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Effect of germination on proximate and phytochemical content of horse gram and green gram malt

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Abstract
The present study was conducted to investigate the effect of germination on proximate and phytochemical content of horse gram and green gram malt. The horse gram and green gram were soaked in water for about 72 hrs to get the sprout. It was cabinet dried to obtain the malt. The proximate and phytochemical content of horse gram reveled that, it had moisture (6.76%), protein (22.31%), fat (1.32%), ash (3.54%) and carbohydrate (62.46%) and phytochemicals such as phenol (2.86 mg GAE/100g), flavonoids (2.45 mg/100g), alkaloids (0.54 g/100g) and tannins (0.102 g/100g). The proximate composition of green gram was found to be moisture (9.86%), protein (23.42%), fat (1.42%), ash (3.52%) and carbohydrate (63.86%). The phenol, flavonoids, alkaloid and saponin content of green gram was found to be 4.14 mg GAE/100g, 3.36 mg/100g, 0.78 and 0.128 g/100g. After germination period of 72 hrs, the moisture, fat and ash content were decreased over germination. The phytochemical content were reduced after germination. Hence it was showed the germination provides the nutritional value to processed products.

Keywords: Horse gram, Green gram, sprout, malt, phytochemical content

Introduction
Cereals and legumes are the foodstuffs for most humans and animals and have been throughout recorded history. To extract "maximum nutrients for minimum costs," the seeds of those plants have usually been treated by germinating, fermenting or selectively heat treating to increase the amount or availability of nutrients. Legumes are among the earliest food crops cultivated by man. Grain legumes or pulses belong to the family *Leguminaceae* and sub family *Papillionaceae,* occupies an important place in the world food. Dependence of humans on plant species is inevitable, but continued dependence on a few crops is risky in terms of evolution of virulence leading to large scale crop failures. Grain legumes constitute an important source of protein for majority of population in India. But over the last two decades, production and productivity of most of the pulse crops have either declined or stagnated. This has resulted a decline in per capita availability of pulses from 64 g (1951) to 47.78 g/day (Anonomous, 1995) [1].

The horse gram, *Macrotyloma uniflorum* (*Fabaceae*) is normally used to feed horses, though it is also commonly used in dishes. In traditional ayurvedic cuisine, horse gram is considered a food with medicinal qualities. It is prescribed for persons suffering from jaundice or water retention and as part of a weight loss diet. Although rich in proteins (20 %), due to less acceptable taste and flavor of cooked products, it is consumed only by the farming community and low-income groups. Thus, it has remained an underutilized food legume. Such grain legumes are however, potential sources for preparation of protein products like concentrates and isolates. The residue left over after separation of proteins can be further processed to obtain starch. The isolated legume starches have variety of applications in food industry. Consumption of seeds and sprouts has become increasingly popular among people interested in improving and maintaining their health status by changing dietary habits. The seeds and sprouts are excellent examples of 'functional food', lowering the risk of various diseases and exerting health promoting effects.

The mung bean (*Vigna radiata*) alternatively known as the moong bean, green gram, or mung, is a plant species in the legume family. Green gram is one of the important pulse crop in India. It has been reported that Green gram has been cultivated in India since ancient times**.**

It disbelieved that green gram is a native of India and Central Asia and grown in these regions since prehistoric times. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, Indonesia, Malaysia, south China, and Formosa. In Africa and U.S.A. it is probably recent. Green gram is a protein rich staple food. It contains about 25 percent protein, which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. It is particularly rich in Leucine, Phenylalanine, Lysine, Valine, Isoleucine, etc.

In the present study, the effects of sprouting on quality parameter such as proximate and phyto-nutrient content of their malt were determined. As, it is necessary to develop the value added food products from locally available legumes.

Materials and Methods

Horse gram (*Macrotyloma Uniflorum*) and Green gram (*Vigna Radiata*) *Var*. i.e. Shining Moong will be procured from the local market of Parbhani.

