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IMPACTS OF VARYING GERMINATION PERIODS ON THE NUTRITIONAL QUALITY AND PROTEIN AVAILABILITY OF FOUR LEGUMES

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Abstract

To improve the quality and digestibility of legumes, and to examine effects of varying germination periods (6, 12 and 18 hrs) on the protein quantity, digestibility, ash, moisture, fibre and fat content of locally available legumes namely green gram, chickpea, lentil and moth bean the present study was undertaken. It was found that the protein content and protein digestibility of legumes increased by 4.87 to 42.79 and 3.48 to 19.77 per cent, respectively depending on the type of legume, and length of germination. The highest per cent increase in protein content was found in chickpea (42.79%) germinated for 18 hrs. Maximum increase in *in-vitro* protein digestibility (19.77 %) was noticed in moth bean, ranging from 5.31 to 19.77 per cent at 6, 12 and 18 hrs of germination. Highest enhancement in protein value was observed in legumes which were germinated for 18 hrs. In comparison to raw, a significant reduction was noted in the ash content of all types of germinated legumes and in terms of per cent, the decrease varied from 2.98 to 8.46. Moisture and crude fibre content increased significantly where fat content was decreased in all legumes. Processed legumes can serve as a functional food for augmenting the nutritional status of vulnerable sections of society.

Introduction

It is an established fact that plant proteins are very valuable and their consumption compared to red meat proteins, confers a number of health promoting effects by controlling inflammatory processes in the body. This nutrient density of legumes not only allows them to be used as meat replacers, but also aid in nourishment. The functional properties of legumes are accounted to for their being rich source of amino acids, complex carbohydrates, slowly digested starch, low glycemic index, dietary fiber both soluble and insoluble, phenolic compounds, etc. (Jeong *et al.* 2019). It is quiet difficult to properly digest and utilize the legumes' protein owing to presence of different types of anti-nutritional factors which interfere with protein hydrolysis (Parmar *et al.* 2017). The optimum utilization of legume nutrition can be achieved through different types of processing techniques i.e. soaking, cooking, heating, roasting, blanching, germination etc., as these techniques not only improve the nutritional quality but also enhance the shelf life, palatability, mouth feel, taste, add variety to plate and influence the functional properties of grains (Drulyte and Orlien 2019).

Germination subsumes multicomplex transformations in the grain constitution, and the modifications in grain structure commence at the cost of energy and nutrients, provided by grain for growth of sprouts and synthesis of new proteins (Mamilla and Mishra 2017). These changes are reminiscent of activation of enzymes, increase in water soluble vitamins, diminished tannins, phytates, etc. during germination. These transformations in legume composition significantly affect the digestibility and availability of proteins and carbohydrates. Now a days the nutritionists' interest is focused more on improving immunity through intake of functional foods including processed legumes which could be one of the best suited foods for people of different age groups

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and economic status. Different legumes exhibit different nutritional quality and availability, depending on their inherent characteristics and structures, inter nutrient interactions also tend to behave differently when subjected to processing (Ohanenye *et al*. 2020, Sofi *et al.* 2020). Much works were carried out related to the effect of germination on nutritional quality of legumes but the impact of germination time on crude protein and *in-vitro* protein digestibility of legumes has not been discussed in detail in any previous studies. Thus the present study was carried out on the impact of varying germination periods on crude protein content and changes in the protein digestibility of different types of locally available legumes.

Materials and Methods

The commonly consumed varieties of whole legumes namely green gram (*Vigna radiata*, var. MH-421), chickpea (*Cicer arietinum* var. HC-1) and lentil (*Lens culinaris*, var. *Sapna*) were procured in a single lot from the Pulses Section, Department of Genetics and Plant Breeding, College of Agriculture, CCSHAU, Hisar. Moth bean (*Vigna aconitifolia*) was purchased from a local market in Hisar (Haryana). Legumes were cleaned, made free of dust, dirt, foreign materials and washed 3-4 times under running water. Grains were soaked overnight (12 hrs) in distilled water at room temperature and germinated (37°C in orbital incubator for 6, 12 and 18 hrs). After germination all grains were put in hot air oven (65°C) and were ground in an electric grinder to make fine powder and kept in airtight plastic containers for further analysis.

The crude protein, ash, moisture, fat and crude fibre in raw and germinated legume samples were determined by AOAC official methods (AOAC 2010). The conversion factor of N × 6.25 was used to estimate the crude protein. *In-vitro* protein digestibility of raw and germinated legume samples were determined by using modified method of Mertz *et al.* (1983). Data obtained were administered to analysis of variance (ANOVA) using completely randomized design (CRD) and the means with critical differences were calculated.

