Effect of soaking, germination and dehydration variables on anti-nutritional factors of horse gram (*Macrotylomauniflorum*)

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Abstract: Horse gram (*Macrotylomauniflorum*) is legume, which has a place with family *Fabaceace*. It is a potential grain legume having excellent wholesome quality and least expensive source of protein. It is rich in calcium and iron. Most extreme use of horse gram is missing because of the presence of anti-nutritional factors like tannin and phytic acid which interferes with the bioavailability of nutrients present in horse gram. The present work was attempted to optimize the soaking, germination and dehydration conditions. Horse gram seeds were soaked for 6, 12 and 18hrs followed by germination for 24, 48 and 72hrs. The soaked and germinated samples were later dried in cabinet drier at 50, 60 and 70℃. Practically all the attributes were significantly influenced by soaking, germination and dehydration. In light of the anti-nutritional properties, it was discovered that treatment having 18hrs soaking, 72hrs germination and drying at 70℃ gave extreme decrease in the anti-nutritional factors i.e. Tannin content reduced up to 53.61% and phytic acid content reduced up to 59.26%.

***Index Terms*- Anti-nutritional factors, drying, germination, horse gram, soaking.**

I. INTRODUCTION

Horse gram has a place with family *Fabaceae* as a potential grain legume having excellent dietary and healing properties with better atmosphere versatility to adjust harsh climatic conditions. It is one of the most significant unexploited food legumes being developed in practically everywhere throughout the world (Bhartiya *et al*., 2015). Horse gram is usually known as *Kulthi* or *Madras Bean*. It is outstanding for its hardness, flexibility to poor soil and adverse climatic conditions. The horse gram is a least expensive source of protein. It is rich in calcium and iron. Most extreme use of horse gram is missing because of the components like tannin, trypsin inhibitor, phytic acid which interfere with the bioavailability of supplements present in horse gram (Haripriya *et al*., 2017). In India, horse gram is cultivated as a pulse crop contributing about 0.33% of total food grain production (Ramteke *et al*., 2016). Use of horse gram can be expanded through an understanding of its physical and chemical properties (Jain *et al*., 2012). In Indian eating routine, grains (lacking in lysine) are generally eaten with pulses (poor source of methionine) to make a complete protein diet (Patil and Kasturba, 2019). Germination assumes to play a significant job in improving nutritive quality of horse gram. Utilization of sprouted seed becomes progressively famous because of the fantastic source to decrease the danger of different diseases and applying health promoting effects (Prasad and Singh, 2015).

Anti-nutritional factors can cause dangerous effects to humans and animal growth and performance by impairing intake, uptake or utilization of other foods components or by causing discomfort and stress to humans and animals (Bora, 2014). Numerous anti-nutrients like hemagglutinin activities, trypsin inhibitor, phytic acid, and tannin present in horse gram can be diminished by soaking, dehusking, germination, cooking and roasting (Borade *et al*., 1984).Anti-nutritional factors not only reduce mineral absorption, protein digestibility, but also create "hard-to- cook" phenomenon of legumes (Moktan and ojha, 2016). Conventional processing method have been appeared to deliver gainful impacts by decreasing the anti-nutrients which results about improved acceptability and nutrition quality in additional to ideal usage of horse gram as human food (Kadam and Salunkhe, 1985). Soaking is a treatment of hydration of seeds in water for any hours to permit the seeds to absorb water**.** Thus, it reduces the anti-nutritional factor present in legumes. Germination procedure triggers the enzymatic movement of growing seeds, which further breaks the starches, proteins and fats into less complex structures (Ismail *et al*., 2003). Hence, the present investigation was intended to examine the impact of soaking and germination (at three different levels) and later drying at three drying temperatures on the anti-nutritional factors of horse gram.

II. RESEARCH METHODOLOGY

2.1 Material

Horse gram (*variety AK 42*) seeds were procured from whole sale vendor, Loni kalbhor, Pune in a bulk amount to ensure uniformity of the raw material all throughout the examination.

