g) and lower in *Vigna radiata* (14.4±0.4 g/100 g) at 72 h of germination. Protein content of the three seeds was found to be significantly increased with the increase in the time of

Table 1: Carbohydrate, protein, fat, ash, ascorbic acid and energy value of dry and germinated seeds of *Vigna radiata*, *Vigna mungo* and *Pennisetum typhoides*

Experiment	Time of germination	Vigna radiata	Vigna mungo	*Pennisetum typhoides*
Carbohydrate (g/100 g)	Dry seed	35.90±2.0	33.70±3.0	44.80±4.0
	24 h	27.20±1.2^{b}	25.60±1.4^{b}	32.20±3.2^{b}
	48 h	19.20±1.0^{c}	21.10±2.0^{c}	25.10±2.0^{c}
	72 h	14.40±0.4^{c}	15.30±0.9^{c}	17.40±0.9^{c}
Protein (g/100 g)	Dry seed	15.00±1.2	17.80±1.1	13.00±1.5
	24 h	19.00±2.0^{a}	22.00±2.0^{a}	16.00±1.6^{a}
	48 h	21.00±1.8^{b}	24.00±1.9^{b}	18.00±1.2^{b}
	72 h	21.70±1.7^{b}	25.30±1.0^{c}	18.90±1.3^{b}
Fat (g/100 g)	Dry seed	0.60±0.02	0.52±0.03	0.54±0.05
	24 h	0.54±0.02	0.50±0.02	0.50±0.01
	48 h	0.50±0.01	0.47±0.01	0.47±0.03
	72 h	0.50±0.01	0.46±0.03	0.45±0.04
Ash (g/100 g)	Dry seed	1.50±0.6	1.30±0.30	1.60±0.9
	24 h	1.60±0.6	1.32±0.40	1.68±0.7
	48 h	1.72±0.8	1.41±0.70	1.70±0.7
	72 h	1.74±1.0	1.40±0.90	1.70±1.0
Ascorbic acid (mg/100 g)	Dry seed	8.80±1.0	5.90±0.50	4.30±0.3
	24 h	11.80±1.2^{a}	11.70±1.12^{c}	8.70±0.2^{c}
	48 h	7.60±1.3^{c}	14.80±1.1^{c}	11.80±0.9^{c}
	72 h	20.60±1.8^{c}	21.00±2.0^{c}	14.70±0.82^{c}
Energy k cal/100 g	Dry seed	209.00±18	211.00±19	236.00±18
	24 h	190.00±17^{c}	195.00±16	197.00±16^{c}
	48 h	165.30±15^{c}	185.00±17	177.00±17^{c}
	72 h	148.30±12^{c}	167.00±12	149.00±12^{c}

p-values are expressed as Mean±SEM for 3 replicates: ^{a} p<0.05, ^{b} p<0.01, ^{c} p<0.001, dry seed vs. 24 h, dry seed vs. 48 h, dry seed vs. 72 h

germination when compared with the dry seed. The level of significance for *Vigna radiata* and *Pennisetum typhoides* at 72h of germination was found to be p<0.01 whereas the level of significance for *Vigna mungo* was p<0.001. Protein content was found to be higher in *Vigna mungo* seeds (25.3±1.0 g/100 g) compared to other seeds. Fat and ash content of the germinated seed varieties did not show much variation when compared with their corresponding dry seed.

Ascorbic acid content of all the germinated seeds showed significant increase with the increase in the time of germination when compared to the dry seed. The level of ascorbic acid was found to be highly significant (p<0.001) at 72 h of germination. Energy value was found gradually decreasing with the increase in the time of germination in all three seeds. Energy value was found to be significantly (p<0.001) low in germinated seeds compared to other seeds.

DISCUSSION

Germination of seeds lead to breakdown of seed reserves (Vanderstoep, 1981)and increased enzyme activity that leads to a loss of total dry 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. Tarasevicience *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.

CONCLUSION

Germination causes alterations in the chemical composition of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides*. Carbohydrate content and energy value was decreased and protein and ascorbic acid content was increased during the process when compared to dry seeds. Germination did not alter the fat and ash content.

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