Results and Discussion

Compared to raw, the crude protein content of green gram increased by 6.5, 14.68 and 23.23 per cent at 6, 12 and 18 hrs of germination (Table 1). Similarly the crude protein content of moth bean (20.72, 22.46, 24.31 and 26.71 g/100 g), chickpea (17.43, 20.94, 22.65 and 24.89 g/100 g) and lentil (21.93, 23.00, 24.89 and 26.33 g/100 g) also amplified on germination. It could be seen that as the period of germination of legumes increased from 6 to 12 and 18 hrs, the protein content also increased significantly and this could be attributed to synthesis of new proteins during sprouting (Khare *et al.* 2021), or some structural changes in cells (Shah *et al.* 2011) or due to consumption of polysaccharides and diminution of grain mass during germination and respiration (Uppal and Bains 2012). When compared to raw, the highest per cent increase in protein content was found in chickpea (42.79%), germinated for 18 hrs followed by that of moth bean (28.90%), green gram (23.23%) and lentil (20.06 %). The differences in the performance of different legumes during germination is explained to the differences in their nature, varieties, three-dimensional structures, nutrient available for hydrolysis to support germination, nutritional worth, grain vigor, region of production etc. (Yu-Wei and Wang 2015, Skylas *et al.* 2018, Dipnaik and Bathere 2017). The increment in protein content was also assumed to loss in dry weight, particularly carbohydrates, through respiration to support germination (Uppal and Bains 2012, Mahmoud and El-Anany 2014).

The protein digestibility of different types of unprocessed legumes was found to range from 60.82 to 63.27 per cent which increased to 62.94 to 74.19 per cent after germination (Table 2). The maximum per cent increase in *in vitro* protein digestibility was noticed in moth bean, ranging from

5.31 to 19.77 per cent at 6, 12 and 18 hrs of germination. As the period of germination enhanced the protein digestibility also increased significantly and this may be explained to exposure of legumes to metabolic and bioprocesses (Ohanenye *et al*. 2020) for longer times and enhanced inter-nutrient interactions, hydrolytic processes, solubilisation of some of the polysaccharides due to action of amylases and release of proteins, softening of grain structure, partial or complete removal of polyphenols, tannins, phytic acid and trypsin inhibitors, peptide breakdown etc. (Ma *et al*. 2017, Thakur *et al*. 2021).

Table 1. Impact of varying germination periods (6, 12 and 18 hrs) on the crude protein content of legumes (g/100 g, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	21.52±0.64	20.72±0.02	17.43±0.03	21.93±0.04
Germination (6 hrs)	22.92±0.09 (+6.50)	22.46±0.23 (+8.39)	20.94±0.05 (+20.13)	23.00±0.10 (+4.87)
Germination (12 hrs)	24.68±0.25 (+14.68)	24.31±0.16 (+17.32)	22.65±0.14 (+29.94)	24.89±0.16 (+13.49)
Germination (18 hrs)	26.52±0.21 (+23.23)	26.71±0.03 (+28.90)	24.89±0.12 (+42.79)	26.33±0.23 (+20.06)
CD (P≤0.05)	1.19	0.49	0.44	1.30

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent increase (+) over the control value.

Table 2. Impact of varying germination periods (6, 12 and 18 hrs) on the protein digestibility (*in-vitro)* of legumes (%, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	63.27±0.52	61.94±0.28	61.91±0.06	60.82±0.12
Germination (6 hrs)	66.61±0.17 (+5.27)	65.23±0.13 (+5.31)	64.17±0.10 (+3.65)	62.94±0.32 (+3.48)
Germination (12 hrs)	70.01±0.05 (+10.65)	70.73±0.16 (+14.19)	68.14±0.13 (+10.06)	68.04±0.18 (+11.87)
Germination (18 hrs)	73.96±0.37 (+16.89)	74.19±0.18 (+19.77)	70.73±0.34 (+14.24)	71.85±0.20 (+18.13)
CD (P≤0.05	1.10	0.78	0.60	1.54

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent increase (+) over the control value.

In comparison to raw, a significant reduction was noted in the ash content of all types of germinated legumes (6, 12 and 18 hrs) and in terms of per cent, the decrease varied from 2.98 to 8.46 (Table 3). In green gram and lentil it was observed that there was non-significant difference

in ash content at 6 hrs (3.58 and 2.67 g/100g, respectively) and 12 hrs (3.56 and 2.60, g/100g, respectively) of germination, but after that a significant decrease occurred. A non-significant decrease was observed in the ash content of all types of germinated moth bean and chickpea. Similar findings were reported by Thakur *et al*. (2021) and it was explained that the decrease in ash content was owing to ejection of minerals in soaking water (Duenas *et al.* 2016).