2.2 Methodology

Seeds were cleaned manually to remove the mud particles and some extraneous matter present alongside horse gram. Seeds were additionally washed with water and divided into three portions (400 gm each), soaked in water (1:5 w/v) for 6, 12 and 18hrs at room temperature. Later, the same samples were used further for germination process. The excess water was drained and each portion of soaked example was again divided into three portions independently and the process of germination was carried out for 24, 48 and 72hrs respectively at room temperature. Germinated samples were dried in a cabinet drier (Labfit, India) at 50, 60 and 70˚C for 6hrs till the accomplishment of consistent moisture content.

2.3 Source of standard procedure

Proximate analysis of raw horse gram was carried out by (Ranganna, 2011) i.e. moisture content by hot air oven method, fat content by extracting sample with petroleum ether, Protein content by micro-kjeldhal method, fiber by using FibroTRON, ash content by muffle furnace, carbohydrate by difference method. The calcium and iron were estimated by titration. Tannin and phytic acid were estimated by (Thimmaiah, 2016). Later on analysis was carried on for germinated samples in context to anti-nutritional factors.

III. RESULTS AND DISCUSSION

3.1 Results of nutritive analysis of raw horse gram

Table 3.1: Nutritive analysis

Parameters	Nutritional composition
Carbohydrate (g)	58.86±0.57
Protein (g)	21.06±1.05
Moisture (%)	10.3±0.21
Ash (%)	3.47±0.01
Crude fibre (g)	5.01±0.19
Fat (g)	1.36±0.05
Calcium (mg/100g)	281±0.37
Iron (mg/100g)	7.69±0.42

Values reported are mean ± SD of three replicates.

Table 3.1 displayed moisture, ash, protein, fat crude fibre, carbohydrate, iron and calcium content of raw horse gram. Horse gram has high healthy benefit equal to other commonly grown pulse crops in all viewpoints and also an excellent source of iron, molybdenum and calcium (Bhokre *et al*., 2012). The carbohydrate content was found to be 58.86±0.57g. Carbohydrates content extents from 50-60% in normally consumed pulses. Carbohydrate content present in horse gram was 58.32±0.0 and 57.12±0.72 % respectively in (Marimuthu and krishnamoorthi, 2013; Abhirami *et al*., 2018).

Horse gram is least expensive source of protein for humans. Averagely, horse gram seed contains 22–24% protein, which is contrast to commonly consumed pulses like chickpea, pigeon pea, green gram and black gram however, due to varietal difference large difference is observed in protein content ranging from 18.5–31.16% in horse gram (Bhartiya *et al.*, 2015). The protein content was found to be 21.06±1.05g (Table 3.1). Protein content present in horse gram was 22.5±1.0% and 22.12±0.11 (Sreerama *et al*., 2012; Marimuthu *et al*., 2013). Protein contains more lysine content than pigeon pea and chick pea making it a great supplement to a grain based eating routine (Prasad *et al*., 2010).

The moisture content was found to be 10.3±0.21% (Table 3.1). The moisture content was about 10.2-13.0% and 10.60%, respectively (Sudha *et al*., 1995; Thirukkumar and sindumathi 2014). However, moisture content in seeds depend upon the stage and time of harvesting of the crop as it is generally on higher side (18-25%) at the time of harvesting and 9-12% is the ideal range for safe storage in pulses (Mohan *et al*., 2011). The ash content was found to be 3.47±0.01% (Table 3.1). Ash content ranges from 3.30% and 3.34%, respectively (Thirukkumar and sindumathi 2014; Ramteke *et al*., 2016). Higher ash content is characteristic of high mineral content. Horse gram is also used as leafy vegetable and its leaves contain generally high content (4.50%) of minerals when compared with other common vegetables (1.5–2.4%) (Mandle *et al*., 2012).

The fiber content was found to be 5.01±0.19g (Table 3.1). Sufficient dietary fiber is basic for appropriate working of the gut and has also been related with hazard decrease for various diseases including coronary illness, certain cancers and diabetes. Fiber incorporates pectin, gum, adhesive, cellulose, hemicelluloses and lignin (Khogare *et al*., 2012). Fiber content present in horse gram was 5.23±0.15g and 5.3g (Moktan and ojha, 2016; Kamboj and nanda, 2017).The fat content was found to be 1.36±0.05g (Table 3.1). The fat content of horse gram ranges from 1.33% and 1.30%, respectively (Sudha *et al*., 1995; Abhirami *et al*., 2018).

