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

Sadawarte SK, Pawar VS, Sawate AR, Thorat PP and J Surendar

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 revealed 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, alkaloids 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) \[2\].

\[C = \frac{c V}{m}\]

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,

\[\text{TFC} = \frac{A \times \text{DF}}{A_{1\%1cm} \times (w - \text{ld})}\]

Where, \(A\) = Absorbance
\(\text{DF}\) = Dilution Factor
\(A_{1\%1cm}\) = Specific absorption by AlCl\(_3\)

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) \[4\].

\[\text{Alkaloids (}% = \frac{W_1 - W_2}{W_1} \times 100\]

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) \[5\].

\[\text{Saponin} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}\]

Preparation of horse gram malt

Flowchart 1: Preparation of horse gram malt
Preparation of green gram malt

Raw green gram grains

Steeping in water (18 hrs at room temperature)

Steeped grains

Germination (Steeped for 72 hrs)

Germinated grains

Drying in a dehydrator

Killed malt

Grinding (To fine powder)

Malt powder

Stored in air tight container

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Horse gram</th>
<th>Green gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>6.76 ± 0.50</td>
<td>9.86 ± 0.50</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>22.31 ± 0.50</td>
<td>23.42 ± 0.50</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.32 ± 0.50</td>
<td>1.42 ± 0.50</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.54 ± 0.50</td>
<td>3.52 ± 0.50</td>
</tr>
<tr>
<td>Crude carbohydrate (%)</td>
<td>62.46 ± 0.50</td>
<td>63.86 ± 0.50</td>
</tr>
</tbody>
</table>

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 nitrates and nitrites (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 phytochemicals 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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Horse gram</th>
<th>Green gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phenol (mg GAE/100g)</td>
<td>2.86</td>
<td>4.14</td>
</tr>
<tr>
<td>Flavonoids (mg/100g)</td>
<td>2.45</td>
<td>3.36</td>
</tr>
<tr>
<td>Alkaloids (g/100g)</td>
<td>0.54</td>
<td>0.78</td>
</tr>
<tr>
<td>Saponins (g/100g)</td>
<td>0.102</td>
<td>0.128</td>
</tr>
</tbody>
</table>

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.
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 (Tatsadju 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 5: Effect of germination on proximate composition of horse gram and green gram

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Horse gram malt</th>
<th>Green gram malt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>5.11</td>
<td>5.91</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>24.99</td>
<td>24.82</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.03</td>
<td>1.37</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.47</td>
<td>2.64</td>
</tr>
<tr>
<td>Crude carbohydrate (%)</td>
<td>64.31</td>
<td>66.36</td>
</tr>
</tbody>
</table>

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 polyphenoloxidases, 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 case 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