The moisture content of unprocessed legumes ranged from 7.04 to 9.28 per cent, which increased in a time-dependent manner after germination (Table 4), as germination hours increased the moisture content which increased gradually and reached the maximum level (9.47 to 11.18%) at 18 hrs of germination. These findings are similar to the results reported earlier in which the moisture content was increased significantly after germination (Bains *et al.* 2011, Kavitha and parimalavalli 2014, Fouad and Rehab 2015),

Table 3. Impact of varying germination periods (6, 12 and 18 hrs) on the ash content of legumes (g/100 g, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	3.69±0.03	3.19±0.08	3.02±0.16	2.78±0.21
Germination (6 hrs)	3.58±0.04 (-2.98)	2.96±0.06 (-7.21)	2.90±0.21 (-3.97)	2.67±0.19 (-4.96)
Germination (12 hrs)	3.56±0.06 (-3.52)	2.94±0.05 (-7.83)	2.87±0.26 (-4.96)	2.60±0.14 (-6.47)
Germination (18 hrs)	3.42±0.07 (-7.31)	2.92±0.16 (-8.46)	2.86±0.35 (-5.29)	2.58±0.12 (-7.19)
CD ($P \leq 0.05$)	0.08	0.09	0.06	0.08

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent decrease (-) over the control value.

Table 4. Impact of varying germination periods (6, 12 and 18 hrs) on moisture content of legumes (g/100 g, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	9.28±0.03	7.04±0.01	8.07±0.04	8.11±0.21
Germination (6 hrs)	9.45±0.05 (+1.83)	8.24±0.07 (+17.04)	8.60±0.18 (+6.56)	8.31±0.08 (+2.46)
Germination (12 hrs)	10.00±0.05 (+7.75)	9.30±0.16 (+32.10)	9.21±0.01 (+14.12)	9.00±0.02 (+10.97)
Germination (18 hrs)	11.18±0.12 (+20.47)	10.82±0.37 (+53.69)	9.47±0.27 (+17.34)	10.18±0.04 (+25.52)
CD ($P \leq 0.05$)	0.23	0.68	0.53	0.17

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent increase (+) over the control value.

The fat content of unprocessed legumes was between the range of 1.02 to 2.87g/100g and that of germinated legumes 6, 12 and 18 hrs ranged from 0.85 to 2.05, 0.70 to 1.82 and 0.56 to 1.54 g/100 g, respectively (Table 5). The present study indicated that the fat content of all the legumes decreased gradually with the increase in germination time. This tendency of decrease in fat content was also supported by Ghavidel and Prakash (2007), Shah *et al*. (2011), Kavitha and Parimalavalli (2014).

Crude fibre content of unprocessed legumes varied from 2.83 to 4.12 g/100 g which increased significantly ($P \leq 0.05$) by 3.75 to 10.24 per cent after germination for 6h and further increased by 15.77 to 20.49 per cent and 19.66 to 37.45 per cent as germination period increased to 12 and 18 hrs, respectively (Table 6). These results are in agreement with those reported earlier (Barakoti and Bains 2007, Uppal and Bains 2012 and Fouad and Rehab 2015)

Table 5. Impact of varying germination periods (6, 12 and 18 hrs) on fat content of legumes (g/100 g, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	1.57±0.35	1.35±0.12	2.87±0.05	1.02±0.21
Germination (6 hrs)	1.28±0.32 (-18.47)	1.03±0.06 (-23.70)	2.05±0.03 (-28.57)	0.85±0.10 (-16.66)
Germination (12 hrs)	1.16±0.08 (-26.11)	0.88±0.30 (-34.81)	1.82±0.06 (-36.58)	0.70±0.30 (-31.37)
Germination (18 hrs)	1.07±0.37 (-31.84)	0.68±0.02 (-49.62)	1.54±0.32 (-46.34)	0.56±0.21 (-45.09)
CD ($P \leq 0.05$)	0.07	0.17	0.15	0.09

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent decrease (+) over the control value.

Table 6. Impact of varying germination periods (6, 12 and 18 hrs) on crude fibre content of legumes (g/100 g, dry weight basis).

Period of germination	Legumes			
	Green gram	Moth bean	Chickpea	Lentil
Unprocessed	3.99±0.02	4.12±0.10	3.81±0.05	2.83±0.03
Germination (6 hrs)	4.14±0.06 (+3.75)	4.42±0.01 (+7.28)	4.04±0.01 (+6.03	3.12±0.01 (+10.24)
Germination (12 hrs)	4.62±0.05 (+15.78)	4.77±0.05 (+15.77)	4.24±0.02 (+11.28)	3.41±0.04 (+20.49)
Germination (18 hrs)	4.79±0.01 (+20.05)	4.93±0.20 (+19.66)	4.71±0.03 (+23.62)	3.89±0.05 (+37.45)
CD ($P \leq 0.05$)	0.13	0.11	0.14	0.21

Values are mean ± SE of three independent determinations. Figures in the parentheses indicate per cent increase (+) over the control value.

The increase in fibre content may be attributed to the fact that part of seed fibre may be solubilized enzymatically during seed germination or could be due to synthesis of structural carbohydrates such as cellulose and hemicellulose, a major constituents of cell walls.

It may be concluded that germination is a sustainable process to advance the nutritional quality of legumes, and can easily be adopted at house hold level. Germination of legumes before consumption is a nutrition intervention to improve the protein quantity, digestibility and availability to body. It is advisable to increase the germination periods for better outcome of germination process as indicated in the present study. There is need to popularize and educate the masses about household germination and increase utilization of nutritious legumes for overcoming malnutrition, at no extra cost.

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