Analysis of Phyto-nutrients

The non-nutrients such as total free phenol, flavonoids, alkaloidss and saponins were estimated from raw and processed horse gram seeds. The methods adopted for phyto-nutrient analysis are as follows:

Determination of total phenolic content

Total phenol content (mg/100g) of sample was determined by spectrophotometric method given by (Pinelo *et al.,* 2005) [2]. 0.2 ml of aliquot was pipette out into test tubes and 3ml volume was made up with water. The 0.5 ml folin ciocalteu reagent was added to each tube. After 3 minutes 2 ml of 20% sodium carbonate solution was added and mixed thoroughly. The tubes were placed for exactly one minute in boiling water followed by cooling. The absorbance was then measured at 650nm using spectrophotometer against a reagent blank. The total phenolic contents in all samples was calculated the using the formula,

$$C = c\ V/m$$

Where, C = total phenolic content mg GAE/g dry extract
c = concentration of gallic acid obtained from calibration curve in mg/mL
V = volume of extract in ml
m = mass of extract in gram.

Determination of total flavonoid content

The total flavonoid content of cold and hot extracts was determined using a slightly modified method reported by Meda *et al*., (2005) [3]. A 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 mol L^{-1} potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using formula,

$$TFC = \frac{A \times DF}{A^{1\%}_{1cm} \times (w - ld)}$$

Where, A = Absorbance
DF = Dilution Factor
$A^{1\%}_{1cm}$ = Specific absorption by $AlCl_3$
w = Mass of plant material
ld = Loss on drying

Determination of alkaloid

Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harborne, 1993)[4].

$$\text{Alkaloids (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

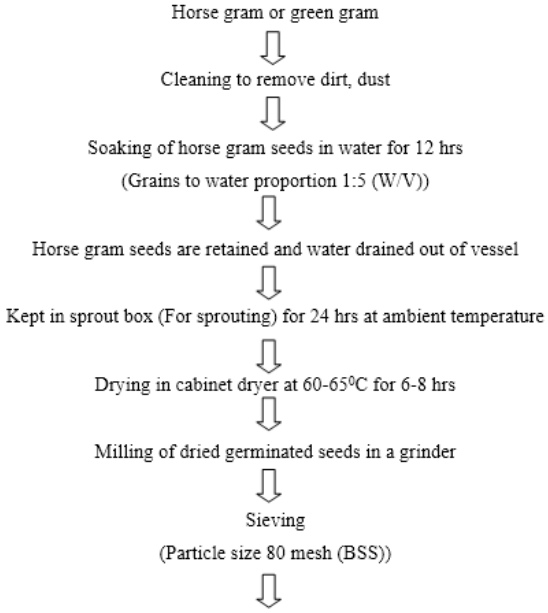
Where, W_1 = Initial weight of sample,
W_2 = Weight of the extract and
W_3 = Final weight of the residue

Determination of saponin

Two gram of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was filtered using Whatman filter paper no.1 into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using Whatman filter paper No. 1 to obtain a clean colourless solution. One ml was added into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm (Bruneton, 1999) [5].

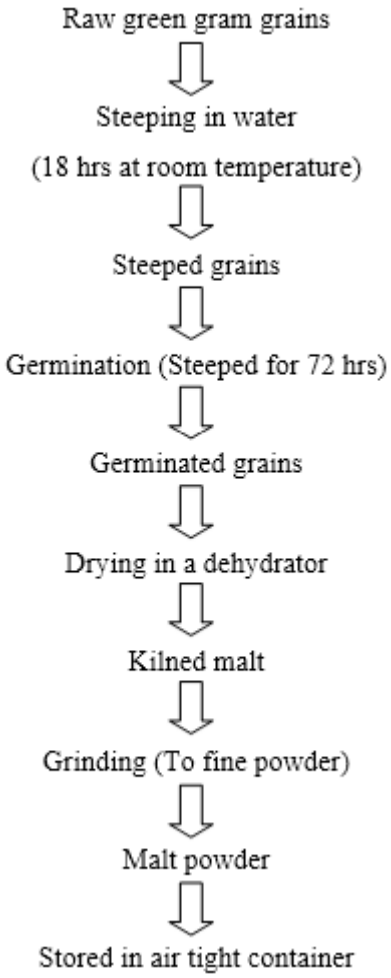
$$\text{Saponin} = \left[\frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}} \right] \cdot 1$$

Preparation of horse gram malt



Flowchart 1: Preparation of horse gram malt

Preparation of green gram malt



Flowchart 2: Preparation of green gram malt

Results

Proximate composition of horse gram and green gram

The nutrient composition of legumes is an important aspect to be considered before its value addition in products. It will enhance the nutritional value of the prepared products. Hence, the nutrient composition of horse gram and green gram was calculated and presented in Table 3.