The calcium and iron content was found to be 281±0.37 and 7.69±0.42mg/100g respectively (Table 3.1). It is a genuinely, rich source of calcium which was 287, 287 and 289 in entire seed (Gopalan *et al*., 1999; Kamboj and nanda, 2017; sadawarte *et al*., 2018). Crude horse gram contains iron content ranging from and 5.89–7.44 mg and 7.03mg (Khatun *et al*., 2013; Moktan and ojha, 2016).

3.2 Results of tannin content (% reduction) after germination in horse gram

Table 3.2: Tannin content

S	G	D	Reduction (%)	S	G	D	Reduction (%)	S	G	D	Reduction (%)
6	24	50	10.97	12	24	50	28.21	18	24	50	40.75
		60	12.22			60	28.84			60	41.37
		70	12.85			70	29.78			70	42.00

	48	50	18.18		48	50	31.97		48	50	45.45
		60	18.80			60	32.91			60	46.08
		70	19.43			70	33.86			70	47.03
	72	50	22.58		72	50	35.11		72	50	51.73
		60	23.83			60	36.06			60	52.67
		70	24.77			70	36.37			70	53.61

S-Soaking time (hrs), G- Germination time (hrs), D- Drying temperature (℃), Reduction- %

Table 3.2 displayed percent reduction of tannin content after soaking, germination and drying at different variables. The tannin content of raw horse gram seeds was found to be 319mg/100g. It was found that treatment having 6hrs soaking, 24hrs germination and drying at 50℃ gave minimum decrease and treatment having 18hrs soaking, 72hrs germination and drying at 70℃ gave maximum decrease in the tannin. Tannin content of raw horse gram demonstrated a decrease with an increase in soaking time (Handa *et. al.*, 2017, Moktan and ojha, 2016). The decrease in tannin content is mostly because of the fact that these compounds are present in their seed coats (Reddy and Pierson, 1994). Polyphenolase activity during germination causes loss of tannins in grains (Reddy *et. al.*, 1985).

3.3 Results of ANOVA for Tannin content (Percent Reduction)

Table 3.3: ANOVA for Tannin content

Source	D.F.	S. S.	M. S.	F_{CAL}	F_{TAB}	S. EM	CD	Test
Soaking (S)	2	11013.507	5506.753	605137.790	3.172	0.018	0.052	**
Germination(G)	2	1342.971	671.485	73789.660	3.172	0.018	0.052	**
S X G	4	74.178	18.544	2037.860	2.544	0.032	0.090	**
Drying (D)	2	36.306	18.153	1994.860	3.172	0.018	0.052	**
S X D	4	0.252	0.063	6.930	2.544	0.032	0.090	**
G X D	4	0.266	0.066	7.330	2.540	0.032	0.090	**
S X G X D	8	1.385	0.173	19.030	2.110	0.055	0.156	**
Error	54	0.491	0.009					
CV%	0.294							

* Significant at 5 % level of significance

Table 3.3 shows ANOVA for Tannin content. After germination and drying significant effect was observed for soaking, germination, drying, interaction of Soaking-Germination, Soaking-Drying, Germination-Drying and Soaking-Germination-Drying on Percent reduction in tannin content at 5 % significance level as their F_{CAL} was more than F_{TAB}.