Table 3: Proximate composition of horse gram and green gram

Parameters	Horse gram	Green gram
Moisture (%)	6.76 ± 0.50	9.86 ± 0.50
Crude Protein (%)	22.31 ± 0.50	23.42 ± 0.50
Fat (%)	1.32 ± 0.50	1.42 ± 0.50
Ash (%)	3.54 ± 0.50	3.52 ± 0.50
Crude carbohydrate (%)	62.46 ± 0.50	63.86 ± 0.50

Table 3 showed the analysed proximate composition namely, moisture, protein, fat, ash and carbohydrate of horse gram and green gram. The horse gram had 6.76% moisture, 22.31% crude protein, 1.32% fat, 2.47% ash and 62.46% crude carbohydrate. Similar results for proximate composition of horse gram were reported by Marimuthu and Krishanamoorthi (2013) [6].

The green gram is found rich in carbohydrate content (63.86%) as a macro nutrient. The crude protein, fat and ash content of green gram were found to be 23.42, 1.42 and 2.64% respectively. The legumes had less moisture was the reason for their long storage at ambient temperature. The green gram was found to contain moisture about 9.86%. Similar results for proximate composition of green gram were also reported by Paul *et al*., (2011) [7].

Protein, fat and carbohydrate are also referred as proximate principles. They are oxidized in the body to yield energy which the body needs. Although protein provides energy, their primary function is to provide amino acids for building body proteins. The higher true protein means that there were lesser amounts of non proteinaceous substances like nitrites and nitrates (Kadwe *et al*., 1974) [8]. Fat is an important component of diet and serves a number of functions in the body. Fats being concentrated source of energy provide per unit weight more than twice the energy furnished by either protein or carbohydrate. Presence of fat in the diet is required for the absorption of fat soluble vitamins like vitamin A and carotene present in the diet (Sood *et al*., 2002) [9].

Phytochemical content of horse gram and green gram

Food contains a wide range of organic chemical compounds, which have no nutritional functions. Some of these compounds however act as phytonutrients. The phytochemcials such as total phenols, flavonoids, alkaloids and saponins are estimated and presented in Table 7.

Table 4: Phytochemical content of horse gram and green gram

Phytochemicals	Horse gram	Green gram
Total Phenol (mg GAE/100g)	2.86	4.14
Flavonoids (mg/100g)	2.45	3.36
Alkaloids (g/100g)	0.54	0.78
Saponins (g/100g)	0.102	0.128

Pulses are good sources of phytonutrients. The total phenol and flavonoid content of horse gram were found to be 2.86 mg GAE/100g and 0.45 mg/100g. Alkaloid and saponin content were less as compared to phenols which is 0.54 and 0.102 mg/100g. The phytochemical content of horse gram was found in similar with the results obtained by Sundaram *et al*., (2013) [10].

The total phenol and flavonoid content in green gram were 4.14 mg GAE/100g and 1.36 mg/100g. Total phenolics and flavonoid contents of green gram were similar with the finding of Aggarwal and Sharma (2017) [11]. Also alkaloid and saponin content were 0.78 and 0.128 respectively.

The phenolic compounds may have high antioxidant activity, which means that they could have positive effects on the preservation of foods quality and human health (Becker *et al*., 2004) [12]. Flavonoid has antioxidant activity and therefore lower cellular stress. A great number of reports have established that plant phenolic compounds including flavonoids are potent antioxidants with reported anti-mutagenic and anti-carcinogenic effects (Middleton and Kandaswami, 1994) [13].

Effect of germination on proximate composition of horse gram and green gram

Germination changes the nutritional composition of legumes with varying the period of germination. Hence the proximate composition such as protein, carbohydrate, fat and ash content were analyzed after germination of horse gram and green gram and obtained results were presented in Table 13.