3.4 Results of phytic acid content (% reduction) after germination in horse gram

Table 3.4: Phytic acid content

S	G	D	Reduction (%)	S	G	D	Reduction (%)	S	G	D	Reduction (%)
6	24	50	22.22	12	24	50	44.54	18	24	50	53.80
		60	22.61			60	44.73			60	54.09
		70	23.20			70	45.12			70	54.38
	48	50	32.36		48	50	46.97		48	50	56.23
		60	32.56			60	47.07			60	56.82
		70	33.05			70	47.47			70	57.01

	72	50	39.09		72	50	50.20		72	50	58.77
		60	39.28			60	50.49			60	58.96
		70	39.66			70	50.69			70	59.26

S-Soaking time (hrs), G- Germination time (hrs), D- Drying temperature (˚C), Reduction- %

Table 3.4 shows that percent reduction of phytic acid content. Raw horse gram seeds contained 10.26mg/g of phytic acid. It was found that treatment having 6hrs soaking, 24hrs germination and drying at 50˚C gave minimum decrease and treatment having 18hrs soaking, 72hrs germination and drying at 70˚C gave maximum decrease in the phytic acid. Phytic acid content of horse gram diminishes with increase in soaking time. During sprouting, phytic acid, a phosphate reserve corrupts because of the activity of phytase which is used by growing seedling (Mamudu *et al.*, 2005). Phytase activity during germination, resulting in hydrolysis of phytate phosphorus, adds to the reduction in phytic acid. The liberated phosphorus is possibly shipped to the embryo for further synthesis of organic phosphates. The rise in phytase action during seed germination could be because of activation of the pre-existing enzyme (Ayet *et al.*, 1997). Simultaneously inorganic phosphorus was liberated because of breakdown of phytic acid (kim *et al.*, 1984).This has been attributed to an increase of phytase activities. In fact, this enzyme makes the phytates soluble and discharges dissolvable protein and minerals (Khattak *et al.*, 2007). The soaking, germination and drying out of horse gram resulted in a decrease in phytic acid content.

3.5 Results of ANOVA for phytic acid content (Percent Reduction)

Table 3.5: ANOVA for phytic acid content

Source	D.F.	S. S.	M. S.	F_{CAL}	F_{TAB}	S.EM	CD	Test
Soaking (S)	2	8667.412	4333.706	335946.200	3.172	0.022	0.062	**
Germination(G)	2	1116.301	558.150	43267.470	3.172	0.022	0.062	**
S X G	4	404.021	101.005	7829.870	2.544	0.038	0.107	**
Drying (D)	2	5.375	2.687	208.330	3.172	0.022	0.062	**
S X D	4	0.197	0.049	3.810	2.544	0.038	0.107	**
G X D	4	0.097	0.024	1.870	2.540	0.038	NS	NS
S X G X D	8	0.226	0.028	2.190	2.110	0.066	0.186	**
Error	54	0.697	0.013					
CV%	0.251							

* Significant at 5 % level of significance

Table 3.3 displayed ANOVA for phytic acid content. After germination and drying significant effect was observed for soaking, germination, drying, interaction of Soaking-Germination, Soaking-Drying and Soaking-Germination-Drying on Percent reduction in phytic acid content at 5 % significance level as their F_{CAL} was more than $F_{TAB.}$ After germination and drying there was no significant effect was observed for interaction of Germination- Drying on percent reduction in phytic acid content at 5 % significance level as their F_{TAB} was more than $F_{CAL.}$

Fig. 1(Soaking 6hrs; Germination 24hrs)

Fig. 2(Soaking 18hrs; Germination 72hrs)

IV. CONCLUSION

Soaking, germination and dehydration by different variations of horse gram seeds impacted the properties of the horse gram. In view, of the anti-nutritional properties, it was discovered that the sample treatment having 6hrs soaking, 24hrs germination and drying at 50˚C gave less reduction in the anti-nutritional factors i.e. tannin content and phytic acid content were reduced by 10.97% and 22.22% respectively and treatment having 18hrs soaking, 72hrs germination and drying at 70˚C was the best where most decrease in the anti-nutritional factors i.e. Tannin content and phytic acid content were reduced by 53.61% and 59.26% respectively. Tannin content and phytic acid content of horse gram showed a decrease with an increase in the soaking, germination time and dehydration temperature. Thus, it is concluded that 18hrs soaking, 72hrs germination and drying at 70˚C can be considered as the ideal conditions to decrease anti-nutritional content of horse gram flour, which can additionally be used for the preparations of different value-added food products. Significant changes induced by soaking and germination would make the horse gram as one of the suitable legume foods to be incorporated in the everyday diet of all age group people.

V. REFERENCES

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