Table 5: Effect of germination on proximate composition of horse gram and green gram

Parameters	Horse gram malt	Green gram malt
Moisture (%)	5.11	5.91
Crude Protein (%)	24.99	24.82
Fat (%)	1.03	1.37
Ash (%)	2.47	2.64
Crude carbohydrate (%)	64.31	66.36

The moisture content of horse gram and green gram was found to be reduced to 5.11 and 5.91% due to the drying. The protein content of horse gram and green gram were increased on germination for a period of 24 hrs and found to contain 24.99 and 24.82%. An increase in protein content might be due to biosynthesis of protein as a result of germination. The marginal increases in the total protein are not in fact real ones but merely the result of dissolution of starch content into the soaking medium. Similarly a decreasing pattern of starch has been reported during soaking/germination of corn at ambient conditions (Sattar *et al*., 1989) [14]. Similar impact of germination on proximate composition of horse gram was also reported by Moktan and Ojha (2016) [15].

Fat content of horse gram and green gram was reduced on germination. The horse gram and green gram was analyzed for fat content and found 1.03 and 1.37%. The decrease in fat content might be due to use of fatty acid for energy for the germination (Hahm *et al*., 2008) [16]. The ash content were found to be decreased on germination. The ash content in horse gram and green gram was found to be 2.47 and 2.64%. The decrease in ash content represents loss in minerals due to rootlet and washing of the rice in water to reduce the sour smell during the period of germination (Tatsadjieu *et al*., 2004) [17]. The carbohydrate content was increased on malting in horse gram (64.31%) and green gram (66.36%). Twice as much energy as carbohydrates, the reduction, in Fat content observed during germination a reduction in the energy value. The result showed that the carbohydrate content was increased with in steeping and germination period. Similar trends for germinated mung bean were also reported by Rusydi *et al*., (2011) [18].

Effect of germination on phytochemical content of horse gram and green gram

The effect of germination on phytochemicals such as total phenol, flavonoids, alkaloids and saponin was studied. The results pertaining to phytochemical content of germinated horse gram and green gram was presented in Table 16.

Table 6: Effect of germination on phytochemical content of horse gram and green gram

Phytochemicals	Horse gram malt	Green gram malt
Total Phenol (mg GAE/g)	1.06	1.024
Flavonoids (g/100g)	5.8	5.5
Alkaloids (g/100g)	0.31	0.44
Saponins (g/100g)	0.064	0.086

The data from Table 16 revealed that, the total phenols were reduced after germination. The total phenol content in horse gram and green gram malt was found to be 1.06 and 1.024 mg GAE/g. Randhir *et al*. (2004) reported that germination causes a decrease of total phenolic content in Green mung. Barroga *et al*. (1985) [19] reported similar total phenolic content values for raw and 24 h germinated Mung bean. Fernandez-Orozco *et al*. (2008) [20] reported that the total phenolic content significantly decreased after 2 days of germination but then increased as germination time increased to 4 days. During germination, the endogenous enzymes of the legumes are activated and the most important enzymes are the hydrolases and polyphenoloxydases, whose activity increases during germination depending on the type of legume.

The intake of foods rich in flavonoid protects human against diseases associated with oxidative stress. The mechanisms of action of flavonoids are through free-radical scavenging or chelating process and protection against oxidative stress (Shahidi and Naczk 1995) [21]. The flavonoid content of germinated horse gram and green gram were found to be 5.8 and 5.5 g/100g.

The alkaloids and saponin content were reduced due to germination which cose leached it into water. The alkaloid and saponin content in horse gram malt were found 0.31 and 0.064 g/100g. Whereas alkaloid and saponin in green gram malt were found 0.44 and 0.086 g/100g. This reduction is directly correlated with the reduction in total phenol. The decrease in saponin level in the legumes during soaking may be attributed to their leaching out into soaking media. Katariya and Chauhan (1988)[22] reported that germination reduces saponin content of various pulses and showed 48 hrs sprouting results in 22% reduction of saponin content in mung bean.

Conclusion

It was concluded from the present study, the sprouting had significant effects on quality characteristics of horse gram and green gram malt. The moisture, fat and ash content were reduced after germination while protein and carbohydrate was found to be increased over sprouting. Hence, it could be concluded that the germination process is helpful in preparation of nutritionally rich malt. The Phytonutrients such as alkaloids, flavonoids, phenol and tannins were found to be decreased over sprouting which includes their anti-nutritional factors.

References

1. Anonymous. Food and Agriculture Statistics Series no. 125, Year Book, 1994. Food and Agriculture organization of United Nations, Rome, 1995, 48.
2. Pinelo M, Rubilar M, Jerez M, Sineiro J, Nunez MJ. Effect of solvent, temperature, and solvent to solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. Journal of Agricultural and Food Chemistry. 2005; 53(6):2111-2117.
3. Meda A, Lamicn CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina fasan honey, as well as their radical scavenging activity. Food chemistry. 2005; (91):571-577.
4. Harborne JB. Phytochemistry. Academic press, London, 1993, 89-131.
5. Bruneton J. Pharmacognosy, phytochemistry of medicinal plants. 2nd edition intercept Limited, Hampshire, United Kingdom, 1999, 385-386.

6. Marimuthu M, Krishnamoorthi K. Nutrients and functional properties of horse gram (*Macrotyloma Uniflorum*), an underutilized south Indian food legume. Journal of Chemical and Pharmaceutical Research. 2013; 5(5):390-394.
7. Paul T, Rubel Mozumder NHM, Sayed MA, Akhtaruzzaman M. Proximate compositions, mineral contents and determination of protease activity from green gram (*Vigna radiata* L.). Bangladesh Research Publications Journal. 2011; 5(3):507-513.
8. Kadwe RS, Thakare KK, Badhe NN. A note on the protein content and mineral composition of twenty-seven varieties of pulses. Indian Journal of Nutrition Dietetics. 1974; 11(2):83-85.
9. Sood M, Malhotra SR, Sood BC. Effect of processing and cooking on proximate composition of chickpea (*Cicer arietinum*) varieties. Journal of Food Science and Technology. 2002; 39(1):69-71.
10. Sundaram U, marimuthu M, Anupama V, Gurumoorthi P. Comparative antioxidant quality evaluation of underutilized/less common south Indian legumes. International Journal of Pharma and Biosciences. 2013; 4(2):117-126.
11. Aggrawal S, Sharma S. Phytrochemical profile and antioxidant activity of green gram whole (Phasiolus aureus roxb), International Journal of Engineering Sciences and Research Technology. 2017; 6(7):657-661.
12. Becker EM, Nissen LR, Skibsted LH. Antioxidaant evaluation protocol: food quality or health effects. European Food Research and Technology. 2004; 219: 561-571.
13. Middleton EJ, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B. (Ed.), The Flavonoids. Chapman & Hall, London. 1994, 619-652.
14. Sattar SK, Durrani F, Mahmood A Ahmad, Khan I. Effect of soaking and germination temperatures on selected nutrients and antinutrients of mung bean. Food Chemistry. 1989; 34:111-120.
15. Moktan K, Ojha P. Quality evaluation of physical properties, antinutritional factors, and antioxidant activity of bread fortified with germinated horse gram (*Dolichus uniflorus*) flour. Food Science and Nutrition. 2016; 4(5): 766-771.
16. Hahm T, Park S, Lo YM. Effects of germination on chemical composition and functional properties of sesame (*Sesamum indicum* L.) seeds. *Bioresources Technology*. 2008; 100:1643-1647.
17. Tatsadjieu NL, Etoa FX, Mbofung CMF. Drying Kinetics, Physicochemical and Nutritional Characteristics of Kindimu, a Fermented MilkBased-Sorghum-Flour. The Journal of Food Technology in Africa. 2004 9(1): 17-22.
18. Rusydi MR, Noraliza CW, Azrina A, Zulkhairi A. Nutritional changes in germinated legumes and rice varieties. International Food research Journal. 2011; 18: 705-713.
19. Barroga CF, Laurena AC, Mendoza MT. Poly-phenols in mung bean (*Vigna radiata* L Wilczek): determination and removal. Journal of Agriculture Food Chemistry. 1985; 33:1006-1009.
20. Fernandez-Orozco R, Frias J, Zielinski H, Piskula MK, Kozlowska H. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emerald, *Glycine max* cv. jutro and *Glycine max* cv. merit. Food Chemistry. 2008; 111:622-630.
21. Shahidi F, Naczk M. Phenolic compounds in grains. In: food phenolics; source, Chemistry Effect Applications, Technomic Publishing Company Inc. Lancaster PA. 1995, 3-39.
22. Katariya A, Chauhan BM. Contents and digestability of carbohydrate of mung bean (*Vigna radiata*) as affected by domestic processing and cooking. Plant Foods Hum Nutr. 1988; 38(1):51-59.